ADVANCES IN FOOD RESEARCH

VOLUME II

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Foreword

In the foreword of Volume I the editors pointed out that food research during the past few years has been accelerating and expanding and that this has been accompanied by the realization of the importance of fundamental as well as applied research. The fields of interest in food research have increased and the number of institutions engaged in these researches is greater than ever before. The results of these researches appeared in a large number and variety of scientific journals. In view of these developments it has been nearly impossible for one to keep informed in more than a very restricted area of food research. *Advances in Food Research* has been offered as a partial fulfillment of the need for coordination, integration and the promotion of orderly and systematic development of scientific knowledge in the general field of food research.

It was further pointed out in Volume I that subject matter areas in food research fall under several headings and that it is the plan of the editors to cover intensively various phases of these areas as successive volumes appear. The subject matter areas are: agriculture, biochemistry and histology, entomology and zoology, food acceptance, food technology and engineering, and commodities. A number of these areas are represented by the contributions in this volume of *Advances in Food Research*. A brief statement concerning each of these reviews is given below.

Marked advances have been made in the engineering, design, operation and theory of spray driers for a variety of food products such as eggs, milk, coffee, yeast, etc. Although some of the information pertaining to these advances has found its way into journals, much of it is generally unavailable. Some of the published material has appeared in little-known Japanese journals, but even more important is the vast amount of information that has reposed in the private files of the manufacturers and users of spray driers. In preparing their review, Seltzer and Settelmeyer have not only included a resumé of published information but also much of the heretofore unavailable information relating to design, operation, costs and use for specific food products. To our knowledge this is the first inclusive review on the spray drying covering engineering as well as technology.

The industrial use of ion exchangers in the food industries has been increasing at a rapid rate. Not only are they suitable for water treatment, but also for the concentration of valuable ions or the removal of small quantities of ionic impurities from larger quantities of non-electrolytes; as in the purification of sugars, pectin and protein solutions. In his review on "Ion Exchange Application by the Food Industry," Felton has

presented information relative to various types of ion exchangers, factors influencing their action and their application to the food industry.

In preserving food by thermal processing there is always the necessity of using a process time and temperature sufficient to preserve the product and to protect the consumer against the possibility of bacterial poisons. At the same time it is almost equally important that over-processing be avoided in order to minimize heat damage to the product. To arrive at such a process requires not only extensive experimentation but a thorough knowledge of the information available and the principles involved. The review by Stumbo on "Thermobacteriology as Applied to Food Processing," does much to fulfill this requirement.

Sanitation in the food industries is an ever-increasing requirement. During the past year at least one book and one new journal relating to sanitation in the food industries have appeared. There are many phases to the subject. One very important one pertains to surface active agents which are used so extensively for cleaning belts, tables, equipment, instruments, hands, etc. The quaternary ammonium compounds constitute a very important group of chemicals used for this purpose. Dunn, in his review, "The Quaternary Ammonium Compounds and Their Use in the Food Industry," has brought together the pertinent information on this subject.

In the course of a few years DDT has become one of the most important insecticides. It has found wide usage in the food industries, ranging from the control of insects and flies in the dairy barn, creameries, or grain storage, to the pear and apple orchard. Knowledge of the pharmacology of DDT and its effect on man has not kept pace with that relating to the control of insects. In his review "The Pharmacology of DDT," Lehman has brought together important published and heretofore unpublished information relating to this phase of the subject.

The area of food acceptance is complicated and may be divided into at least four phases; namely, difference testing, preference (quality) testing, consumer testing, and the physiology of acceptability. The general field of food acceptance is at present receiving considerable attention from food manufacturers. The philosophy of meeting consumers' desires rather than those of brokers, occupies greater attention from the manufacturer now than a few years in the past. Of equal importance to the food producer is to know if a change in his manufacturing process has resulted in an appreciable or detectable change in his product. To determine this requires the use of difference testing procedures which, if conducted properly, will give reliable answers, but if conducted improperly will give unreliable answers. The review by Boggs and Hanson, "Analysis of Foods by Sensory Difference Tests" summarizes the litera-

Spray Drying of Foods

By Edward Seltzer, *Continental Foods, Inc., Thomas J. Lipton, Inc., Hoboken, New Jersey*, and James T. Settelmeyer, *Maxwell House Division, General Foods Corporation, Hoboken, New Jersey*

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Ion Exchange Application by the Food Industry

By GEORGE E. FELTON

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I. Introduction

Ion exchange, defined by Walton (1941) "as a reversible interchange of ions between a liquid phase and a solid body which does not involve any radical change in the solid structure," was first observed by Way (1850). He noted that on passing a potassium chloride solution through soil, potassium was taken up and an equivalent amount of calcium and sodium was released to the solution. In addition to soil, many colloidal systems such as proteins, humic acids, pectin, hydrous oxides, soap, aluminosilicates and synthetic resins exhibit the phenomena of ion exchange in varying degrees of capacity.

The study of ion exchange in soils and other natural materials has led to the accumulation of a considerable fund of information concerning the controlling factors in this process. The commercial exploitation of naturally occurring zeolites was most important in the softening of hard waters, but the usefulness of the natural or synthetic aluminosilicates was limited since they were not stable under acidic or alkaline conditions.

Adams and Holmes (1935) demonstrated that condensation products of phenols with formaldehyde gave insoluble resins containing hydroxyl groups which were free to ionize and to react in the usual manner. This discovery has led to the development of a number of resinous products which contain reactive groups. These materials have become commonly known as ion exchangers and they have already found many uses in the laboratories and factories of the food industry.

The synthetic resin exchangers that have been developed since 1935 are stable over a wide range in pH and their various acidic or alkaline groups have greatly extended the practical limits of ion exchange applications. The desirable properties of these materials have even led to a considerable replacement of the older type zeolites in water softening installations. The use of ion exchange resins for water treatment has recently been reviewed by Myers (1946b) and Bauman (1945).

Ion exchangers are particularly suitable for the concentration of valuable ions which are present in solutions in such small amounts that they cannot be economically recovered by precipitation or evaporation procedures. The ions which have been concentrated by the exchanger can be recovered by conventional methods. The removal of small quantities of ionic impurities from larger quantities of nonelectrolytes is another important application of ion exchange materials. Examples of this type of use are in the purification of sugar, pectin, and protein solutions.

Ion exchangers are also finding many laboratory applications. They are valuable tools for the separation and fractionation of natural or synthetic basic or acid compounds, such as the amino acids and the rare

earth elements. Their uses in analytical chemical methods are continually increasing. They also may serve as catalysts for reactions such as esterification or hydrolysis. The general theory and application of ion exchangers has been reviewed by Nachod (1949).

II. Cation Exchangers

The principal cation exchangers may be divided into three types. These are the aluminosilicates, sulfonated coals, and synthetic resin exchangers. The physical and chemical properties of these materials are quite variable and limit the applications for which each are suited. In spite of these differences, the controlling factors in exchange reactions undoubtedly apply to all types. The information which has been accumulated since 1850 in the study of soils and natural zeolites is useful in interpreting the action of the more recently developed resinous exchangers. These synthetic organic resins are chemically homogeneous materials which makes them better suited for fundamental studies than the variable natural products.

1. Aluminosilicates

The various aluminosilicates which show ion exchange properties have been described by Walton (1941). He has pointed out that openness of structure in these materials is required if they are to possess appreciable exchange capacity. It is necessary that ions be able to move in and out of the solid freely if the mineral is to effect ion exchange. Although the importance of the openness of structure in resinous exchangers has not been stressed in the recent literature, it is a vital factor and will be discussed in detail in a later section. The degree of openness in silicate exchangers is fairly well shown by their volumes per structural oxygen atom which are given in Table I.

Volume per Structural Oxygen Atom in Silicate Exchangers [a]

Mineral	Formula	Volume per oxygen atom ($A.^3$)	Cation exchange
Garnet	$Mg_3Al_2Si_3O_{12}$	15.4	None
Muscovite (a mica)	$K_2Mg_6Al_2Si_6O_{22}\cdot H_2O$	19.2	Slight
Orthoclase (a felspar)	$K\ Al\ Si_3O_8$	23.0	Slight
Nosean	$Na_6Al_3Si_3O_{12}(SO_4)$	23.1	Fair
Ultramarine	$Na_6Al_3Si_3O_{12}(SO_4)$	23.1	Very Good
Apophyllite	$K\ Ca_4Si_8O_{20}F\cdot 8H_2O$	26.8[b]	Good
Natrolite (a zeolite)	$Na_2Al_2Si_3O_{10}\cdot 2H_2O$	28.0	Very Good
Glauconite	$K(Fe,Al)Si_2O_6$	about 28.0	Excellent

[a] Walton, (1941). [b] Fluorine atom is counted as an oxygen atom.

In addition to the natural inorganic ion exchangers, various synthetic aluminosilicates have been made by fusion or precipitation. The fusion products were prepared from quartz, clay, and soda ash. The precipitation products were made from mixtures of sodium silicate with sodium aluminate or aluminum sulfate. They were produced preferably as gels which could be dried to horny lumps that disintegrate into small granules on wetting with water. The formula of the gel type aluminosilicates is $Na_2O \cdot Al_2O_3 \cdot nSiO_2 \cdot H_2O$. The value of n is usually 5 or 6.

The gel type aluminosilicates are easily permeable to small ions. Walton (1941) has estimated that the pores of the gel are 4-5 A. in diameter. Trimethylammonium ions of 3.9 A. can enter them only with great difficulty and they are not permeable to sucrose molecules which have a diameter of about 5 A.

2. Sulfonated Coals

The treatment of bituminous coal with concentrated sulfuric acid or sulfur trioxide produces sulfonated coal which has an appreciable cation exchange power. The reactive groups appear to be not only sulfonic but also carboxylic acids. The treated product has about the same appearance as the original coal, however, it is hygroscopic and will pick up about 25% of moisture from the atmosphere. The sulfonated coals swell in water, with an increase in volume of 30 to 40% and further increase in volume about 40% on treatment with alkaline solutions, and are easily permeable to water and ions with a base exchange capacity of about 1.5 to 2.5 meq./g. They are insoluble in acids and therefore can be used to exchange hydrogen ions for other cations. The sulfonated coals are not entirely stable in alkaline solutions although their rate of deterioration is quite slow.

3. Resinous Cation Exchangers

a. Chemical Constitution. Synthetic resins which show cation exchange have been made from a wide variety of materials. The exact methods of preparation for many of the important cation exchangers have not been published. However, they were presumably largely made by various modifications of the phenol-formaldehyde condensation reaction until an aromatic hydrocarbon polymer containing nuclear sulfonic acid groups was developed (Bauman and Eichhorn, 1947).

The strong sulfonic acid group can be incorporated in the resin structure in three different ways: (a) by after treatment of a standard phenol-formaldehyde resin (Wassenegger and Jaeger, 1940); (b) by condensing phenols and formaldehyde in the presence of sodium sulfite to incorporate methylene sulfonic acid groups, $-CH_2SO_3H$ (Boyd *et al.*, 1947c); (c)

by the condensation of formaldehyde with *o*- and *p*-phenolsulfonic acid to give a nuclear sulfonic acid type with the $-SO_3H$ groups attached directly to the benzene ring (Bauman, 1946).

Examples of the methylene sulfonic acid type are presumably Amberlite IR-1 (Boyd *et al.*, 1947c) and the German exchanger Wolfatit P (Myers, 1946a). A large number of the other commercial cation exchangers are probably also of this type. The basic group in the structure will be shown by A in Fig. 1.

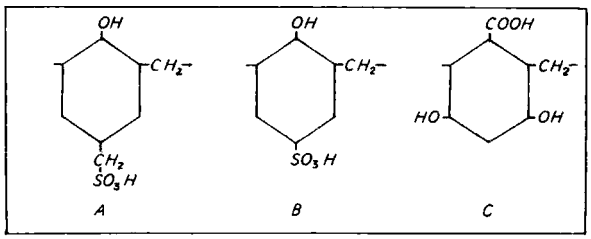

Fig. 1. Structure of cation exchangers.

The exchanger Dowex-30 is an example of the nuclear sulfonic acid type (Bauman, 1946) with basic structure B in Fig. 1. The production of this polymer has been described by Wassenegger and Jaeger (1940).

Cation exchangers containing carboxylic acid groups have also been prepared. An example is the German product, Wofatit C (Myers, 1946a), which is prepared by condensing resorcylic acid with formaldehyde. It has a basic structure which is shown by C in Fig. 1. Amberlite IRC-50 is a recent addition to the exchangers which contain carboxylic acid groups. This product is prepared as white spherical beads and has a total exchange capacity of about 10 meq./g. The titration curves for various types of cation exchangers were determined by Griessbach (1939) and are shown in Fig. 2.

In the various formulas, a portion of the four positions on the benzene ring are substituted by $-CH_2-$ cross links. The function of the cross links in the exchanger structure is to give insolubility and stability to the polymer. In many ion exchange applications the most important property of the exchanger is its capacity per unit of volume. If additional sulfonic acid groups are added to an exchanger without corresponding increase in cross links, the product may swell more so that the net effect is an actual decrease in capacity per unit of volume. The nature of the acidic groups will also have a bearing on the amount of cross linking required to give an insoluble product.

The organic cation exchangers are all decomposed by strong oxidizing agents such as chlorine, bromine, and chromic acid. It is therefore im-

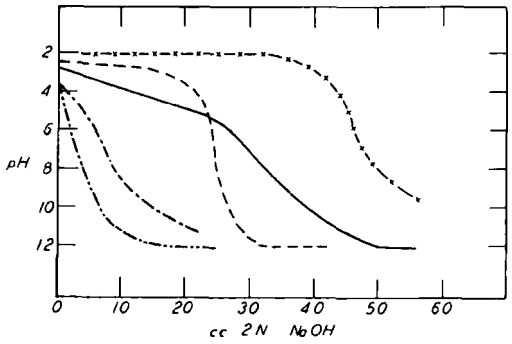

Fig. 2. Titration curves for cation exchangers (Griessbach, 1939).

	Designation *	*Active Group*
— x — x — x —	K Resin	—SO_3H
— — — — — —	A Resin	—CH_2SO_3H
————————	C Resin	—COOH
——— - - ——— - - ———	R Resin	—OH
——— — ——— — ———	Decationized greensand	

* Five grams of resin and 20 g. of greensand were used.

portant to avoid the use of such chemicals in an ion exchange system. There is a great variation among the exchangers with regard to their stability towards weaker oxidizing agents such as dilute nitric acid and oxygen. Many cation exchangers are rapidly attacked by dilute nitric acid with the evolution of gas. However, Dowex 30 has been reported (Bauman, 1946) to be unaffected by 10% concentrations of this acid at room temperature. Dowex 50 is even more stable and appears resistant to dilute nitric acid even at elevated temperatures.

The capacities of the resinous exchangers vary from about 1.5 to 5.0 meq./g. In view of the greater density of some of these exchangers, their capacities per unit of volume may be as much as five or six times that of the sulfonated coals.

b. Physical Structure. The resinous exchangers are usually regarded as being homogeneous gels (Bauman and Eichhorn, 1947; Boyd *et al.*, 1947c). The water of gelation is a vital part of the structure and its removal by excessive drying will greatly lower the adsorptive capacity. Remoistening of the dried exchanger will bring back its exchange power. The gel structure of most exchangers must be arranged so that reactive groups in the interior are available and the exchange capacity is almost independent of particle size. According to Boyd *et al.* (1947a), x-ray diffraction data show the reactive groups to be randomly dispersed throughout the interior of the usual cation exchangers.

The cation exchangers are usually supplied in granular particles rang-

ing from 10 to 70 mesh. Dowex 50, Amberlite IR-105 and Amberlite IRC-50 are available as spherical particles ranging from 12 to 50 mesh. One advantage of spherical particles is that void space is less with this uniform product. The densities of the exchangers as shipped may vary over a range of from 30 to 50 lb. per cubic foot.

III. Anion Exchangers

The anion exchangers are all organic amines, either primary, secondary, tertiary or quaternary. The various commercially available cation and anion exchangers are listed in Table II.

Table II

Commercial Cation and Anion Exchangers

Cation exchangers	Anion exchangers	Producers
Ionac C-284	Ionac A-293 Ionac A-300	American Cyanamid Company, New York, N. Y.
Duolite Expanded Cation Exchanger	Duolite A-2	Chemical Process Company, San Francisco, Calif.
Duolite C-3 Duolite Cation Selector No. 1	Duolite A-3 Duolite A-4	
Dowex 30 Dowex 50		Dow Chemical Company, Midland, Mich.
Zeo-Karb Zeo-Rex Permutit Q	De-Acidite	Permutit Company, New York, N. Y.
Amberlite IR-100 Amberlite IR-105 Amberlite IRC-50	Amberlite IR-4 B Amberlite IRA-400	Resinous Products and Chemical Company, Philadelphia, Pa.

Several types of basic polymers that show anion exchange properties have been patented. Condensation products of aromatic amines and formaldehyde have been described by Kirkpatrick (1938), Melof (1941; 1942), and Griessbach (1941). Aliphatic amines have also been used with formaldehyde and other materials to produce anion exchangers (Myers and Eastes, 1944; Bock, 1944). Swain (1941) produced basic polymers by condensing guanidine derivatives with formaldehyde and urea or melamine. The replacement of chlorine in a polymer by an acyclic ammonia-type organic compound was patented by Hardy (1942).

The anion exchangers are generally heat sensitive and are usually employed at temperatures below 100°F. However, Duolite A-3 is recom-

mended for use up to 140°F. and Ionac A-300 is reported to be stable even in boiling acid or alkaline solutions.

Although some earlier investigators thought that the amine type resins adsorbed acids molecularly instead of exchanging anions, it is now generally accepted (Kunin and Myers, 1947) that the anion resins react as true exchangers. The reaction of an anion exchanger in the hydroxide form with a hydrochloric acid solution is shown by the following reaction:

$$\text{R-OH} + \text{HCl} \rightarrow \text{R}\cdot\text{Cl} + \text{H}_2\text{O} \qquad (1)$$

The anion exchangers vary in their basicity and this factor may affect the adaptability of the resins for particular uses. Most anion exchangers are weak bases, and they are not able to adsorb anions in appreciable quantities from neutral or alkaline solutions. The recent introduction of Amberlite IRA-400 has added a strongly basic anion exchanger that has a practical capacity for adsorbing acids from neutral or mildly alkaline solutions. This exchanger will effectively remove from solution even such weak acids as silicic and hydrogen sulfide.

IV. Controlling Factors in Exchange Reactions

1. Rate of Diffusion

The exchange of a monovalent cation, such as sodium, for hydrogen from the acid form of the resin R is shown by the following reaction:

$$\text{Na}^+ + \text{HR} \rightarrow \text{NaR} + \text{H}^+ \qquad (2)$$

Boyd *et al.* (1947a) have pointed out that the completion of this exchange reaction may be divided into five steps: (1) diffusion of the sodium ion through the solution to the exchanger particle; (2) diffusion of the sodium and its accompanying anion through the adsorbent particle; (3) chemical exchange between sodium ion and hydrogen resin at the exchanging positions within the particle; (4) diffusion of the displaced hydrogen ion to the surface of the exchanger particle; (5) diffusion of the hydrogen ion away from the adsorbent particle. The slowest step in this series will determine which factor controls the rate of the exchange reaction. The experimental results of Boyd *et al.* (1947a) and Bauman and Eichhorn (1947) show that for solutions of 0.1 *M* or greater concentration the rate of diffusion through the exchanger particles is rate controlling. In more dilute solutions Boyd *et al.* (1947a) consider that permeation through a thin, enveloping liquid film is the controlling factor; however, Bauman and Eichhorn (1947) and du Domaine *et al.* (1943) consider the rate at low concentrations to be controlled by the chemical exchange reaction.

The rate of diffusion through the exchanger particles is much slower than through aqueous solutions. Bauman and Eichhorn (1947) found the rate of diffusion of hydrochloric acid and sodium chloride through Dowex 50 to be about one-fifth as great as in dilute aqueous solutions. Boyd *et al.* (1947a) found rates of diffusion from one-fifth to one-tenth as great through particles of Amberlite IR-1 as for the same ions in aqueous solution.

2. Electrical Charge and Radius of Hydrated Ions

The adsorption affinities of various ions have been shown to be determined largely by the magnitude of the charge and the radius of the hydrated ions in solution (Boyd *et al.*, 1947c; Gieseking and Jenny, 1936). The importance of charge indicates that the ion exchange phenomenon is largely controlled by electrostatic forces. The trivalent ions are held more firmly than the divalent ions which in turn are adsorbed to a greater extent than the monovalent ions.

In comparing ions of the same charge the adsorbability increases with a decrease in the radius of the hydrated ion. The crystal radii of the ions are not necessarily similar to the hydrated radii of the ions in solution. The hydrated radii have been correlated by Boyd *et al.* (1947c) with the experimentally determined activity coefficients of these ions. From the activity coefficient data the following series of decreasing adsorbability have been predicted: for the trivalent ions of the third group of the periodic table, lanthanum^{+++} > cerium^{+++} > praseodymium^{+++} > neodymium^{+++} > samarium^{+++} > europium^{+++} > yttrium^{+++} > scandium^{+++} > aluminum^{+++}; for the alkaline earth cations, barium^{++} > strontium^{++} > calcium^{++} > magnesium^{++}; for the divalent ions of the transition metals, zinc^{++} > copper^{++} > nickel^{++} > cobalt^{++} > iron^{++}; for the monovalent cations, cesium^{+} > rubidium^{+} > potassium^{+} > ammonium^{+} > sodium^{+} > hydrogen^{+} > lithium^{+}.

The position of hydrogen in the series of adsorbability varies with the type of exchanger. With the synthetic resin exchangers that contain sulfonic acid groups, the hydrogen ion is one of the most weakly adsorbed. With weakly acidic exchangers whose adsorptive properties are due to carboxylic or silicic acid groups, the hydrogen ion is one of the most strongly adsorbed cations. Boyd *et al.* (1947c) have advanced the hypothesis that this variation in the position of the hydrogen ion is due to the varying acidity of the structurally bound anionic groups responsible for the base exchange reaction. They feel that the sulfonic acid exchangers adsorb hydrogen as the stable hydronium ion H_3O^+, whereas

the weakly acidic exchangers nearly completely dehydrate the hydrogen and adsorb it as H^+.

The adsorbability of anions also depends upon structure and valence of the ion as well as the ionization constant of the corresponding acid. The following list of decreasing adsorbability has been reported by Kunin and Myers (1947); hydroxide> sulfate chromate> citrate> tartrate> nitrate> arsenate> phosphate> molybdate> acetate = iodide = bromide> chloride> fluoride.

3. Effects of Concentration on Cation Exchange

In the exchange reactions between ions of different valence the concentration on the exchanger of the ion of higher valency is greater the more dilute the solution. Examples of this generalization are shown in the data of Patton and Ferguson (1937) in Table III and of Melsted and Bray (1947) in Table IV. Resinous cation exchangers give results similar to those with aluminosilicates (Bauman and Eichhorn, 1947).

TABLE III

Exchange of Ca^{++} from Solution for Na^+ on Exchanger *

(1.2 meq. of Ca^{++} as $CaNO_3$ to 1 meq. of Na^+ on gel type aluminosilicate exchanger)

Concentration of $CaNO_3$ solution N	Na^+ exchanged at 21°C. %
0.25	50
0.10	57
0.02	67
0.005	75
0.001	80

* Patton and Ferguson (1937).

TABLE IV

Exchange of K^+ from Solution for Ca^{++} on Exchanger *

(1 meq. K^+ as KCl to 1 meq. of Ca^{++} on soil composed largely of montmorillonite-beideilite clay minerals)

Concentration of KCl solution N	Ca^{++} concentration as % of total cations on soil at equilibrium
0.026	63
0.00065	78

* Melsted and Bray (1947).

Concentration has a much smaller effect upon the relative adsorbabilities of ions of the same valence than it does between ions of different valence.

4. Equilibrium Concentrations

The actual ion-exchange reactions are usually considered to be analagous to an ordinary metathesis reaction and the equilibrium concentrations obey the mass law. The exchange reaction for sodium and hydrogen between solution and cation exchanger, R, was shown in equation 2. The equilibrium constant K_{Na} for this reaction would be

$$K_{Na} = A_{H^+} A_{NaR} / A_{Na^+} A_{HR} \qquad (3)$$

in which A_{H^+} and A_{Na^+} are the respective activities of hydrogen and sodium ions in aqueous solution and A_{NaR} and A_{HR} are the activities in the solid state. This equation has been used in various modifications by many investigators (Myers, 1942). An excellent discussion of the appropriate activity coefficients to use in calculating equilibrium constants is given by Boyd *et al.* (1947c).

5. Flow Rate

The flow rates used in exchange treatments will vary with the objective. The resinous cation exchangers reach equilibrium in a short time. Bauman (1946) showed that the sodium form of Dowex 30 came to equilibrium with 0.01 *N* hydrochloric acid in about 2 minutes. It is accordingly possible to use relatively rapid rates for normal demineralizing operations. A contact time of as little as 3 minutes may be used in commercial work. However, Boyd *et al.* (1947b) have shown that desorption rate is dependent upon flow and that sharper fractionations may be obtained at slower flow rates. In fractionation procedures, the sharper separations obtained at slow rates must be balanced against the greater output that may be realized with faster rates.

6. Temperature

The effects of temperature upon exchange reactions have not been thoroughly studied but in reported observations the effects have not been great (Nachod and Wood, 1944). Ketelle and Boyd (1947) obtained sharper fractionations of the rare earth elements at 100°C. than at room temperature. This result was due to the increase in rate of reaction at the elevated temperature.

7. Size of Organic Ions

The effect of size on the adsorption of organic cations is of great importance. Gieseking (1939) found that ions like brucine, aniline, naphthylamine, and methylene blue were very strongly adsorbed by clay. These compounds could not be displaced by small ions like hydrogen

but could be replaced by ions of about the same size. Thiamine is also held very tenaciously by cation exchangers and is difficult to elute. According to Herr (1945) about twenty volumes of concentrated hydrochloric acid are required to effect recoveries approaching 100%.

Firmly held organic cations may be of considerable importance in the use of ion exchangers with some food products. The juice from pineapple waste contains compounds that will adsorb on cation exchangers and cannot be eluted by regeneration with sulfuric acid. The practical importance of such a material is shown by the fact that it may reduce the exchange capacity of a new cation resin by 25% in one cycle. On continued use there are still further capacity decreases and also the exchanger loses its ability to remove this class of impurity. These compounds can be largely removed by alkaline solutions. The main constituent of this group of impurities was isolated from an alkaline regenerant. It had a nitrogen content of about 14.5% and gave positive protein color reactions. It appears that it can be classified best as a polypeptide fraction.

The ability of various cation exchangers to adsorb the polypeptide fraction is related to the density of the exchanger. The capacity of the exchanger for this fraction appears to depend on the penetration through the gel to the reactive acid groups. Since the high capacity resins are very dense they show a low ability to pick up this polypeptide fraction as is shown by Fig. 3. It has also been noted that with two exchangers of approximately the same density the one with the lower exchange capacity will adsorb more of these large molecules.

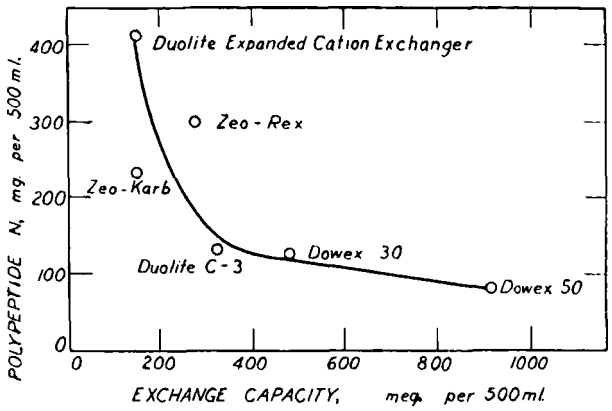

Fig. 3. Relationship of exchange capacity to adsorption of polypeptides by cation exchangers.

The deleterious effects of the adsorbed polypeptides appears to be due to a blocking of the gel pores. The loss in capacity experienced in one cycle is much greater than can be accounted for by the free amine groups of the polypeptide material adsorbed. The effectiveness of alkali in removing this fraction may be in part due to a slight swelling of the gel structure. It has been noted that the exchangers with high capacity for large molecules are more easily and completely regenerated by the caustic treatment.

V. Industrial Applications

The development of a new ion exchange application follows the usual course of laboratory, pilot plant and commercial steps. Some of the possible uses which are considered in the following discussion have not yet passed beyond the pilot plant stage. Undoubtedly, some of these proposed applications offer no advantages over older methods of accomplishing the same results. In other fields such as sugar recovery, the commercial applications are still of such short duration that the exact place of this new tool can not be evaluated accurately.

In all stages of development the exchanger material is most commonly utilized in beds through which the various liquids are passed. These beds in the laboratory are usually contained by glass tubes of from 1 to 4 inches in diameter. The pilot plants (see Fig. 4) commonly consist of rubber lined steel vessels or open wooden tanks of from 1 to 2 feet in diameter. In a few of the pilot plants much larger exchanger cells are used and may approach the commercial units in size. Deionization equipment varies considerably in size with some cells as large as 24 feet in diameter. However, most units are less than 12 feet in diameter.

Flow of the liquid through the bed may be accomplished by gravity in open tanks, although it is more common to use pressure flow in closed vessels. This type of operation not only minimizes contamination but also makes possible much faster flow rates through deep beds.

In all except the simplest processes, the use of automatic control offers many advantages. In cases of short cycles and complicated regeneration procedures, automatic control becomes a virtual necessity. The benefits are derived from uniform operation, labor saving, and reduction in chance for error in manipulation of the valves. The control panel on a commercial installation is shown in Fig. 5.

The steps in the normal operating cycle are as follows: (1) "sweetening on," in which the exchanger particles are covered by water that must be displaced by the solution to be treated; (2) the reaction between exchanger bed and solution; (3) "sweetening off," in which the liquid being treated, if it is of value, is displaced by water before the regeneration is

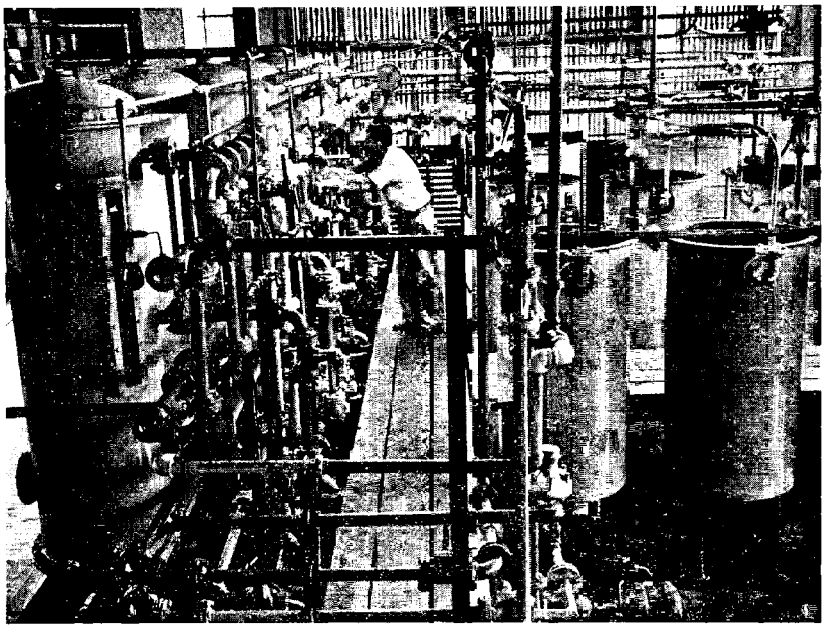

Fig. 4. Ion exchange pilot plant.

Pilot plant of the Hawaiian Pineapple Company built by the Illinois Water Treatment Company, Rockford, Illinois.

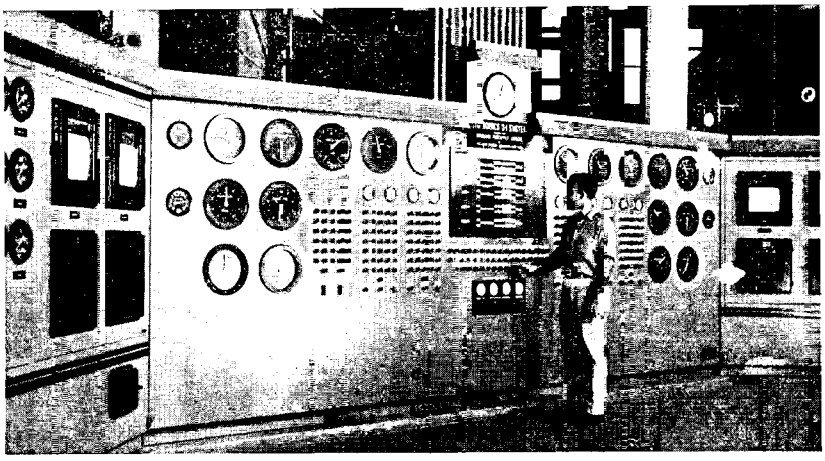

Fig. 5. Control panel of commercial ion-exchange plant.

Hawaiian Pineapple Company installation designed by The Dorr Company, New York.

started; (4) backwash to remove insoluble foreign material and to reclassify the bed in order to assure uniform flow; (5) regeneration of the exchangers to remove adsorbed compounds; (6) rinse of beds to free them of regenerant chemicals.

1. Apple

Apple sirups, prepared by concentrating juice that has been neutralized with lime, have a bitter flavor due to their calcium malate content. Buck and Mottern (1945) used ion exchangers to produce apple sirups of improved quality. This result can be accomplished either by removing the malic acid on an anion exchanger or by complete demineralization with both cation and anion exchangers.

Anion exchangers have the capacity to treat 15 to 20 volumes of juice containing 0.3 to 0.4% of free acid. This treatment prevents the formation of calcium malate, thereby avoiding the production of a bitter flavor. Although treatment by combined anion and cation materials effects a more complete removal of both ash and malic acid, there is only a slight improvement in quality over the single treatment.

The removal of considerable quantities of lead and arsenic was also achieved by the exchange treatment. Lead was reduced by about 50% by treatment either with an anion or cation exchanger. It has been suggested that the low cation removal of 50% of the lead may be due to its presence in an un-ionized form. Since the lead can be almost completely removed by liming, it is not a major problem. The removal of arsenic was more effective and about 96% could be adsorbed by the anion, cation, and anion system.

The malic acid adsorbed on an anion exchanger may be recovered (Buck and Mottern, 1947). The sodium malate can be obtained in about 5% concentration. This solution is evaporated to about 20% malate and then precipitated with a 10% excess of calcium chloride. The calcium malate is decomposed with sulfuric acid in order to obtain free malic acid.

2. Grape

When tartaric acid became scarce during World War II, the possibility of using ion exchange resins for the recovery of tartrates from grape wastes was investigated at the Western Regional Research Laboratory. The tartrates occur in the pomace from grape juice manufacture and also in the pomace and still slop from wine and brandy making. It is estimated that there are annually about ten million pounds of tartaric acid in the grape wastes in the United States.

The still slop contains from 0.1% to 0.4% of tartrate. One may adsorb this acid by either a two-step process which consists of removing

the bases with a cation exchanger and then adsorbing the free acids on an anion exchanger, or by the single process of exchanging the tartrate for chloride on a chloride saturated anion exchanger.

The two-step process has been described by Matchett *et al.* (1944). The cation exchanger, Amberlite IR-100, was used in the usual manner. It was kept in still slop until the pH rise denoted a cation break-through. It was then washed and regenerated with mineral acid. The capacity of the cation exchanger was sufficient to treat about 10 volumes of still slop per volume of exchanger as compared to 30 volumes that could be treated by the anion exchanger, Amberlite IR-4.

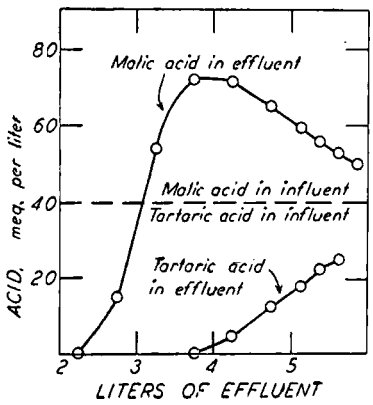

Fig. 6. Adsorption of tartaric and malic acids from a solution of the mixed acids (Matchett *et al.*, 1944).

The anion exchanger could be loaded to a capacity of about 5.9 lb. of tartaric acid per cubic foot of resin at the break-through point. The load of tartaric acid could be increased to about 9.1 lb. per cubic foot of resin at 100% tartaric leakage. It is not feasible to operate beds beyond a point of about 25% leakage due to the slow rate of adsorption.

In addition to tartaric acid, grape wastes also contain malic, acetic, sulfuric, phosphoric, and other acids. The acetic and malic acids are held less firmly than the tartaric acid. This is shown by Fig. 6, which gives results from a run using pure solutions of malic and tartaric acids.

The possibility of adsorbing the tartaric acid directly from nonacidified slop was also investigated. It can be considered that the tartaric acid is present primarily as potassium acid tartrate. The results on passing potassium acid tartrate through an anion exchanger regenerated with sodium hydroxide in one case, and sodium carbonate, in a second case, are shown in Fig. 7. It is interesting to note that the adsorption was greater from the exchanger regenerated with carbonate. This result is due to an anion exchange reaction in which carbonate is liberated from the resin and replaced by tartrate. This reaction is shown in the following equation:

$$KHC_4H_4O_6 + AnCO_3 \rightarrow KHCO_3 + AnC_4H_4O_6 \qquad (4)$$

The sodium hydroxide regenerated resin picked up exactly one-half of the tartaric acid from the potassium acid tartrate solution. The effluent

would therefore be neutral potassium tartrate. The equation for this reaction is as follows:

$$2KHC_4H_4O_6 + AnOH \rightarrow K_2C_4H_4O_6 + AnC_4H_4O_6 + 2H_2O \qquad (5)$$

In view of the low efficiency or recovery of tartrate in the presence of appreciable quantities of base, the direct adsorption on an anion exchanger was not considered to be feasible.

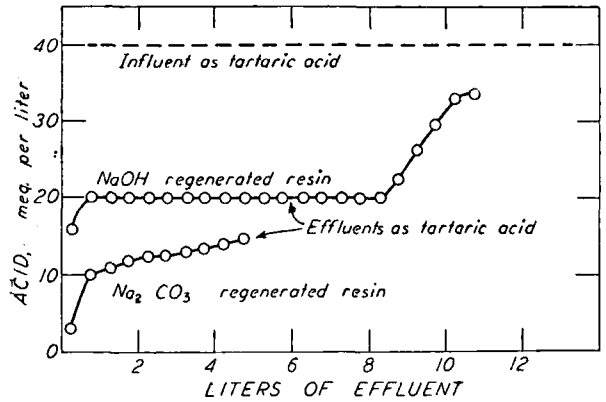

Fig. 7. Adsorption of tartarate ion from 0.02 *M* potassium bitartrate solution on anion exchanger regenerated with sodium carbonate and with sodium hydroxide solution (Matchett *et al.*, 1944).

The regeneration of the anion exchanger was accomplished by a four-stage treatment with sodium carbonate. These four solutions contained about 3%, 1%, 0.1%, and 0.0% tartaric acid before the regeneration started, if they had been used previously long enough to reach an equilibrium condition. The corresponding concentrations after regeneration were 6%, 3%, 1%, and 0.1%. The 6% tartaric acid sample was treated with a 10% excess of calcium chloride to precipitate the calcium tartrate. The other three portions were used in the next cycle.

The anion exchanger, Amberlite IR-4, showed a capacity drop of about 25% after being used for 80 cycles. The replacement of the anion exchanger would be a large cost in the operation of a plant for tartaric acid recovery from grape wastes, if the losses continued at this rate.

The one-step process for the recovery of tartrates from grape wastes is also the result of work at the Western Regional Research Laboratory (Anonymous, 1943). This process consisted of loading an anion exchanger with chloride ions by treating it with a solution of hydrochloric acid. The nonacidified still slop was then passed through the resin and the tartrate was exchanged for chloride. Recoveries in excess of 85%

were experienced by this method. The regeneration of the exchanger was accomplished by passing a strong sodium chloride solution through the bed. It is necessary to use a strong brine in order to drive the reaction in the reverse direction. This procedure is similar to that which has been commonly used for regenerating cation exchangers that have been used in the sodium form for water softening. The tartrate for chloride exchange reaction is shown by the following equation:

$$KHC_4H_4O_6 + AnCl \rightarrow AnHC_4H_4O_6 + KCl \qquad (6)$$

The sodium chloride regeneration was carried out in stages similar to that described for the sodium carbonate solutions. The first portion was used for tartrate recovery. It was treated with carbon in order to remove impurities before the addition of calcium chloride to precipitate the tartrate. It was necessary to wash and dry the precipitate promptly in order to avoid losses due to bacterial action.

The tartrate recovery by ion exchange has not been carried beyond the pilot plant stage, apparently because direct precipitation with calcium chloride and lime is a simpler and cheaper process. It should be pointed out that the function of the anion exchanger in the two processes used for tartrate recovery has been to concentrate the tartaric acid. It served to raise the acid level from a few tenths of a per cent up to about 6%. However, the acid still had to be separated as its calcium salt. The solubility of calcium tartrate has been given by Halperin (1945) as approximately 0.1 lb. per gallon which corresponds to about 0.07% tartaric acid. From a 0.4% solution of the acid it would be possible to recover almost 85% of the tartrate by direct calcium precipitation. It is therefore apparent that it is only in the very dilute solutions that ion-exchange recovery offers any advantage over direct calcium precipitation.

3. Pineapple

Ion exchange resins are being used in the recovery of citric acid and sugar sirups from pineapple waste (Anonymous, 1946). This waste is derived from the skin of the fruit and other inedible parts, and yields more than 50 gallons of juice per ton of fruit processed. This juice has a solids content of about 11%, of which 80-85% is sugar and 7-9% is citric acid.

The pineapple waste juice goes through several purification steps before the ion exchange treatment. It is first heated to precipitate albumin and then filtered to remove this precipitate and other insoluble solids.

As it is more economical to remove citric acid by lime precipitation than it is by ion-exchange treatment, the filtered juice is limed to pH 5.2 and heated to precipitate calcium citrate. This process decreases the

anion exchanger load by amounts up to 50%. The excess calcium added by the liming increases the cation load but in a much smaller amount than the decrease in the acid content. In view of the fact that cation exchangers are much cheaper and also more stable, the ion exchange installation is appreciably less expensive for operating on limed juice than it is for treating the filtered acid juice.

After the calcium citrate has been precipitated, it is separated by filtration. The juice is then cooled before being introduced into the ion exchange cells. In this application, the juice is passed through two pairs of cells arranged in the order cation-anion-cation-anion. This double pass is advantageous in that it gives better removal of impurities than can be accomplished by a single pair. Since sucrose will not be crystallized from the treated juice, the slight inversion that takes place in the second pair is of minor consequence. This is in contrast to the application of this process to the purification of sugar-beet or cane juice where additional purification is obtained for the major product by the crystallization step that follows. In this instance, the two-pair treatment would greatly increase the amount of sugar inverted and decrease the yield of crystalline sugar. It is accordingly considered by most investigators in this field that the use of a single pass system is more desirable.

The cation exchanger used in the pineapple installation is Duolite C-3. This exchanger has a good capacity for removal of mineral constituents and at the same time is satisfactory for the removal of organic impurities. The latter is considered of much greater importance in the purification of pineapple juice than is the removal of ash constituents.

The anion exchanger used on pineapple is Duolite A-3. The sugars in the waste juice are about half sucrose and half invert. Many of the commercially available anion exchangers show rapid decreases in capacity when used with reducing sugars. Duolite A-3 is stable to reducing sugars and also has good color removing properties.

In the demineralization step, juice is passed through two pairs of exchangers until the first pair is exhausted. This pair is then cut out for regeneration and the second pair becomes the first pair in the next juice cycle. Since the exhausted pair is covered with full strength juice, the first step in regeneration is to displace this juice with water. The recovered juice is returned to the raw juice supply. The cut-off point on the sweetening off operation is controlled by conductivity. There is a close correlation between the conductivity and Brix of the juice. In view of the fact that conductivity can be more accurately and easily measured than density, it can be used to advantage in controlling the sweetening-off end point.

The next step in the regeneration consists of passing a strong sodium

chloride solution through both the cation and anion cells. This treatment serves to displace much of the calcium from the cation exchanger and the citric and other organic acids from the anion. The first part of the brine solution is then heated in order to precipitate an additional quantity of calcium citrate. There are also appreciable quantities of malic acid in this regenerant and if additional calcium is added, it can be precipitated in increasing amounts along with the calcium citrate. The calcium citrate is filtered from the brine which is then returned and used in subsequent cycles. Part of the brine is discarded in each cycle in order to prevent the building up of excessive concentrations of impurities.

Following the brine treatment, the cation exchanger is rinsed with a caustic solution in order to displace organic impurities that can not be removed by sulfuric acid. The nature of one of these impurities has been discussed in the section dealing with the effect of size of ions on exchange reactions. The alkali rinse is followed by warm water which facilitates the removal of these organic compounds.

The cation exchangers are next regenerated by sulfuric acid in three stages. The acid is held in three batches which are designated twice used, once used, and fresh. The twice-used acid is passed through the cation exchanger first and sent to the sewer. The once-used and fresh acid are returned to the twice-used and once-used acid tanks. The first part of the rinse water is sent to the fresh acid tank where concentrated sulfuric acid is added to it. Staging of the acid regenerant is economically advantageous because of the high cost of chemicals in the pineapple producing region.

The anion exchangers are regenerated by a 2% solution of sodium hydroxide. It is necessary to use about 10% excess of caustic in order to secure satisfactory removal of colored impurities. It is possible to recover citric acid from the anion exchanger by omitting the brine step and regenerating the exhausted bed directly with sodium hydroxide. This regenerant solution can be treated with calcium chloride to precipitate calcium citrate. This procedure, however, yields a much poorer quality of calcium citrate since some of the organic impurities that are not removed by salt come off with the caustic and are then precipitated by the calcium.

The demineralized juice is almost water white in color. It is concentrated to about 30° Brix and mixed with cane sugar for the production of the sirups used in canned pineapple. During the concentration the sirup acquires a light yellow color. The flavor of this sirup is quite bland although it is still possible to detect that it was prepared from pineapple.

The citric acid is recovered from the calcium citrate by the usual pro-

cedure of decomposing with sulfuric acid followed by concentrating and crystallizing the acid liquor.

4. Pectin

The cation exchanger, Zeo-Karb, has been used commercially in the extraction of pectin from grapefruit peel. This process is described by Myers and Rouse (1943). In this process the grapefruit peel and rag are ground in a hammermill into approximately ¼-inch particles. The peel is washed several times with water at 175°F. It is then mixed with Zeo-Karb and water and extracted at 196°F. for ½ to 1 hour. The magnesium, calcium, and other metal cations that are combined with the pectin are exchanged for hydrogen from the Zeo-Karb. The pH of the mixture is lowered to about 2.7-2.8 and the pectin goes into solution in the water. This solution is then separated in a centrifuge from the mixture of peel and cation exchanger. The solids are found on the wall of the centrifuge in two layers. A partial separation for the recovery of the Zeo-Karb can be obtained in the cutting of these layers separately. The exchanger is also recovered from the peel fraction by screening and backwashing. This process is reported to give a better yield of a higher grade pectin than is obtained by the conventional methods.

5. Milk

One of the first applications of base exchange materials to the solution of a food problem was in the readjustment of salts in milk (Lyman *et al.*, 1933). The milk of each species is adapted to the needs of its young. In view of the differences in the digestive systems and rates of growth between calves and children, it is not surprising that cow's milk contains twice as much protein and three times as much ash as does human milk. The dilution of cow's milk with water and the addition of lactose and fat increases the similiarity between these two milks. However, there are still important physical differences. Cow's milk forms large tough curds which digest slowly and lead to imperfect absorption. Human milk forms a much finer curd that digests rapidly and with nearly complete absorption. The nature of the curd formed in cow's milk can be varied by altering its calcium ion content. If 20% or more of the total calcium in the milk is removed, no curd can be formed by the addition of the enzyme rennin. A process for producing soft curd milk by zeolite removal of calcium and phosphorus has been described (Lyman, 1934; Lyman *et al.*, 1934; Otting, 1936; Otting *et al.*, 1937; Otting and Browne, 1937). The zeolite treatment is carried out on milk which has been acidified to a level of 0.3% (as lactic) by the addition of citric, hydrochloric, or lactic acids.

Passing neutral cow's milk through a sodium zeolite removes very

little calcium. The removal of calcium is greatly increased by acidification. It is felt that it is present in a combination in which its ionization is depressed. A water solution containing calcium would be completely exchanged for sodium under the same conditions which give only 3 to 5% calcium removal from milk. Table V shows the effect of variations in acidity on calcium and phosphorus removal by zeolite.

TABLE V

Effect of Degree of Acidity of Milk on Calcium and Phosphorus Removal by Zeolites *

Milk	Lactic acid	Wt. of moist zeolite	Ca removed	P removed
cc.	%	g.	%	%
600	0.16	180 Greensand	5	17
600	0.16	90 Crystalite	3	14
600	0.30	180 Greensand	15	24
600	0.30	90 Crystalite	22	22

* Lyman *et al.* (1933).

The acidified milk is raised to a temperature of about 18°C. (69°F.) before passing through the sodium exchanger. This operation is continued until the desired removal of calcium and phosphate is no longer obtained. The milk is then drained from the exhausted zeolite. The exchanger bed is washed with water, preferably upflow, in order to remove in part the milk fat, protein, and other organic matter which still adheres to the silicate particles. Water at a temperature of 100-105°F. is used for this operation. The bed may then be washed with sodium hydroxide containing sodium silicate. This alkali treatment is used to remove milk fat and protein that the warm water wash has failed to dislodge. It also serves to maintain the phosphate adsorbing power of the zeolite.

In spite of the fact that phosphoric acid is an anion, a considerable amount of the phosphate is removed by mineral zeolites during the treatment of milk. It has been reported by Lyman *et al.* (1933) that there is no phosphate removal by the base exchange silicates unless calcium or other di- or trivalent ions are also present in solution. The ability of the zeolite to remove phosphate and calcium is not restored by the normal salt regeneration. It is necessary to give the exchanger a preliminary alkali or detergent treatment (Hull, 1944) in order to make the salt effective. The exact mechanism of phosphate removal is not known but it may be as a complex with calcium or other basic compounds.

An improvement on the original sodium hydroxide treatment for the removal of fat, protein and absorbed phosphorus from the zeolite has been described by Hull (1944). In place of the solution of caustic soda

and silicate, a wetting agent such as sodium lauryl sulfate, is recommended. The wetting agent may be used alone in concentrations from 0.005% to 0.1% or mixed with 0.1% to about 1.0% of borax. This detergent solution not only gives a better removal of fat, protein and phosphorus from the surface of the zeolite, but also leaves the calcium in a condition in which it is more easily removed in the subsequent salt regeneration. The exchanger bed is left substantially free of all milk fat and protein by this treatment. The bed is washed with water before proceeding to the salt regeneration step.

In order to maintain the sodium and potassium content of the milk in approximately the same ratio as before zeolite treatment, it is necessary to regenerate the exchanger with a mixture of sodium and potassium chlorides. The effect of the alkali chloride used on the mineral composition of the milk is shown in Table VI.

TABLE VI

Effect of Kind of Alkali Metal Chloride Used in Reviving Zeolite upon Alkali Metals in Treated Milk *

Treatment	Composition of milk			
	Ca	P	Na_2O	K_2O
	%	%	%	%
Untreated milk	0.1390	0.1067	0.0710	0.1840
Milk plus crystalite revived with NaOH and NaCl	0.1016	0.0789	0.1230	0.0315
Milk plus crystalite revived with NaOH and NaCl plus KCl (1:1 by wt.)	0.1049	0.0837	0.0782	0.1618

* Lyman *et al.* (1933).

The calcium removal by the salt solution is aided by the addition of an acid such as acetic which may be buffered with sodium acetate. This treatment removes some alkali from the zeolite. This loss is made up by a final wash with an alkali solution that also may contain a soluble aluminate in order to restore alumina lost by the exchange material during the revivification process. The zeolite bed is washed with water after this alkali treatment and is then again ready for use on milk.

After zeolite has been operated on milk it is necessary to use great care in order to avoid bacterial growth. This can be accomplished by circulating through the bed a solution containing from 1 to 5 parts of formaldehyde per 1000 parts of water. It is necessary to thoroughly wash the bed free of formaldehyde before it is used on another milk cycle.

The zeolites tend to deteriorate on continued use. This deterioration results in a softening of the material and also a decrease in the effectiveness of phosphate removal. Since it is desirable to maintain a constant

ratio of calcium and phosphate in the treated milk for dietetic reasons, it is desirable to reactivate the bed. This restoration can be accomplished by treatment with sodium silicate or sodium aluminate solutions in order to maintain the desired silica to alumina ratio.

The production of soft curd milk has always been carried out with zeolite type exchangers. At the present time there are approximately twenty-four companies manufacturing and distributing soft curd milk (Garrett, 1947).

Another application of zeolites by the milk industry is in the production of soluble alkali caseinate, which has been described by Otting (1940). In this process the casein is prepared by acid precipitation. The casein is then washed and finely suspended before passing through the exchanger. The zeolite bed will remove a considerable quantity of calcium and phosphate from the casein suspension. The casein is converted by the sodium exchanger into the soluble alkali caseinate. This product may be used directly or after drying. The alkali caseinate is very low in inorganic material.

A third application of ion exchange to the milk industry is the treatment of acidified whole or skim milk which changes its character so that greater quantities can be used in ice cream or sweetened condensed milk without the precipitation of lactose (Otting and Quilligan, 1941). The procedure used is similar to that described above for the production of soft curd milk. The removal of calcium and phosphate would not seem to explain the fact that the lactose concentration can be built up to above 7.8 to 8.5%, whereas in untreated-milk products concentrations above 5.9 to 6.5% will produce crystallization of lactose. It is felt that this different effect is due to changes in the colloidal materials in the milk, which effectively prevent precipitation and crystallization of the lactose.

It has been recently recorded by Josephson and Reeves (1947) that the incorporation of a small amount of cation-exchanger-treated skim milk will stabilize evaporated milk so that there is no coagulation during heat sterilization. This result is apparently due to the fact that coagulation results from a high calcium and magnesium ion content with respect to citrates and phosphates present. It has been standard practice to stabilize evaporated milk when required by the addition of a salt such as sodium citrate or disodium phosphate.

The cation exchanger used in this work was Amberlite IR-100, which removed about 60% of the calcium without materially affecting the anion concentration. In view of the mechanical and technical difficulties encountered, these investigators preferred to use exchange-treated skim milk rather than whole milk. The quantity of exchange-treated milk required was less than 5% of the evaporated milk. Although the work

did not definitely establish the mechanism by which stabilization was obtained, it was felt that it could be attributed to the removal of the excess calcium and magnesium ions, thereby effecting a more favorable ionic equilibrium in evaporated milk.

6. Sugar Beet

The objective in ion exchange treatment for sucrose production is to remove nonsugar solids in order to increase the recovery of crystalline sugars and to simplify the production of a high quality product. The most rapid progress in this field has been made in the sugar beet industry, which produces refined sugar in a single process. The application to the sugar cane industry is complicated by the fact that the cane mills produce raw sugar which is then shipped to a central refinery. A combination of the two operations is required if the sugar cane industry is to take full advantage of ion-exchange treatment.

The first commercial operation in the sugar beet industry was begun in 1941 at the Isabella Sugar Company at Mount Pleasant, Michigan (Weitz, 1943; Gutleben and Harvey, 1945). The gravity system and open tanks used in this installation have only slight resemblance to the equipment which is now being installed. In addition to the cation and anion cells, this original sugar beet installation included a granular carbon bed. The purpose of the carbon was to remove colored anionic impurities from the cation-exchanger-treated juice. This treatment served to lighten the load on the low capacity anion exchanger used in this installation. The availability of high capacity anion exchangers with good color removing capacities led to the elimination of the carbon bed in sugar beet exchange work.

The ion exchange treatment can be applied either to raw diffusion juice or to juice following defecation. The use of beet juice before defecation appears to offer some advantages and it may be possible to remove sufficient impurities by the ion exchange process to entirely eliminate the defecation step (Riley and Sanborn, 1947). The elimination of defecation is desirable because it usually increases the ash content of the juice; also, heating under alkaline conditions causes the destruction of reducing sugars.

One disadvantage in operating with juice before defecation has not as yet been satisfactorily overcome in all cases. The cation exchanger treatment lowers the pH of the juice to a point where considerable precipitation of colloidal material may occur. This precipitate may clog both the cation and anion beds and also be difficult to remove in backwashing. Gustafson (1946) patented a method of avoiding precipitation in the beds in which a portion of the cation effluent is mixed with the raw juice.

The acid in the cation effluent will precipitate the colloidal material, which is then removed by filtration before passing into the cation cell. Another practice that has been tested is to filter the cation effluent so that the anion cell and treated juice would not be contaminated by precipitated material (Gustafson and Paley, 1946). The filtration costs introduced by these two methods may more than offset the advantages to be gained by operating with raw juice.

The change in composition produced by ion exchange treatment of beet juice is shown for two typical examples in Table VII. The large decrease in Brix is due primarily to removal of nonsugars and to dilution. Riley and Sanborn (1947) pointed out that a typical raw juice containing about 12 lb. of solids per 100 lb. will contain 1.9 lb. of nonsugars. The dilution is caused by mixing with water during the sweetening-on and sweetening-off periods. It is usually estimated to be about 10%.

TABLE VII

Composition of Ion Exchange Treated Beet Juice

Analysis	Second carbonation juice[a]		Raw juice[b]	
	Feed	Ion exchange treated	Feed	Ion exchange treated
Brix	12.2	10.45	12.75	10.11
Purity	91.5	97.14	87.45	96.41
Reducing sugar as % dry substance	0.5	0.34	0.80	2.06
Total Sugars	91.55	97.48	88.25	94.47
% Ash eliminated		92.5		98.3
% Nonsugars eliminated		72.6		87.0

[a] Haagensen (1946b).
[b] Riley and Sanborn (1947).

The increase in reducing sugars is due to inversion. It has been pointed out by Haagensen (1946a; 1946b) that most of the inversion that takes place on treating sugar beet juice in a cation exchanger is due to the catalytic effect of the resin itself. He found that beet juice on being passed through a 6-ft. deep exchanger bed, obtained a pH of 2.0 and was in the acid state for less than 3.5 minutes. At the temperature used and a pH of 2.0 an inversion of only 0.02% would be expected. However, he obtained inversions of from 0.09 to 0.54%. A great deal of this inversion was attributed to bacterial action due to contamination in the filter press and cooler that preceded the ion exchange installation. If bacterial contamination was completely eliminated, inversions of about 0.10% were obtained. The difference in inversion between 0.02% and 0.10% was due to the catalytic action of the acid groups on the exchanger.

The amount of inversion will depend primarily upon temperature, bacterial contamination, and time in acid condition or in contact with the regenerated cation exchanger. It is usually considered desirable to operate at temperatures of 20°C. (68°F.) or less. Even at this temperature Riley and Sanborn (1947) found an inversion of 0.3% on the sugar when operating with a multiple pass system. Inversion due to bacterial action has been reported in several instances and may be of a greater magnitude than that resulting from acid hydrolysis.

Normal regeneration procedures are usually applied to exchangers operating on beet juice. The cation exchangers are regenerated with sulfuric acid of from 2% to 8% concentration. If the juice contains large quantities of calcium, it may be desirable to use a salt regeneration (Rawlings and de Geofroy, 1945; 1947), before the acid. This treatment will remove the calcium and prevent the precipitation of calcium sulfate in the bed.

The anion exchangers are most commonly regenerated with ammonium hydroxide. This alkali is the cheapest per equivalent in many locations. It also offers the possibility of recovering the ammonium salts from the anion which may be used for fertilizer (Anonymous, 1947d). The anion regeneration may be carried out also with caustic soda or soda ash. The alkalies are usually used in 2% to 4% solutions.

The quantities of regenerants required will vary with the composition of the beets. Rawlings and Shafor (1942) have estimated that 12 lb. of soda ash and 11 lb. of 93% sulfuric acid are needed per ton of beets. Riley and Sanborn (1947) found that 18 lb. of caustic soda and 18 lb. of sulfuric acid were used per ton of beets. Haagensen (1946b) reported that 1 lb. of 60°Bé. sulfuric acid and 0.6 lb. of soda ash are required for the removal of 1 lb. of nonsugars.

In some cases special regeneration procedures may be desirable. Porter (1947) recommends the use of water at 160°F. for backwashing the beds in order to facilitate the removal of organic impurities. Occasional alkali rinses may be helpful in maintaining the capacity of the cation exchangers although this operation is not required with the frequency that is needed after contacting some natural products.

The sweetwaters that result from sweetening on and sweetening off the exchanger beds may be disposed of in several ways. The dilute sweetwaters of from 0 to 0.5° Brix are usually sent to the sewer. Sweetwaters of from 1 to 5° Brix may be used entirely in the diffusion battery or in part for the start of the sweetening off operation in place of water. The 1 to 5° Brix sweetwater from the sweetening on operation is completely demineralized and it may be included in the treated juice fraction and thus sent directly to the evaporators.

The advantages that can be obtained by the use of ion exchange purifi-

cation in sugar beet factories have been summarized by Haagensen (1946b). They are as follows: (1) increased recovery of 10.5% on sugar processed, (2) low ash white sugar, (3) higher operating efficiencies in the sugar end, (4) elimination of the use of sulfur and carbon, (5) elimination of boiling out during campaign, (6) higher quality final molasses.

The fact that the regenerant solutions contain valuable fertilizer materials make possible another advantage in the use of exchangers in sugar beet processing. Although the installations to date have not taken advantage of this they will undoubtedly do so in the future. The cation regenerant solutions will contain potassium, sodium, calcium, and magnesium. The anion regenerant solutions will contain ammonium salts if ammonia was used as the regenerating alkali. The ammonia and potassium would be of considerable value as fertilizer constituents.

Numerous patents have been issued on various phases of the ion exchange treatment of sugar solutions. Some of the most important of these patents are those of Dahlberg (1944); Vallez (1945); Rawlings (1947); Shafor (1947); Behrman (1945).

The economic aspects of the ion exchange treatment in the beet industry are hard to evaluate accurately. Many optimistic reports have been published (Anonymous, 1947a; 1947d; Haagensen, 1946b; Riley and Sanborn, 1947) and the process appears to offer a real advance in sugar technology. However, longer periods of operation are required in order to establish the exact costs of this treatment. It is also probable that the recovery of fertilizers, amino acids, betaine, and possibly other compounds may be incorporated into the process.

7. Sugar Cane

The process for demineralizing cane juice is essentially the same as has been described for sugar beet juice, although there are some significant differences in composition and processing conditions that vitally affect the utility of the process. The cane mills are located in warm climates which do not have natural low temperature cooling water available. They must either operate at higher temperatures with greater inversion losses or install expensive refrigeration equipment. The composition of cane juice, before and after demineralization, is given in Table VIII. The higher reducing sugar content of cane juice as compared to beet juice is especially significant.

The reducing sugars (commonly referred to as glucose) in cane juice are normally looked upon as undesirable constituents since they increase the amount of sucrose lost in the molasses. The demineralization treatment increases the usefulness of this fraction and should make its value intermediate between that of molasses and cane sugar. The more com-

plete the impurity removal has been, the closer its value should approach that of the crystalline sugar.

TABLE VIII

Composition of Ion Exchange Treated Cane Juice

Raw cane juice *

	Feed	Ion exchange treated
Brix	15.5	13.2
Purity	82.5	90.75
pH	5.2	8.5
Reducing sugars	6.11	8.4
Total sugars	88.61	99.15
Ash eliminated %		99.2
Nonsugars eliminated %		92.85

* Riley and Sanborn (1947).

Extensive pilot plant operations have been carried out on cane juice demineralization. The results from some of these operations have been reported by Riley and Sanborn (1947) and Mindler (1948). Commercial scale operations on cane sirups and molasses were carried on for a short time and the results are described by Bloch and Ritchie (1947). The results have indicated that the increased yields of raw sugar obtained will not pay for the treatment (Fitzwilliam and Yearwood, 1947) and that the economic success of this application depends upon the increased returns from the sale of the ion exchange molasses and also the savings that would result from the elimination of the raw sugar step in the process. The recovery of by-products such as aconitic acid and fertilizer salts may also increase the value of the ion exchange treatment.

8. Miscellaneous Sirup and Sugar Products

The organic exchange resins have been used in the production of sirups or sugar from a wide variety of products. Although the general treatments are the same as have been described for sucrose and pineapple sirup production, in almost every instance there are interesting variations that illustrate the versatility of ion exchange treatments.

The production of artichoke sirup has been described by Englis and Fiess (1942). An extract of the Jerusalem artichoke was treated with a cation exchanger which formed acid from the salts in the solution. This liquid was then separated from the exchanger and heated in order to utilize the acid produced to hydrolyze the levulose containing polysaccharides. The free acid left after the conversion was adsorbed by an anion exchanger. The treated solution was then concentrated to yield a

palatable sirup. This process illustrates the possibility of taking advantage of combinations of the demineralization treatment with other operations in order to obtain a more economical process.

The pilot plant production of levulose has been improved by a demineralization step (Hockett, 1947). The levulose containing solutions were prepared by the lime precipitation process of McGlumphy *et al.* (1931). Following the decomposition of the lime-levulose suspension with carbon dioxide and filtration of the levulose solution, it was found that passage through a cation and an anion exchanger removed the ash and greatly improved the crystallization of the fructose. Undoubtedly this procedure should be of great value in the laboratory and pilot plant production of many other rare sugars.

The free acids in orange or grapefruit juice may be removed by an anion exchanger before concentrating to a sirup. According to Gore (1947) the quality of these sirups is much superior to products that have been made by neutralizing the free acids with an alkali metal. The grapefruit sirups were bitter apparently due to naringin which is not removed by the exchanger treatment. Less than 20% of the ascorbic acid in orange juice was adsorbed by the anion exchanger treatment.

The preparation of sirups from the juice pressed from citrus peel has also been reported (Anonymous, 1946). This nonpotable juice contains about 9% solids, of which two-thirds are sugar. In addition to ion exchange treatment a carbon adsorption step is also used in order to remove the naringin.

Another commercial application of ion exchangers is in the demineralization of sorbitol solutions (Porter, 1947). The sorbitol is prepared by the catalytic reduction of corn sugar. In this reduction some of the nickel catalyst is dissolved. The removal of this metallic contamination is of great importance since it inhibits the activity of the bacteria that oxidize sorbitol to sorbose. The production of sorbose for use in the synthesis of ascorbic acid is one of the most important uses for sorbitol.

Extensive investigations have been carried out on the ion-exchange treatment of corn sirup and corn sugar liquors. Patents have been issued on some phases of this work (Behrman *et al.*, 1947; Walsh, 1943; Walsh and Dudicker, 1943). The removal of organic impurities which contribute to color formation during the storage has been one of the objectives in the treatment of the starch conversion products. The removal of ash from the corn sugar liquor also makes possible the recovery of greater amounts of dextrose. The mother liquors from this process may be converted repeatedly and additional dextrose obtained.

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9. Pharmaceutical

a. Alkaloids. The fact that zeolites could adsorb alkaloids has been known for a long time (Ungerer, 1925). The adsorption by carbonaceous exchangers and the use of the resulting product as an insecticide was later disclosed (Higgins, 1938; Riley, 1940). The use of cation exchangers to adsorb alkaloids and to recover these materials from them has been a later development. The adsorption of nicotine by a sulfonated carbonaceous exchanger and its subsequent elution with acid as a nicotine salt has been patented (Tiger and Dean, 1942).

The elution of some alkaloids by acid is not satisfactory due to the fact that many of these compounds have a limited solubility in acid solution. The use of hot acid may increase the solubility but it is still not a satisfactory process. A more satisfactory system has been developed using alkali to liberate the alkaloid (Sussman *et al.*, 1945).

The alkaloids liberated with alkali are extracted from the exchanger with an organic solvent. Two variations of this method for alkali and solvent recovery of alkaloids have been developed. In dealing with fairly pure materials it is satisfactory to elute the alkaloid with an alkaline solvent such as ammoniacal alcohol. In dealing with crude extracts a better procedure is to liberate the alkaloid with aqueous alkali. This treatment serves to wash out of the exchanger a great deal of the adsorbed colored impurities without removing much of the alkaloid. The alkaloid may then be extracted with a solvent and recovered in a pure condition.

In the commercial production of alkaloids by the use of cation exchangers the first step is the extraction with acid of the alkaloid bearing material. This acid extract is then passed through the cation exchanger, which will strip out the alkaloid. The solution may be used for repeated extractions of the bark or other plant material. The adaptation of this method to the production of totaquine from cinchona bark has been described by Applezweig (1944).

Portable equipment for the recovery of totaquine directly in the forest has been developed (Anonymous, 1945). Cation exchangers have also been used for the commercial production of scopolamine from Datura plants (Sussman *et al.*, 1945).

A carbonaceous exchanger such as Zeo-Karb will pick up about 8% of its dry weight of quinine or nicotine. This quantity represents a capacity of about 2.4 lb. per cubic foot. It has been estimated that the chemical costs for the recovery of a pound of alkaloid by the alkali solvent process is about thirty-nine cents.

b. Antacid. Numerous neutralizing agents have been tried in the treatment of stomach ulcers. The compounds used include bismuth sub-

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carbonate, sodium bicarbonate, magnesium oxide, calcium carbonate, the tertiary phosphates of magnesium and calcium, aluminum hydroxide, aluminum phosphate, and magnesium trisilicate. The acid adsorbing properties of anion exchangers led to biological tests on Amberlite IR-4 (Segal *et al.*, 1945) and Amberlite XE-43. These tests demonstrated that they were nontoxic (Segal *et al.*, 1947) and rapidly adsorbed acids especially when finely ground (Martin and Wilkinson, 1946). The phosphates and chlorides adsorbed in the stomach were eluted in the intestines. It therefore did not disturb the metabolic balance of these ions. The exchanger caused neither diarrhea nor constipation, which may result from use of magnesium and calcium compounds. There was also no danger of alkalosis as neutralization is obtained without introducing a soluble alkali.

The observation that small amounts of the exchanger would give almost instantaneous relief from pain to the ulcer victim has led to a change in the concepts of its mode of action (Spears and Pfeiffer, 1947). Relief of pain was secured with doses which would fill only a fraction of the requirement for raising the pH above the inactivation point of pepsin. These results have been confirmed by clinical tests (Kraemer and Lehman, 1947). In the light of these results, Martin concluded that the resins' effectiveness could not be measured in terms of its acid neutralizing powers alone but that it adsorbed and inactivated the pepsin directly. The inactivation of pepsin by this treatment has been demonstrated in the laboratory (Wilkinson and Martin, 1946).

c. Streptomycin. Streptomycin is an organic base and it is adsorbed by cation exchangers (Anonymous, 1947c). The exchanger streptomycin salt is so stable that concentrated eluates have not been obtained from it. Cation exchangers have, therefore, not proven useful in the recovery of this antibiotic. Anion exchangers, however, have been useful in the conversion of streptomycin sulfate to hydrochloride. A solution of the sulfate is passed through an anion exchanger which has been treated with hydrochloric acid. The sulfate ions form a more stable salt with the exchanger than the chloride ions, therefore, the sulfate remains on the exchanger and streptomycin hydrochloride appears in the effluent. This effluent will be slightly acid due to hydrolysis of the resin chloride salt. It can be neutralized by passing through an alkali regenerated anion exchanger.

VI. Laboratory Uses

1. Fractionations

a. Rare Earths. The fractionation of rare earths is not directly related to the use of ion exchange resins in food products. However, a new fractionation technique has been developed that might be adapted to the separation of organic bases or acids. This technique consists in the selective removal of an adsorbed cation by treating with a solution containing a compound which will form a complex with that cation. An example of the effect of complex formation is described by Tompkins *et al.* (1947). "When equivalent molar concentrations of zirconium and hydrogen ions compete, the resin is largely in the zirconium form. If oxalate is added to form the negatively-charged zirconium oxalate complex, the resin is converted nearly quantitatively to the hydrogen form."

The reactions concerned in complex formation can be illustrated for the citrate elution of a small amount of cation M^{+n} which is adsorbed on a resin column. This resin is held largely in the ammonium form, NH_4R. If an ammonium citrate solution, at a pH that will support complex formation, is passed through the column, ammonium ions will exchange reversibly for M^{+n} according to the reaction.

$$n\ NH_4^+ + MR_n \rightleftarrows M^{+n} + nNH_4R \qquad (7)$$

The cation, M^{+n}, then enters into the complex formation according to the reaction

$$M^{+n} + x\text{Cit.}^{-m} \rightleftarrows \text{MCit.}_x^{n-xm} \qquad (8)$$

The removal of M^{+n} from reaction (7) will promote the elution of this cation from the resin. Harris and Tompkins (1947) have pointed out that it is the competition between the complex and the resin for the cation that accounts for the separation of the ions. It had been earlier shown by Russell and Pearce (1943) that an ion exchange mechanism alone gave only a slight fractionation of the rare earths.

In passing down through a column of resin the cations may be adsorbed and eluted a number of times. The action in a column of resin is similar to that which is obtained in a packed distilling column. The best conditions for separating two similar cations are long resin columns, slow flow rates, fine resin particles and small cation-to-resin ratios.

Excellent separations of the rare earths and other cations have been obtained by Harris and Tompkins (1947), Spedding *et al.* (1947a; 1947b; 1947c), Ketelle and Boyd (1947), Ayers (1947), Mayer and Tompkins (1947), Marinsky *et al.* (1947), and Tompkins and Mayer (1947). The

method is used in the separation of the various fission products being distributed from the Clinton Laboratories.

The separation of radium and barium by a multistage countercurrent elution technique has been described by Reid (1948).

b. Amino Acids. Sulfonic acid exchangers in the hydrogen form will adsorb all types of amino acids (Englis and Fiess, 1944). The dicarboxylic and neutral amino acids are readily removed by displacement with inorganic cations. The basic amino acids can also be removed by inorganic cations but require much higher concentrations. The basic amino acids are more difficult to displace with an acid regenerating solution than are inorganic ions, such as ammonium (Block, 1946).

The tenacity with which the basic amino acids, histidine, lysine, and arginine, are held by cation exchangers has been used by Block (1942; 1945; 1946) to effect a separation of this class from the neutral and acidic amino acids. A mixture of amino acids is passed through a cation exchanger which is in the hydrogen form until there is an appreciable leakage of the basic amino acids. The adsorbed nitrogen compounds may then be eluted with strong mineral acid solutions (4% sulfuric or constant boiling hydrochloric acid). About 70% of the recovered nitrogen is present as basic amino acids in the regenerant. The adsorbed basic amino acids may also be eluted with alkaline solutions.

Freudenberg *et al.* (1942) first demonstrated that neutral and acidic amino acids could be eluted from a cation exchanger by treating with dilute pyridine without displacing the basic fraction. Block (1946) reported that 5 to 50% aqueous solutions of pyridine would remove histidine as well as the monoamino acids. A separation of histidine from arginine and lysine may therefore be obtained.

Anion exchangers will adsorb only the dicarboxylic amino acids (Cannan, 1944; Englis and Fiess, 1944; Cleaver *et al.*, 1945). The acidic amino acids in protein hydrolyzates have been determined quantitatively by adsorbing on Amberlite IR-4. In order to completely adsorb the dicarboxylic amino acids it is necessary to reacidify and treat two or more times with an anion exchanger. Only traces of the other types of amino acids are included with the acidic fraction. The glutamic and aspartic acids may be recovered from the exchanger by displacement with hydrochloric acid.

The problems involved in the use of exchangers to separate the amino acids are discussed in detail by Cannan (1946) and Cleaver *et al.* (1945). It is pointed out by Cannan that a weakly acidic cation exchanger should be useful in separating the basic amino acids. However, Block (1946) tested two carboxylic acid exchangers at pH 6.0 and found that one concentrated the basic fraction from dilute influents but the

other failed to do so. The manufacturer of Amberlite IRC-50 reports that this exchanger is capable of fractionating the basic amino acids. The amino acids can be easily and efficiently recovered from the carboxylic exchangers by acid regenerants.

Sperber (1946) used an anion exchanger in the middle cell in the electrophoresis of amino acid mixtures in order to maintain a constant pH and prevent the migration of the neutral amino acids to the cathode.

Cation exchangers have been used by Bennett (1942) and Nees and Bennett (1945) to aid in the separation and purification of betaine and glutamic acid from sugar beet molasses.

2. Separation and Concentration

The ion exchange resins are finding laboratory uses in the separation, purification, and concentration of acid or basic chemicals. The preparation of glucose l-phosphate has been shortened and improved by McCready and Hassid (1944) with the aid of ion exchange adsorbents. The glucose l-phosphate was obtained by the action of phosphorylase on starch in the presence of inorganic phosphate. The excess phosphate was removed by precipitation as magnesium ammonium phosphate. The salts remaining in solution were converted into acids by treating with a cation exchanger. The acids were then adsorbed on an anion exchanger and subsequently eluted in a volume only one-sixteenth as large as the original solution. The glucose l-phosphate was separated from the eluate as its dipotassium salt.

An anion exchanger has been employed by Barnes (1947) for the separation of the reaction products of glucose and glycine. This reaction was carried out under mild conditions in order to simulate the changes which may occur in the storage of foodstuffs due to the condensation of reducing sugars and amino acids. The combination of the amino group in the glycine with the glucose makes the reaction products more acidic than the starting materials. It is possible to adsorb these acid derivatives on an anion exchanger and, although they are present in a quantity amounting to less than 1% of the glycine used, they can be separated from the mixture of sugar and amino acid.

The recovery of the reaction products from the anion exchangers was accomplished by a novel procedure. They were eluted with an aqueous solution of trichloroacetic acid. The excess trichloroacetic acid was separated by extraction with ether. It was therefore possible to obtain a relatively pure solution of the reaction products.

The exchange resins have also been used by Haas and Stadtman (1949) in studies on the changes which take place during the storage of dried fruit products. These investigators divided an apricot concentrate into

three fractions. The cation fraction was adsorbed on passing the concentrate through a cation exchanger. It was eluted with hydrochloric acid. This fraction contained the inorganic bases and about 81% of the nitrogenous constituents. The anion fraction was adsorbed by an anion exchanger and eluted with sodium hydroxide solution. This fraction contained most of the acid constituents. The neutral fraction was not adsorbed by either exchanger. This fraction consists largely of sugars.

The various fractions of the apricot concentrate were stored alone or in combinations and their rates of darkening determined. The cation, anion, and neutral fractions darkened only slightly when stored alone. The three fractions darkened at the rate of unfractionated concentrate when recombined in their original proportions. Combinations of any two fractions darkened at rates faster than the individual fractions but much slower than the total mixture.

Cation exchangers can be conveniently used in the laboratory for the preparation of organic acids from their salts. In the past it has been difficult to prepare acids from their alkali salts. Metals that give insoluble precipitates with sulfuric acid or hydrogen sulfide could be conveniently removed from solution, and thus alkali salts have often been converted to alkaline earth or heavy metal salts before liberating the free acids. It is now possible to adsorb sodium or ammonium ions from solutions and allow for the direct preparation of the acid from the solution of the alkali metal salt.

3. Catalysts

Acid-regenerated cation exchangers can be used as catalysts for many of the reactions which normally are carried out with mineral acids. Puri and Dua (1938) demonstrated that acid extracted soils hydrolyzed ethyl acetate or sucrose at about the same rate as a mineral acid with an equal hydrogen ion activity. The use of Zeo-Karb as a catalyst for esterification, acetal synthesis, ester alcoholysis, acetal alcoholysis, alcohol dehydration, ester hydrolysis and sucrose inversion was reported by Sussman (1946). The commercial production of butyl alcohol esters of fatty acids has also been announced (Anonymous, 1947a).

The advantages of cation exchangers over mineral acids as catalysts are largely due to their easier separation from the reaction products and their milder action upon easily resinified compounds. The resin can be separated from the other reactants by filtration or decantation. It is also possible to use it repeatedly without reactivation. This multiple usage offsets the higher initial cost of the exchanger. It has been possible to prepare esters with furfuryl alcohol that could not be obtained using mineral acids due to resinification. The yields, however, of the furfuryl esters were only 10 to 20%.

Thomas and Davies (1947) found the rate of a resin-catalyzed re-

action was dependent to some extent upon the adsorption of the reactants by the resin surface. Levesque and Craig (1948) found the rate of esterification of butanol and oleic acid was directly proportional to the surface area of the cation exchanger catalyst.

4. Purification

The removal of lead and arsenic have been mentioned in the production of apple sirup (Buck and Mottern, 1945). A similar use, the removal of lead from maple sirup, has been proposed by Willits and Tressler (1939). They were able to reduce the lead content from 36 p.p.m. to less than 1 p.p.m. by contacting the maple sirup with the calcium form of Zeo-Karb for 1 minute.

Cationic exchange treatments are a convenient method for reducing the metallic ion content of fermentation media. Perlman *et al.* (1946a) obtained a threefold increase in yield of citric acid from commercial sugar by passing the sugar solution through a cation exchanger. The yield of citric acid from cane molasses could also be greatly increased by a similar process (Perlman *et al.*, 1946b; Karow and Waksman, 1947; Woodward *et al.*, 1944). The use of an anion exchanger did not add to the benefits of the treatment.

McColloch and Kertesz (1945) used a cation exchanger to remove pectase from commercial pectinase preparations. The enzymes would not be expected to be adsorbed above their isoelectric points. However, below their isoelectric points they will be present as cations and might be removed. The pectase, pectin methylesterase, was completely adsorbed below pH 3.9 in one sample and 6.1 in another sample. Only 20 to 40% of the activity of the pectinase (polygalacturonase) was lost in this pH range. It was suggested that different isoelectric points could account for this separation. However, a difference in molecular size may be responsible for the separation which was obtained.

In order to obtain water for plant growth work, ion-exchange treatment has been investigated (Liebig *et al.*, 1943; Hewitt, 1946). A product which is comparable to distilled water and suitable for many investigations has been prepared. However, the boron is only slightly removed and ion-exchange treatment is not suitable for water intended for boron deficiency studies (Schroeder *et al.*, 1946). The use of ion exchanger salts as plant nutrients has also been investigated (Arnon and Grossenbacher, 1947). This method was not satisfactory in all respects as some of the nutrients, such as calcium, are so firmly held by the exchanger that they are not available in adequate quantities to support plant growth.

In order to prepare gelatin of a high degree of purity, Holmes (1940)

demineralized solutions of this protein by passing them through cation and anion exchanger beds.

5. Analytical

The uses of ion exchangers in analytical chemistry have been increasing rapidly. Consideration of these uses is often of value in suggesting new larger scale applications for these materials.

Gaddis (1942) saturated the anion exchanger, Amberlite IR-4, with hydrogen sulfide. It held about 12% of its weight of hydrogen sulfide. This saturated exchanger was then used as a precipitant for the group II ions in the inorganic qualitative separation.

Ion exchange adsorption has been used by Riches (1946) in place of ashing in the preparation of samples for polarographic analyses. For this analysis it is necessary to have the inorganic ions free of organic matter. There may be appreciable losses of volatile inorganic ions in the ashing procedure. This loss was avoided by the ion exchange technique and good recoveries were obtained.

A rapid and convenient method for measuring the total base in serum has been developed by Polis and Reinhold (1944). The serum sample is treated with a cation exchanger that adsorbs the bases and allows for their simple measurement by titration of the acids liberated. It is necessary to titrate to the same pH as that of an untreated sample which has been similarly aerated. This method is as precise as and much more rapid than the previously used electrodialysis method.

In the determination of pyridoxine, interfering compounds have been removed by adsorption on Amberlite IR-4. According to Brown *et al.* (1945), this step greatly simplifies the purification procedure. With short periods of contact very little pyridoxine is adsorbed by the anion exchanger.

Dubnoff (1941) used a silicate cation exchanger to separate arginine from glycocyamine. Arginine was completely adsorbed from a 0.5% sodium chloride solution. The glycocyamine was not adsorbed from this solution. The adsorbed arginine could be recovered by eluting with a stronger salt solution (2 to 5%). Sims (1945) improved upon this method by using a resinous rather than a silicate exchanger.

In the determination of citrulline and allantoin a separation has been obtained with Amberlite IR-100 according to Archibald (1944). At a pH of 6 to 7 the allantoin is not adsorbed and the citrulline is completely removed. The difference in colorimetric determinations before and after adsorption gives a measure of the citrulline content.

A cation exchanger has been used by Cranston and Thompson (1946) in the determination of copper in milk products. The function of the

exchanger is to concentrate the copper which may be present in concentrations of less than 1.0 p.p.m. The copper is eluted from the exchanger with acid and measured by polarographic or spectrophotometric methods. This application illustrates the usefulness of exchangers both in separating and concentrating ions that may be present in only trace amounts.

A semimicro ion exchange column has been described by Applezweig (1946). This type of column should be useful in preliminary investigations with small quantities of materials.

The determination of phosphorus in phosphate rock has been simplified by the use of an exchanger to remove the cations after the fluoride and silica were removed by evaporation. The phosphate is determined by titration between pH 4.63 and 8.98 as it is converted from primary to secondary phosphate. Helrich and Rieman (1947) developed this method which is not only accurate but also more rapid than previous procedures.

Other analytical uses are covered in Kunin's (1948) review on ion exchange.

VII. Summary

The industrial applications of ion exchange resins have been increasing at a rapid rate. This development has proceeded from the original water softening and water demineralization uses to the application of deionization techniques to solutions containing complex organic mixtures. Among the many problems which have been introduced, bacterial contamination has been a serious form of trouble. The exchangers may also become fouled by organic materials that are not adequately removed by normal operating procedures. In these cases special regeneration treatments have been developed. The short cycle and complicated regeneration systems have greatly increased the complexity of operating the ion-exchange plants dealing with organic solutions. These plants require a high degree of skill on the part of the operators in order to maintain efficient production.

The future industrial developments should see a more complete utilization of the fractions obtained from the ion-exchange processes. The recovery of amino acids from cation regenerant solutions and other organic acids from the anion regenerants is being extensively investigated. Valuable fertilizer constituents, such as potassium and ammonia, may also be profitably utilized. The ion exchangers make possible the separation of natural products into different components which may then be diverted to the use for which each is most valuable. The availability of this technique means that all industrial food wastes must be re-evaluated. Reducing sugars in cane molasses can not be looked upon as an almost worthless by-product if, by removing impurities with exchangers,

they approach the major product, sucrose, in value. The removal of impurities from inedible products, such as extracts of fruit peelings, produces edible fractions that should materially increase the efficiency of food processing.

Ion exchangers have proven of great value in the fractionation of closely related compounds. Attention has been directed largely to the separation of the rare earth elements, other atomic fission products, and the amino acids. The techniques developed in this work should prove useful in many future investigations.

Although a considerable number of analytical chemical applications of ion-exchange resins have already been announced, undoubtedly a much wider use can be made of this convenient tool. Laboratory exchanger columns are inexpensive and simple to set up and to operate. The excellent fundamental information published recently should further assist in the development of this branch of analytical methods.

The usefulness of ion exchangers in the separation of acidic or basic compounds from natural or synthetic mixtures has been demonstrated. This technique is of special value when dealing with a component which is present in low concentrations and can be concentrated on a cation or anion exchanger. Cation exchangers have proven to be useful substitutes for mineral acids as catalysts in some reactions.

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Thermobacteriology As Applied to Food Processing

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I. Introduction

Most foods are very complex materials and the solving of virtually any major research problem concerning them seldom involves the application of only a single science; usually, fundamental information from

several sciences must be applied in the solution of a single problem. The past 50 years has been a period of rapid growth for the sciences, especially for bacteriology, chemistry and physics. As the store of fundamental information has grown through basic research in these and other sciences, more and more scientifically trained workers have become interested in the complex problems relating to food preservation. The correlative advancement of the food preservation industry has been gratifying. Nowhere is the interrelationship of basic research, applied research and industry progress more striking than in the history of thermobacteriology as it has been applied to food processing. To one not familiar with the subject it might seem that the application of thermobacteriology to food processing involves only the science of bacteriology. Actually it involves bacteriology, chemistry, physics, mathematics and, to a lesser degree, other sciences.

The most widely used agent to accomplish food preservation today is heat. The primary object of thermal-processing foods is to free the foods of microorganisms which might cause deterioration of the foods or endanger the health of persons who eat the foods. However, if freeing foods of microorganisms were the only consideration involved in thermal-processing them, their preservation would be relatively a simple matter. Unfortunately many of the organoleptic and nutritive properties of foods are also affected by heat. For this reason it is imperative to the preservation of food quality that heat treatments given are very little more severe than just adequate to free the foods of undesirable microorganisms. Therefore thermal resistance of bacteria which may occur in foods is of primary concern. Information gained through basic research in thermobacteriology is essential to the establishment of scientific methods of food processing. Studies relating to factors which influence thermal resistance of bacteria in foods, relating to variations in thermal resistance among species of bacteria which are of concern in food preservation, and relating to mechanisms by which heat destroys bacteria yield information necessary to the formulation of applicable methods for evaluating the lethality of thermal processes for foods. That fundamental information of this type was very meager 30 years ago is believed to be the basic reason for so little real scientific progress in the art of thermal processing of foods prior to that time. Though presently available information of this type is far from complete, the past three decades of basic and applied research has made some remarkable contributions—sufficient to permit of notable refinements in the thermal processing of foods.

With respect to evaluating thermal processes for foods from the standpoint of their capacity to destroy bacteria, fundamental information concerning the effects of heat on bacteria, though of primary concern, is

far from sufficient in itself. Of equal importance is basic information concerning rates of heating of the different foods during process. Mechanisms of heat transfer within the food itself during process must be considered also. Again it should be noted that presently available information of this type is inadequate in many respects, though a great amount has accumulated during the past 30 years. Integration of lethal effects, determined from a consideration of bacteriological and physical data, involves the application of basic mathematical principles. Evaluation of heat processes with respect to their effects on nutritive qualities of foods involves studies in biochemistry and nutrition.

Thermobacteriology as applied to food processing embraces a diversity of considerations, foremost among which is the evaluation of thermal processes with respect to their capacity to destroy bacteria in foods. Since most foods are hermetically sealed in containers (metal and glass chiefly) prior to their being heat-processed, chief concern has been evaluation of thermal processes for canned foods. The first scientific approach to this problem of applying bacteriological and physical data to evaluation of thermal processes for foods was the General Method described by Bigelow *et al.* (1920). Simpler and more versatile methods involving mathematical integration of heat effects were developed by Ball (1923; 1928). These methods have been used so extensively in the canning industry that no discussion of thermobacteriology as applied to food processing could be considered complete unless it included some description of them. Prior to the development of the methods, time-temperature requirements of thermal processes for foods were determined almost entirely by "trial and error." This in itself is sufficient to account for the slowness of progress in refinement of the art of food processing prior to 1920.

It seems best to begin this discussion of thermobacteriology as it is applied to food processing with a brief description of the methods of process evaluation, special attention being given to fundamental concepts on which development of the methods was based. It is hoped that the following discussion will clearly indicate some of the many problems still existent with regard to further refinement in the art of thermal processing of foods.

II. Thermal Process Evaluation

1. The General Method

This method described by Bigelow *et al.* (1920) is essentially a graphical procedure for integrating the lethal effects of various time-temperature relationships existent in a container of food during process. The time-temperature relationships for which the lethal effects are integrated

are those represented at the point of greatest temperature lag during heating and cooling of the product. (This point was found to be at or near the geometric center of the container.) Heating and cooling curves are constructed to represent the temperatures existent during process (see Fig. 1). Each temperature represented by a point on the curves is considered to have a sterilizing, or lethal, value.

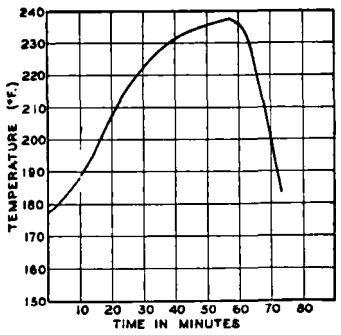

Fig. 1. Heating and cooling curves representing temperatures existent at center of container of product (pureed spinach in 8-ounce glass container) during process (RT = 240°F.).

Thermal resistance of bacteria is represented by thermal death time curves obtained by plotting time required to kill the spores of a given microorganism against temperature of heating (see Fig. 2). From time-temperature relationships represented by the thermal death time curve, it is possible to determine a lethal rate value for each temperature represented by a point on the curves describing heating and cooling of a product during process. The lethal rate value assigned to each temperature represented is equal to the reciprocal of the number of minutes required to destroy the organism in question at this temperature, destruction time corresponding to any given temperature being ascertained from the thermal

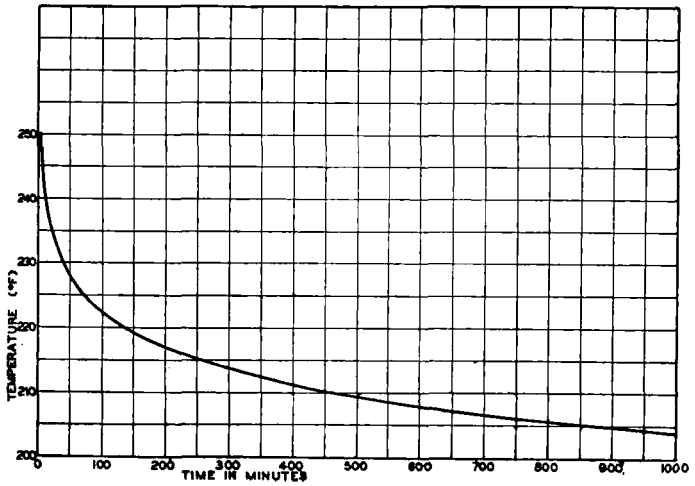

Fig. 2. Hypothetical thermal death time curve typical in form of curves obtained for spores of *Cl. sporogenes* and related organisms.

death time curve for the organism. For example if the thermal death death time curve indicated that 10 minutes were required to destroy the spores of a given organism at 115°C. (239°F.), the lethal rate value assigned to this temperature would be 0.1. Lethality then is equal to the product of lethal rate and time, a process of unit lethality being that process which is just sufficient to sterilize a food.

According to concepts on which the method was based, it may be said that each point on the curves describing heating and cooling of a container of food during process represents a time, a temperature and a lethal rate. By plotting the times represented against corresponding lethal rates represented, a lethality curve representing the process is obtained. Figure 3 shows such a curve on plain coordinate paper, lethal rate being represented in the direction of ordinates and time in the direction of abscissae. Since the product of lethal rate and time is equal to lethality, the area beneath the lethality curve may be expressed directly in units of lethality. To determine what process time must be employed to give unit lethality (sterility), the "cooling" portion of the lethality curve is shifted so as to give an area beneath the curve equal to one. When the area is equal to 1, process time required to accomplish sterilization is represented by the intersection of the "cooling curve" and the x-axis. This is a trial-and-error procedure, and for this reason the method is sometimes referred to as the "graphical trial-and-error method."

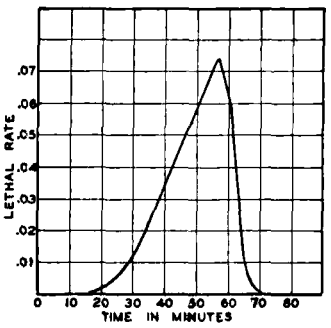

Fig. 3. Lethality curve based on values from curves in Figs. 1 and 2.

Notable improvements in the General Method were made by Schultz and Olson (1940). A special coordinate paper for plotting lethality curves was described. Use of this paper considerably reduces the effort required for making calculations and reduces the chances of misplotting points. Formulae were introduced for converting heat-penetration data obtained for one condition of initial food temperature and retort temperature to corresponding data for different retort and initial temperatures. These improvements greatly increased the applicability of the General Method; however, the method is still laborious and is ordinarily used only for calculation of processes which are not readily calculated by the simpler mathematical procedures developed by Ball (1923; 1928).

The basic concepts on which the General Method was developed are worthy of note. Time-temperature relationships which would account

for complete destruction of the spores of a given type of bacteria, were considered to exist. That is, it was believed that if the spores were exposed to a lethal temperature for some given length of time all of them would be destroyed. The thermal death time curve was considered to represent end-points of destruction. The influence of number of spores on severity of process required was accounted for only through the resistance values employed to construct thermal death time curves. No given number of spores was specified for obtaining these resistance values.

Another important concept concerns the integration of lethal effects produced, during process, at a single point in the container of food—the point of greatest temperature lag. Since food at all other points in the container was considered to receive more severe heat treatments, it was assumed that the point of greatest temperature lag was the only point of concern with respect to calculating a process to accomplish sterilization.

The probable validity of these concepts will be discussed later. Suffice it to say at this point that the General Method has been abandoned, for the most part, because it is far more laborious to use than the simpler and more versatile mathematical methods. It should be pointed out, however, that the fundamental concepts on which the General Method was based also served as the basis for development of the mathematical methods.

2. Mathematical Methods

These methods, developed by Ball (1923; 1928) mathematically accomplish integration of the lethal effects produced by time-temperature relationships existent at the point of greatest temperature lag in a container of food during process. The formulae developed for use are relatively simple and constitute a great improvement over the General Method for calculation of processes for most foods. In the words of Olson and Stevens (1939), "These formulas can be applied to any case wherein the major portion of the heating curve on semi-logarithmic paper approximates a straight line or two straight lines, and wherein the thermal death-time curve on semi-logarithmic paper is, or can be assumed to be, a straight line. Ball's work not only greatly extended the scope of process calculations but simplified them as well. Less time was consumed in calculating processes, and provision was made for applying the given heat-penetration data to all can sizes and retort temperatures."

Familiarity with the meaning and significance of certain terms proposed and defined by Ball is essential to a clear understanding of the formulae developed and the concepts on which development of the methods was based.

a. Slope of the Thermal Death Time Curve. Studies reported by Bigelow (1921) indicated that thermal death time curves, for certain

important food spoilage bacteria, approximated straight lines when plotted on semi-logarithmic paper—time being plotted in the direction of ordinates and temperature in the direction of abscissae. Ball's methods were based on the assumption that thermal death time curves are straight lines when so plotted.

The slope of a line is usually expressed as the tangent of the angle between the line and the x-axis. However, the slope of the thermal death time curve was found to be more conveniently expressed as the number of degrees Fahrenheit on the temperature scale required for the curve to

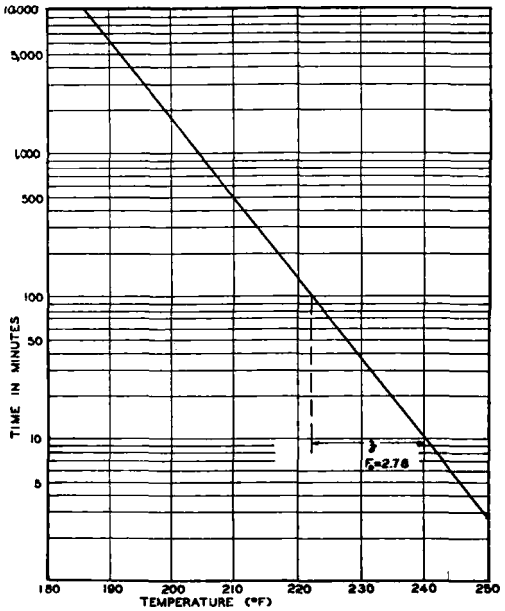

Fig. 4. Hypothetical thermal death time curve plotted on semi-logarithmic coordinates.

traverse one logarithmic cycle on the time scale. The slope of the thermal death time curve expressed in this way was given the symbol z (See Fig. 4). The value of z varies with certain factors. These factors and the significance of variations in value of z caused by them will be considered later in the discussion.

b. Heat Penetration Factors "j," "I," and "f_h." Heating curves constructed by plotting, on semi-logarithmic paper, temperature on the log scale and time on the linear scale are generally straight lines. However, instead of food temperature being plotted on the log scale, values representing differences between retort temperature and food temperature are

plotted. In practice, the equivalent is accomplished by a very simple procedure. The semi-logarithmic paper is rotated through 180°. The log scale then increases from top to bottom. With the paper in this position, the top line is given a value 1 degree below that of retort temperature. Figure 5 shows a heat penetration curve so plotted.

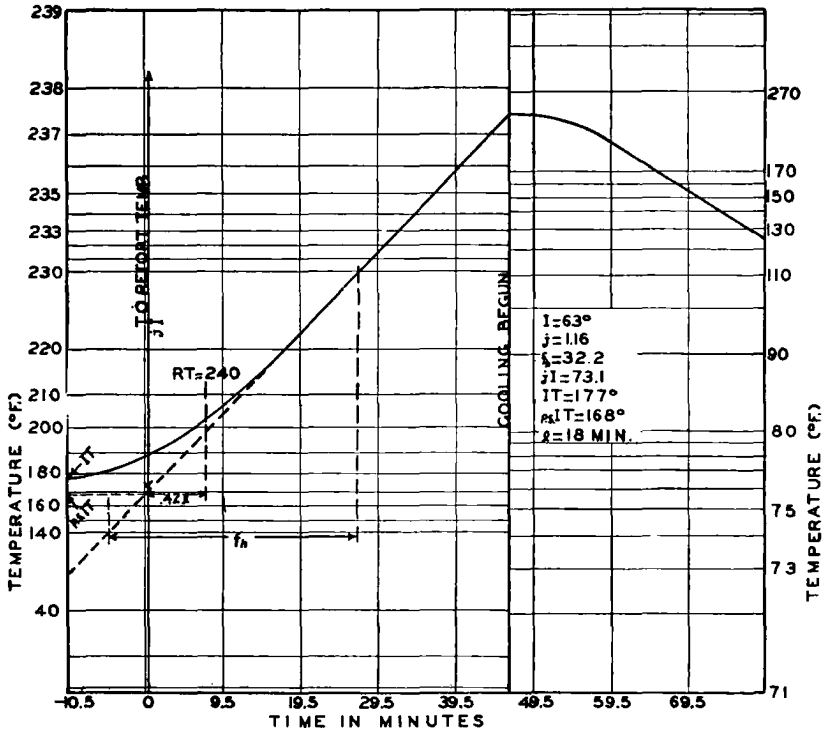

Fig. 5. Heating and cooling curves, plotted on semi-logarithmic coordinates, representing temperatures existent at the center of container of product (pureed spinach in 8-ounce glass container) during process (RT = 240°F.).

The factor j was introduced by Ball to locate the intersection of the extension of the straight line portion of the heating curve and the vertical line representing the beginning of a process, when no time is consumed in bringing the retort to holding, or processing temperature (see Fig. 5). The value of j is obtained by dividing the difference between retort temperature and the theoretical (or pseudo) initial food temperature by the difference between retort temperature and the actual (or real) initial food temperature. The initial temperature (real) is the temperature of the food at the time steam begins to enter the retort (see Fig. 5). The

pseudo-initial temperature is ascertained as follows: (1) A vertical line is drawn so that it passes through a point 0.42 of the distance from the vertical line, representing the time retort temperature was reached, to the vertical line representing the time when steam was turned into the retort (this line so drawn represents the beginning of the process and is given the value 0 time); (2) The straight line portion of the heating curve is extended to intersect the line representing beginning of process or 0 time; (3) The temperature indicated by this point of intersection is the pseudo-initial temperature (see Fig. 5). Considering the vertical line (representing 0 time) as the beginning of process amounts to including 42% of the time required to bring the retort to processing temperature as processing time at retort temperature. As expressed by Ball (1923), "The time taken to bring a retort to processing temperature after steam has been turned on is time during which heat is entering the can, and therefore this period must have some time value as a part of the process. This value may be expressed in per cent of the actual length of time consumed. That is, the period of increasing the temperature of the retort, will shorten the length of time necessary to process a can of food after the retort has reached processing temperature, by a certain percentage of the period." Ball experimentally established that 42% of the "coming up" time should be considered as process time at retort temperature.

The value of j is conveniently calculated by use of the following equation (see Olson and Stevens, 1939).

$$j = \frac{RT - ps.IT}{RT - IT}$$

In this equation:

RT = Retort temperature (holding temperature).
$ps.IT$ = Pseudo-initial temperature.
IT = Initial temperature (real).

For example: When $RT = 240$; $ps.IT = 168$; $IT = 177$.

$$j = \frac{240 - 168}{240 - 177} = \frac{72}{63} = 1.14.$$

The difference in degrees between retort temperature and the initial temperature of the food is designated by the letter I; or, $I = RT - IT$. When multiplied by I, the factor j designates the point of intersection of the vertical line, representing the beginning of a process, with the extension of the straight portion of the semi-log heating curve, when no time is consumed in bringing the retort to processing temperature (see

Fig. 5). The factor j has a similar application with reference to the cooling curve when it is multiplied by the quantity "m," m being defined as the difference in degrees between cooling water temperature and the maximum temperature attained by the food (slowest heating portion) during process.

Since jI represents a point on the heat penetration curve, and since the position of a straight line curve may be fixed by one point on the curve and the slope of the curve, all that is required to fix the position of the heat penetration curve is jI and the slope of the curve. The slope of the heat penetration curve is represented by the symbol f_h which is defined as the number of minutes required for the straight portion of the heat penetration curve to traverse one log cycle; f_h is most easily ascertained by plotting the heat penetration data on semi-log paper (see Fig. 5) though it may be calculated (Ball, 1923).

As pointed out in the above discussion concerning the General Method each temperature existent during the thermal processing of a food may be assigned a lethal rate value which is equal to the reciprocal of the number of minutes required to destroy a given bacterium at the respective temperature. It therefore follows that any method which sums up the values obtained when lethal rate values, representing all temperatures existent during the process, are multiplied by the times during which the respective lethal temperatures were operative, will express the total lethal value of a process. The General Method accomplishes this graphically. The method developed by Ball (1923) accomplishes the summation mathematically. It is not within the scope of this review to give more than a brief description of how the formulae were developed and how they may be applied in solving processing problems; however, some description seems pertinent to a full understanding of the concepts on which development of the methods was based. These concepts are to be considered further in other sections of this paper.

Theoretical heating and cooling curves were drawn, on semi-log paper, representing the center temperatures of a can of food during thermal processing. According to these heating and cooling curves the food attained a temperature, during process, g degrees below retort temperature. It was further assumed that the container, immediately after steam was turned off, was plunged into cooling water m degrees below the maximum temperature reached by the food at the center of the container during process, or $m + g$ degrees below retort temperature.

Three equations were derived to describe the theoretical curves representing center temperature of food during process: (1) an equation of the heating curve (logarithmic), (2) an equation of the first part of the cooling curve (hyperbolic) and (3) an equation of the cooling curve

(logarithmic). Having established equations to describe the curves, calculus was then applied and the lethal effects represented by the process thereby summed up by integration. The heating and cooling curves were visualized as being composed of elements, each element being infinitesimal in width. Each of these elements was multiplied by the lethal rate corresponding to the position of the element on the curve; the elements were then summed up by integration between limits. Since three equations were necessary to describe the heating and cooling curves, as mentioned above, it was necessary to integrate first along a logarithmic curve, then along a hyperbola, and finally along another logarithmic curve. The limits of integration were taken as g°F. and 80°F. on the very safe assumption that any temperature more than 80°F. lower than the processing temperature, has, in any case, a lethal rate value which is negligible with regard to its effect on the lethal value of the entire process. The formula derived for application took the form:

$$\frac{f_h C}{t} = A = \text{total lethal value of process.}$$

Or, for the condition of sterility:

$$\frac{f_h C}{t} = 1$$

or

$$\frac{t}{f_h} = C$$

in which, f_h represents slope of the heating curve and is equal to the number of minutes required for the curve to traverse one log cycle,
C is an arbitrary constant,
t is the time in minutes required to destroy an organism at the highest food temperature attained, at the point of greatest temperature lag in the container, in a process the length of which is to be calculated.

The value of C, being a function of g, m and z, was tabulated for all necessary values of the variables g, m and z. From these tabulations $C : g$ curves were constructed for different values of z and $m + g$. To apply the formula, f_h is obtained from the heating curve. A value of g is obtained corresponding to a certain value of t on the thermal death time curve, and to a certain value of C on the $C : g$ curves (values of t and C thus obtained will satisfy the above equation). The value of g obtained, referred to the heating curve, gives the length of process necessary. For a detailed discussion of solution of the equation the reader is referred to Ball's treatise (1923).

The next important step in arriving at a convenient formula for

calculating processes for canned food was establishing the relationships existing between all varying processing conditions. Through use of the above formula in calculating process times, Ball found that, when considering a single value of z and a single value of $m + g$, any given value of the ratio $\frac{f_h}{U}$ has a value of g corresponding to it. (U was defined as the number of minutes required to destroy an organism at retort temperature.) On the basis of these findings he constructed graphs in which $\frac{f_h}{U}$ values were plotted against corresponding g values giving $\frac{f_h}{U}$: g curves for the different values of z and $m + g$.

By substituting values in the equation of the heating curve, the following equation was obtained:

$$\frac{B}{f_h} = \log \frac{jI}{g}.$$

This equation as it is generally used in process caluculation takes the form,

$$B_B = f_h \log \frac{jI}{g} = \text{length of process in minutes.}$$

This latter equation is considered valid for processes for which g is greater than 0.1°—that is, for processes during which the center temperature of the food does not come within 0.1° of retort temperature. If g is less than 0.1, it is necessary to use a slightly different equation. This equation may be written as follows:

$$B_B = U + f_h (\log jI - T + 1)$$

Values of T for different values of z and $m + g$, given by Ball (1928, p. 49), satisfy this equation.

c. Sterilizing Value of a Process. The above equations were derived for calculation of the time required, at a given retort temperature, to accomplish sterilization of the product with respect to an organism of known resistance. It is not convenient, however, to express relative sterilizing values of different processes from information obtained by the use of the equations. In comparing the sterilizing capacities of processes it is helpful if values expressing the sterilizing capacites are all referable to a common base value—in which case any two values may be compared directly.

Ball (1928) introduced the symbol F to designate the time in minutes required to destroy an organism at 121.1°C. (250°F.). He then de-

veloped formulae for calculating the sterilizing capacity of any thermal process in terms of F. On this basis, a process having an F value of two or any other number is considered as equivalent to that number of minutes at 121.1°C. (250°F.) with regard to its capacity to destroy bacteria. For example then, an organism, the actual thermal death-time curve of which passed through the point representing 2.78 minutes at 121.1°C. (250°F.), would require a process having an F, or sterilizing, value of 2.78 to destroy it. With reference to F values it is well to remember this fact: According to concepts on which the methods are based, all processes having the same F value are considered to be equivalent with respect to their capacity to destroy a given organism in a given product.

In deriving an equation to evaluate the sterilizing capacity of processes, Ball (1928) substituted equivalent values in the equation for the thermal death-time curve. The resulting equation may be written:

$$\frac{250^\circ - RT}{z} = \log \frac{U}{F}$$

or,

$$U = F \log^{-1} \frac{250^\circ - RT}{z}.$$

In this equation U is defined as the number of minutes necessary to destroy a given organism at retort temperature. The symbol F_i was introduced to represent the number of minutes required to destroy a given organism at retort temperature when F is equal to one. Then by definition, $U = FF_i$. It follows from the two equations expressing the value of U that,

$$F_i = \log^{-1} \frac{250 - RT}{z}.$$

Ball (1928) compiled $F_i : z$ tables which may be used for obtaining values of F_i necessary in solving all but unusual processing problems.

Though the methods used in arriving at formulae for calculating processes may be a bit difficult to comprehend, the application of the formulae to the simpler processing problems is very easy and solution of the problems may be obtained in far less time than is required by use of the General Method. For calculating processes for foods which exhibit straight line semi-logarithmic heating curves, the following equations are adequate according to the methods developed by Ball (1923, 1928).

(1) $F_i = \log^{-1} \frac{250 - RT}{z}$

(2) $U = FF_i$

(3) $I = RT - IT$

(4) $j = \frac{RT - ps.IT}{RT - IT}$

(5) $B_L = f_h \log \frac{jI}{g}$, or when g is less than 0.1, $B_R = U + f_h$ (log $jI - T + 1$)

Use of these equations may be best understood from a sample calculation of a typical problem. A problem and calculation for solution follow (after Ball, 1928).

Typical Problem. Calculate length of process when:

(1) $RT = 244°$F. (Retort temperature or holding temperature).

(2) $f_h = 62$ (Obtained from heat penetration curve plotted on semi-log paper).

(3) $RT - CW = (m + g) = 174°$ (Retort temperature minus cooling water temperature).

(4) $F = 15$ (Number of minutes required to destroy organism at 250°F.).

(5) $j = 1.41$ (Obtained by formula 4 above).

(6) $IT = 185°$F. (Initial temperature of food).

(7) $z = 18°$ (Slope of thermal death time curve for organism to be destroyed by process).

Solution.

$F_i = \log^{-1} \frac{250 - RT}{z}$

$= \log^{-1} \frac{250 - 244}{18} = \underline{2.154}$ (F_i may be obtained directly from tables—see Ball, 1928).

U $= FF_i = 15 \times 2.154 = \underline{32.31}$

$\frac{f_h}{U} = \frac{62}{32.31} = \underline{1.92}$

$g = \underline{1.82}$ (Obtained from $\frac{f_h}{U}$: g curves—Ball, 1928).

$I = RT - IT = 244 - 185 = \underline{59}$

$B_R = f_h \log \frac{jI}{g}$

$= 62\ [\log\ (1.41 \times 59) - \log\ 1.82]$

$= \underline{102.92}\ \underline{\text{minutes}} = \underline{\text{process}}\ \underline{\text{time}}$

It is obvious from the above example that, if the conditions were identical except that the process time were known but the F (sterilizing value) of the process were unknown, F could be readily obtained as it would be the only unknown. Therefore, by use of the above formulae, it is possible to determine the F value of a given process or determine

the time required at any retort temperature to obtain a given F value (other conditions in either case being known).

The methods just described are very convenient for calculation and evaluation of processes for foods the heating curves for which can be represented by one straight line. The mathematical methods for calculating processes, for foods the heating curves for which are more complex, though correspondingly more complicated, are based on the same principles.

3. Improvements in Methods of Process Evaluation

As discussed above, Schultz and Olson (1940) improved the General Method to the extent that it is now quite applicable for calculation and evaluation of processes for foods exhibiting the more complex heating curves. Olson and Stevens (1939) described a series of nomograms for the graphic calculation of thermal processes for non-acid foods exhibiting straight-line, semi-logarithmic heating curves. Use of these nomograms greatly shortens the time required for calculations. A method was presented by Schultz and Olson (1938) for converting heat-penetration data obtained for one can size to the equivalent for another can size when the can contents heat mainly by convection.

These improvements constitute further development of the methods in regard to "mechanics" of operation. They do not involve alteration of the original concepts on which the methods, both graphical and mathematical, were based. Stumbo (1948a) discussed the concept regarding the effect of heat on bacteria in light of present knowledge concerning the order of death of bacteria when subjected to heat. Because of the practical implications involved it seems appropriate to treat this subject in greater detail here.

III. Order of Death of Bacteria and Process Evaluation

1. Concept of Bacterial Death on Which Methods of Process Evaluation are Based

The concept regarding the order of bacterial death, on which development of process evaluation methods described above was based, can best be visualized by reviewing the definitions of certain terms employed in the mathematical procedures. Though all these terms are not employed in the General Method, the equivalent of their use is accomplished in making calculations by the method. The terms and their definitions as given by Ball (1923, 1928) are as follows:

F—Number of minutes required to destroy organism at 121.1°C. (250°F.).

$F_t = \frac{U}{F}$*—Number of minutes required to destroy organism at retort temperature* (RT) *when* $F = 1$.

U—Number of minutes necessary to destroy organism at retort temperature (RT).
z—Represents the slope of the thermal death time curve, its value being the number of degrees passed over by the curve in traversing one logarithmic cycle.

When applying the mathematical methods in the calculation of time and temperature specifications of a process for a given food, a value is used to represent each of these terms. If the values used are valid, a process meeting the calculated specifications should be sufficient to accomplish sterilization of the food, assuming of course that the heat penetration data employed in the calculations are accurate. (With methods now available, very accurate heat penetration data can be obtained for most food products.) Are the values, ordinarily used to represent these terms relating to thermal resistance of bacteria, valid? Definitions given for the terms imply end-points of destruction for bacteria, that is, definite time-temperature relationships which will destroy all cells (or spores) of a given organism. Is this concept compatible with present knowledge concerning the order of bacterial death? It is believed that a review of present knowledge concerning the order of bacterial death and an analysis of methods in use to obtain thermal resistance data will satisfactorily answer these questions.

2. Order of Death of Bacteria

From the standpoint of food sterilization, bacteria may be considered dead if they have lost their powers of reproduction. Using failure of reproduction as the criterion of death, numerous studies have been made of the rate of death of bacteria when subjected to moist heat. The quantitative studies by Chick (1910) indicated the death of bacteria to be logarithmic in order. Literature appearing since is replete with results of studies confirming that the order of death of bacteria is logarithmic (among others, Weiss, 1921; Esty and Meyer, 1922; Viljoen, 1926; Watkins and Winslow, 1932; Rahn, 1932, 1943, 1945a). Many explanations have been offered to account for the logarithmic order of death. The most plausible of the explanations given would seem to be that offered by Rahn (1929; 1934; 1945b), namely, that loss of reproductive power of a bacterial cell when subjected to heat is due to the denaturization of one gene essential to reproduction. Rahn reasons that since the death of bacteria is a first order reaction, death of a single cell must be due to the denaturization of a single molecule; and, since the size of a gene (Fricke and Demerec, 1937) is that of a small protein molecule, a gene would consist of only one or two molecules.

What the true explanation is as to the cause of the logarithmic order of death of bacteria does not alter the fact that it exists and should be fully considered in the evaluation of thermal processes for foods. In the words of Rahn (1945a), ". . . , it permits us to compute *death rates* and to draw conclusions from them which are independent of any explanation. Death rates make it possible to compare the heat resistance of different species at the same temperature, or the heat resistance of one species at different temperatures. It also enables us to describe in quantitative terms the effect of environmental factors, such as concentration of the medium or its pH, upon heat sterilization."

Since the death of bacteria is logarithmic in order, death rate may be computed by the following formula:

$$K = 1/t \log \frac{\text{initial number}}{\text{number of survivors}}$$

in which K represents the death rate constant and t represents time in minutes.

A typical rate of destruction curve plotted on semi-logarithmic paper is shown in Fig. 6. According to Ball (1943), Baselt suggested the symbol

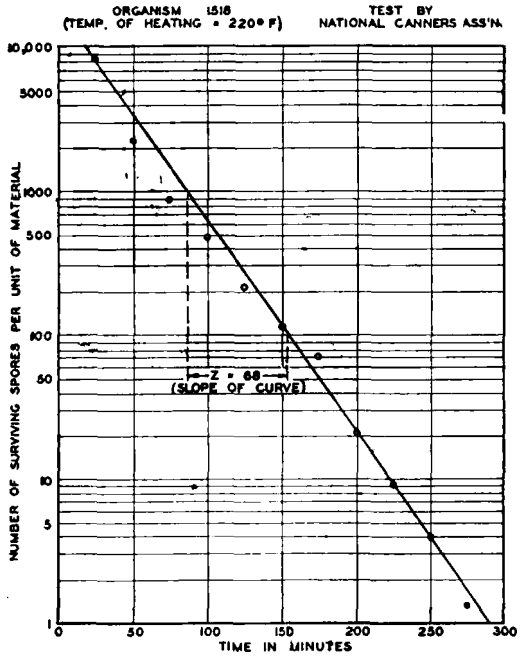

Fig. 6. Rate-of-destruction curve on semi-logarithmic coordinates (Zeta = 68)—Taken from Ball (1943).

Z to represent the slope value of the rate of destruction curve, Z being defined as the number of minutes required for the curve to traverse one log cycle. Considering Z as the unit of time, it follows that 90% of the organisms subjected to a given lethal temperature are killed during each unit of time. Starting with 1,000,000 organisms, the rate of destruction may be depicted as follows:

Time in terms of Z units	Number of organisms surviving
0Z	1,000,000
1Z	100,000
2Z	10,000
3Z	1,000
4Z	100
5Z	10
6Z	1

It should be noted that the value of Z is not constant except for a given set of conditions. It will depend on temperature applied, kind of bacteria to be killed, nature of the medium in which the bacteria are suspended, and possibly other factors.

Stumbo (1948a) presented the following equation to express the time (in minutes) required at a given temperature to reduce a given number of a given species of bacteria to any other number.

$$U = Z\ (\log\ a + P)$$

In this equation,

Z = slope of rate of destruction curve for the organism subjected to the given temperature.
a = initial number of organisms concerned.
P = logarithm of the reciprocal of the number of organisms remaining viable at the end of heating time U.

This definition of the factor U was suggested to replace that given for the factor by Ball (1923; 1928). Ball defined U as the number of minutes required to destroy an organism at retort (process) temperature. Ball's definition is misleading because it implies the existence of thermal death points for bacteria. Further, it does not account for the influence of the initial number of organisms on the time required to accomplish any given degree of reduction. U as defined by the equation above may be used directly in Ball's mathematical procedures for process calculation and modifies them to account for the logarithmic order of death of bacteria. The equation given for calculation of U also has useful application in the analysis and interpretation of thermal resistance data.

3. Factors Influencing Thermal Resistance of Bacteria in Foods

Much of the thermal death time data reported in the past was collected under conditions highly artificial compared with those existing in foods. With the growing knowledge concerning factors which influence thermal resistance there has been a growing tendency, in making thermal death time determinations, to suspend the test bacteria in the food for which a heat process is to be calculated. Attempts have been made to find a reference medium for thermal death time determinations. That is, a simple readily reproducible medium in which the bacteria could be suspended for heating, and in which the resistance of the bacteria would bear a definite relationship to their resistance in a given food. Neutral phosphate solutions, peptone solutions, etc. have been tried. Townsend *et al.* (1938) discussed the resistance ratio known as the phosphate factor. Its value is expressed as follows:

$$\text{Phos. factor} = \frac{\text{Resistance in food}}{\text{Resistance in standard phosphate}}$$

It was pointed out that use of this phosphate factor is justified only when the z values for an organism in the phosphate solution and in a food are identical.

It will be recalled that z, when employed in process calculation, accounts for the relative resistance of an organism at different temperatures existent during the process. Variations in its value are very important therefore and must be fully considered if greatest accuracy is to be attained in process calculations. Since z is an expression of thermal resistance, variations in its value are caused by a factor or factors influencing thermal resistance of an organism to a greater extent at certain temperatures than at others. The times, required to destroy a given number of spores of a given organism suspended in two media, may be identical for one temperature but different for every other temperature. Therefore, from the standpoint of process calculation, factors which influence either the value of F or the value of z must be considered; and, until more information is available concerning these factors thermal resistance data used in process calculation should whenever possible be that for the organism suspended in the food for which a process is being calculated. Some factors are known to cause variations in values of F and z. These factors will be discussed only briefly because most of the information concerning their influence is qualitative in nature. There is a great need for quantitative studies designed to evaluate the importance of these and other factors for different bacteria in the different foods.

a. Number of Cells. The importance of this factor cannot be too strongly emphasized. The order of increase in resistance with increase in number of cells per unit quantity of product has been discussed above. Other things being equal the F value required of a process will depend on the number of cells to be destroyed; or, in other words, the severity of a thermal process adequate to accomplish sterilization of a food is directly related to the number of cells (or spores) of the most resistant species of bacteria present. If ultimate refinement had already been attained in methods of process evaluation, greatest practical value of these methods could not be realized until far greater effort is expended in keeping to a minimum the number of bacteria in foods prior to their being heat-processed. Handling of foods prior to processing has improved markedly during recent years. Improvements in food-plant sanitation, temperature control and humidity control have resulted in general improvement throughout the food industry; however, further application of knowledge concerning the influence of these factors on growth of bacteria in foods could result in further marked improvement in quality of heat-processed food by virtue of allowing less severe processes to be employed. Improvement in the handling of low-acid foods sufficient to allow virtually all such foods to be sterilized by heat processes based on maximum resistance values for *Cl. botulinum* would seem to be well within the realm of future possibilities. Improvement in the handling of acid foods presents equal possibilities.

There is urgent need of further study concerning the influence of various factors on growth and sporulation of bacteria in foods, concerning methods of pre-sterilization of various ingredients employed in the manufacture of many food products, and concerning the discovery and development of agents which could be added to foods to inhibit growth and sporulation of certain types of bacteria in them.

b. Nature of Medium in Which Bacteria Have Grown. There is limited evidence to indicate that the nature of the medium in which spores are produced may significantly influence their resistance to heat. Williams (1929) observed wide variations in the thermal resistance of spores of *Bacillus subtilis* produced in media of different compositions. These studies indicated that, among other things, the kind of peptone used in the medium influenced spore resistance. A casein digest medium was shown to produce spores of relatively high resistance to heat. Sommer (1930) reported results showing that the nature of the medium in which spores of *Cl. botulinum* were produced, markedly influenced resistance of the spores to heat. The addition of phosphate to a peptone medium was shown to increase resistance. Vinton *et al.* (1947) demonstrated

that spores of a mesophilic anaerobe (No. 3679) were more resistant to heat when produced in cooked meat than when produced in raw meat.

It is not surprising that the chemical environment of the organism during its growth would influence its resistance to heat. Studies demonstrating such should serve to emphasize the importance of exhaustive studies relative to the thermal resistance of bacteria as they naturally occur in foods. They should also serve to emphasize the possible relative importance of different sources of contamination. Most of the information now available on these points is qualitative in nature. Extensive studies are needed to furnish quantitative information essential to evaluation of the importance of the different influencing factors.

c. Nature of Medium in Which Bacteria are Suspended When Heated. The chemical environment of the bacterial cell at the time it is subjected to heat has a marked influence on its resistance (Weiss, 1921; Dickson *et al.*, 1922; Esty and Meyer, 1922; Viljoen, 1926; Murray, 1931; Baumgartner and Wallace, 1934; Fay, 1934; Townsend *et al.*, 1938; Tanner, 1944; Rahn, 1945; Stumbo *et al.*, 1945; Jensen, 1945; and others). Many factors have been shown to be important. The following are cited in the approximate order of their importance.

1. pH of medium.
2. Salt (NaCl) concentration.
3. Concentration of sugars and other carbohydrates.
4. Concentration of fats.
5. Agents used in curing meats, especially sodium nitrite.
6. Water content.

It should be noted that variations in heat resistance, which could not be explained by variations in the above factors, have been observed quite frequently by different investigators. Because so many of the studies relative to the factors named have been qualitative in nature, only general statements concerning the influence of these factors can be made at this time. Increased acidity usually has the effect of lowering the resistance of bacteria to heat. Low concentrations of salt (up to about 4%) tend to increase the resistance of many organisms, whereas higher concentrations tend to decrease resistance. High sugar concentrations (as in syrups) tend to protect bacteria from heat injury. High concentrations of fat have in some instances been shown to increase resistance. The importance of this factor is probably minor for most foods. The influence of curing agents, other than salt, in the concentrations used probably have only minor effects on thermal resistance of bacteria in meats. These agents in water or prepared culture media may have pronounced effects. Water content is probably of minor importance in most foods. It is known that bacteria are more resistant to heat when dry

than when moist, but since most foods which are heat-processed have relatively high water contents, variations would not be expected to affect thermal resistance of bacteria to any great extent.

4. Methods of Measuring Resistance of Bacteria to Heat

Methods now in use for studying thermal resistance of bacteria may be roughly classified as follows:

1. The thermal death time (TDT) Tube Method (Bigelow and Esty, 1920).
2. The thermal death time (TDT) Can Method (American Can Company, 1943).
3. Rate of Destruction Method (Williams *et al.*, 1937).

These methods are all in use at present for obtaining data for use in process calculation. The TDT tube and can methods are designed to obtain end-points of destruction of bacteria or their spores. Organisms studied are usually those isolated from foods and most important from the standpoint of food sterilization. With respect to data obtained by these methods, thermal death time is usually defined as the time necessary to destroy a known number of spores at a given temperature. Usually temperatures ranging from about 100°C. (212°F.) to 121.1°C. (250°F.) at 5- to 10-degree intervals are employed for obtaining data upon which to base construction of thermal death time curves. It should be noted that, though known concentrations of spores are usually employed, there has been very little consistency among the various investigators with regard to actual concentrations employed.

As the TDT tube and TDT can methods are now used, spores of the test bacteria are suspended in food, food juices or phosphate buffer solutions. For the tube method, the inoculated product is distributed in small test tubes (7 to 10 mm. in diameter) which are subsequently sealed, near the mouth, in the flame of a blast burner. The volume of product used per tube by different investigators is often not the same. Some investigators have not accurately measured the volume of material placed in each tube when employing foods which heat by conduction. Since the spore concentration is usually expressed in number of spores per unit volume or weight of product, it is obvious that the number of spores employed per tube of product has been subject to considerable variation. The sealed tubes of inoculated product are usually heated in a thermostatically controlled bath of mineral oil, lard, Crisco, propylene glycol, butyl phthalate or some other suitable medium. Subsequent to the heat treatment, the tubes are cooled by plunging them into water at or below 21.1°C. (70°F.). After cooling they are usually opened aseptically and their contents transferred to tubes of culture medium favorable

for growth of the organism being studied. However, if the medium in which the bacteria were suspended for the heat resistance test is favorable for growth, the tubes may be incubated directly without subculture. End-points of destruction are ascertained from data relative to growth of the test organism upon incubation at a favorable growth temperature.

The TDT can method is similar to the tube method with respect to preparation of food samples, inoculation, etc.; however, the inoculated product is distributed in small specially constructed cans (2½ in. in diameter and ⅜ in. high) instead of in glass tubes. Approximately 13 g. of product are placed in each can. The cans are then closed under vacuum with metal closures. The closed cans of product are heat-processed in steam under pressure in small specially constructed retorts (see American Can Co., 1943). If the food or medium is a favorable one for the growth of the organisms being studied the processed cans of product are usually incubated directly, after processing, and end-points of destruction ascertained from data relative to swelling of the can ends. In certain cases the contents of the processed cans of product are transferred, after opening the cans aseptically, to a more favorable medium for bacterial growth. In these cases end-points of destruction are ascertained in the same manner as they are in the tube method. Subculturing of cans is a laborious job which is usually avoided if possible.

In the rate of destruction method described by Williams *et al.* (1937), the inoculated food or other medium is heated in a small steam jacketed tank, of about 900 ml. capacity. The food is mechanically stirred during heating. Specially constructed outlet ports for withdrawing samples during process project from the food tank. Bacterial (spore) counts are made on samples withdrawn periodically during heating at a given temperature. By plotting per cent survival of bacteria after given intervals of time against time of heating, rate of destruction curves for the bacteria are established. The data are usually plotted on semi-log paper, time being plotted on the linear scale and per cent survival on the log scale. So plotted, the rate of destruction curve is usually a straight line or very closely approximates a straight line. To establish thermal death time curves from which to ascertain lethal rate values and z values for process calculation, time values representing some given per cent survival at several different temperatures are employed (the times required to reduce the number of organisms to 0.01% of the number present before heating has been commonly employed). When these time values are plotted, on semi-log paper, against corresponding temperatures, the thermal death time curve is obtained, time being plotted on the log scale and temperature on the linear scale.

Ball (1943) suggested plotting slope values for rate of destruction curves, obtained for a given organism subjected to different temperatures, against corresponding temperatures to obtain the thermal death time curve, slope (Z) of a rate of destruction curve being taken as the number of minutes required for the curve to traverse one log cycle. (Values of Z are plotted on the log scale and corresponding temperatures on the linear scale.) A thermal death time curve constructed in this manner was called a "phantom" thermal death time curve because it is in reality a curve with direction, but not position—that is, position with respect to time or end-point of destruction. Its position may, however, be located to represent any given per cent destruction. Without locating it with regard to position, z values to be used in mathematical methods of process calculation are obtainable; but it must be located in order to obtain U values for the mathematical methods and lethal rate values for the General Method. Formulae presented by Ball (1943) may be used for calculating z values from rate of destruction data, or these values may be obtained from thermal death time curves constructed as described above.

5. Interpretation of Thermal Resistance Data for Process Calculations

a. Thermal Death Time Data. Interpretation of thermal resistance data must be done in light of the method employed to collect the data. The TDT tube method and the TDT can method are employed to obtain thermal death times of bacteria. Heretofore the end-point of destruction at a given temperature has generally been considered as the shortest heating time employed which allowed no viable bacteria to remain in any of the replicate samples of food. As expressed by Rahn (1945a), "The thermal death times are not precise values. Between the last sample that showed viable bacteria and the first that showed none, some time has passed. During this interval, the number of survivors was reduced to less than 1 per sample. In all experiments, the number of survivors was identically the same at some moment between these two critical times, but the exact moment is not known. All death time data have a certain range of possible error, the magnitude of which depends upon the spacing of the time intervals. The number of survivors is never zero, but becomes very small e. g., 1 in 100 liters, 1 in 1,000 liters, etc."

The magnitude of errors may be greatly reduced if the thermal death time determination is properly carried out and the data properly interpreted. Obtaining sufficient data for interpretation depends on multiplicity of replicates as well as proper spacing of time intervals. This can best be demonstrated by example. The data appearing in Table I

TABLE I

Thermal Resistance of Spores of a Putrefactive Anaerobic Bacterium[a] in Pureed Canned Peas

	Heated at 250°F.[b]		Heated at 260°F.[c]	
Number of samples subjected to each time-temperature relation	Heating time in minutes	Number of samples showing growth when cultured after heating	Heating time in minutes	Number of samples showing growth when cultured after heating
12	1.00	12	0.30	12
12	2.00	12	0.60	12
12	3.00	12	0.90	12
12	4.00	10	1.20	8
12	5.00	3	1.50	1
12	6.00	0	1.80	0
12	7.00	0	2.10	0
12	8.00	0	2.40	0
12	9.00	0	2.70	0

a Strain of National Canners Association No. 3679.
b Each sample initially contained 6,250 spores.
c Each sample initially contained 5,000 spores.

were obtained in this laboratory by means of a newly developed thermal death time method. The method will be described in some detail in another section of this paper. Though these data are not adequate to support final conclusions with respect to the thermal resistance of the organism employed, they are sufficient to demonstrate the method of treating data.

Interpretation, in the usual manner, of the data in Table I would be somewhat as follows: Destruction time at 121.1°C. (250°F.) = 6 minutes; destruction time at 126.7°C. (260°F.) = 1.8 minutes—or, by some investigators: destruction time at 121.1°C. (250°F.) = 5.5 minutes; destruction time at 126.7°C. (260°F.) = 1.65 minutes. However, applying the equation,

$$U = Z(\log a + P)$$

or,

$$Z = \frac{U}{\log a + P},$$

more exact end-points may be determined. Using the data for samples heated at 121.1°C. (250°F.) the slope of the rate of destruction curve may be calculated. Since 12 samples were subjected to each time-temperature relationship and each sample initially contained 6,250 spores, the total number of spores subjected to each time-temperature relationship was 75,000. Then,

$$Z = \frac{U}{\log 75{,}000 + P}$$

For a heating time of 5 minutes, P is equal to log 1/3;
and,

$$Z = \frac{5}{\log 75{,}000 + (\log 1 - \log 3)}$$
$$= 1.137 \text{ minutes} = \text{slope of rate of destruction curve.}$$

Substituting this value back in the same equation, the number of spores surviving after any other interval of time may be ascertained. For 6 minutes heating time,

$$6 = 1.137\ (\log 75{,}000 + P)$$
$$P = 0.404 = \log \text{ of reciprocal of number of survivors.}$$

Then, reciprocal of number of survivors is equal to 2.536, and number of survivors equal to 1/2.536. This latter value would be interpreted as meaning that one spore should remain viable per 2.536 volumes of food, each volume of which contained 75,000 spores initially and was heated virtually instantaneously to 121.1°C. (250°F.), held at this temperature for 6 minutes, and cooled virtually instantaneously to a non-lethal temperature.

The data appearing in Table I for a heating temperature of 126.7°C. (260°F.) may be treated in the same manner. Since in this case 5,000 spores per sample were employed, the total number subjected to each time-temperature relationship was 60,000. Taking the relationship allowing spores to remain viable in only 8 of the 12 replicate samples, or theoretically one spore in each of 8 samples of the 12,

$$Z = \frac{1.2}{\log 60{,}000 + (\log 1 - \log 8)} = 0.31$$

Accordingly, for a heating time of 1.5 minutes,

$$1.5 = 0.31\ (\log 60{,}000 + P)$$
$$P = 0.06064$$

Reciprocal of number of survivors = 1.15
Number of survivors = 1/1.15

Interpreting the meaning of this latter value as in the case of heating at 121.1°C. (250°F.), it may be said that one viable spore should remain in 1.15 volumes of product, each volume of which initially contained 60,000 spores. The data show that one spore survived in one such volume.

Z values calculated as above, when plotted on semi-log paper in the direction of ordinates against corresponding temperatures in the direction of abscissae, yield "phantom" thermal death time curves from which values of z are obtainable for process calculation. A thermal death time curve should be established from rate of destruction values for at least

four temperatures over the range of lethal temperatures which will obtain in the food at sometime during process.

b. Initial Concentration and End-Point of Destruction. In applying thermal death time data to process evaluation, two values must be chosen almost arbitrarily, namely, one to represent the initial number of organisms and one to represent the end-point of destruction. The number of organisms to be considered as the initial concentration should be arrived at with judgment even though any value chosen corresponds to a special set of conditions only. If sterilization is the aim the number chosen should refer to the number of cells, or spores, of the most resistant type or types of bacteria per given volume of food which the process is being designed to sterilize and not to the total number of cells of all types which might be present. Many types of bacteria may be present simultaneously in a given food product prior to its being heat-processed. The order of resistance of many of these types may be very low compared with that of the most resistant type present, and a heat process designed to free the food of the most resistant type will usually be sufficient to sterilize the food. There are exceptions to this condition, however, which should be fully considered. For example, if a process is designed to free a container of food of 100 spores of a given organism, the process could well be inadequate to free the food of 10,000 spores of another organism of appreciably lower resistance. Therefore, both the number and kind of bacteria to be destroyed should be considered when choosing a value to represent initial concentration in equations for calculating thermal process specifications.

There probably is no generally applicable rule which could be followed in choosing a value to represent the number of cells (or spores) of any given species of bacteria likely to occur in a given volume of any food product. The number depends on many variable factors. Certain species of bacteria, for example, *Clostridium sporogenes,* when growing in pure culture may produce several million spores per gram of food. The total number of cells, vegetative and spore, per gram is large (usually several billion) for this condition. Growth to this extent usually results in detectable spoilage of the food and should not be given consideration in predicting the number of spores likely to occur in foods to be heat-processed. Moreover, a given species probably never occurs in pure culture in such foods. Ordinarily the microflora, of a food prior to its being heat-processed, is made up of many different species which under favorable conditions for growth are constantly in competition with each other. Consequently the number of cells of any one species usually represents only a small fraction of the total number of microbial cells present. The number of heat-resistant spores of any one species represents a still

smaller fraction of the total. It may be said therefore, that though a food prior to heat-processing may contain several million bacterial cells, the number to be considered with respect to establishing a heat process is relatively small.

Assume for the sake of illustration that a certain food contains 100,000,000 bacterial cells per gram. In all probability there will be at least 100 different species of bacteria represented. Unfortunately the literature is extremely lacking in this respect. Because there have been so few systematic studies relative to the number of different species of bacteria occurring simultaneously in foods prior to their being heat-processed, a more definite statement in this regard would have little meaning. But, it may be said, chiefly on the basis of unpublished data, that among the species which do occur the highly heat-resistant spore-forming species are rarely predominant. This problem is in need of a vast amount of careful study. Many refinements in food processing are dependent on information to be gained from such study. But, to continue with the initial assumption, one would not expect more than a few thousand of the 100,000,000 cells to be of the most heat-resistant species present. Of these only a small percentage would be expected to be in the spore state. Taking the several factors into consideration we may say that a properly handled fresh food product should probably contain per gram no more than a few hundred spores of the most resistant species present. However, any value chosen to represent the initial concentration of any given species of bacteria in foods should be one chosen in view of the value to be used to represent the end-point of destruction.

Since bacterial death as the result of the application of heat is logarithmic in order, there can be no such thing as an absolute end-point of destruction for bacteria. However, some end-point must be established in order to locate the position of a thermal death time curve and establish the value of U for calculating thermal processes for foods. Should such an end-point represent survival of 1 organism in 1 unit of food, 1 in 10 units, 1 in 100 units, 1 in 1,000 units, or what? Available information is not adequate to support a logical answer to this question. If only one organism remains per container of food, will it grow and reproduce? The nature of foods influences the capacity for organisms to grow in them, especially if the organisms occur in small numbers. There may be many foods, however, which would permit the growth of even a single cell. Such must be assumed to be the case at least until more information is available concerning the factors influencing growth of bacteria in the many different foods. It would seem therefore that an end-point of destruction chosen at this time should be such as to make the chance for survival ex-

tremely remote, especially when calculating processes for foods which are known to support good growth of the organisms in question.

Any choice of the end-point of destruction must be arbitrary. It should be made, however, in consideration of the maximum number of spores of the type to be destroyed which are likely to occur in each unit of the food. It should also be made in consideration of the number of organisms occurring per container of food, which are subjected to heat treatment no more severe or very little more severe than the heat treatment calculated for the point of greatest temperature lag in the container (Stumbo, 1948a). The values for U and F employed in process calculation, and therefore the severity of the calculated processes, depend upon the magnitude of values chosen to represent initial concentration and end-point of destruction. This can be best depicted by constructing an example.

Assuming 100 per container as the initial concentration and 0.000001 as the number permitted to remain viable, U and F values to be used in process calculation may be obtained, if the slope Z of the rate of destruction curve for the organism at the retort temperature and the slope (z) of the thermal death time curve for the organism are known. Taking RT (retort temperature) = 121.1°C. (250°F.), $Z = 1.00$ and $z = 10$°C. (18°F.) calculations to obtain F and U values would be as follows:

$$U = Z(\log a + P)$$

According to values chosen,

$$Z = 1.00,\ a = 100,\ P = 6,$$

and

$$U = 1.00\ (2+6) = 8.$$

According to definition,

$$U = FF_i$$

$$F_i = \log^{-1}\frac{250 - RT}{z}.$$

Substituting,

$$F_i = \log^{-1}\frac{250 - 250}{18} = \log^{-1} 0 = 1.00$$

$$F = \frac{U}{F_i} = 8.$$

Any values so obtained for U and F may be used in the mathematical methods (Ball, 1923; 1928) for process calculation. Other data required are obtainable by heat penetration studies. For products which exhibit straight line semi-log heating curves, the factors required are f_h, I and j (see definitions under discussion of mathematical methods for

process calculation). Process time is given by the following formulae in which B_B is equal to process time in minutes.

$$B_B = f_h \log \frac{jI}{g},$$

or when g is less than 0.1,

$$B_B = U + f_h (\log jI - T + 1).$$

The value of g is obtained from $\frac{f_h}{U}$: g curves and the value of T from tables (see Ball, 1923; 1928). The factors, to account for thermal resistance of bacteria, employed in more complex formulae for calculating processes, for foods which do not exhibit straight line heating curves, are identical and need not be discussed further here.

5. *Nature of Thermal Death Time Data Used in the Past to Establish Requirements of Commercial Processes*

Cl. botulinum is the only bacterium known which produces highly heat resistant spores and is greatly significant from the standpoint of food consumption and public health. This organism is widely distributed in nature and the presence of its spores in foods prior to processing must be assumed. Many foods of the low-acid type will support its growth. If it is not destroyed by the thermal process it may grow in the foods and produce toxin which if ingested would generally prove fatal to the food consumer. Therefore, knowledge concerning the maximum resistance of spores of *Cl. botulinum* in foods is extremely important to the establishment of adequate thermal processes for those foods which will support its growth.

Esty and Meyer (1922) reported the results of studies in which the thermal resistance of many strains of *Cl. botulinum* had been determined. In these studies, the TDT tube method was employed. An "ideal" thermal death time curve for spores of *Cl. botulinum* in neutral phosphate solutions was suggested. This curve was designated by the values $F = 2.78$ minutes and $z = 18°$F. Townsend *et al.* (1938) reported corrected values for these factors, namely, $F = 2.45$ and $z = 17.6$. The maximum resistance values reported by Esty and Meyer were obtained for suspensions containing billions of spores. The F value, 2.45, is higher than the F values generally reported by other workers for this organism (see Townsend *et al.*, 1938; Tanner, 1944). As shall be shown later, this, in certain cases at least, is due to the lower number of spores employed by the others. These higher values are still employed as the basis for establishing safe commercial processes throughout the canning industry. When the necessary data are available the F value is multiplied by a

factor (the phosphate factor) to correct it to refer to the resistance of the spores in the specific food concerned. In view of the fact that bacterial death is generally logarithmic in order, this procedure of using these maximum resistance values would seem to be wholly justified. The resistance values reported were generally considered to represent end-points of destruction. Their use has possibly been based on the hope of covering the worst possible condition which could exist in a food rather than on the realization that bacterial death is logarithmic in order and use of such values accordingly reduced the probability of a viable spore of *Cl. botulinum* remaining in a container of product after heat process. However, results of studies concerning the rate of destruction of spores of *Cl. botulinum* were reported by Esty and Meyer (1922) as indicating logarithmic order of death. These data were also shown by Rahn (1945b) to represent death of bacteria as occurring logarithmically.

Regardless of the premise upon which the maximum resistance values reported by Esty and Meyer became established standards, analysis shows the procedure of using the values to be sound practice. In view of the logarithmic order of death of bacteria, it has been difficult for some investigators to understand how the use of even these standards would protect against the occasional survival of a botulinum spore in some of the billions of cans of food placed on the market. Speaking of foods which are known to support the growth of *Cl. botulinum,* it has been suggested that if viable organisms do remain in the food after processing, they are in such a state that they can do no harm (Ball, 1943). Let us analyze the probability of survival and assume that if a botulinum spore remains in a food it will germinate and subsequent growth will cause damage. From results of later work Townsend *et al.* (1938) it is obvious that the strain of *Cl. botulinum* for which Esty and Meyer observed maximum resistance is among the most resistant strains thus far discovered. The probability of spores of as resistant a strain occurring in foods would seem to be relatively remote. Though important, this probability cannot be mathematically evaluated from information available.

The fact that *Cl. botulinum* is difficult to isolate from foods suggests that its spores occur in very low concentrations, if at all, in most foods. However, assume that on the average botulinum spores of the resistance observed by Esty and Meyer occur in foods to be canned at the rate of 10 spores per gram of food. If we assume that the resistance values observed were for a concentration of 60,000,000,000 spores per tube reduced to a concentration of one spore per 10 tubes we may calculate the probability of survival of similar spores in commercially canned foods. It seems fair to assume that, on the average, no more than 10 g. of food

per container would be subjected to heat treatments as low in severity as that given the point of greatest temperature lag in the container, the point which processes employed in the past have been designed to sterilize. On the basis of these assumptions it may be said that the processes employed were effectively dealing with about 100 spores per container. Then applying the equation,

$$Z = \frac{U}{(\log a + P),}$$

Z = slope of rate of destruction curve for *Clostridium botulinum* spores heated at 250°F.,

$U = 2.45$ = corrected F value of Esty and Meyer,

$a = 60{,}000{,}000{,}000$ and

$P = \log 10 = 1$.

Then,

$$Z = \frac{2.45}{10.778 + 1} = 0.208$$

and, for commercially canned foods,

$$0.208 = \frac{2.45}{\log 100 + P}$$

$P = 9.7788$, logarithm of reciprocal of number of survivors, and number of survivors = 1/6,000,000,000.

Interpreting this latter value we find that, on the basis of assumptions made, one botulinum spore should remain viable in every six billion containers of commercially canned foods. Actually when we consider all factors, reason tells us that the probability is far less than indicated by this figure, undoubtedly one in many hundred billions. Comparing this with the many hazards of our present day life, we realize that commercially canned foods as they are now processed cannot, by any stretch of the imagination, be considered a health hazard from the standpoint of their containing viable spores of *Cl. botulinum*. This is borne out by the fact that during the past 20 years and more not a single case of botulism has been attributable to the consumption of commercially canned foods.

It was noted above that the resistance values reported by Esty and Meyer (1922) were higher than those reported by others. Esty and Meyer employed sixty billion spores per tube. Townsend *et al.* (1938) employed a maximum of two hundred million spores per tube. Townsend *et al.* reported a maximum resistance value of $F = 1.90$ or a destruction time of 1.90 minutes at 121.1°C. (250°F.) for spores of *Cl. botulinum* in neutral phosphate. Since essentially the same methods were employed in the two studies, let us assume that the end-point of destruction, to which

the value of Townsend *et al.* applies, represents survival of 1 spore in 10 tubes (same as assumed above for the Esty and Meyer data). U and F are identical at 121.1°C. (250°F.), hence,

$$F = Z(\log a + P),$$

and for data of Townsend *et al.*,

$$1.90 = Z(\log 200{,}000{,}000 + 1),$$

or,

$$Z = 0.204$$

(value for Esty and Meyer data was 0.208).

Using this value of Z to determine what F value Townsend *et al.* should have obtained if sixty billion spores had been used instead of two hundred million, we find,

$$F = 0.204\ (\log 60{,}000{,}000{,}000 + 1),$$

or,

$$F = 2.41.$$

It may be said, therefore, that the maximum resistance value reported by Townsend *et al.* is in reality virtually as high as that reported by Esty and Meyer. This clearly shows that resistance values must be interpreted in light of the number of spores employed, and in light of the degree of reduction in number accomplished at the so-called end-point of destruction. In comparing the resistance to heat, of different species or strains of bacteria, the resistance values compared should be for equal numbers reduced to the same extent.

From the above analysis it is obvious that values, to represent initial concentration and end-point of destruction, employed by Esty and Meyer were essentially equivalent to the following:

Initial concentration = 100 spores per container
End-point of destruction = 1.66×10^{-10}

Though these values are reasonable for establishing processes for foods when public health is a primary consideration, they are perhaps a great deal more severe than would be practical when economic considerations only are involved. Since *Cl. botulinum* may not be the most resistant organism occurring in foods, and since many foods will not support its growth, resistance values for *Cl. botulinum* have been used primarily for establishing minimum process requirements for low-acid canned foods.

Spores of certain mesophilic and thermophilic anaerobes are more resistant to heat than are the spores of *Cl. botulinum*. The mesophilic

anaerobe 3679[a] produces spores which are significantly more resistant to heat than are the spores of *Cl. botulinum*. This organism is also different from *Cl. botulinum* in that it does not produce toxin. Therefore, its presence in food is important only from the standpoint of food spoilage. Since it will readily grow in most low-acid foods, it has been the cause of important losses from spoilage of foods which had been given processes sufficiently severe to destroy *Cl. botulinum*. How prevalent it and other organisms similar to it in resistance are in foods prior to canning and heat processing is not known. How many related species of similar resistance to heat have been isolated but not described in literature cannot be determined. However, the fact that many foods are now given processes designed to free them of bacteria of similar heat resistance, indicates that these undescribed species of bacteria are considered to have a great deal of economic importance from the standpoint of their causing losses due to food spoilage.

Many processes in use today for a variety of food items have been based on resistance values observed for the spores of the putrefactive anaerobe 3679. Since the order of resistance of its spores is 1.5 to 5.0 times that of spores of *Cl. botulinum*, it goes without saying that processes based on resistance values for spores of P. A. 3679 reduce still further the probability that *Cl. botulinum* will survive in foods given such processes. However, it should be noted that, though resistance values observed for spores of P. A. 3679 are now widely used for establishing process specifications, there has been very little consistency with respect to the resistance values employed. Values observed for spore concentrations ranging from a few hundred to several thousand per unit volume of product are commonly employed. For example, one laboratory may employ resistance values observed for 10,000 spores per container of a given product, another may use values observed for 200 spores per container of the same product, and each may employ values based on still other concentrations for establishing processes for another product. Such practices no doubt have their specific value, but they do not yield results which are readily interpreted.

There is an urgent need for a standard procedure to be followed in reporting thermal resistance data. It is impossible to ascertain the significance of many thermal resistance values reported for various organisms because the values have been reported without adequate description of the conditions under which the values were obtained or, more generally, because resistance data from which the values were computed have not been reported in full. For example, reporting that an

[a] Non-toxic putrefactive anaerobe designated by National Canners Association as No. 3679.

organism survived 10 minutes and was destroyed in 15 minutes at 115.6°C. (240°F.) is not only reporting an approximation but, in most cases, doing an injustice to the thermal resistance data from which such values were ascertained. Data reported in the following manner are far more valuable.

Thermal resistance of spores of N.C.A. organism 3679 in neutral phosphate buffer (pH 7.0) at 240°F.

Process time Min.	Survival
14′ 18″	+ + +
17′ 54″	+ + −
21′ 24″	+ + −
25′ 6″	− − −

Spore concentration 2.5×10^6 spores per ml. (Taken from American Can Company, 1943.)

Data so reported, if sufficient information concerning the methods employed is given, are subject to interpretation and analysis.

Suffice it to say that most data reported relative to the thermal resistance of P. A. 3679 are such as to make conclusions concerning the exact thermal resistance of the organism in any food product virtually impossible. It can be said, however, on the basis of studies reported that the spores of P. A. 3679 are in general much more resistant when suspended in a variety of food products than are the spores of *Cl. botulinum* (see Townsend *et al.*, 1938). Consequently, processes for low-acid canned foods based on resistance values for P. A. 3679 should be adequate to destroy all spores of *Cl. botulinum* likely to occur in the foods.

Various species of thermophilic bacteria which are more resistant to heat than either *Cl. botulinum* or P. A. 3679 may occur in certain low-acid foods. However, resistance values for these organisms are seldom used as the basis for determination of process specifications. In other words, processes for low-acid foods are seldom designed to free the foods of highly heat resistant thermophilic bacteria. Rapid cooling of the products subsequent to processing and storage of the products at temperatures inimical to growth of thermophilic bacteria are usually relied upon to prevent growth of any of the bacteria which may have survived the processes given. In addition, special precautions are usually taken to keep thermophilic contamination of product ingredients and products to a minimum prior to thermally processing the products.

Resistance values for other bacteria have been employed for establishing requirements of processes for canned foods other than those of the low-acid type. Resistance values for *Bacillus acidurans* and *Clostridium pasteuranum* are commonly employed for establishing specifications of

processes for certain acid type foods, e.g., certain tomato products (Berry, 1933, Townsend, 1939, Wessel and Benjamin, 1941, Sognefest and Jackson, 1947).

Many important food items which are not canned (hermetically sealed in containers) are thermally processed to free them of a portion of their microflora. What organisms are employed to establish resistance values on which to base requirements of these "pasteurization" processes depends usually upon the nature of the products concerned and upon the conditions to which the products are to be subjected subsequent to thermal processing. Pasteurization processes for milk are usually based on resistance values for certain pathogenic bacteria, i.e., *Mycobacterium tuberculosis, Brucella abortus, Brucella suis, Brucella melitensis, Eberthella typhosa,* and others which may occur in milk prior to pasteurization. Thermal resistance values for *Trichinella spiralis* are frequently used as a basis for establishing "pasteurization" processes for pork or pork-containing products such as cured ham and certain sausages. Resistance values for certain *Staphylococcus* species are frequently employed for establishing specifications of thermal processes for various bakery products (see Dack, 1943).

Whether the thermal process is to destroy all or a part of the microorganisms present in a product, the problems connected with calculating a process adequate to accomplish either objective are very similar—that is, the process employed must be adequate to sterilize the food with respect to those microorganisms to be eliminated. Obviously, resistance values employed as the basis for establishing process specifications should be those for the most resistant organism to be destroyed by the process.

7. Common Errors in Thermal Death Time Data

Three types of errors are common in much of the thermal death time data reported, namely, (1) errors resulting from short-comings of methods employed to obtain the data, (2) errors resulting from failure of the investigator to appreciate the limits of methods employed to obtain the data and (3) errors resulting from improper interpretation of the results of experiments conducted. Failure to report data in full, though it cannot be considered an error in the data, is often just as serious.

Perhaps the most common errors in data reported for studies employing the TDT tube and TDT can methods, are due to failure of the investigator to apply proper corrections for heating lags for products heated in the cans or tubes, or due to the use of these methods under conditions to which they do not apply. Methods for arriving at corrections were reported by Sognefest and Benjamin (1944). In summary they state, "It has been demonstrated that when making thermal death-time tests

involving relatively short times, the heat-penetration lag and the retort come-up time take up an appreciable percentage of the total thermal death time. Correction factors for these lags have been determined for a number of products. When the factors are used in correction of come-up and heat penetration lags in thermal death-time studies, lower F and z values are obtained than when instantaneous heating and cooling is assumed without applying the corrections."

In view of available information there is reason to doubt the validity of this method for arriving at corrections for heating lags. In fact, there is reason to question whether or not the TDT tube and TDT can methods are applicable for obtaining true thermal resistance values under conditions necessitating significant corrections in the data. Such corrections are based on time-temperature relationships at the point of greatest temperature lag in the tube or can. The point of greatest temperature lag is usually the point in the container of product most remote from the surface. Any appreciable temperature lag at this point indicates that the lethality of the heat treatment given food at this point is less than the lethality of the heat treatment given food at any other point in the container. In other words the calculated lethality applies only to food at this one point in the container—all other food in the container receives heat treatments of greater lethality.

In consideration of the logarithmic order of destruction of bacteria, this question immediately presents itself: How many bacteria are being subjected to the heat treatment for which the lethality is calculated? If there were a million present in the container, there could well be only a few at the point of greatest temperature lag. In which case, all the rest in the container would be subjected to heat treatments of greater lethality than that represented at the point of greatest temperature lag; and, consequently, they would die at a more rapid rate than those located at that point. The result in such cases would obviously be that of obtaining thermal death times for bacteria subjected to temperature relationships not considered in the test. Therefore, corrections based on time-temperature relationships at the point of greatest temperature lag would in reality be greater than they should be.

Until more information is available, concerning evaluation of temperature effects at different locations in the container, it would seem best to consider the TDT tube and TDT can methods inapplicable for the determination of accurate thermal death times if conditions are such as to require significant corrections to be made for temperature lags. Proper corrections undoubtedly could be made if sufficient information were available, but to arrive at proper corrections on the basis of present knowledge seems quite beyond reach. When the difference in lethality

of heat treatments at the center and near the surface of a food in TDT tubes or cans is small in comparison to the total lethality of the process given the error in thermal death time should not be great. It is doubtful if either method should be considered reliable for determining thermal death times shorter than 15 or 20 minutes. Since corrections range from about 0.5 to about 2 minutes, depending on the food studied, they would constitute a significant percentage of shorter times. If the thermal death time were 20 minutes at 115.6°C. (240°F.) for an organism the z value for which were 18°, a correction time of 2 minutes would be interpreted as follows:

F value of process given,
Organism at surface = 6.12
Organism at center = 5.56

Errors in thermal death time of the magnitude of those resulting from lack of uniform heating in TDT tubes or cans might not be too serious if the errors were identical for all heating times; however, the magnitude of the errors increase as the heating times decrease in length. Since thermal death times are employed for establishing thermal death time curves, the z (slope) values of which curves are used directly in formulae for process calculation, it is obvious that errors for heating times at one temperature larger than the errors for heating times at another temperature would cause serious errors in values obtained from process calculations. In essence, the errors should be progressively larger as the temperatures tested increase. If no corrections were applied to the heating times, z values arrived at from the data would be greater than the actual; whereas, if corrections suggested by Sognefest and Benjamin were applied, the z values arrived at would be smaller than the actual. The magnitude of the effects on z would obviously depend on the temperature range concerned. It should be noted that corrections suggested by Sognefest and Benjamin are based on assumed values for z. To establish such corrections, z values must be assumed, because to determine z values by the TDT tube and can methods, the true corrections must be known.

Another common error in thermal death time data is its incompleteness. This may be considered by some as merely a weakness, but analysis would indicate that it should be considered a grave error. Incompleteness in thermal death time data is usually due principally to one of two things, namely, lack of sufficient replicates per test or the time intervals between time-temperature relationships employed being too great, or both. There is no set rule as to the number of replicate samples which should be subjected to each time-temperature relationship studied.

Some workers suggest that the number should be as great as 25 (see Tanner, 1944). Though the greater the number, the more reliable the results, it is often physically impossible to employ 25 replicates per time-temperature relationship without sacrificing accuracy elsewhere in the experiment. It is suggested that this be left to the discretion of the investigator, though it may be said that results from less than 6 replicates per test would probably be subject to considerable error.

With reference to time intervals between time-temperature relationships employed, there is a definite rule to be followed. It may be stated as follows: Time intervals should be such that, for some given time-temperature relationship employed, a fraction of the number and not all of the replicate samples subjected to this relationship should be sterilized. For example, suppose that 12 replicate samples are subjected to each time-temperature relationship. The following table shows the type of results which should be obtained if the time intervals are correct.

For a heating temperature of 250°F.

Heating time in minutes	Number of nonsterile samples after heating
3	12
4	12
5	5
6	0
7	0

Data of this sort are subject to analysis. For example, if each sample initially contained 5,000 spores of a given organism, 60,000 spores were subjected to each time-temperature relationship and theoretically only 5 of the 60,000 spores subjected to 121.1°C. (250°F.) for 5 minutes survived. Then, since

$$U = Z\ (\log\ a + P),$$

$$Z = \frac{5}{\log 60{,}000 - \log 5} = 1.225$$

By substituting values back in the same equation the time required for any degree of reduction in number of spores may be readily calculated.

Suppose the above data had been as follows:

Heating time	Nonsterile samples
3	12
4	12
5	12
6	0
7	0

This would have indicated that the time intervals employed were too great and the experiment should have been repeated. In lieu of repeating

the experiment, assumed values for survival would have to be employed for further interpretation of the data.

8. Recent Improvements in Thermal Death Time Methods

In view of the limitations of the TDT tube and TDT can methods for studying thermal resistance, the rate of destruction method reported by Williams *et al.* (1937) may, in principle, well be considered a notable advancement. This method eliminates the necessity of employing corrections for heating lag and its applicability, for thermal death time measurement would appear to be limited only by the physical limits of the method. However, these limits are such as to confine the use of the method to a study of temperatures not to exceed about 121.1°C. (250°F.) depending on the resistance of the organism studied.

At 126.67°C. (260°F.) heating times employed, even when studying the more resistant bacteria, may be as short as 5 seconds. If bacteria are distributed in the food before it is placed in the heating chamber, it is obvious that the food might well be virtually sterile by the time the food temperature reached 126.67°C. (260°F.). For any heating time at this temperature, withdrawing a number of samples (8 or 10) periodically, cooling them rapidly, and quantitatively estimating the size of the samples would present grave experimental difficulties. If the bacteria were introduced after the food reached 126.67°C. (260°F.) a certain amount of time would be required for the stirring mechanism to distribute the bacteria uniformly throughout the food, and the same difficulties would exist with respect to rapid withdrawal of samples.

Suffice it to say that this method as well as any other has its limitations, but within the limits of applicability the method is a great improvement, in many respects, over the TDT tube and TDT can methods. It should be noted that the method employed by Gilcrease and O'Brien (1946) is the same in principle as the method of Williams *et al.*, except that this method is employed for studying thermal resistance of bacteria to temperatures attainable at atmospheric pressure.

Stumbo (1948b) developed a method which, though it has been in use for only a few months, promises to be an improvement over other methods for studying thermal resistance of bacteria to temperatures above 118.33°C. (245°F.). The method may be employed for studying the effect of temperatures as high as 132.22°C. (270°F.). The principle on which the method is based is virtually instantaneous heating of the food samples to process temperature and virtually instantaneous cooling of the samples from process temperature to a temperature having comparatively no lethal value.

Rapid heating is accomplished by introducing small samples of in-

oculated food (about 0.02 g.) into an atmosphere of saturated steam at the temperature the effect of which is being studied. The samples are carried in small metal cups approximately 7 mm. in diameter and 1 mm. deep. The cup is not covered and, since the layer of food never exceeds 0.5 mm. in depth, heating is extremely rapid. At the end of any designated heating time, steam pressure in the heating chamber is released by simultaneously closing a valve in the steam supply line and opening a valve in the exhaust line. Cooling of the food sample to about

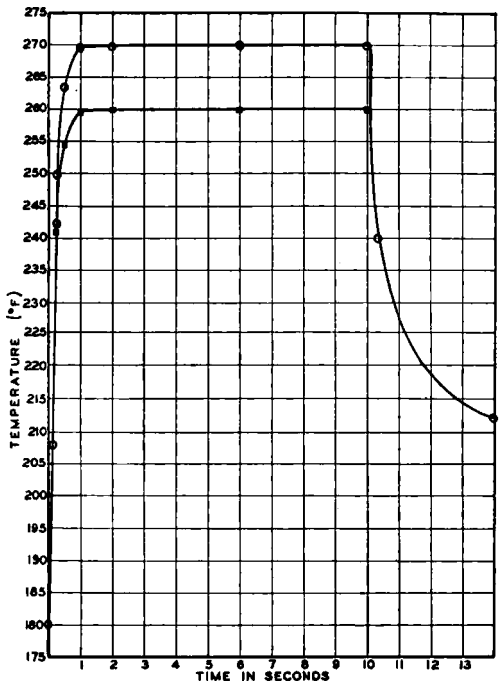

Fig. 7. Calculated heating and cooling curves representing temperatures existent in food sample, during thermal process, at point most remote from surface. Note rapidity of heating and cooling.

101.67°C. (215°F.) is thus accomplished very rapidly. When the pressure in the heating chamber has fallen to about ½ pound, the samples are withdrawn from the heating chamber and fall directly into sterile tubes of culture media. The cultures are then incubated in the usual manner. Figure 7 shows calculated heating and cooling curves for samples given 10-second processes at 126.67°C. (260°F.) and 132.22°C. (270°F.). Corrections calculated in the usual manner, for heating lag amount to less than 5% of the process time for all processes employing temperatures

up to 129.44°C. (265°F.) and having F values of 1 and greater. The correction for processes employing 132.22°C. (270°F.) is about 0.3 second or 7% of the heating time for a process having an F value of 1, 3.5% for a process having an F of 2, etc. Process time in minutes is indicated by an electric time clock which is automatically started and stopped by two micro-switches. Errors in timing and errors due to heating lags are believed to be well within experimental errors involved in preparation of spore suspensions etc., and probably could be ignored for studying the effects of temperatures up to 132.22°C. (270°F.).

From data obtained by this method, Z values for rate of destruction curves for different temperatures may be calculated. Thermal death time curves may be established from Z values so calculated. A thermal death time curve thus obtained for the putrefactive anaerobe 3679 in pureed canned peas is shown in Fig. 8. Because of the simplicity of the method in operation there is a great saving in time for making determinations of thermal death time at temperatures above 118.33°C. (245°F.). On the average, determinations by this method require about ⅕ as much time as is required for determinations by the TDT tube or TDT can method.

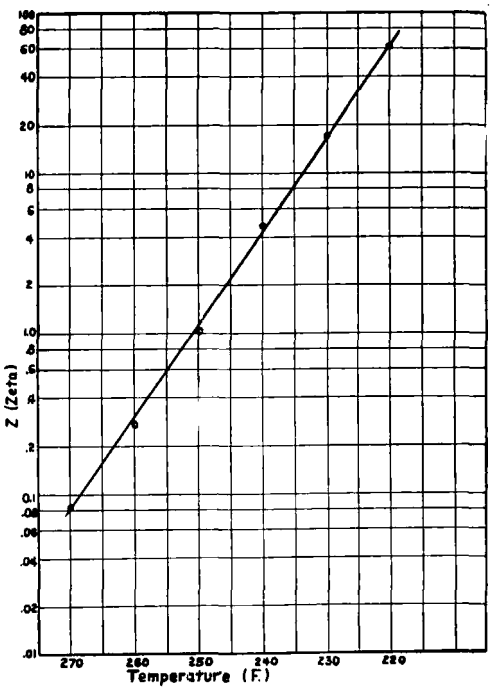

Fig. 8. "Phantom" thermal death time curve for spores of P. A. 3679 suspended in pureed canned peas.

IV. Mechanism of Heat Transfer and Process Evaluation

Stumbo (1948a) presented a critical analysis of process evaluation methods in which it was shown that mechanism of heat transfer within the food container during process must be considered if greatest accuracy in process evaluation is to be attained. A method was suggested for ascertaining the location in the container where probability of bacterial survival is greatest. A portion of this analysis is virtually reproduced here because of its bearing on preceding discussions in this review.

The General Method and Ball's mathematical methods of process evaluation discussed in the earlier sections of this paper are based on the concept that a thermal process adequate to accomplish sterilization of the food at the point of greatest temperature lag in a container during process is adequate to sterilize all the food in the container (Bigelow *et al.*, 1920; Ball, 1923, 1927, 1928, 1943, 1948; Olson and Stevens, 1939; Schultz and Olson, 1938, 1940; Jackson, 1940; Jackson and Olson, 1940; and Sognefest and Benjamin, 1944). This concept does not properly account for the influence of number of bacteria on the lethality of the process required to sterilize all the food in a container.

Jackson (1940) on the basis of data accumulated over a number of years covering heat penetration tests in a large number of food products, classified the products according to the mechanism of heat transfer within the food container. Six main classes of products are listed, ranging from those which heat by rapid convection throughout the process to those which heat by conduction throughout the process. For the sake of simplicity, discussion here will be confined to the two classes representing the two extremes with respect to mechanism of heat transfer, namely, foods which heat primarily by convection and foods which heat primarily by conduction.

Jackson (1940) and Jackson and Olson (1940) reported results of a series of studies concerning the mechanism of heat transfer in bentonite suspensions during process in No. 2 (307 x 409) and No. 10 (603 x 700) cans. A suspension of 5% bentonite in water gave heat penetration curves quite typical of those usually obtained for certain food products which heat by conduction. A suspension of 1% bentonite gave curves typical of those for certain convection-heating products. A suspension of 3.25% bentonite gave broken heating curves, indicating convection heating changing to conduction heating during the process. The latter suspension need not be discussed here beyond saying that the nature of heat transfer in it is similar to the nature of that observed for certain foods and ultimately should receive consideration, with respect to process evaluation, similar to that given the other types of heating.

On the basis of results of these studies with bentonite, mechanisms of heat transfer may be described in general as they relate to calculating adequate processes for food sterilization.

1. Conduction-Heating Products

Products heating by conduction, if the container is stationary, do not move within the container during process. During heating, heat from the surrounding medium (usually steam or hot water) is transferred to the outermost layer of food in the container, thence inward toward the center of the food mass without any of the food, and bacteria in the food, changing location within the container. When a container of food is placed in a heating medium such as steam under pressure it may be quite safely assumed that a very thin layer of food next to the container wall assumes the temperature of the steam almost instantaneously. Heat is then transferred inward toward the center of the food mass from all points at the container wall. During the initial phase of heating there is a constant temperature portion of food near the center of the container. The temperature of the food near the container wall rises during this lag period. Subsequent to the lag period the temperature from the center to the can wall rises on a smooth curve (Fig. 9). If heating is allowed to continue long enough, the entire contents will eventually reach the temperature of the surrounding steam; however, the last portion of food to reach this temperature will be that at the geometric center of the container (the point of slowest heating).

If at the end of any given heating time, the container is plunged into cooling water [say at a temperature of 21.1°C. (70°F.)] heat transfer within the container is reversed in direction and the contents cool until equilibrium is reached with the surrounding medium. Because temperature rise during heating and temperature drop during cooling are logarithmic in order, cooling to a non-lethal temperature is accomplished in considerably less time than is required to heat the food, from the lowest temperature which is lethal, to the highest temperature attained during process. Therefore, even though the temperature drops less rapidly at the center than at other points in the container during cooling, there is a small volume of food at the center which receives a less severe heat treatment than any other food in the container. Then it may be said that the severity of heat treatment increases progressively from the center, in any direction, to the wall of the container.

If we visualize a series of cylindrical containers ranging in size from one the size of the food container to one the size of a short piece of thin pencil lead we can picture this decrease in severity of heat treatment from outside to center of the mass of food. If the containers are con-

sidered to decrease in size progressively in accordance with a uniform decrease in length and diameter we may picture them being placed one inside the other from largest to smallest such that the geometric center of each imaginary cylindrical container is common with the geometric center of the real container. It can be shown that the surface area of each of these imaginary containers would represent a number of bacteria bearing the same relation to the number of bacteria represented by the surface area of the real container as the surface area of the imaginary container bears to the surface area of the real container. Also the surface area of one imaginary container would bear the same relation to the surface area of another as the number of bacteria at the surface of one bears to the number of bacteria at the surface of the other. The decrease in severity of heat treatment from the outside to center of the real container is also related to the decrease in surface area of the imaginary cylindrical containers; and therefore, it is related to the decrease in number of bacteria from outside to center of the real container. It should be noted here that the relationships pictured are not exact; but their divergence from the more complicated relationships which truly exist during process is believed to be so small that errors induced from use of the assumed relationships in methods of process evaluation would be negligible. The true relationships could be visualized by picturing the imaginary containers gradually changing from cylindrical in shape to ellipsoidal or spherical in shape from outside to center of the real container. Differences in container size and shape would influence these relationships, but the total influence of all these factors is believed minor and justifiably neglected in the following considerations.

2. Location in Container Where Probability of Survival is Greatest

Assume that a conduction heating food is placed in a No. 10 (603 x 700) can and the mechanism of heat transfer within this food during process will be identical with that described by Jackson and Olson (1940) for a 5% bentonite suspension. Assume further that this can of food contains 10,000 spores of *Cl. botulinum* uniformly distributed in the food mass. At what location in the container would the probability of spore survival be greatest? What would be the F value of the heat treatment at the geometric center of the container when the probability of survival is 1 in 1,000,000,000, or some other given value, in the location where probability of survival is greatest?

For a condition of 10,000 spores per No. 10 can of food, there would be one spore per approximately 0.3 g. of product. Also, there would be approximately 1,215 spores in a layer of food, 0.3 cm. in depth, next to the can wall; 860 spores in a layer of equal depth next to the wall of an

imaginary container 5 in. in diameter and 6 in. high; 567 spores in a similar layer next to the wall of an imaginary container 4 in. in diameter and 5 in. high; 347 in the corresponding layer for an imaginary container 3 in. in diameter and 4 in. high; 162 in the corresponding layer for an imaginary container 2 in. in diameter and 3 in. high; and, 50 in the corresponding layer for an imaginary container 1 in. in diameter and 2 in. high. Assuming one spore to be located at the geometric center and plotting the number of spores at the surface of the various containers against the radius of the corresponding containers a spore distribution curve is obtained describing the increase in number of spores from geometric center to wall of the No. 10 can for a condition of 10,000 spores distributed uniformly in the food mass (Fig. 9).

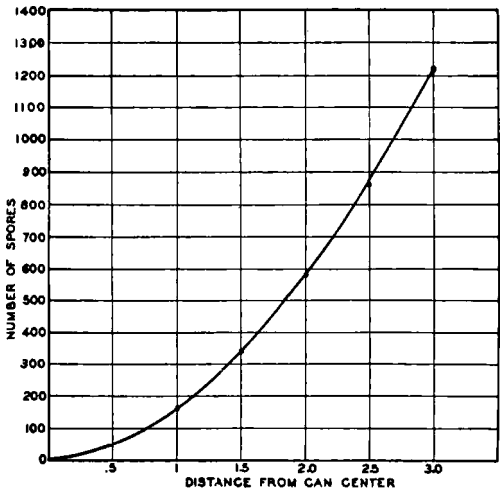

Fig. 9. Spore distribution from geometric center to wall of No. 10 (603x700) can, when 10,000 spores are distributed uniformly in food mass. Distances from can center (radii of imaginary cylinders of food) plotted against number of spores lying at surface of respective imaginary cylinders—see text.

The severity of heat treatments to reduce to some given level the number of spores in the different locations in the container may be calculated. Taking the values computed to represent the number of spores at the center, at the surface of each of the imaginary containers, and at the surface of the real container, we find heat treatments having the following F values required to reduce the number in each location to 0.000000001 spore.

Initial number of spores	F value required
1 (center)	1.953
50 (0.5 inch = radius of container)	2.322
162 (1.0 inch = radius of container)	2.433
347 (1.5 inch = radius of container)	2.504
567 (2.0 inch = radius of container)	2.550
860 (2.5 inch = radius of container)	2.589
1,215 (3.0 inch = radius of container)	2.622

These F values were calculated using $Z = 0.217$ for slope of rate destruction curve for spores of *Cl. botulinum* heated at 250°F., and using the following equations:

$$U = Z\ (\log a - \log b)^a$$

$$F = \frac{U}{F_i}$$

$$F_i = \log^{-1}\frac{250 - RT}{z}$$

RT was taken as 250°F. The value of z was assumed to be 18° though its value does not alter the above calculated values of F. The value 0.217 used for Z is about average for values computed from published work of Esty and Meyer (1922) and Townsend *et al.* (1938).

If the F values listed above as required for the different numbers of spores are plotted on the linear scale against the respective numbers of spores on the log scale a straight line curve is obtained. This may be termed the F requirement curve (Curve No. 1, Fig. 12) for *Cl. botulinum* spores. In practice, the F requirement curve should be established from resistance values obtained for botulinum spores suspended in the food for which a process is to be calculated.

Heat penetration curves representing heat treatments at various points in the food from the geometric center to the can wall may be estimated from the temperature distribution curves, reported by Jackson and Olson (1940), for a 5% bentonite suspension (see Fig. 10). On the basis of these heat penetration curves, F values for the heat treatments at various points in the container may be calculated. Assuming that one single spore would be located at the point representing the geometric center of the container, the minimum F value required to reduce the probability of survival to 1 in 1,000,000,000 at this point should equal 1.95. The time requirement (191 minutes) for a thermal process (RT = 250°F.) was therefore calculated which would result in this F value at the center of the container. (A z value of 18°F. was assumed to repre-

[a] This is simply another form of the equation $U = Z\ (\log a + P)$. In this equation b is equal to the number of organisms (spores) remaining viable at the end of heating time U.

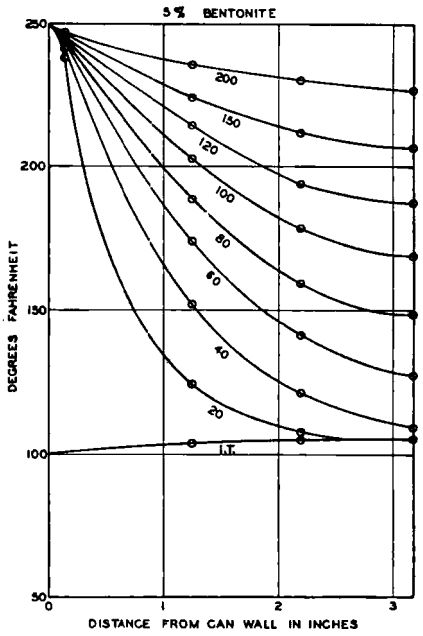

Fig. 10. Temperature-distribution pattern across central horizontal plane in No. 10 can. (The curves represent temperature distribution at various designated minutes during the process.) Taken from Jackson and Olson (1940).

sent the slope of the thermal death time curves for spores of both *Cl. botulinum* and P.A. 3679.) *F* values for heat treatments at other points various distances from the center were then calculated for the process of 191 minutes in length. The *F* value of the heat treatment for food next to the can wall was assumed to be 191. Then each *F* value calculated was for food at a point some given distance from the geometric center of the container and lying in a horizontal imaginary plane bisecting the container midway between the top and bottom. Each point was visualized as also lying at the surface of a cylinder of food the radius of which would equal the distance from the center to the respective point. The height of each cylinder was considered to be as much less than the height of the container as the diameter of the cylinder was less than the diameter of the container. By plotting the number of spores at the surface of each imaginary cylinder against the *F* value for any point at the surface of each respective cylinder, an *F* distribution curve was obtained which described the *F* value of the heat treatment to which any given number of spores were subjected.

The *F* distribution curve plotted on the same coordinates as the *F* requirement curve for 10,000 spores uniformly distributed in the food mass determines the location in the container where probability of spore survival is greatest. Figure 12 shows the *F* requirement curve (Curve No. 1) for 10,000 spores of *Cl. botulinum* as they are distributed in the food and the *F* distribution curve (Curve No. 2) for a hypothetical conduction-heating food which heats during process similarly as did the 5% bentonite suspension studied by Jackson and Olson (1940). With regard to *F* requirement and *F* distribution curves so plotted, it may be said that if, and only if, all points on the *F* distribution curve fall on or above all points on the *F* requirement curve, the probability of spores

surviving in any location (designated layer 0.3 cm. thick) in the container is as low or lower than the probability of survival for which the F requirement curve was established (1 in 1,000,000,000 for the F requirement curve in Figure 12). It may be noted that, in this case, the probability (1 in 1,000,000,000) would be greatest at the geometric center.

3. Convection-Heating Products

Foods which heat by convection exhibit much more rapid heating than do foods which heat by conduction. In the case of convection heating, transfer of heat in the food mass is aided by product movement within the container. For a condition of ideal convection heating, temperatures throughout the container of food during process would be identical at all times. Then it may be said that for ideal convection heating, heat treatments at all points throughout the container would have identical lethality (F) values. Though such an ideal condition of heating is probably never realized, it is obvious that movement of product within the container during heating to any great extent would result in more nearly uniform heating. How nearly ideal heating would be approached would depend, other things being equal, on the extent of product movement, and therefore, indirectly on the nature of the product being heated.

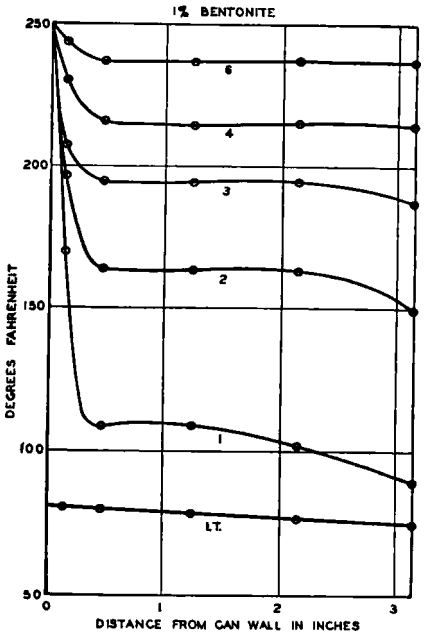

Fig. 11. Temperature-distribution pattern across central horizontal plane in No. 10 can. (The curves represent temperature distribution at various designated minutes during the process.) Taken from Jackson and Olson (1940).

In their studies with 1% bentonite suspensions, Jackson and Olson (1940) determined temperature distribution curves for the product during heating in No. 10 (603 x 700) cans. Figure 11 shows a series of curves representing temperatures across a central horizontal plane in the No. 10 can. The curves represent temperature distribution at the various designated minutes during process at a retort temperature of 121.1°C (250°F.). Estimating values from these curves, an F distribution curve was established for this convection-heating product on the basis of

considerations identical with those on which establishment of the F distribution curve for the conduction-heating product (5% bentonite) was based. This curve (Curve No. 3) appears in Fig. 12. Observing Fig. 12, it

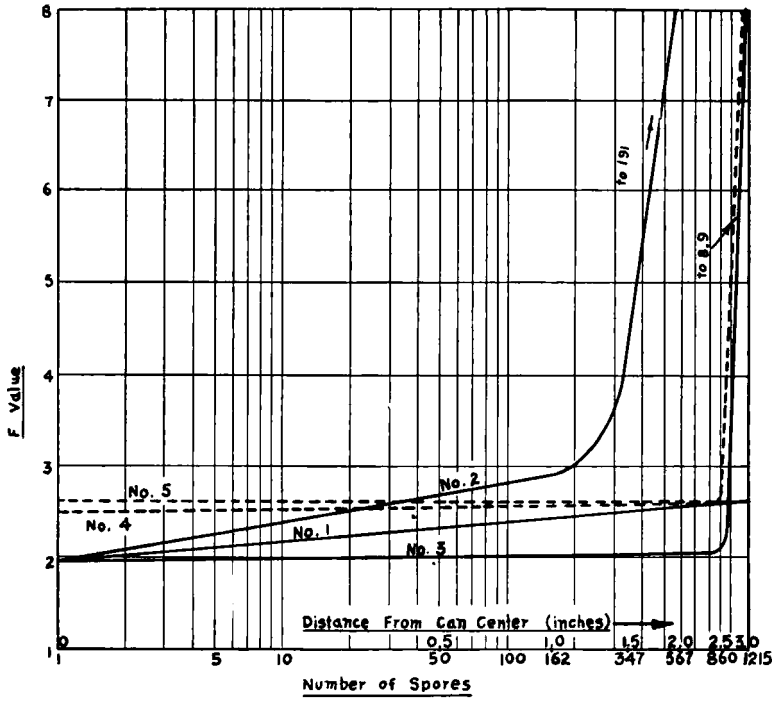

Fig. 12. Graphical depiction of relative capacities of heat treatments, for different locations in the container, to reduce the number of *Cl. botulinum* spores in these locations.

Curve No. 1—F requirement curve for spores of *Cl. botulinum*.

Curve No. 2—F distribution curve for conduction-heating product (5% bentonite in water).

Curve No. 3—F distribution curve for convection-heating product (1% bentonite in water).

Curve No. 4—F distribution curve for convection-heating product when probability of survival is one in one billion or less in all locations in container.

Curve No. 5—F distribution curve for product which heats ideally by convection.

will be noted that a considerable portion of Curve No. 3 lies below Curve No. 1, the F requirement curve for *Cl. botulinum*. This indicates that the process (8.9 minutes at 250°F.) calculated to give an F value of 1.95 at the center of the container is inadequate to reduce the probability of

survival to 1 in 1,000,000,000 in all locations in the container. It may be noted that the probability of survival is greatest in locations quite remote from the geometric center (in this case, at the surface of an imaginary cylinder the radius of which is about 2.5 in. and the geometric center of which is common with the geometric center of the real container). Curve No. 4 is Curve No. 3 moved upward in the direction of ordinates to a position at which all points on the curve lie on or above the F requirement curve. The F value (2.50) indicated by the intersection of Curve No. 4 and the y-axis represents the F value which would be obtained at the center of the container for a process adequate to reduce the probability of survival of *Cl. botulinum* spores to 1 in 1,000,000,000 in the location in the container where probability of survival is greatest. Curve No. 5 is the F distribution curve for a product which would heat ideally by convection. It may be noted that the F value indicated by the intersection of this curve and the y-axis is equal to 2.62, or that value required to reduce the probability of survival to 1 in 1,000,000,000 in the location where probability of survival is greatest.

It may be said that foods in No. 10 cans exhibiting temperature distribution patterns during process identical with those of 1 and 5% bentonite suspensions would require thermal processes adequate to give food at the center of the containers heat treatments having the following F values, if the probability of survival in all locations in the containers is to be one in one billion or less.

Conduction-heating product	1.95
Convection-heating product	2.50

This is considering 10,000 spores of *Cl. botulinum*, of the assumed resistance, are present per container.

4. Influence of Resistance of Organism to be Destroyed

The magnitude of the difference in F values required, at the center of the container for conduction and convection-heating products, to accomplish comparable reduction in number of viable organisms present should logically be influenced by the resistance of the organism concerned. The relative magnitude of the influence may be pictured by comparing Figs. 12 and 13. Curves in Fig. 13 were constructed in the same manner as were the curves in Fig. 12. The F values for the F requirement curve were determined for the putrefactive anaerobe 3679 by use of the following values in calculations.

$Z = 1.0$

$z = 18.0$

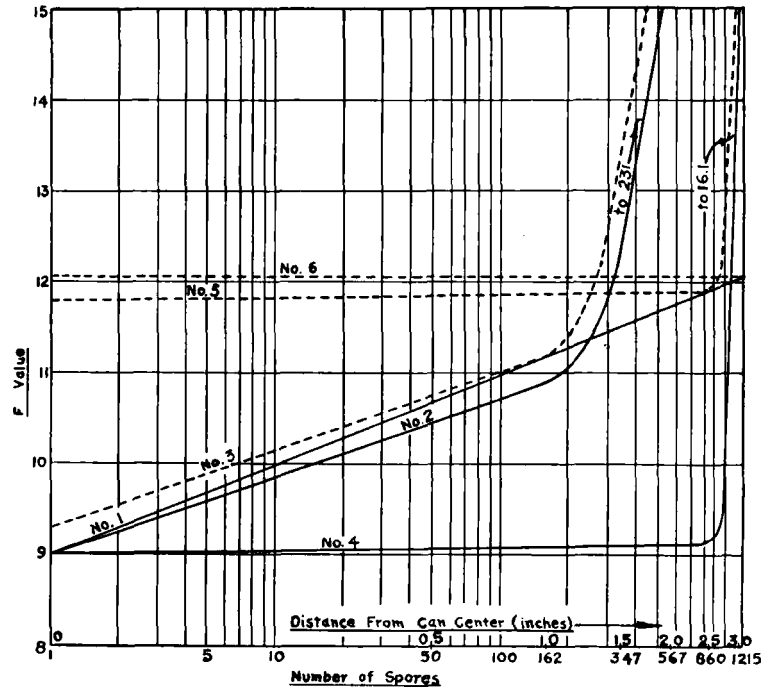

Fig. 13. Graphical depiction of relative capacities of heat treatments, for different locations in the container, to reduce the number of P. A. 3679 spores in these locations.

Curve No. 1—*F* requirement curve for spores of P. A. 3679.

Curve No. 2—*F* distribution curve for conduction-heating product (5% bentonite in water).

Curve No. 3—*F* distribution curve for conduction-heating product when probability of survival is one in one billion or less in all locations in container.

Curve No. 4—*F* distribution curve for convection-heating product (1% bentonite in water).

Curve No. 5—*F* distribution curve for convection-heating product when probability of survival is one in one billion or less in all locations in container.

Curve No. 6—*F* distribution curve for product which heats ideally by convection.

End-point of survival = 0.000000001 spore.

F values required therefore were determined to be as follows:

Number of spores	*F* value
1	9
50	10.7
162	11.21
347	11.54
567	11.75
860	11.93
1,215	12.08

Curve No. 1 (Fig. 13) is the *F* requirement curve for spores of P.A. 3679. Curve No. 2 is the *F* distribution curve for the conduction-heating product. Since a considerable portion of this curve lies below the *F* requirement curve, it is obvious that, in this case, a process adequate to accomplish the desired survival probability (1 in 1,000,000,000) at the center of the container would not be adequate to accomplish this result in all the designated locations in the container. It may be noted that the probability of survival is greatest in locations quite remote from the center (at the surface of an imaginary cylinder the radius of which is approximately 1 in. and the geometric center of which is common with the geometric center of the real container). Curve No. 3 is obtained by shifting Curve No. 2 upward until all points lie on or above the *F* requirement curve. Curve No. 3 intersects the *y*-axis at the point representing an *F* value of 9.30. Therefore, a process adequate to reduce the probability of survival in all locations in the container to 1 in 1,000,000,000 or less would result in a heat treatment, at the center of the container, having an *F* value of 9.30.

Curves No. 4 and No. 5 are similar curves constructed for heating relationships characterizing the 1% bentonite suspension (convection-heating). Curve No. 5 intersects the *y*-axis at the point representing an *F* value of 11.81. An adequate process in this case then would result in a heat treatment, at the center of the container, having an *F* value of 11.81. Curve No. 6 is the theoretical *F* distribution curve required of a product which heats purely by convection, if probability of survival is reduced to one in one billion or less in all locations in the container.

Based on the values assumed and estimated, processes required to give the desired reduction in survival probability of spores of *Cl. botulinum* and P.A. 3679 in the location where probability of survival is greatest may be specified as follows:

	F value for heat treatment at center of container		
Organism	Conduction	Convection	Ideal Convection
Cl. botulinum	1.95	2.50	2.62
P. A. 3679	9.30	11.81	12.08

5. *Discussion*

The above considerations, concerning the influence of mechanism of heat transfer and the order of bacterial death on F values required for thermal processes for foods, have been presented in evidence that the factors must be considered if greatest accuracy in process evaluation is to be attained. It is not the intention to imply that temperature distribution curves for bentonite suspension should be employed in evaluating processes for foods. Such curves were used here for the purpose of demonstrating considerations that should be given similar relationships which undoubtedly obtain for food products. With reference to mechanism of heat transfer within the container, the basic principles involved are virtually the same for suspensions of bentonite and for many canned food products manufactured commercially. Sufficient quantative information, concerning temperature distribution in canned foods during heat processing, to support the present analysis could not be found in literature. It should be noted that considering the point of greatest temperature lag as occurring at the geometric center of the container for products which heat by convection is believed to be a justifiable procedure until more exact information is available. The point of greatest temperature lag for many such products has been found to lie somewhat below the geometric center. However, the rate of heating at the center is usually somewhat less than the rate of heating at points above the center, and the error introduced above by considering slowest heating as occurring at the center should be small.

The more rapid the heating the smaller is the f_h value characterizing the heat penetration curve representing rise in temperature at the point of slowest heating. Relatively small f_h values characterize heat penetration curves for products heating primarily by convection, values in the range of 5 to 15 for foods in No. 10 cans being quite common. (The f_h value determined for the heat penetration curve representing rise in center temperature of the 1% bentonite suspension in the No. 10 can was about 4.3, that for the curve representing rise in temperature at a point 1.5 in. above the bottom was about 4.8, and that for the curve representing temperature rise at a point 1.5 in. below the top was about 4.0). The f_h values characterizing heat penetration curves for products which heat primarily by conduction are relatively large, values in the range of 150 to 200 for foods in No. 10 cans being common. (The f_h value determined for the heat penetration curve representing rise in center temperature of the 5% bentonite in the No. 10 can was about 174.) Between these two rather well-defined classes of foods, with re-

spect to mechanism of heat transfer within the container, there are foods which heat by an admixture of convection and conduction.

A method has been presented for ascertaining the location in the container where probability of bacterial survival is greatest and for arriving at processes which will reduce the probability of survival in this location to any desired level. To arrive at an exact method of process evaluation which will determine the probability of survival in the entire container of food will require a great deal of information concerning temperature distribution in many foods during process.* The influence of container size, temperature of processing, etc. on temperature distribution in the container will have to be studied thoroughly. That information of this nature is not available at present is undoubtedly due to the fact that its importance in process evaluation has not been realized heretofore. However, the logarithmic order of death and mechanisms of heat transfer within the container would seem to have been accounted for, to an extent at least, in commercial processing of foods.

6. Theory and Practice

In January 1930 the National Canners Association issued a bulletin (26-L) titled, "Processes for Non-Acid Canned Foods in Metal Containers." The sixth edition of this bulletin appeared in 1946. Processes recommended in the bulletin have been arrived at through consideration of scientific data and information which major food packing industries

* Subsequent to the preparation of this review the author developed a graphical procedure for integrating probabilities of survival throughout the container of food, thereby making possible the calculation and evaluation of thermal processes in terms of number of spores surviving per container. This method of process calculation has been published in *Food Technology* (Stumbo, 1949) under the title, "Further Considerations Relating to Evaluation of Thermal Processes for Foods." The accompanying analysis clearly demonstrates that there is no one location, within the food container, the sterility of which denotes sterility of all other locations. The probability of survival in the entire container can be determined only by integrating the probabilities of survival in all the designated locations (imaginary layers of food in the container according to method described). In this manner thermal processes may be specified which are equivalent with respect to their leaving the same number of spores surviving per unit volume or per container of product. Applying the integration method developed to evaluation of thermal processes for conduction-heating and convection-heating foods, the author concluded that if thermal processes for all foods were adjusted so as to give the same probability of survival in the location in the container where probability of survival is greatest, the processes would from the practical standpoint be virtually equivalent with respect to allowing the same number of spores to remain per container or per any given number of containers. If processes were adjusted in this manner, the more laborious task of integrating probabilities of survival in all locations within the container would be unnecessary except for the purpose of arriving at reference processes.

have gained by experience. Many processes suggested are more severe than those which have generally been considered necessary to safeguard against botulism; the severity of these processes has for the most part been dictated by commercial experience.

Though the processes listed in Bulletin 26-L are given in terms of times and temperatures for the different products in various sized cans, F values may be calculated, from heat penetration and bacteriological data, and assigned to these processes. If the z is assumed to be 18°, the F values are designated as F_0 values to describe this special condition. This is a convenient assumption to make for the purpose at hand. Two groups of products may be chosen, one representing products which heat primarily by conduction and the other representing products which heat primarily by convection. Table II lists six products of each type and

TABLE II

Approximate F_0 Values of Processes Used in Industry

Conduction heating			Convection heating		
Product	Approx. F_0 value No. 2	No. 10	Product	Approx. F_0 value No. 2	No. 10
Corn (cream style)	5.00	2.50	Corn (whole kernel) in brine	9.00	14.50
Pumpkin or squash	2.25	2.00	Carrots and peas	12.00	16.00
Sweet potatoes (solid pack)	3.50	3.00	Peas	8.00	11.00
Sweet potatoes (syrup pack)	3.00	3.00	Beans (green and wax) brine packed	3.50	6.00
Hash (corned beef, roast beef, and ham)	5.00	5.00	Beans (shelled type, succulent)	11.00	18.00
Spaghetti and meat balls	5.50	5.50	Onions in brine	4.00	7.00
Averages	4.04	3.50		8.25	12.08

the approximate F_0 values calculated for the processes recommended for processing the foods in No. 2 and No. 10 cans. Most of the processes for which the approximate F_0 values are listed in Table II, appear in Bulletin 26-L; but for the purpose of completeness, a few were selected from other reliable sources as representative of those which are being used successfully by industry in general. No attempt was made to select only those products the processes for which would support the contentions made here; but rather to select products which it was thought would exhibit similar influences on bacterial resistance even though some heat by

convection and some by conduction. Other products could be added to the list, but in reviewing the processes employed for most all low-acid foods it was found that processes given those products listed in Table II were sufficiently representative to give a true picture of the point in question. It would be expected that exceptions could be found, but the general picture must be considered rather than exceptions in this case.

With reference to the F_0 values listed in Table II for the various products, obvious questions immediately present themselves. It is very doubtful if the differences in severity of processes, indicated by the table, for conduction- and convection-heating products, can be explained on the basis that different bacteria occur in the two types of products, or that the same bacteria are consistently more resistant in products which heat by convection. If so, how can the fact be explained that more severe processes are considered necessary for the convection-heating products in No. 10 cans than are considered necessary for these products in No. 2 cans? In general, the differences noted are logically explained by considerations discussed above, namely, that bacteria die according to the logarithmic order and that convection-heating results in a larger percentage of the product in the container receiving a uniform or nearly uniform heat treatment of the order of that received by food at the point of greatest temperature lag in the container.

7. Product Agitation During Process

Upon inspection of Figs. 12 and 13 it becomes obvious that, especially in the case of conduction-heating products, food next to the container wall and for a considerable distance toward the center receives heat treatments far more severe than would be necessary to accomplish the same reduction in number of bacteria as is accomplished by an adequate heat treatment at the center of the container. Many foods which heat almost entirely by conduction, and many others which heat partially by conduction when not agitated during processing, will heat almost entirely by convection when sufficiently agitated. Agitation of product therefore should result in less food in the cotnainer being over-processed. For this reason, interest in agitation of product during process is now increasing rapidly.

In establishing process specifications for food which is agitated during process full consideration should be given to the influence of temperature distribution in the container on the F value required of the process. By agitation it should be possible to shorten considerably the time required at a given temperature to accomplish sterilization of the product. However, the F value required of the heat treatment at the geometric center of the container would, on the basis of considerations discussed above,

have to be somewhat higher. *F* values of processes comparable in sterilizing capacity to those employed for identical products not agitated during process could be established through studies relating to temperature distribution in the container during process.

8. High-Temperature Short-Time Processes

Ball (1938) makes the following statements concerning the relation of processing temperature, heat penetration, and quality.

"Foods that have a high rate of heat penetration, whether it be produced by convection currents in the food or by agitation of the food during heating, will usually have better quality after processing if they are sterilized at temperatures in the higher range, e.g., above 121.1°C. (250°F.), than they will if sterilized at a lower temperature. Conversely, articles having slow heat penetration will usually have better quality after processing if they are sterilized at a temperature below 121.1°C. Furthermore, foods having rapid heat penetration will usually have better quality after being sterilized at a high temperature than will foods having slow heat penetration after being sterilized at a low temperature."

As pointed out by Ball, these facts led to the conclusion that, by increasing the rate of heat penetration into a food that is impaired in quality by the ordinary process, and by raising the processing temperature, improvement in quality of the finished product would be attained. During the past 20 years there has been a rapidly growing interest in high-temperature short-time sterilization of foods. Numerous patents have been issued covering various methods and equipment for accomplishing this type of processing of many different foods (see Ball, 1938).

In the determination of process specification for these high-short sterilization methods, the fact that more nearly uniform heating of the product is being accomplished during process should be fully considered. Though quality improvement is attainable for many foods processed in this manner, it would be reasonable to expect that somewhat higher *F* values would be required for sterilization than are required of processes characterized by slower rates of heating.

V. Summary and Discussion

It will have been noted that no attempt is being made to review all the important contributions relating to the application of thermobacteriology to food processing. During the past three decades several hundred papers and several books have been published which have dealt wholly or in part with this subject. Obviously, adequate treatment of each of these is beyond the scope of this brief review. However, a great many of these works, though not mentioned here, have contributed greatly toward shaping the course of the evolutionary developments which have

characterized the past thirty year period of rapid advancement. A great amount of scientific research, the results of which have not been published, and the invaluable experience of the food industry have likewise contributed generously. The course of developments discussed above, though admittedly it describes a narrow path through a diverse and complex field, has been chosen as much because of the many important unsolved problems it points up as because of the advancement it exemplifies.

With respect to calculating processes for foods, attention in the past has been confined to collecting information which would permit of arriving at thermal processes adequate to free food, at the points of greatest temperature lag in containers, of certain microorganisms considered to be of greatest importance from the standpoint of food spoilage and consumer health. With few exceptions time-temperature relationships at the point of greatest temperature lag only have been studied. This has undoubtedly been due to the fact that methods of process evaluation which have been in use since 1920 were based on the concept that a thermal process adequate to sterilize a food at the point of greatest temperature lag in the container would be adequate to sterilize all food in the container. Yet these methods, especially the mathematical methods developed by Ball (1923; 1928), undoubtedly constitute one of the greatest contributions ever made to the art of thermal processing of foods. The methods have allowed a high degree of refinement to be made in food processing. However, on the basis of considerations brought out in the foregoing discussion, modification of the methods to account for the logarithmic nature of bacterial death should permit of still further refinement. Analysis has shown that the concept regarding the point of greatest temperature lag in the container as being the only point of concern with respect to arriving at adequate and desirable processes should be revised in light of present knowledge concerning the influence of mechanism of heat transfer on process requirements.

It is now apparent, since it is realized that probability of bacterial survival is often not greatest at the point of greatest temperature lag, that temperature distribution throughout the container during process will have to be considered if greatest accuracy is to be attained in thermal process evaluation. It is firmly believed that marked refinement in food processing methods and therefore in quality of thermally processed foods is attainable through such consideration. The refinement, however, is dependent upon the accumulation of a great deal of information through organized studies concerning temperature-distribution patterns characterizing the heating and cooling of many different foods in containers of various sizes. The influence of retort (processing) temperature, nature

of product, product agitation and other factors on these temperature-distribution patterns will have to be studied thoroughly before greatest refinement can be expected. Mathematical analysis should play a major role in guiding these studies, as well as in interpreting the results obtained from them. The ultimate goal should be the development of mathematical methods for predicting the temperature-distribution patterns which would characterize the heating and cooling of all important types of food during process; in this case, since heating and cooling are physical phenomena, it should be possible, eventually, to replace experiment with mathematics. This goal will be reached only through several years of carefully organized and patiently executed research. However, if this goal had already been attained, the task with regard to accumulating sufficient information to permit of greatest refinement in food processing through the application of thermobacteriology would be scarcely more than begun.

To be of greatest practical value from the standpoint of commercial food processing, knowledge concerning time temperature relationships existent throughout the container of food during process must be interpreted in terms of the possible effects of these relationships on bacteria which may be present in the food. Modification of the mathematical methods for process evaluation to account for the logarithmic order of death of bacteria has been suggested. It has been shown by various investigators that, in general, the death of bacteria as the result of the application of heat may be described as occurring logarithmically. Deviations from a true logarithmic order have been noted; however, the occurrence of deviations does not mean that the law governing the rate of a monomolecular reaction may not be basically applicable for describing the rate of death of bacteria. It is known that many laws which are fundamentally applicable for describing physical and chemical reactions are subject to correction because of the influence of extraneous factors. The gas law commonly expressed as $pv = nRT$ is a fitting example. Until Budde corrected the gas law equation to account for volume occupied by the gas molecules and Van der Waals corrected it to account for molecular attraction, the equation was not strictly applicable. Deviations in this case were logically explained; corrections applied to the basic equation greatly increased its applicability.

With regard to methods of process evaluation, it may be possible to account for all deviations in the order of death of bacteria when the causes of the deviations are fully understood. Many of the deviations noted are undoubtedly attributable to inadequate methods of study; others may be due to inherent differences in the bacterial cells within a group or to the influence of extraneous factors such as constituents of

the medium in which the bacteria are suspended during heating. Rahn (1945a; 1945b) summarized available information relative to conditions resulting in a non-logarithmic order of death of bacteria. He states (1945b) that, "The evidence on hand is overwhelmingly in favor of logarithmic order of death in bacteria, and isolated exceptions could be disregarded. However, there are some consistent exceptions which cannot be disregarded although they are unexplained." As an example of the latter he discusses the order of death of bacteria treated with chlorine solutions. Suffice it to say that further studies may reveal consistent exceptions when heat is used as the killing agent, though present evidence does not indicate that such exceptions will be numerous.

Extensive studies concerning factors influencing death rates of bacteria in foods subjected to heat are immediately suggested upon considering death of bacteria in the foods as occurring logarithmically. Results of a large majority of the thermal resistance studies conducted in the past are not such as to give reliable information relative to rates of destruction of important bacteria in the various food products. Most of the data presented have been qualitative in nature. There is a very urgent need for studies designed to give quantitative data relative to rates of destruction by heat of spores of *Cl. botulinum, Cl. sporogenes,* P.A. 3679, *Cl. pasteurianum, B. thermoacidurans,* and other important food spoilage bacteria in a wide variety of food products. These studies should include investigations designed to evaluate the influence of various factors on rate of destruction of the bacteria at the various temperatures employed in processing foods. The following factors are known to be important but the magnitude of their importance has not been well established.

1. Kind of food.
2. pH of food.
3. Medium in which bacteria have grown prior to thermal resistance tests.
4. Added ingredients such as salt (NaCl), sugars, curing agents in the case of meats, etc.
5. Age of bacteria (state of maturity of cells, or spores, subjected to heat).

Delayed spore germination, though not a factor influencing thermal resistance, may influence the results observed for thermal resistance tests. Very limited evidence has been presented from time to time indicating that germination of spores given very severe heat treatments may require a longer time than is required for germination of similar spores given less severe heat treatments. It is logical that such should be the case, but the phenomenon is often not adequately accounted for in thermal resistance studies. In the bacteriological sense a cell is considered

dead when it fails to reproduce. Using failure of reproduction as the criterion of death, the logarithmic order of death of bacteria can be explained by assuming that death of a bacterial cell is due to the denaturization of a single molecule (gene) responsible for reproduction (Rahn, 1945b). It is quite logical to believe however that a severe heat treatment could cause a great deal of cell injury (enzyme destruction, coagulation of protoplasm, etc.) without inactivating the reproductive gene. It is also logical that cell injury of this type would have to be repaired before normal reproduction could proceed—in the case of the spore, before spore germination and normal reproduction could proceed. Recent studies (unpublished) in this laboratory strongly support the explanation that cell injury is at least a contributing factor to delayed spore germination.

The significance of delayed spore germination as it influences rate of destruction data can be very serious. Cell injury being less for shorter heating times, the surviving spores may germinate in far less time than spores of equal resistance which have survived longer heating times. If the incubation period after which growth readings, or spore counts, are taken is short, the rate of destruction curve obtained will usually be concave downward. The survivor curve, plotted on semi-log paper, will usually more nearly approach a straight line as the incubation period is increased unless conditions in the subculture medium become unfavorable for cell repair and bacterial growth. It is often difficult when studying thermal resistance of spores of anaerobic bacteria to maintain anaerobiosis in the subculture medium sufficiently long to permit of germination of spores which have survived comparatively severe heat treatments. That delayed germination and growth is, in part at least due to cell injury, is supported by the fact that survivor curves for bacteria treated with chlorine are usually concave downward (see data of Mallmann and Audrey, 1940). In this connection, Rahn (1945b) states, "It is conceivable that chlorine, being such a very active chemical reagent, kills bacteria by destroying the membrane, or the protoplasm, or the enzymes before attacking the mechanism of reproduction." However, other factors are known to cause delays in spore germination.

Certain chemical and physical environmental factors have been shown to influence markedly the time required for spores to germinate (see Wynne and Foster, 1948a, 1948b). A vast amount of further study will be required before definite conclusions can be made concerning the number of factors contributing to delay in spore germination, or concerning the magnitude of effect of any single factor. The problem is one of the most important from the standpoint of evaluating thermal processes for foods, and warrants the serious attention of many research workers. In

studies relating to rate of destruction of spores by heat, limited results in this laboratory indicate that divergence of the rate of destruction curve from a straight line on semi-logarithmic coordinates is in some cases a good indication that delayed spore germination is being encountered.

In order to apply rate of destruction data in evaluation or calculation of a thermal process for a food, it is necessary to know the rates of destruction of the organism in question when subjected to several different temperatures over the range of temperatures which obtained or which will obtain in the food during the thermal process, and to know the rate of destruction of the organism when subjected to the temperature used or to be used as the retort (process) temperature. Z (Zeta) values are obtainable by calculation or from rate of destruction curves constructed from such data. The value of z may be ascertained from the curve obtained by plotting Z values on the log scale against corresponding temperatures on the linear scale.

To establish a value for the factor "U," two additional values must be chosen. At least until more information is available these must be chosen arbitrarily. First, a value to represent the number of cells which are to be allowed to remain viable, in the location in the container where probability of survival is greatest, must be decided upon. Second, a value to represent the number of cells (spore or vegetative) of the organism in question which are likely to be present in the food product must be chosen.

It is suggested that, in cases where minimum processes are being set to safeguard against botulism, the value chosen to represent the number of spores surviving in the location where probability of survival is greatest should not exceed one billionth spore per container (one spore remaining viable, in one of every one billion containers, in the location in the container where probability of spore survival is greatest). According to the best estimate that can be made at this time, a probability of survival of one in one billion in the location in the container where probability of survival is greatest would represent a probability of survival, considering all locations in container, not greater than three in one billion (one spore surviving in each of three containers in every billion). This may, until other factors are considered, appear to be a rather high probability of survival. Undoubtedly *Cl. botulinum* spores are absent from many low acid foods even prior to their being heat processed. It is reasonable to believe that, under present methods of food handling, the number per unit volume of any of the foods is never large, probably seldom if ever exceeding ten spores per gram. If the number per gram were taken as ten, to represent the average condition, it is believed that

a considerable margin of safety would be represented. By assuming that the ten spores per gram would belong to a strain as resistant as the most resistant strain of *Cl. botulinum* ever studied, the margin of safety in using calculated processes would be increased still further. Considering all factors involved, the probability of survival on the basis of assumptions made should be as low as 1 in several billion.

What would be involved in adjusting processes for canned foods on this basis? Stepwise for each product, the procedure may be outlined as follows:

1. Collect rate of destruction data for spores of *Cl. botulinum* dispersed in the food product. From these data establish values for Z and z.

2. Assuming ten spores to be present per gram of food calculate the total number which would be present per container.

3. Assuming one spore to be located at geometric center of container, construct a spore distribution curve representing increase in number of spores from geometric center to wall of container.

4. Calculate the F value required of a process to reduce to 0.000000001 the number of spores at the geometric center and in each of a number of locations between the center and the wall of the container. The equations used would be as follows:

$$U = Z(\log a - \log b)$$

$$F = \frac{U}{F_i}$$

$$F_i = \log^{-1} \frac{250 - RT}{z}$$

If the Z value represents the slope of the rate of destruction curve obtained for heating at 121.1°C. (250°F.), U would be equal to F and the "use" equation would be written as follows:

$$F = Z(\log a - \log b)$$

5. By plotting F values, required for the different numbers of spores, on the linear scale against the respective numbers of spores on the log scale, construct an F requirement curve for the *Cl. botulinum* spores as they would be distributed in the container of food.

6. Collect data relative to temperature distribution in the container during process at the retort temperature to be employed in practice. From these data construct heating and cooling curves for food at the geometric center and for food in various locations between the geometric center and the can wall.

7. Calculate the length of a process adequate to give an F value, for the heat treatment at the geometric center of the container, equal to the

F value determined as necessary to reduce one spore to 0.000000001 spore. Then calculate the F value which would characterize the heat treatments for the different chosen locations between geometric center and can wall if the container were given a process of the calculated length. The equations used, if each of the heating curves is represented by one straight line, would be as follows:

$$B_B = f_h \log \frac{jI}{g},$$

or,

$$B_B = U + f_h \ (\log \ jI - T + 1).$$

$$U = FF_i.$$

8. From the F values calculated under step 7 construct an F distribution curve representing heat treatments which would be given food at various locations from center to wall of the container by a process of the calculated length. This curve should be constructed on the same coordinates as were used for constructing the F requirement curve, that is, the F values representing lethality of heat treatments for the various locations in the container should be plotted on the linear scale against the numbers of spores, in the respective locations, on the log scale.

9. If all points on the F distribution curve lie on or above the F requirement curve, the process calculated as adequate to reduce one spore to 0.000000001 spore at the geometric center of the container should be adequate to accomplish the desired reduction in number of spores.

10. If any portion of the F distribution curve lies below the F requirement curve, relocate the F distribution curve such that all points on it lie on or above the F requirement curve and such that at least one point on it lies on the F requirement curve. The point of intersection of this curve and the Y-axis represents the F value required of the heat treatment given food at the center of the container during process.

11. Calculate the length of the process necessary to give this required F value at the center of the container. A process of this length should be adequate to accomplish the desired reduction in number of spores.

When organisms other than *Cl. botulinum* are used for establishing resistance values on which to base calculation of processes for canned foods, the F requirement curves probably should be based on a greater probability of survival. Economic considerations would be involved in this case and it is likely that the canner would be willing to sacrifice several containers per billion in order to produce the better quality products obtainable by employing less severe heat processes. To establish pasteurization processes the primary object of which is to free foods of the less resistant pathogenic microorganisms, the values used to repre-

sent probability of survival should be such as to make probability of survival very remote. When pasteurization is the aim, the same principles are involved in setting process requirements as are involved when sterilization is the aim. However, the values employed in the calculations to represent initial number of microbial cells and end-point of survival should be chosen in full consideration of whether or not the most resistant organism to be destroyed is pathogenic. Values to be used in calculating process requirements for each individual product, should be chosen in so far as possible, on the basis of information available. Information from laboratory study and commercial processing experience should be fully considered.

There is a great need for additional information relative to the prevalence of many bacteria of both the pathogenic and food spoilage types in virtually every food product manufactured commercially today. Extensive studies to determine how often certain important bacteria occur in the different foods, and in what numbers, should yield information of great value with respect to further improvement in methods of food processing. The importance of studies relative to the thermal resistance of bacteria such as *Cl. botulinum* and *Cl. sporogenes* as they occur in the different foods at the time of prccessing would be equally great. Factors influencing growth of bacteria, which survive thermal processes adequate to free the food of all but a few bacteria, should be subjected to a great deal of further study. The improved methods which are now available for studying thermal resistance of bacteria should be of great value in solving many of the problems the solution of which is important to improvement in existing methods of processing and to development of new methods of processing.

The growing interest in high-temperature short-time sterilization of foods makes study of thermal resistance of bacteria to temperatures in the range of 121.1°C. (250°F.) to 148.9°C. (300°F.) imperative. Since methods employing these processing temperatures involve more nearly uniform heating of the products, the influence of this difference in the nature of heating on the F value required of processes adequate to accomplish sterilization must be fully considered. With reference to conventional methods of canning foods, there is a great need for re-evaluation and adjustment, of processes now in use, based on full consideration of the logarithmic order of bacterial death and mechanism of heat transfer within the food container. Process evaluation on this basis will undoubtedly require the development of new factors for converting heat-penetration data obtained for one container size to the equivalent for another container size. It may also require special factors for converting

the heat-penetration data obtained for one retort temperature to the equivalent for another retort temperature.

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The Quaternary Ammonium Compounds and Their Uses in the Food Industry

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I. Introduction

The quaternary ammonium compounds are surface-active agents which, on account of their particularly desirable attributes, are finding greatly increased usage in the food industries, eating and drinking establishments, medical and other fields as sanitizing agents, disinfectants and germicides.

The compounds have been referred to under various names, for example as cationic compounds, soaps-in-reverse, invert soaps, synthetic detergents, wetting agents and surface-interface modifiers. In aqueous solutions the quaternary ammonium compounds ionize into two portions, one known as the cation which is positively charged and represents the bulk of the compound in respect to the molecular weight and the germicidal activity, and the other known as the anion which is negatively charged and represents usually only a small portion of the molecular weight of the compound and exerts but little influence on the germicidal activity. Hence, quaternary ammonium compounds are cationic compounds, but they are not the only ones and therefore the term is not a distinguishing one.

The quaternary ammonium compounds are sometimes called soaps-in-reverse or invert soaps, for chemically they are a reversed or inverted type of surface-active agent in relation to soaps. In the case of soap (formed usually by the saponification of fats), the main portion of the molecule, which contains the alkyl group, is negatively charged and constitutes the anion. Solutions of the quaternaries usually foam or

froth when agitated, but this is not an indication that they may be used to replace soaps or other detergents for cleaning.

Quaternary ammonium compounds possess some of the properties of detergents, such as good wetting action, but they are, with few exceptions, rather poor detergents. Hence, although they have been referred to as synthetic detergents in the literature occasionally, this is not a good term to use for classifying them.

General reviews on synthetic detergents, wetting agents and surface-active compounds have been made by Snell (1943a, b), Fischer (1943, 1944), Sunde (1943), Van Antwerpen (1943), Epstein *et al.* (1943), Taube (1944), Valko (1946), McCutcheon (1946, 1947), Ackley (1947), Glassman (1948) and others.

General reviews of the quaternary ammonium compounds have been presented by Miller and Baker (1940), Baker *et al.* (1941a, b), Bartlett (1944), Marshall (1944, 1947), DuBois (1944, 1947a), Mueller *et al.* (1946), Vignolo (1946b), Botwright (1946), Rahn and Van Eseltine (1947), Varley (1947), Lawrence (1947a), Johnson (1947), Hucker *et al.* (1947), and others. Klarmann (1946, 1947) reviewed the advances made in the field during the years 1944, 1945 and 1946.

II. General Description of the Compounds

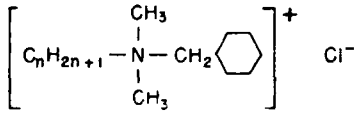
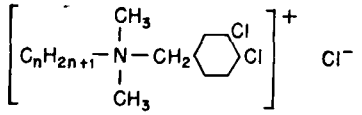
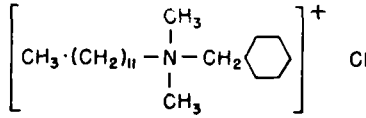
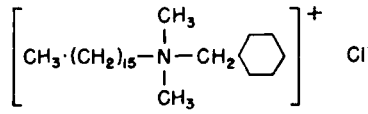
1. General Chemical Structure of the Quaternary Ammonium Compounds

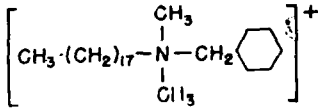
The quaternary ammonium salt is built around the nitrogen atom. This atom contains five valence bonds, four of which are attached directly to adjacent carbon atoms of organic radicals, forming the main part of the compound as far as the molecular weight and germicidal activity are concerned, and one of which is attached to an inorganic or organic radical, usually of low molecular weight. The nucleus of the salt may be represented by $\equiv N \cdot X$, where X is an inorganic or organic radical.

When the quaternary ammonium compound dissociates in aqueous solution, the main part of the molecule becomes positively charged and is referred to as the cationic portion or the cation, while that part attached to the fifth nitrogen valence bond (represented by X above) becomes negatively charged and is designated as the anionic portion or the anion. Since the germicidal and surface active properties are confined almost exclusively to the cationic portion, the quaternary ammonium compounds are frequently referred to as cationic germicides. However, it should be pointed out that they are not the only germicides which may be so classified.

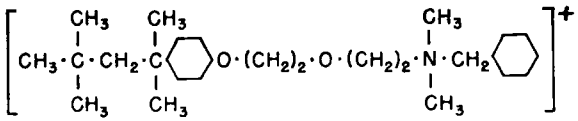
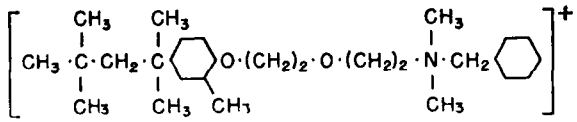
TABLE I

Data Concerning Some Commercially Available Quaternary Ammonium Compounds

Chemical name of quaternary ammonium compound	Chemical structure of quaternary ammonium compound	Trade name of commercial product	Manufacturer of product
1. Long-chain dimethylbenzylammonium compounds			
Alkyldimethylbenzylammonium chlorides		No-Roma	Baird & McGuire, Inc.
		Onyx BTC	Onyx Oil & Chemical Co.
		Roccal	Winthrop Chemical Co.
		Roclina	Winthrop Chemical Co.
		Rodalon	Rhodes Chemical Corp.
		Zephiran	Winthrop Chemical Co.
		Zephirol	
Alkyldimethyl 3, 4-dichlorobenzylammonium chlorides		Tetrosan	Onyx Oil & Chemical Co.
Lauryldimethylbenzylammonium chloride		Nopco QCL	National Oil Products Co.
		Ster-Bac	Klenzade Products, Inc.
		Thoral	Turco Products, Inc.
Cetyldimethylbenzylammonium chloride		Triton K-12	Rohm & Haas Co.

Stearyldimethylbenzylammonium chloride		Triton K-60	Rohm & Haas Co.

2. Dimethylbenzylammonium compounds containing aromatic ring in the long-chain radical

Diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride		Amerse Hyamine 1622 Phemerol Polymine D	Vestal Laboratories, Inc. Rohm & Haas Co. Parke, Davis & Co.
Diisobutylcresoxyethoxyethyldimethylbenzylammonium chloride		Hyamine 10X	Rohm & Haas Co.

3. Compounds containing one long alkyl radical and three short radicals

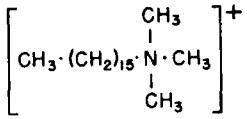
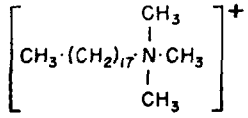
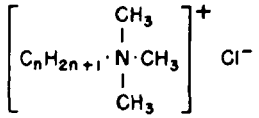
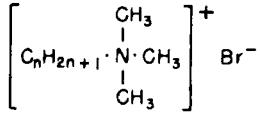
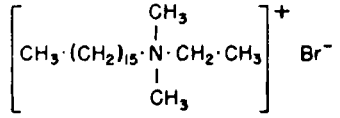
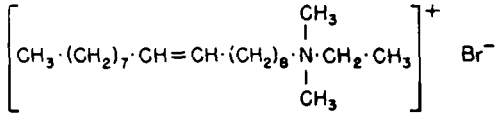
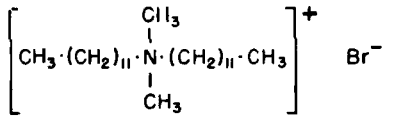
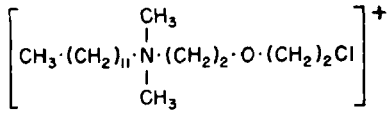
Cetyltrimethylammonium bromide		CTAB Cetavlon CETAB	Rhodes Chemical Co.
Stearyltrimethylammonium bromide		Retarder LA	E. I. DuPont de Nemours & Co.

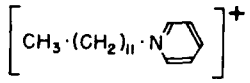
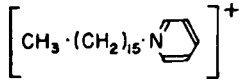
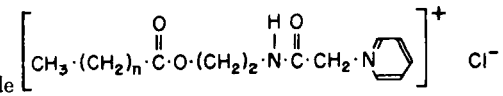
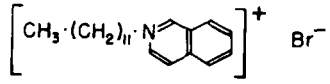
TABLE I (Cont.)

Chemical name of quaternary ammonium compound	Chemical structure of quaternary ammonium compound	Trade name of commercial product	Manufacturer of product
Alkyltrimethylammonium chloride		Arquads	Armour & Co.
Alkyltrimethylammonium bromide		Product QB	E. I. DuPont de Nemours & Co.
Cetyldimethylethylammonium bromide		Ethyl CETAB	Rhodes Chemical Co.
Oleyldimethylethylammonium bromide		Amerse Onyxide Quartol	Vestal Laboratories, Inc. Onyx Oil & Chemical Co.

4. Dialkyldimethylammonium compounds

Dilauryldimethyl-ammonium bromide		Isonol DL–1	Onyx Oil & Chemical Co.
Lauryldimethyl-chlorethoxyethyl-ammonium chloride		Isothan OX	Onyx Oil & Chemical Co.

5. Compounds containing heterocyclic nitrogen

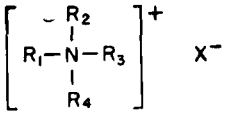
Laurylpyridinium bromide		Isothan Q4	Onyx Oil & Chemical Co.
Cetylpyridinium chloride		Ceepryn	Wm. S. Merrell Co.
N(higher acyl esters of colamino formylmethyl) pyridinium chloride		Emulsept	The Emulsol Corp.
Laurylisoquinolinium bromide		Isothan Q15	Onyx Oil & Chemical Co.

In the quaternary ammonium compound, the anion may be a bromide, chloride, iodide, fluoride, formate, acetate, citrate, lactate, alkyl sulfate, phosphate, salicylate, thiocyanate or other inorganic or organic radical. However, most of the quaternary ammonium salts being manufactured for use as germicides, disinfectants and sanitizing agents are bromides, chlorides or iodides (refer to Table I).

In the cationic portion of the quaternary ammonium salt, each valence bond of the nitrogen may be attached to a separate radical or chain, or three of the bonds may be attached to two carbon atoms of a cyclic compound and the other one to a radical or chain. The number of compounds capable of synthesis is thus very large.

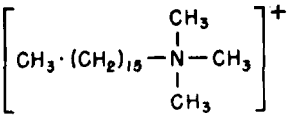
Illustrations of the general chemical structure of some of the quaternary ammonium compounds now being manufactured for the trade are presented in the following paragraphs:

A. The general chemical formula for the noncyclic types of quaternaries may be indicated as follows:

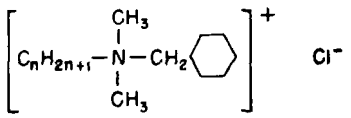


In this formula, R_1, R_2, R_3, and R_4 represent organic radicals or chains and X represents the anion, usually an halide.

In the simplest series of the quaternary ammonium compounds, R_1 represents a long-chain aliphatic group and R_2, R_3, and R_4 represent methyl or ethyl radicals (or other short-chain group). Cetyltrimethylammonium bromide, which has the following structural formula, is illustrative of this series:

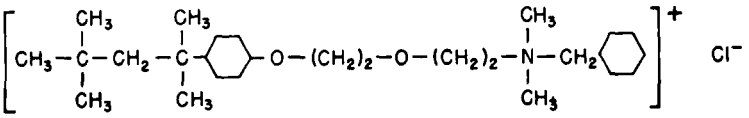


When R_1 is a long-chain aliphatic group, R_2 and R_4 are methyl groups, and R_3 is a benzyl group, salts such as alkyldimethylbenzylammonium chlorides result.

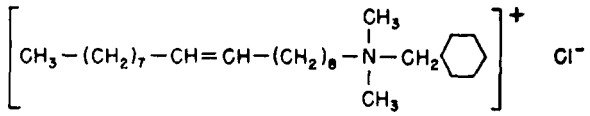


In the above formula, C_nH_{2n+1} represents a mixture of fatty acid radicals ranging from C_8H_{17} to $C_{18}H_{37}$.

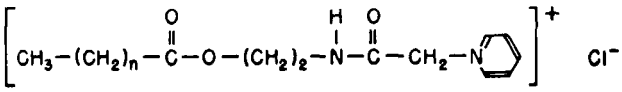
B. Quaternary ammonium compounds containing an aromatic ring in the long chain are illustrative of another series, an example of which is diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride:



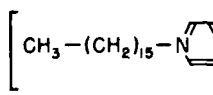
C. In another related series of quaternary ammonium compounds, the long-chain alkyl groups contain unsaturated linkages. An example of this series is 9-octadecenyldimethylbenzylammonium chloride:

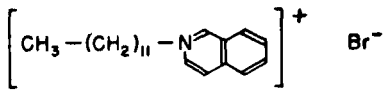


D. Oxygen atoms are introduced as amide linkages in the long-chain alkyl group in still another series of quaternary ammonium compounds. *N*(higher acyl esters of colaminoformylmethyl) pyridinium chloride is illustrative of this series.



E. A cyclic type of quaternary ammonium results when three of the nitrogen valence bonds form part of a cyclic compound, as for example cetylpyridinium chloride and laurylisoquinolinium bromide, the structural formulas of which follow:





These types are representative of the series of quaternary ammonium compounds derived from the cyclic amines, such as lutidine, pyridine, picolines, quinoline, and isoquinoline.

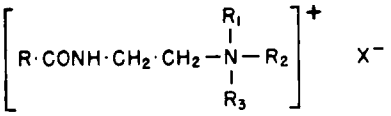
2. Some Studies Concerning Structure

A great deal of information concerning the chemical structure of the quaternary ammonium compounds may be obtained by reference to selected papers. Some of these deal particularly with the preparation and description of the compounds; some, with general formulas and suggested uses; and some, with the relationship between chemical structure and germicidal action.

Jacobs and Heidelberger (1915a, b) reported on the preparation of many quaternary salts of hexamethylenetetramine. Jacobs (1916) studied the bactericidal properties of these salts in relation to the problem of chemotherapy of experimental infections of a bacterial nature. The relationship between constitution and bactericidal action of substituted benzylhexamethylenetetramine salts and in the quaternary salts obtained from halogenacetyl compounds was investigated by Jacobs and Heidelberger *et al.* (1916a, b).

Browning *et al.* (1922) carried out the same type of research on compounds of the pyridine, quinoline, acridine and phenazine series, using *Staphylococcus aureus* and *Escherichia coli* as the test organisms. Later, Browning *et al.* (1926) prepared and determined the antiseptic properties of a large number of amino derivatives of styryl and anil quinoline compounds.

Hartmann and Kagi (1928) studied compounds of the general formula:



Harris (1933a, b, c, d) obtained several patents on quaternary ammonium compounds relating to their use in emulsions, for example, in producing a nonspattering margarine. A later patent (Harris, 1935) relates to nitrogen-containing esters, which have interface modifying properties. These are represented by the formula $L \cdot O \cdot C(:O) \cdot CH_2 \cdot N \equiv$, where L is a lipophile group containing at least four carbon atoms in the form of an alkyl, ether or ester radical; X is an anion, for example, bromide, chloride, hydroxide, acetate, sulfate or other inorganic radical; and N is pentavalent nitrogen, the valence bonds of which are satisfied by alkyl, aryl or cyclic radicals.

Domagk (1935) showed that quaternary ammonium salts other than the derivatives of hexamethylenetetramine were good germicides. He worked with alkyldimethylbenzylammonium chloride and aroused a great deal of interest in the medical and other fields concerning its use. This worker (Domagk, 1938) obtained a patent relating to quaternary ammonium compounds suitable for use in the disinfection of table utensils, medical instruments, floors and other items. He pointed out the particular effectiveness of the quaternary ammonium halide substituted by two lower alkyl radicals, one benzyl radical and a high molecular aliphatic radical containing from eight to eighteen carbon atoms,

selected from a group consisting of higher alkyl, hydroxyalkyl, halogenalkyl, alkylmercaptoalkyl, aminoalkyl and alkylaminoalkyl radicals.

In a series of patents, Bruson (1937a, b; 1938a, b; 1939; 1941) has described the preparation of a large number of quaternary ammonium salts and bases, also amines and aromatic polyether chlorides which may be used in their preparation. His invention relates primarily to the water-soluble salts and bases which have been found to be excellent wetting, emulsifying, dispersing and cleaning agents. Certain of the compounds prepared by him were later studied by Rawlins *et al.* (1943) to determine the relationship between chemical structure and germicidal activity.

In a series of publications, Shelton *et al.* (1939; 1940; 1946a, b, c) have discussed the correlation of structure and germicidal activity of a large number of quaternary ammonium compounds. For example, they studied this relationship for nonacylated quaternary ammonium salts derived from aliphatic amines, for acetoxy and carbethoxy derivatives of aliphatic quaternary ammonium salts, and for salts derived from cyclic amines.

The quaternary salts of aminophenol ethers were studied by Kuhn and Jerchel (1940). Dialkylmethylbenzylammonium chloride was investigated by Kuhn *et al.* (1940).

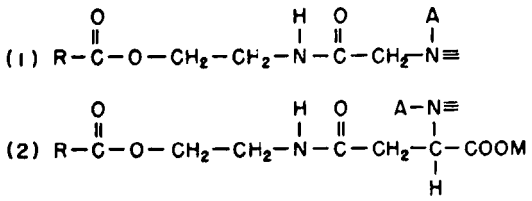
Quaternary ammonium compounds with interface modifying properties were the subject of a patent by Katzman (1940). The compounds described by him are covered by the following general formulas:

$$(1)\quad R\cdot O\cdot(alk\cdot N\cdot Y)_m\cdot C(:O)\cdot Z\cdot(Q)_w$$

$$(2)\quad R\cdot O\cdot(alk\cdot N\cdot Y)_m\cdot C(:O)\cdot Z\left[\begin{matrix}N\equiv\\ \dot{A}\end{matrix}\right]_w$$

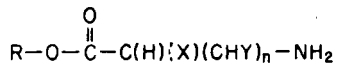
in which R represents an organic radical containing at least four carbon atoms; alk, a hydrocarbon (for example, alkylene); Y, hydrogen, alkyl, cycloalkyl, alkoxyl, aralkyl, aryl or alkylol; Z, a hydrocarbon residue preferably; A, an anion, preferably of the solubilizing type; m and w, whole numbers, and w, one or two preferably; and Q, a quaternary ammonium radical. Another specification is that at least one of the three valence bonds attached to the nitrogen is satisfied by a radical belonging to the class including alkyls, cycloalkyls, alkylols, aralkyls, aryls, aralkylols and the radical of an heterocyclic ring of which nitrogen is a member.

Epstein and Harris (1942a) described quaternary ammonium compounds with germicidal and antiseptic properties. These compounds were of the nature represented by the following general formulas.



where R is a fatty acid hydrocarbon radical containing five to thirteen carbon atoms, M is a cation, A is an halogen, and the three valence bonds of the nitrogen are attached directly to carbon atoms. Fifty-nine examples are given in the patent.

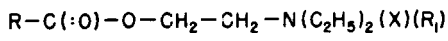
In another patent, Epstein and Harris (1942b) described compounds of the general formula:



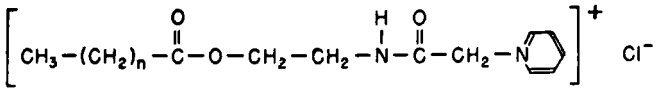
where R is an organic radical, preferably containing eight to eighteen carbon atoms; X and Y are hydrogen, alkyl, cycloalkyl, alkoxyl, aryl, or alkylol radicals; and n equals zero or a small whole number.

Kolloff *et al.* (1942) described the germicidal activity of quaternary ammonium salts of the alkyl pyridinium and alkyl α-and γ-picolinium bromide series.

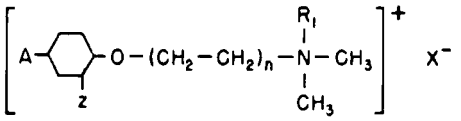
A study of the germicidal activities of the following series was made by Woodruff *et al.* (1942):



Epstein *et al.* (1943) investigated the relationship between bactericidal potency and the length of the fatty acid radical in the series:



The relationship between germicidal activity and structure was studied by Rawlins *et al.* (1943). They investigated compounds of the following general formula:

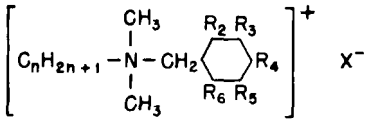


In this formula, A represents an alkyl, aryl, aralkyl, or cycloalkyl radical; z, an halogen, or alkyl, aryl, or cycloalkyl radical; R_1, hydrogen, methyl or substituted methyl; X, chloride or other anion; and *n*, two or three.

Valko and DuBois (1945) reported on researches concerning the correlation between antibacterial action and chemical structure of series of quaternaries closely related to dodecyldimethylbenzylammonium chloride. Among the series studied were higher aliphatic dimethybenzylammonium chlorides, higher aliphatic dimethyl ethyl ammonium bromides, higher aliphatic dimethyl allyl ammonium bromides, and higher aliphatic trimethyl ammonium bromides.

The germicidal action of alkyldimethylbenzylammonium chlorides which contained chlorine substituents in the 3,4 positions on the benzene nucleus was described by DuBois and Dibblee (1946b, c).

Lawrence *et al.* (1947) studied the effect of chain length in the alkyl group and chlorine substituents in the benzene nucleus of quaternary ammonium compounds of the general formula indicated below:



In this formula, n equals 6, 8, 10, 12, 14, 16 or 18; and R_2, R_3, R_4, R_5, and R_6 represent positions in the benzene nucleus in which substitutions were made.

3. General Properties of the Quaternary Ammonium Compounds

a. Requirements for Ideal Germicides. Some of the requirements for the ideal germicide, sanitizing agent, or disinfectant are as follows: (1) freedom from color, taste and odor; (2) solubility in water (for most purposes); (3) noncorrosive and nonstaining to metals, rubber and other surfaces; (4) freedom from toxicity; (5) stability over a wide range of temperature and to light over a relatively long period of time; (6) freedom from incompatibilities; (7) of high germicidal activity to microorganisms, including spores; (8) effectiveness in the presence of organic matter; (9) economical to use. Obviously no chemical sanitizing agent or disinfectant is known at the present time which meets all of the requirements of the ideal disinfectant. However, a number of the quaternary ammonium compounds appear to meet a large proportion of these requirements.

b. Freedom from Organoleptic Defects. Most of the quaternary ammonium compounds marketed for use as germicides, sanitizers or disinfectants are relatively free of color, taste and odor in the dilutions ordinarily employed. This fact has been reported by a large number of investigators.

c. Solubility. Although the quaternaries differ considerably in respect

to their solubility in water, those marketed for use as general sanitizers are freely soluble. Most of the compounds are also soluble in mixtures containing alcohol, acetone and water. Some are soluble in oils; for example, the high molecular dialkyldimethylammonium bromides are insoluble in water, but soluble in isopropyl alcohol and water. The latter agents may be used in emulsifying mineral and vegetable oils, where germicidal or fungicidal action is desired.

d. Corrosive Action. The quaternary ammonium compounds are relatively noncorrosive, as has been pointed out by Mueller *et al.* (1946) and by Hucker *et al.* (1947), who concluded that they have little, if any, corrosive action on the metals and alloys ordinarily used in the fabrication of dairy and other food processing equipment.

4. Toxicity

Quaternary ammonium compounds, such as alkyldimethylbenzylammonium chlorides (Zephirol, Zephiran), cetylpyridinium chloride (Ceepryn), diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride (Phemerol) and cetyltrimethylammonium bromide (CTAB, Cetavlon) have been used in hospitals in this country, Europe and elsewhere on tens of thousands of individuals. These compounds are generally nontoxic in the dilutions employed. They do not interfere with healing processes nor do they ordinarily cause irritation. In a very small percentage of cases, sensitivity to some of these agents has been reported. However, even penicillin affects some sensitive individuals.

Numerous tests have been carried out on animals to determine the toxicity of the various compounds (reference is made to some of the tests employed in a later section). In general, the products have been found to be of low toxicity when injected into animals. Domagk (1935), Walter (1938), Deskowitz (1937), and others have supplied the 1:1,000 aqueous solution of alkyldimethylbenzylammonium chlorides to guinea pigs for weeks as the only source of fluid without apparent deleterious effects. Maier (1939) found that a 1:4,000 solution of alkyldimethylbenzylammonium chlorides was safe for the eyes of albino rabbits. Harshbarger (1942) fed white rats a diet containing 3% of Zephiran without apparent ill effects, based on gains in weight in comparison with control rats.

Welch and Brewer (1942) found the quaternaries examined by them by a tissue toxicity method to be of low toxicity, for example, the toxicity indices of some germicides were as follows: Zephiran, 0.48; E-605, 0.3; E-607, 0.5; Chloramine-T, 1.16, and formaldehyde, 30.0 (The lower the toxicity index, the lower the toxicity towards tissues.)

The use of quaternary ammonium compounds in the medical field

has been carefully studied by physicians, surgeons and others. Brief reference to a few of these reports follows. Additional data along these lines will be found elsewhere in this report. Wright and Wilkinson (1939) described the uses of Zephiran in connection with amputations, abdominal laparotomy, other surgical conditions and burns. The agent was without toxic effect when used on large denuded surfaces and did not cause discomfort to patients. Clarke (1942) used patch and scratch tests on 600 persons with a 1:200 tincture and aqueous solutions of cetylpyridinium chloride. Observations were made at the end of 24 hours, 48 hours and 7 days. There were no reactions.

Whittet (1943) reported on the use of cetyltrimethylammonium bromide, crude and purified, in a hospital in England covering a period of about a year and found no cases of skin reaction or sensitivity. Kramer and Sedwitz (1944) stated that cetylpyridinium chloride was not irritating when used for preoperative preparation in combination with a special soapless technique. Brown *et al.* (1944) reported that the 1:500 tincture of Phemerol was a good bactericidal and bacteriostatic agent, which did not produce skin irritation or interfere with the healing of wounds. They found that this product was suitable for use as a skin antiseptic in surgery and obstetrics and superior to some of the mercurials and other antiseptics tested. Helmsworth and Hoxworth (1945) showed that a 5- to 10-minute scrubbing of the operative field with a 1:100 aqueous solution of cetylpyridinium chloride was an effective method for degerming of the skin, as determined by swab and pinch graft procedures. Hagan *et al.* (1946) found that the 1:1,000 tincture of cetylpyridinium chloride compared favorably with other cutaneous antiseptics. On the other hand, Hughes and Edwards (1946) found lesions on cows' teats which had been treated for over 3 months with a 1% solution of cetyltrimethylammonium bromide in a lanette wax-oil base.

5. Incompatibilities

Knowledge concerning the incompatibilities of the quaternary ammonium compounds is essential for the intelligent application of them. In general it may be stated that these compounds are incompatible with soaps, soap solutions, phospholipides, anionic wetting agents and detergents, some alkaline detergents, iodine and certain other special compounds. The incompatibility of soap with quaternary ammonium compounds has been pointed out by Walter (1938), Baker *et al.* (1941b), Joslyn *et al.* (1943), Hauser and Cutter (1944), DuBois (1944), and many others.

The inhibition of bacterial metabolism by cationic and anionic detergents is prevented by phospholipides, such as lecithin, cephalin and

sphingomyelin, according to Baker *et al.* (1941c). However, in order to prevent the inhibitory or antibacterial action, it is necessary to treat the bacteria with phospholipides before they are subjected to the action of the cationic and anionic agents, or at the same time. Bacteria exposed to phospholipides and then washed are protected against the action of these surface active agents also. Thus, in the presence of phospholipides, concentrations of quaternary ammonium compounds ordinarily germicidal fail to accomplish the same degree of destructive action towards bacteria. Gershenfeld and Ibsen (1942) suggest that the lipoids alter the affinity of the cationic and anionic agents for bacteria on account of their physico-chemical action.

Neufeld *et al.* (1940), Grumbach (1941), Miller *et al.* (1943) and Valko and DuBois (1944) have reported that the antibacterial action of cationic germicides may be reversed under certain conditions by anionic agents.

DuBois and Dibblee (1946) showed that the pretreatment of bacteria with anionic compounds delayed but did not prevent the action of cationic germicides. They suggested that, following the addition of a cationic germicide to a medium containing an anionic compound, there is at first a combination of the cations with the anions present on or in the bacteria. The combined portion was thus inactivated, but the excess of uncombined cations were free to act germicidally.

Klein and Kardon (1947) studied the ability of anionic agents, such as sodium lauryl sulfate (Duponol PC) to reverse or neutralize the germicidal action of cationic compounds, such as Zephiran and Phemerol. They found that the action of these compounds towards Gram-positive and Gram-negative bacteria could not be reversed by sodium lauryl sulfate or sodium decyl sulfate. However, when an anionic agent was added to an inoculum containing Gram-negative bacteria before all of the organisms were inactivated by the quaternary ammonium compound, the anionic compound could neutralize the action of the quaternary and prevent further bactericidal action on its part. But anionic agents did not neutralize the bacteriostatic action of Zephiran against Gram-positive bacteria.

Some degree of caution must be exercised in connection with the use of quaternary ammonium compounds in the presence of alkaline detergents or on surfaces cleaned by them but not rinsed. Excessive quantities of some alkaline detergents may seriously reduce the germicidal efficiency of the quaternaries. Powney (1943) investigated the effect of various combinations of cation-active salts, such as cetylpyridinium bromide, and phosphates. He observed that intense turbidity and occasionally complete precipitation occurred when cation-active, long-

chain salts were treated with sodium hexametaphosphate. Tetrasodium pyrophosphate produced slight turbidity and trisodium phosphate, disodium hydrogen phosphate and sodium dihydrogen phosphate were without effect. Sodium metasilicates are highly incompatible with some of the quaternaries.

Further information concerning incompatibilities will be found under the descriptions of some of the quaternary ammonium compounds which follow later, also in the sections concerned with analysis and uses.

6. Compatibilities

In general, quaternary ammonium compounds are compatible with other quaternaries, nonionic wetting agents and detergents, acriflavine and proflavine dyes, fatty acid amine acetates and hypochlorites.

Some alkaline detergents are more compatible with quaternary ammonium compounds than others. Varley (1947) stated that, in general, trisodium phosphate, borax, sodium bicarbonate, tetrasodium pyrophosphate and sodium sesquicarbonate may be used for cleaning items which are to be disinfected with the quaternary ammonium salts with little or no reduction of germicidal efficacy. Varley pointed out that trisodium phosphate appeared to be most compatible with quaternaries, possibly because this agent raised the pH of the germicidal solution to one optimum for germicidal action. However, he cautioned against the use of excessive amounts of alkalies or phosphates since they may greatly reduce the germicidal potency of the solution.

Huyck (1944) showed that a 0.5% solution of cetylpyridinium chloride, containing 50% alcohol and 10% acetone, was compatible with a large number of pharmaceutical compounds including ephedrine hydrochloride, picric acid, sulfathiazole, sulfanilamide, benzocaine, procaine hydrochloride, and cocaine hydrochloride in 1% concentration. Benzyl alcohol, glucose and 1:1,000 adrenaline chloride were compatible in 10% concentrations.

7. Factors Affecting Germicidal Activity

The germicidal activities of the quaternary ammonium compounds are influenced by the pH, temperature, organic matter, concentration used, potentiation and other factors.

a. pH. As a group, the quaternary ammonium compounds are considerably more active germicidally in neutral and especially alkaline than in acid solutions (Table II). Dunn (1936) showed that alkyldimethylbenzylammonium chlorides were much more effective as a sporicide against *Bacillus subtilis* in the alkaline than in the acid range. Miller and Baker (1940) stated that the effectiveness of the cationic compounds

TABLE II

Effect of pH and Concentration on Germicidal Power of Three Cationic Detergents at 37°C. in Absence of Serum [a]

Compound	pH	2	3	4	5	6	7	8	9	10
					Staphylococcus aureus					
Cetyl pyridinium chloride	Dilution	1:66,000	1:66,000	1:58,000	1:54,000	1:60,000	1:66,000	1:66,000	1:58,000	1:54,000
	p.p.m.	15	15	17	18.5	16.5	15	15	17	18.5
					Eberthella typhosa					
Cetyl pyridinium chloride	Dilution	1:40,000	1:36,000	1:24,000	1:24,000	1:36,000	1:38,000	1:40,000	1:36,000	1:32,000
	p.p.m.	25	28	42	42	28	26	25	28	31
					Staphylococcus aureus					
Zephiran [b]	Dilution			<1:5,000	<1:5,000	1:20,000	1:70,000			
	p.p.m.			>200	>200	50	14			
					Staphylococcus aureus					
Triton K-12 [b]	Dilution			<1:100	1:100	1:400	1:900 [c]	1:12,000 [d]	1:20,000	
	p.p.m.			>10,000	10,000	2,500	1,111	83	50	
					Eberthella typhosa					
Triton K-12	Dilution			1:100	1:300	1:500	1:900 [c]	1:14,000 [d]	1:20,000	
	p.p.m.			10,000	3,333	2,000	1,111	71	50	

[a] Quisno and Foter, 1946.
[b] Data from Gershenfeld and Milanick (1941), and Gershenfeld and Perlstein (1941).
[c] pH 7.2.
[d] pH 8.2.

increases progressively as the pH is shifted toward the alkaline side of neutrality. Baker *et al.* (1941) showed that the maximum activity of the cationic compounds was displayed in the alkaline range and that of the anionic compounds in the acid range. Gershenfeld and Witlin (1941) demonstrated that Zephiran was more effective as a bactericide (to *Staphylococcus aureus*) in alkaline and neutral solutions. Gershenfeld and Milanick (1941) reported that cetyldimethylbenzylammonium chloride was several times more effective as a germicide against *S. aureus* and *Eberthella typhosa* at a pH of 9 than at a pH of 4. Similar trends were reported by Gershenfeld and Perlstein (1941) and Gershenfeld and Ibsen (1942). Hoogerheide (1945) demonstrated that the bactericidal and bacteriostatic powers of cetyltrimethylammonium bromide against both Gram-positive and Gram-negative bacteria were increased by raising the pH. Quisno and Foter (1946), on the other hand, showed that cetylpyridinium chloride was as effective towards *S. aureus* and *E. typhosa* at pH 2 as at pH 8. Apparently this quaternary ammonium compound is the exception to the general rule that the germicidal activities of the group as a whole are considerably greater in alkaline or neutral solutions than in acid solutions.

b. Temperature. Quaternary ammonium compounds are effective in general over a relatively wide range of temperature. They are nonvolatile and withstand ordinary autoclaving processes. As a matter of fact, Brekenfeld (1938) reported that boiling instruments with a 1% solution of alkyldimethylbenzylammonium chlorides was as effective as heating with superheated steam. The compounds may be frozen without subsequent deleterious effect on their germicidal activity. They are effective at low temperatures. Dunn (1936) reported that *S. aureus* was destroyed in 10 but not 5 minutes by a 1:4,500 dilution of alkyldimethylbenzylammonium chloride at less than 1°C. (33.8°F.). Under similar conditions, *E. coli* was destroyed by a 1:1,000 dilution of the compound. Kliewe and Althaus (1937) showed the relationship between the concentrations of various disinfectants required to destroy staphylococci within 5 to 10 minutes at 37, 20 and 5°C. They reported the following decreases in germicidal action from 37 to 5°C.: Zephirol, 73%; phenol, 91%; Creolin, 93%; and Lysol, 95%. The decreases in six other disinfectants were 88 to 95%. Hence, at lower temperatures it is necessary to use larger concentrations of a disinfectant if it is desired to accomplish results during the same time as at body temperature, or to use a longer time.

Quaternary ammonium compounds as a group are quite stable during storage. Mueller *et al.* (1946) studied forty-two surface-active agents, which included quaternary ammonium compounds, phosphonium com-

pounds, alkyl-aryl sulfonates, and substituted phenols. Only the quaternary ammonium and phosphonium compounds were of sufficient stability and germicidal effectiveness to be classified as good sanitizers for dairy use. Five out of the six quaternary ammonium salts, originally grouped as effective germicides, and the phosphonium compounds remained effective after storage for 2½ years; the sixth quaternary compound was moderately effective. Dunn (1936) found that the germicidal properties of alkyldimethylbenzylammonium chlorides were not adversely affected by storage at 50°C. (122°F.) for 18 days nor by storage at room temperatures for over 2 years.

c. Organic Matter. As is true of most chemical germicides, the efficacy of quaternary ammonium compounds is reduced by the presence of blood serum. Dunn (1936) reported that alkyldimethylbenzylammonium chlorides destroyed *S. aureus* in less than 30 seconds at 20°C. and in less than 15 seconds at 37°C. in a 1:200 dilution and in 10 but not 5 minutes in a 1:1,200 dilution at 20°C., all in the presence of 50% serum, which is five times the concentration commonly employed in carrying out such tests. Heineman (1937), Warren *et al.* (1942), Hoogerheide (1945), Eckfeldt and James (1947), and many others have studied the effect of blood serum on the quaternary ammonium salts.

Barnes (1942) stated that a 1% solution of cetyltrimethylammonium bromide precipitated serum proteins.

In the presence of milk, quaternary ammonium compounds lose considerable of their efficacy, according to Hoogerheide (1945), Rahn (1945), DuBois and Dibblee (1946b) and others. However, Mull and Fouts (1947) have shown that Roccal does reduce the numbers of bacteria in milk when used in suitable concentration (see section on applications).

The germicidal activity of quaternary ammonium compounds is reduced by agar, according to reports from Sherwood (1942) and Quisno and Foter (1947).

d. Concentration. The concentration of the quaternary ammonium compound used will depend upon a number of factors, among which are the following: the nature of the surface being treated, the amount and kind of organic matter present, the amount of moisture present, the temperature, and the time available for treatment.

e. Potentiation. The use of small quantities of an agent to potentiate or enhance the germicidal activity of a disinfectant is well known. Hill and Hunter (1946) reported that the addition of extremely small amounts of sodium sulfate to cetyltrimethylammonium bromide halved the concentration of the cationic agent required to produce a given effect toward *S. aureus* (pyogenic strain). The use of Triton K-12

(cetyldimethylbenzylammonium chloride) in a 1:10,000 dilution was found to have a pronounced synergistic effect (enhancement) on the antibacterial action of sulfaguanidine and succinylsulfathiazole against *Eberthella typhosa* and *Escherichia coli*, according to Gershenfeld and Sagin (1946).

8. Bactericidal and Bacteriostatic Action

As a group, the commercial preparations of the quaternaries are active in rather high dilution as germicides and inhibitory agents (see Table III). Baker *et al.* (1941) pointed out that all of the cationic detergents studied by them were efficient inhibitors of bacterial metabolism in dilutions of 1:3,000, whereas some were active in dilutions of 1:30,000. Only a few of the anionic detergents investigated by them were as effective. The cationic agents appeared to be equally effective towards Gram-positive and Gram-negative organisms, according to Miller and Baker (1940) and Baker *et al.* (1941).

TABLE III

Germicidal Activity of Some Quaternary Ammonium Compounds at 20°C.[a]

Name of Compound	Dilution destroying organism in 10 but not in 5 minutes	
	Staph. aureus	*E. typhosa*
n-Dodecyldimethylbenzylammonium chloride	1:20,000	1:16,000
n-Tetradecyldimethylbenzylammonium chloride	1:21,000	1:17,000
n-9-Octadecenyldimethylbenzylammonium chloride	1:29,000	1:10,000
n-Hexadecyldimethylethylammonium bromide	1:20,000	1:12,000
n-9-Octadecenyldimethylethylammonium bromide	1:28,000	1:19,000
n-Tetradecyldimethylallylammonium bromide	1:15,000	1:15,000
n-Hexadecyldimethylallylammonium bromide	1:25,000	1:11,000
n-Tetradecyltrimethylammonium bromide	1:12,000	1:11,000
n-Hexadecyltrimethylammonium bromide	1:14,000	1:10,000
Dodecyldimethyl α-menaphthylammonium chloride	1:19,000	1:19,000
9-Octadecenyldimethylethylammonium bromide	1:28,000	1:19,000
9-Octadecenyldimethylpropylammonium bromide	1:31,000	1:15,000
9-Octadecenyldimethyl γ-phenylpropylammonium bromide	1:18,000	1:18,000

[a] Tabulated from data of Valko and DuBois (1945).

Bacterial spores are considerably more resistant to the quaternary ammonium compounds than are the vegetative cells. Likewise there are occasionally marked differences among the quaternaries in respect to their germicidal action towards spores of different species, as has been pointed out by Hucker *et al.* (1947). Dunn (1937a), Heineman (1937), Deskowitz (1937), DuBois and Dibblee (1946), Hucker *et al.* (1947), and others have studied the action of the quaternary ammonium com-

pounds on bacterial spores, more detailed data of which are presented in the section concerned with descriptions of some of the commercially available compounds.

9. Fungicidal and Fungistatic Action

Some of the quaternaries have displayed fungicidal and fungistatic action towards some of the pathogenic fungi in fairly high dilution. Reports on fungicidal activities of these compounds have been presented by Heineman (1937), Dunn (1937a), Howard and Keil (1943), Lawrence (1947) and others.

10. Virucidal Action

Krueger *et al.* (1942) showed that Zephiran in a concentration of 1:10,000 failed to inactivate test influenza virus preparations (types A and B) after exposure for 1 hour. One per cent soap solutions acting upon dense lung suspensions of type B influenza virus rendered them noninfectious within 40 minutes but only partially destroyed the type A virus preparation. However, Knight and Stanley (1944) found Phemerol and Roccal to be highly virucidal *in vitro*. These products caused immediate inactivation of purified influenza virus at 0.5 and 0.05 *N* concentrations and complete inactivation after a period of 2 weeks in 0.005 and 0.0005 *N* solutions. Klein and Stevens (1945) reported that 1:2,000 dilutions of three cationic compounds, cetylpyridinium chloride, Phemerol and Zephiran, and 1:500 dilutions of four anionic compounds, Tergitol 7, Triton W-30, sodium lauryl sulfate and sodium myristyl sulfate and Hexylresorcinol (1:200) completely inactivated influenza A virus in 60 seconds at room temperature *in vitro*. None of these materials protected mice against influenza A virus infection when administered intranasally by installation or spray. Klein *et al.* (1945) showed that Phemerol, cetylpyridinium chloride, and Zephiran inactivated vaccinia and influenza A virus in dilutions of 1:4,000 or higher.

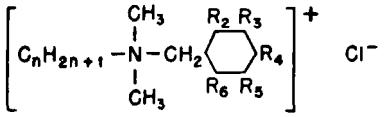
11. Relationship of Chemical Structure to Germicidal Activity

Systematic studies concerning the relationship of chemical structure and germicidal activity have been carried out by a number of scientists. Among these, may be mentioned the researches of Jacobs (1916), Jacobs and Heidelberger (1915a, b; 1916a, b), Browning *et al.* (1922, 1926), Hartmann and Kagi (1928), Domagk (1935), Shelton *et al.* (1939; 1940; 1946a, b, c), Kuhn and Dann (1940), Kuhn and Jerchel (1940, 1941), Kuhn and Westphal (1940a, b), Eldred and Niederl (1941), Westphal (1941), Kolloff *et al.* (1942), Woodruff *et al.* (1942), Jerchel (1942, 1943), Rawlins *et al.* (1943), Epstein *et al.* (1943), Valko and DuBois

(1945), and Lawrence (1947). References to some of these studies follow.

In the series of higher aliphatic dimethylbenzylammonium chlorides, Valko and DuBois (1945) found that the maximum antibacterial activity towards *Staphylococcus aureus* and *Eberthella typhosa* at 20°C. (68°F.) was demonstrated by the *n*-dodecyl and myristyl compounds (twelve and fourteen carbon atoms, respectively) which were approximately equally effective against the two test organisms. Lawrence *et al.* (1947) observed that when there were six, eight, and in some cases, ten carbon atoms in the alkyl group, the germicidal activity was low. However, increasing the chain length to twelve carbon atoms abruptly increased the germicidal value as was shown by the phenol coefficient. Extension of the chain length to fourteen carbon atoms caused further increases in the phenol coefficient in some salts, but when the chain length was increased to sixteen and eighteen carbon atoms, there was a drop in phenol coefficient values.

Lawrence *et. al.* (1947) described the results of their research to determine the effects on germicidal activity of substituents in the aromatic nucleus of some benzyl quaternary ammonium compounds. Bactericidal activity was determined by the Food and Drug Administration Method, as described by Ruehle and Brewer (1931), using *S. aureus* and *E. typhosa* as the test organisms and at 20°C. The general chemical structure of the compounds studied by them may be represented by the following formula:



wherein R_2, R_3, R_4, R_5 and R_6 are the positions in which the substitutions were made in the benzene nucleus, and wherein *n* equalled 6, 8, 10, 12, 14, 16 or 18. Quaternary ammonium salts which contained two or three chlorine atoms in the benzene nucleus were generally more active germicidally than those which contained only one. Lorol (a mixture of alkyl groups from C_8H_{17} to and including $C_{18}H_{37}$, with an average of $C_{13}H_{27}$) dimethyl 2,4-dichlorobenzylammonium chlorides showed a phenol coefficient of 600 at 20°C., when *S. aureus* and *E. typhosa* were used as the test organisms.

Valko and DuBois (1945) reported on research carried out on series of quaternary ammonium compounds which were closely related to dodecyldimethylbenzylammonium chloride. The purpose of their studies was to determine the influence of the lengths of the long hydrocarbon

chains and of the lower alkyl and aralkyl groups on the germicidal activities of the compounds towards *S. aureus* and *E. typhosa* at 20°C. (68°F.). In the series of higher aliphatic dimethylbenzylammonium chlorides, the unsaturated *n*-9-octadecenyl compound was about six times as active against *S. aureus* as the *n*-octadecyl compound, but the difference was less pronounced with *E. typhosa*. In the series of higher aliphatic dimethyl ethyl ammonium bromides, maximum activity was observed when the chain length was fourteen to sixteen carbon atoms. This was true also of the series of higher aliphatic dimethyl allyl ammonium bromides. In the higher aliphatic trimethyl ammonium bromides series, greatest germicidal activity was obtained with compounds containing *n*-tetradecyl and *n*-hexadecyl chains. Of the *n*-dodecyl ammonium derivatives examined, dodecyldimethylbenzylammonium chloride and dodecyldimethyl α-menaphthylammonium chloride were two of the most active towards *S. aureus* and *E. typhosa*. Hexadecyldimethylethylammonium bromide and hexadecyldimethylallylammonium bromide were the most active of the various *n*-hexadecyl ammonium derivatives investigated by Valko and DuBois (1945), whereas 9-octadecenyldimethylethylammonium bromide and 9-octadecenyldimethylpropylammonium bromide were two of the most germicidal of the *n*-9-octadecenyl ammonium derivatives.

Kuhn *et al.* (1940) studied the effect of varying the length of the alkyl radicals from four to six to eight to twelve to sixteen carbon atoms, respectively, in the series dialkylmethylbenzylammonium chloride. They found that the maximum germicidal activity towards staphylococci, *Escherichia coli* and *Corynebacterium diphtheriae* was effected by a chain containing eight carbon atoms; and towards organisms of the paratyphoid group (*Salmonella sp.*), by a chain containing eight or twelve carbon atoms.

Shelton *et al.* (1939; 1940; 1946a, b, c) also have studied the influence of chemical structure upon germicidal activity. They prepared a series of quaternary ammonium salts of the type represented by the formula $R \cdot (CH_3)_3 \cdot N \cdot Br$, in which R stood for a straight-chain alkyl group containing from six to eighteen carbon atoms. The germicidal activities of the various compounds were compared on the basis of the highest dilutions which destroyed the test organisms, *Staphylococcus aureus* and *Eberthella typhosa*, in 10 but not 5 minutes at 37°C. (98.6°F.), using the Food and Drug Administration technique. The salt possessed very little germicidal activity when the long-chain alkyl group contained less than eight carbon atoms. However as the chain length was increased the germicidal activity was also increased attaining a maximum in the compound cetyltrimethylammonium bromide. However, when R repre-

sented a stearyl or oleyl group, the germicidal activity was less than when it represented a cetyl group. Replacement of one of the methyl groups in the formula with a benzyl radical enhanced the bactericidal activity of the lower members of the series somewhat but did not increase the activity of the cetyl compound. Replacement of the methyl groups in cetyltrimethylammonium bromide with ethyl groups had no adverse effect on the germicidal activity, but replacement of them with more than one butyl group lowered the activity. Introduction of hydroxyl groups tended to reduce the bactericidal activity of the series.

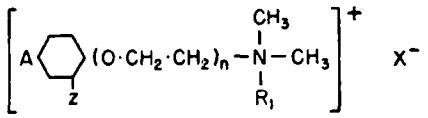
During a study to determine the effect of the anion upon the germicidal activity of the cetyltrimethylammonium salts, Shelton and his associates (*loc. cit.*) found that it exerted but little influence. The phosphate, laurate and salicylate salts had a lower activity than the bromide, chloride, iodide, nitrate, sulfate, methosulfate, acetate, benzoate, cyanide, hydrocinnamate and fluosilicate salts; the halides and the sulfate were somewhat more active than the remainder of the salts.

In further research, Shelton *et al.* (1946b) determined the effects of substitutions in the formula $R \cdot R_1 \cdot (R_2)_2 \cdot N \cdot X$ upon germicidal action towards *S. aureus* and *E. typhosa*. In this formula, R represented a cetyl or lauryl group; R_1, a carbethoxymethyl, β-acetoxyethyl or carbamylmethyl group; R_2, a methyl, ethyl or *n*-butyl group; and X a monovalent anion. The substitution of one of the methyl groups of lauryltrimethylammonium with the N-carbethoxymethyl group resulted in about twice as much germicidal action towards the two test organisms. The substitution of one of the methyl groups with the β-acetoxyethyl group in the lauryl compound yielded a product with somewhat less activity than the foregoing carbethoxy derivative towards *E. typhosa*. The substitution of one of the methyl groups in the cetyltrimethylammonium compound with the carbamylmethyl group resulted in an 80 to 90% loss in germicidal action; and substitution with the carbethoxymethyl group or with the β-acetoxyethyl group resulted in losses of about 50% or more.

In another paper, Shelton *et al.* (1946c) reported the results of studies with quaternary ammonium salts derived from cyclic amines, which included picoline, pyridine, lutidine, quinoline, piperidine, pipicoline and morpholine. The highest germicidal activity towards *S. aureus* and *Eberthella typhosa* in the series prepared from the unsaturated cyclic amines was demonstrated by the cetylpyridinium salts; in the series prepared from the saturated cyclic amines, by cetylmethylpiperidinium bromide. In the alkyl pyridinium and the alkyl α and γ picolinium bromide series, Kolloff *et al.* (1942) discovered that the C_{16} derivatives were more active germicidally against *S. aureus* than the C_{12} and the

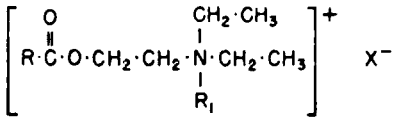
C_{14} derivatives. In the alkyldimethyl sulfonium iodide series, Kuhn and Dann (1940) found that the maximum chain length for optimum germicidal activity was C_{12} for *Escherichia coli* and C_{16} for *S. aureus*.

The relationship between the chemical structure and germicidal action towards *S. cureus* and *Eberthella typhosa* at 20°C. of quaternary ammonium salts of the general formula indicated below was studied by Rawlins *et al.* (1943):



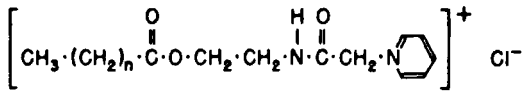
They reported that there should be one long alkyl or alkylarpoly (oxyalkyl) chain, one short aralkyl group, and two lower alkyl groups in the cationic portion of the salt and that the long chain should contain twelve to sixteen carbon atoms (counting the benzene ring as four atoms). Appreciable increases or decreases in the chain length interfered seriously with the antibacterial activity. The substitution of halogen in the aryl group did not increase the bactericidal activity and under some circumstances decreased it. In a series of diethoxy compounds, the greatest germicidal action was demonstrated by diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride. Substitution of a triethoxy group for the diethoxy group decreased germicidal activity. Likewise, substitution of chlorine in the benzene radical attached to the nitrogen lowered bactericidal action towards *E. typhosa*. The use of closed ring substituents on the aromatic nucleus was definitely inferior to the use of alkyl groups for enhancing germicidal activity.

Woodruff *et al.* (1942) studied the series:



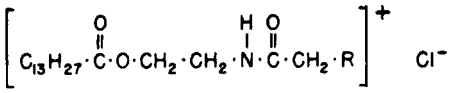
and found that the maximum germicidal activity was obtained when the carbon atoms were sixteen, R_1 represented a methyl group and X, iodide; and when the carbon atoms were twelve where R_1 represented an allyl group and X, the iodide.

Epstein *et al.* (1943) investigated the effect of the length of the fatty acid radical on bactericidal activity as determined by the Food and Drug Administration Method, using *S. aureus* and *E. typhosa* as the test organisms at 20°C. A compound of the structure indicated below was studied:

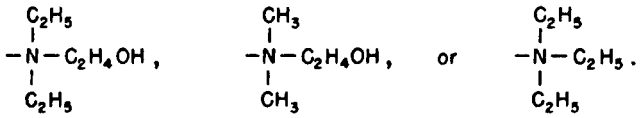


The chain length of the fatty acids was varied from C_8 to C_{18} in the $CH_3 \cdot (CH_2)_n \cdot \overset{\overset{O}{\|}}{C} \cdot$ grouping. It was found that the optimum bactericidal activity resulted when the fatty acid radical contained fourteen carbon atoms and the lowest activity when the fatty acid radical contained eight carbon atoms. Bactericidal action in the descending order, was as follows: $C_{14}>C_{12}>C_{16}>C_{18}>C_{10}>C_8$.

The effect of substitutions of R in the following general formula was also investigated:



Some of the best results were obtained when R represented:



12. Therapeutic Effectiveness

The therapeutic effectiveness of cationic compounds, as well as others, cannot be predicted accurately on the basis of their germicidal action, according to Green (1944). For example, he found that the therapeutic action of cetyltrimethylammonium iodide (CTAI) was much less than that of cetyltrimethylammonium bromide (Cetamium) and of stearyltrimethylammonium bromide (STAB), as determined by the use of the chorioallantoic membrane of the developing chick embryo. The iodide of the cetylpyridinium compound was less effective therapeutically than the chloride and bromide. Fourteen out of seventeen cationic agents showed definite therapeutic action against *Staphylococcus* infections of the chorioallantoic membrane of the developing chick embryo. However, Emulsol 606, Emulsol 607 and Catol 2 demonstrated little action, according to Green (1944). In general, cationic compounds were much superior to anionic compounds in respect to their action on the tissue infected with *Staphylococci.* As a result of further work along these lines, Green and Birkeland (1944) found that penicillin, Ceepryn, C Y B (carbomethoxypentadecylmethylpiperidinium chloride), Cetamium, Zephiran and Phemerol were effective therapeutically against staphylo-

coccal infections. Azochloramide, iodine, Metaphen, Merthiolate, and phenol proved to be ineffective therapeutically.

13. Film Formation

Miller *et al.* (1943) found that a film is formed on the hands as the result of the use of quaternary ammonium salts. This film, which was very resistant to mechanical trauma, retained bacteria beneath it. Although the outer surface of the film exerted strong germicidal action, the inner surface showed low bactericidal action. Films were formed as the result of the use of Phemerol, Emulsol 605, Emulsol 607, cetylpyridinium chloride, Catol, Damol, Zephiran and other cationic compounds. Rahn (1946) reported that the formation of the film possessing the properties described above was due to the oriented absorption of the compounds on an organophilic surface such as skin, fat or paraffin.

14. Mechanism of Action

The mode or mechanism by which the surface-active germicides destroy microorganisms has been discussed by Baker *et al.* (1941a, b, c), Albert (1942), Dubos (1942), Randles and Birkeland (1944), Valko (1946), Hotchkiss (1946) and others. Baker *et al.* (1941a) stated that the action on bacterial metabolism was influenced by the specific chemical structure of the compound, characteristics of the microorganisms, pH of the medium, charge of the ion containing the hydrophilic group, and hydrophilic-hydrophobic balance of the molecule. Baker *et al.* (1941b, c) suggested that the cell membrane was disorganized by the surface-active agent and that the proteins necessary for growth and metabolism were denatured.

Randles and Birkeland (1944), on the basis of studies made concerning the inhibition of Gram-negative bacteria in synthetic media which contained different sources of nitrogen, advanced the suggestion that the inhibition of growth by detergents was due to the selective adsorption of the cation at the sites on the cell where the cations of nitrogen compounds essential in nutrition were normally adsorbed. The detergent cation possessing a structural relationship to the essential nutrient was more readily adsorbed and thereby prevented the adsorption and the utilization of the required nitrogen-containing ion.

Valko (1946) felt that the biochemical changes incidental to the destruction of the cells could be explained in part at least by the following: The surface-active compounds combine with the proteins of the cell on account of their strong affinity for them, thereby altering the

balance of the electrostatic forces and the non-Coulombic cohesion of the molecule and disturbing the intermolecular structure of the proteins. Disruption of the bonds between components of the conjugated proteins may occur following the interaction of the proteins with the solvent molecules. Denaturation of the protein is one of the changes.

Hotchkiss (1946) concluded that destruction of the cells resulted from a combination of the surface-active ions with sites on the bacterial surface which bore an opposite charge, adsorption of a small fraction of the agent, production of irreversible damage to the cellular membrane, loss of soluble nitrogen and phosphorous compounds from the cell and subsequent autolysis of the cell contents.

15. Commercial Preparations

Quaternary ammonium compounds are usually marketed as solids or solutions. In solid form, they are distributed as crystalline or amorphous powders, as soap-like products, or as tablets. Solutions are aqueous, tinctures, or in oil. Aqueous solutions usually contain 7.5, 10, 12.8, 25, 33, 50, 62, 75 or 90% concentrations of the quaternary. Highly purified solutions, tinted or untinted, are sold for use by the medical profession or for first aid purposes in 1:100, 1:200, 1:500 or 1:1,000 dilutions as aqueous solutions or as tinctures containing 50% ethyl alcohol and 10% acetone. Highly purified aqueous concentrates (12.8%, etc.) are also sold to hospitals, which may be used in making up the specific concentration desired.

The liquid quaternaries are ordinarily distributed in glass containers. Liquid concentrates are customarily packaged in pint, quart and gallon glass bottles, or in larger glass carboys. Bottled products sold for medical uses come in the usual drug store sizes. Purchases of this class of disinfectants by government agencies are made on the basis of specifications, one of which is Navy Department Specification 51 D 6 (and 51 D 6a).

According to Lawrence (1947a), two quaternary ammonium compounds are listed in New and Non-official Remedies. One is described in the U. S. Pharmacopoeia (XIII Ed.).

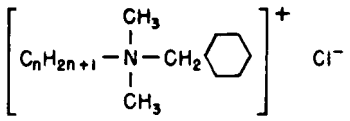
16. Economics

The cost of quaternary ammonium compounds in relation to some of the older types of disinfectants has been discussed by Botwright (1946), Lawrence (1947a) and others.

III. Descriptions of Some Commercially Available Compounds

1. Alkyldimethylbenzylammonium Chlorides

Alkyldimethylbenzylammonium chlorides (ADBACl) * have the general structural formula:



wherein C_nH_{2n+1} represents a mixture of alkyl radicals (C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$ and $C_{18}H_{37}$), which are derived from the fatty acids of coconut oil. The average molecular weight of the mixture is maintained at 357.5 as a result of the standardized methods used in its manufacture, according to Auerbach (1943).

ADBACl in solid form are amber-colored and have soap-like consistency (Dunn, 1936). Domagk (1935), Dunn (1936), Deskowitz (1937) and others have reported that the solid is freely soluble in water and that in the dilutions normally employed, the solution is clear, colorless, and practically odorless. It is soluble in acetone and ethyl alcohol, but only slightly soluble in benzol and insoluble in ether (Heineman, 1937). The aqueous solution, which possesses a slight aromatic odor, foams when shaken. Dunn (1936) has reported that the 10% aqueous stock solution has a surface tension of less than 36 dynes per cm.

The product and its aqueous solutions are stable over a fairly wide temperature range. Freezing or storage at 50°C. (122°F.) apparently has no adverse effects upon the germicidal activity of the compound, according to Dunn (1937). Deskowitz (1937) has reported that it does not deteriorate upon exposure to heat and sunlight or during long storage. It has been used successfully at the boiling temperature to sterilize instruments by Brekenfeld (1938).

a. Early Studies. Without a doubt, ADBACl have been investigated more intensively and extensively than any other of the quaternary ammonium compounds to date. First studies concerning the germicidal properties and uses of this compound were carried out in Germany. Publications on the product, known as Zephirol, first appeared in 1934 and 1935. These included a report by Meissner (1934) on the use of Zephirol as a disinfectant for instruments and a thesis by Cremer (1934). During 1935, there appeared the epochal paper by Domagk as well as the reports of Caesar, Eschenbrenner, Hornung, Lübben, Rode-

* This abbreviation will be used in this section.

curt, Schmidt, Schneider and others. During the following year, 1936, other reports appeared in Germany, as for example those of Hochmuth, Hornung, Jötten and Schön, Kliewe and Maier, Maier and Müller, and Seeman. The first of the published reports on the compound in this country appeared in the same year (Dunn, 1936). During 1937, there followed other reports in this country by Deskowitz, Heineman, and Dunn and in Germany by Kaiser, and Kliewe and Althaus. In 1938 there were reports by Cutler and Zollinger; Dunn; White *et al.*; Vierthaler and Shaw; and others. Subsequently, the results of many other investigations and observations have been published. Many of these have been concerned with applications of the germicide (uses in the food industries and in the medical field), some with methods of testing and assaying it, and some with other aspects.

b. Germicidal Action. The germicidal action of ADBACl has been demonstrated by a large number of workers. Domagk (1935) reported on the concentrations of the product which were effective in destroying various pathogenic microorganisms in short periods of time. The bacteria included both Gram-positive and Gram-negative types. Dunn (1936; 1937a; 1938a, b) has shown that the salts are effective against pathogenic bacteria, yeasts and molds in high dilutions. Table IV presents average phenol coefficients and the highest dilutions capable of destroying the indicated test organisms in 10 but not 5 minutes at both 20 (68°F.) and 37°C. (98.6°F.).

TABLE IV

Germicidal Action of Alkyldimethylbenzylammonium Chlorides[a]

Organisms	Average phenol coefficients		Average highest dilution destroying organism in 10 but not 5 min.	
	20°C.	37°C.	20°C.	37°C.
S. aureus	279	407	1/20,000	1/35,000
Eberthella typhosa	250	429	1/20,000	1/70,000
Escherichia coli	160	358	1/12,000	1/40,000
Str. hemolyticus	435	579	1/40,000	1/95,000
Str. viridans	384	434	1/35,000	1/65,000
Cryptococcus hominis	214	395	1/24,000	1/70,000
Monilia albicans	111	274	1/10,000	1/35,000
Trichophyton interdigitale	30.8	—	1/2,000	—
Microsporon lanosum	400	—	1/40,000	—

[a] Dunn, 1937.

Data relating to the germicidal action of ADBACl in the presence of 20% sterile horse blood serum are shown in Table V.

TABLE V

Germicidal Action of Alkyldimethylbenzylammonium Chlorides in the Presence of 20 per cent Serum [a]

Test organism	Tests at 20°C. Average phenol coefficients	Tests at 20°C. Highest dilution destroying in 10 but not in 5 min.	Tests at 37°C. Average phenol coefficients	Tests at 37°C. Highest dilution destroying in 10 but not in 5 min.
S. aureus	54.2	1/3,250	72	1/5,500
Eberthella typhosa	23.0	1/1,900	39.8	1/5,750
Escherichia coli	17.9	1/1,250	31	1/3,250
Str. hemolyticus	63.6	1/5,000+	61.5	1/8,750
Str. viridans	57.5	1/4,000	48.9	1/5,750

[a] Dunn, 1937.

The efficacy of this compound in the presence of 50% serum has already been referred to on page 136.

ADBACl appeared to possess marked bacteriostatic action towards *S. aureus* and *Bacillus subtilis*, according to Dunn (1937a). Both of these organisms were inhibited completely in dilutions of 1:200,000 at 37°C. The bacteriostatic action towards *Escherichia coli*, a Gram-negative organism, was of a much lower order.

ADBACl (Zephiran) in a dilution of 1:80,000 caused complete inhibition, and in a dilution of 1:400,000 retardation of the growth of a nonvirulent Novy strain of tubercle bacilli, according to Freelander (1940). Nacconol NR and Aerosol OT-100 prevented growth of these bacteria in a dilution of 1:5,000 and retarded them in a dilution of 1:40,000. Growth occurred in a 1:1,000 dilution of Aerosol OS; however, a dilution of 1:10,000 was slightly inhibitory. The bactericidal activity of Zephiran towards the tubercle bacilli was low.

Miller *et al.* (1940) have discussed the inhibitory action of Zephiran on dental plaque material. They showed that Zephiran in 1:3,000 concentration completely prevented acid production by the plaque material when it was suspended and incubated in glucose solution. Plaque material treated for 2 minutes *in situ* with a 1:500 solution of the germicide and removed 10 minutes later and placed in glucose solution failed to induce the production of appreciable quantities of acid.

This compound was not so rapid in germicidal action towards bacterial spores as against the vegetative cells; this is generally true of most chemical germicides. Deskowitz (1937) reported that a 1:6,000 dilution of the compound destroyed *Clostridium welchii* in 10 minutes and a 1:2,500 dilution destroyed *B. subtilis* in 95 minutes at 37°C. Hucker *et al.* (1947) showed that dilutions greater than 1:1,000 destroyed the

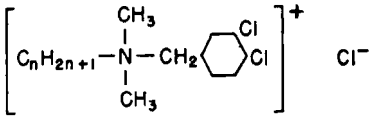
spores of *B. subtilis* during an exposure of 10 minutes; that a dilution of 1:500 destroyed the spores of a facultative thermophilic flat sour organism (National Canners Association No. 1518) and of an obligate thermophilic flat sour strain (N. C. A. No. 1503) within 10 minutes; and that a dilution of 1:1,500 destroyed the spores of a mesophilic flat sour strain (N. C. A. M-23) within 10 minutes. Dunn (1937a) found that a 1:10 aqueous solution of ADBACl destroyed the spores of *B. subtilis* in 30 but not 15 minutes at 37°C. in a solution at pH 8.64. Alkaline solutions of the compound, of a pH range of 8.64 to 10.68, were considerably more effective against the spores of *B. subtilis* than acid solutions.

Gershenfeld and Witlin (1941) demonstrated that the addition of Aerosol OT (sulfonated ester of dicarboxylic acid) to ADBACl greatly decreased the bactericidal efficacy because of the chemical incompatibility.

ADBACl are widely used for their germicidal and antiseptic action in hospitals and in the food industries. The more highly refined product is used for medical and surgical purposes, one such product being Zephiran. The technical grade of the compound is distributed as a disinfectant and sanitizing agent and widely used for these purposes. It is sold under a number of trade names, for example, BTC, Roccal, Rodalon and Roclina. For more details on this subject the reader is referred to page 171.

2. Alkyldimethyl 3,4-dichlorobenzylammonium Chlorides

This compound is closely related in structure and general properties to alkyldimethylbenzylammonium chlorides. It has the following chemical structure.



The product has been studied by DuBois and Dibblee (1946c), Hucker *et al.* (1947), Lawrence *et al.* (1947) and others. DuBois and Dibblee (1946c) found that 60 to 75% of the spores of *Bacillus metiens* are destroyed almost immediately in 1:5,000 and 1:20,000 dilutions of the compound. Hucker *et al.* (1947) reported that the product was effective as a sporicide and that it destroyed other resistant bacteria, including a capsulated strain of *Aerobacter aerogenes*, in relatively high dilutions in short periods of time.

One commercial product is marketed under the trade name of Tetrosan.

3. Cetyltrimethylammonium Bromide

In purified form, this compound is a white, crystalline powder, somewhat astringent and with a slightly bitter taste. Hoogerheide (1945) reports that it may be prepared from cetyl bromide and trimethylamine as indicated by the following reaction:

$$CH_3 \cdot (CH_2)_{15} \cdot Br + (CH_3)_3 \cdot N \rightarrow CH_3 \cdot (CH_2)_{15} \cdot (CH_3)_3 \cdot N \cdot Br$$

It may be purified by repeated recrystallization. The solubility in water under 20°C. is not high, being less than 0.5%. Gold chloride, mercuric chloride and picric acid precipitate it from solution; tannic acid and gelatin do not.

Williams *et al.* (1943) have reported that its germicidal efficacy is reduced by blood serum, milk and certain other organic material including dead cells. Barnes (1942) has shown that a 1% solution precipitates serum proteins and a 1:1,000 solution destroys leucocytes *in vitro*. The bactericidal and bacteriostatic activities towards both Gram-positive and Gram-negative bacteria increased as the pH was increased, according to Hoogerheide (1945).

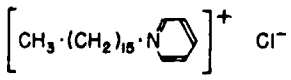
Cetyltrimethylammonium bromide, frequently referred to as CTAB or Cetavlon, was developed in England. First studies on it were carried out by Barnes (1942), who found that the product materially reduced the number of bacteria on the hands, that it did not produce pain or injury to raw flesh surfaces and that it cleansed bowls and baths. Whittet (1942) described the crude product and its use in a hospital at Manchester, England. The crude product occurred as a brown powder which was greasy to the touch and readily soluble in warm water. It possessed a fishy odor due to trimethylamine hydrobromide. A 1% solution of the product was used in the hospital for cleansing wounds and burns with "very good results." It did not interfere with healing processes. It was found to be useful in plastic surgery in removing bandages and in cleansing. It did not interfere with freshly grafted skin.

Cetavlon, the name given to the purified substance in 7.5% aqueous solution, is whitish and translucent and almost free of odor. Solutions of the purified and crude compound were used in a hospital for a year; no cases of skin reaction or sensitivity were observed (Whittet, 1943). A 1:10 dilution was found to be particularly effective for cleansing dirty and infected wounds. Williams *et al.* (1943) used the product in gloveless surgery. They found that only a very low percentage of persons demonstrated irritation to any degree. They recommended the use of a 1% solution for cleansing and sterilizing utensils and instruments.

Forman (1943) used a 2% solution for cleaning and disinfecting wash basins, bath tubs, etc. It was also used for removing scabs and crusts from impetiginized areas and for cleaning areas which had been covered with ointment. Hughes and Edwards (1946) described the use of a 1% concentration in a lanette wax-oil base to control *Streptococcus agalactiae* in a dairy herd. Results of their findings are reported elsewhere in this review. Green (1944) and Green and Birkeland (1944) reported that Cetamium exerted high therapeutic effectiveness against staphylococcal infections as determined by the chick embryo method. The germicidal action of CTAB against spore-formers and some other bacteria has been reported by Hucker *et al.* (1947).

4. Cetylpyridinium Chloride

Cetylpyridinium chloride (CPCl) * is a heterocyclic compound, as is indicated by the following structural formula:



The pure compound, a monohydrate, is a white crystalline solid with a melting point at 80°C. (176°F.). It is quite soluble in water (1:5), acetone, ethyl alcohol and chloroform, but is less soluble in fatty oils and is insoluble in ether (Warren *et al.*, 1942). Aqueous solutions are clear, colorless and practically odorless.

CPCl has been studied by Shelton *et al.* (1939; 1940), Blubaugh *et al.* (1939; 1940; 1941), Green and Birkeland (1941; 1942), Clarke (1942), Warren *et al.* (1942), Reed *et al.* (1943), Kramer and Sedwitz (1944), Huyck (1944a, b; 1945), Green (1944), Helmsworth and Hoxworth (1945), Hagan *et al.* (1946), Kenner *et al.* (1946), Quisno and Foter (1946), Hucker *et al.* (1947), Dyar (1947) and others. Next to alkyldimethylbenzylammonium chlorides, this compound has been studied more extensively than any of the other quaternaries, based on published reports.

a. Germicidal Action. As a result of studying over 100 compounds, Shelton *et al.* (1939, 1940) discovered that the alkyl pyridinium salts were most interesting and that of these CPCl exhibited optimum germicidal activity. Blubaugh *et al.* (1939, 1940, 1941) found that CPCl was highly bactericidal for pathogenic organisms even in the presence of organic matter, when using the Food and Drug Administration (F. D. A.) technique for testing germicidal action. Quisno and Foter (1946) studied the germicidal properties of CPCl rather extensively following

* This abbreviation will be used in this section.

the procedure described in Circular No. 198 of the U. S. Department of Agriculture, with modifications when required. Representative results of some of their tests are shown in Table VI.

TABLE VI

Germicidal Activity of Cetyl Pyridinium Chloride In Aqueous Solution [a]

Organism	No. strains tested	Average critical killing dilution in terms of active ingredients at 37°C. [b] No serum	10% bovine serum
Staphylococcus aureus	5	1:83,000	1:12,500
Staphylococcus albus	1	1:73,000	1:12,000
Streptococcus viridans	1	1:42,500	1:12,000
Streptococcus hemolyticus	2	1:127,500	1:17,000
Neisseria catarrhalis	2	1:84,000	1:13,000
Diplococcus pneumoniae I	1	1:95,000	1:14,000
Diplococcus pneumoniae III	1		1:20,000
Pseudomonas aeruginosa	2	1:5,800	<1:1,000
Klebsiella pneumoniae	2	1:49,000	1:5,500
Corynebacterium diphtheriae	1	1:64,000	1:14,000
Mycobacterium phlei	1	1:1,500	1:1,000
Eberthella typhosa	5	1:48,000	1:3,000
Escherichia coli	2	1:66,000	<1:1,000
Proteus vulgaris	2	1:34,000	1:2,000
Shigella dysenteriae	1	1:60,000	1:5,000
Shigella paradysenteriae (Flexner)	2	1:52,000	1:3,500
Shigella paradysenteriae (Hiss)	1	1:49,000	1:2,000
Shigella sonne	2	1:68,000	1:6,500
Lactobacillus acidophilus	1		1:16,500
Brucella abortus	1		1:19,500
Trichomonas vaginalis [c]	1		1:3,000 [d]
Candida albicans	1	1:37,000	1:3,500
Cryptococcus neoformans	1	1:61,000	1:6,000
Trichophyton mentagrophytes	1	1:36,000	1:3,000
Microsporum canis	1	1:34,000	1:5,000

[a] Quisno and Foter, 1946.
[b] The highest dilution which destroyed the test organism in ten but not five minutes.
[c] Obtained from Dr. Garth Johnson of the State University of Iowa.
[d] Twenty-five per cent human serum.

In general, they found CPCl to be effective germicidally in high dilutions against both Gram-positive and Gram-negative bacteria, pathogenic yeasts and fungi, and *Trichomonas vaginalis*. The compound was active under acid and alkaline conditions and at room and body temperatures. Although serum reduced its efficacy, the compound was still active in fairly high dilutions.

CPCl destroys bacterial spores, but higher concentrations are required than for vegetative cells. Green and Birkeland (1941) determined the action of the compound on the spores of *Clostridium perfringens, Cl. sporogenes, Cl. tetani, Bacillus anthracis* and *B. subtilis* in a liquid medium, using essentially the F. D. A. procedure, and on contaminated instruments. The results obtained indicated that the compound is an effective germicide. Hucker *et al.* (1947) described the action of the compound against a number of spore-formers and other resistant organisms. CPCl destroyed the spores of a facultative thermophilic flat sour organism (N. C. A. No. 1518) in a dilution of 1:1,500; of an obligate thermophilic flat sour strain (N. C. A. No. 1503) in a dilution of 1:1,000; and of a mesophilic flat sour strain (N. C. A. No. M-23) in a dilution of 1:2,000 in less than 10 minutes. Against *B. subtilis,* the action was of relatively low efficacy.

Huyck (1945) investigated the effect of CPCl on bacterial growth in the oral cavity. He found that a 1:4,000 (0.025%) concentration of the product was bacteriostatic and bactericidal towards the bacteria associated with the oral cavity. Employing the Hanke (1940) method, he discovered that a 1:4,000 concentration of the product in a base containing 18% alcohol, 10% glycerine and approximately 70% distilled water had a bactericidal efficiency of 90.64% after 3 hours in an average of twenty-one cases.

The lactic dehydrogenase of *S. aureus* was shown to be more susceptible to CPCl and the dioctyl ester of sodium sulfosuccinate than that of *E. coli* (Ordal and Borg, 1942). The inhibitory effect disappeared rapidly with dilution in the case of *S. aureus* but there was quite a wide range of dilutions over which there was a relatively little change in inhibition in the case of *E. coli.*

A 5- to 10-minute scrubbing of the operative field with a 1:100 aqueous solution of CPCl provided effective degerming of the skin, as determined by swab and pinch graft procedures (Helmsworth and Hoxworth, 1945).

Hagan *et al.* (1946) found that the 1:1,000 tincture of CPCl compared favorably with other cutaneous antiseptics.

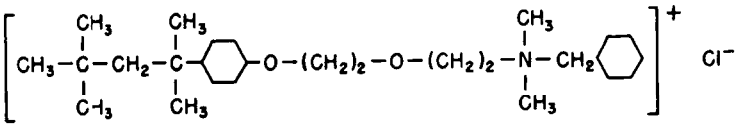
b. Toxicity. A number of studies have been made to determine the toxicity of CPCl. Warren *et al.* (1942) have studied the acute and chronic toxicity of the compound when administered intravenously, intraperitoneally, subcutaneously and orally. The test animals used were mice, rats, guinea pigs, rabbits and dogs. With rabbits, the minimum lethal dose (MLD) of CPCl was 20 mg. per kg. body weight and the LD_{50} was 35 mg. per kg. when injected intravenously. The compound was much less toxic when administered orally to rabbits than when in-

jected intravenously. However, when injected intraperitoneally, CPCl was more toxic than when injected intravenously. Animals dying often did not show toxic symptoms for 6 hours to 3 or 4 days after injection. CPCl produced a "curare-like" action when the dose was sufficiently large. When applied to the scarified skin of rabbits in a concentration of 1:200 to 1:250, the compound caused some reddening; however, greater dilutions had no effect. Clarke (1942) found that both the 1:200 tincture and aqueous solutions of CPCl produced no skin reactions following their uses in scratch and patch tests. The compound was not irritating when used for preoperative preparation in combination with a special soapless technique, according to Kramer and Sedwitz (1944).

Additional facts concerning this interesting compound will be found elsewhere in this report.

5. Diisobutylphenoxyethoxyethyldimethylbenzylammonium Chloride

This compound has the following structural formula:



Rawlins *et al.* (1943) found that this compound possessed the maximum germicidal activity of a series of diethoxy compounds studied by them and offered promise for use as a general germicide.

According to Joslyn *et al.* (1943), the crystals of this compound are colorless and odorless and melt at 165°C. (329°F.); it is extremely soluble in water. The aqueous solution has a pH of 5 to 6 and possesses a bitter taste. The compound is unaffected by dilute acids and alkalies but is precipitated by concentrated solutions of mineral acids. Aqueous and alcoholic solutions are stable to light, air and temperatures up to 100°C. (212°F.). The salt is not compatible with soaps and iodides. It has no deleterious action on rubber, plastics, fabrics and metals with the exception of copper and brass.

Joslyn and his associates found Phemerol to be effective germicidally against ten pathogenic test organisms at both 20 and 37°C. (68 and 98.6°F.) and effective as a fungicide *in vitro*. Hucker *et al.* (1947) determined the germicidal efficacy of diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride in respect to a number of resistant spore-formers and other bacteria. It destroyed the spores of *B. subtilis* and of a facultative thermophilic flat sour strain (N. C. A. No. 1518) in a dilution of 1:750 and of a mesophilic flat sour strain (N. C. A. No. M-23) in a dilution greater than 1:1,000 in less than 10 minutes. It

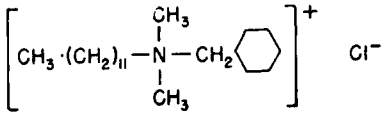
likewise destroyed a capsulated (slimy) strain of *Aerobacter aerogenes* in a dilution of about 1:12,000 in less than 10 minutes.

A 1:500 tincture of the compound (Phemerol) proved to be a good bactericidal and bacteriostatic agent; it did not cause skin irritation or interfere with the healing of wounds (Brown *et al.*, 1944). It was suitable for use as a skin antiseptic in surgery and obstetrics and was found to be superior to several mercurial and other skin antiseptics.

Diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride has been used successfully in veterinary medicine, as well as in surgery and as a first-aid germicide. Bryan *et al.* (1944) treated cows infected with chronic streptococcic mastitis with good results, while Bryan and Young (1945) cured ringworm in calves.

6. Lauryldimethylbenzylammonium Chloride

This compound has the following structural formula:

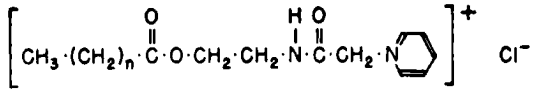


Hucker *et al.* (1947) found this compound to be effective germicidally against several resistant spore-formers and other bacteria. It destroyed the spores of *B. subtilis* and of a facultative thermophilic flat sour strain (N. C. A. No. 1518) in a dilution greater than 1:1,000; of an obligate thermophilic flat sour strain (N. C. A. No. 1503) in a dilution of 1:750; and of a mesophilic flat sour strain (N. C. A. No. M-23) in a dilution of 1:1,500, all in less than 10 minutes. It was also effective towards *Streptococcus cremoris, Micrococcus aureus* and a capsulated strain of *Aerobacter aerogenes* in dilutions of 1:4,000 or greater.

It is distributed under the trade names of Nopco QCL, Ster-Bac and Thoral for use as disinfectants or sanitizing agents.

7. N(Higher Acyl Esters of Colamino Formylmethyl)-Pyridinium Chlorides

These compounds have the following general structure:



They are thus related to the compounds described in the patents of Harris (1933, a, b, c, d; 1935), Katzman (1940) and Epstein and Harris (1942a, b).

The 10% aqueous solution of this compound (Emulsept) is buffered to pH 5.0 to 5.5. It is water white to faint straw in color, has a specific gravity of 1.0 and a low surface tension. In the dilutions used, it is no more corrosive to most metals than water. It is stable when stored at room temperature. The product possesses good detergent properties and is a good wetting agent, according to Epstein *et al.* (1943) and Bernstein and Epstein (1948).

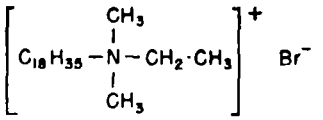
Like other quaternary ammonium compounds, *N* (higher acyl esters of colamino formylmethyl) pyridinium chlorides are effective germicides against both Gram-positive and Gram-negative, non-spore-forming bacteria in relatively high dilutions. It is less effective against spore-formers, as has been pointed out by Hucker *et al.* (1947).

The toxicity of N(higher acyl esters of colamino formylmethyl) pyridinium chlorides appear to be very low in comparison with that of most antiseptics and germicides. As determined by the method of Welch and Brewer (1942) the toxicity index was 0.5; by the method of Hirsch and Novak (1942), 0.6. The maximum nonirritating concentration of the substance was 1:2,000 as determined by Whitehill (1945). Oral administration of the compound to albino rats as the sole source of drinking water apparently produced no ill effects (Vivino and Koppanyi, 1946). Subcutaneous injections of 200 mg. per kg. of body weight of white mice, intraperitoneal injections of 100 mg. per kg. of body weight of albino rats and 125 mg. per kg. of body weight of rabbits are not fatal and appeared to be tolerated well (Anon., 1947a).

A more detailed discussion of the uses of this compound will be presented later, but mention will be made here of some of the special applications. Penniston and Hedrick (1944, 1945, 1947) described its use in the washing of eggs in the frozen and dried egg industries. Bernstein and Epstein (1948) reported on the use of this compound to reduce spoilage and to improve the quality of pasteurized pickles or "overnight dills." Kalter *et al.* (1946) stated that the agent may be used in the isolation of the gamma bacteriophage of *Escherichia coli* from sewage in a final dilution of 1:5,000.

8. N-9-Octadecenyldimethylethylammonium Bromide

The chemical structure of this compound is shown below:



Valko and DuBois (1945) found that the compound was active as a germicide in high dilution against *S. aureus* and *E. typhosa* at 20°C. (68°F.).

Information concerning the toxicity, germicidal activities and uses of this compound was furnished by Botwright (1946). He found that when a 1% solution of Amerse (the trade name of a commercial product) was applied to the forearms of eighteen persons for 24 hours, only two gave slight reactions and that the 1:1,000 solution caused no reactions on twenty-five individuals. He reported that Amerse was equal to or superior to chlorine as a germicidal rinse for glasses.

Lehn and Vignolo (1946a) described the use of this compound as a sanitizer in breweries. They stated that it may be used effectively with hot or cold water or with steam.

IV. Methods for Evaluating Germicidal Activity and Toxicity

A rather wide variety of tests (Table VII) have been used by various investigators to evaluate quaternary ammonium compounds. These have included germicidal, infection-prevention, toxicity index, and other toxicity tests; *in vitro* and *in vivo* methods; laboratory and performance tests. Human beings, monkeys, dogs, rabbits, guinea pigs, rats, mice, chick embryos and phagocytes have been used in determining the toxicity of the products. The compounds have been used in drinking water, fed as a constituent of the diet, tested on unbroken and abraded skin, and injected into animals subcutaneously, intramuscularly, intraperitoneally and intravenously.

Table VII

Some Methods Used for the Evaluation of the Quaternary Ammonium Compounds

Method	Reference
Germicidal Tests	
Phenol coefficient	Ruehle and Brewer (1931)
"Semi-micro" technic	Klarmann and Wright (1948)
Filter-paper	Ruehle and Brewer (1931)
Agar cup-plate	Ruehle and Brewer (1931)
Metabolism inhibition	Baker *et al.* (1941a)
Spores	Weber and Levine (1944)
Glass slide	Johns (1947)
Oval tube	Barker *et al.* (1947)
Use	Mueller *et al.* (1947)
Practical performance	Weber and Black (1948a, d)
Fungicidal	McCrea (1931)

TABLE VII (cont.)

Infection-prevention Tests	
Infection prevention	Nungester and Kempf (1942)
In vivo	Sarber (1942), Kenner *et al.* (1946)
Chick embryo	Green and Birkeland (1942, 1944)
Toxicity Index Tests	
Tissue culture	Salle *et al.* (1937, 1938)
Phagocyte	Welch and Hunter (1940), Welch and Brewer (1942)
Tissue	Hirsch and Novak (1942)
Other Tests	
Nonirritating concentration	Whitehill (1945)
Gain	Harshbarger (1942)
Patch and scratch	Clarke (1942)

It is obviously impossible to learn all about a germicide by the use of a single test no matter how good it may be. The fact that the ideal test, or combination of tests, has not yet been found or agreed upon is indicated merely by reference to the rather long list of tests shown in Table VII. Performance or use tests must remain the criterion of whether an agent is suitable for a given application or not. However, it is essential in addition to be able to obtain information regarding a compound's capabilities by readily carried-out laboratory tests. Although such tests have limitations, they may serve for screening purposes and provide means for indicating the relative values of related compounds.

Among the germicidal tests used for the evaluation of quaternary ammonium compounds are the phenol coefficient, filter paper, agar cup plate, metabolism inhibition, spore, glass slide, oval tube, use and fungicidal tests.

The phenol coefficient test of the Food and Drug Administration (see Ruehle and Brewer, 1931), has been widely used, with and without modifications, in determining and comparing the germicidal activities of the quaternary ammonium salts. The test organisms used are *Eberthella typhosa* and *Staphylococcus aureus*. Results are usually expressed in terms of the phenol coefficient and of the highest dilutions which destroy the test organism in 10 but not 5 minutes. However, modifications of the test in respect to the organism used, the subculture media, the time intervals and other factors have frequently been made.

The limitations of the phenol coefficient test have been discussed by many workers, including Leonard (1931), Reddish (1937; 1946; 1947), Brewer (1943; 1944), Klarmann and Wright (1946a, b; 1947), Bernstein *et al.* (1946), Pressman and Rhodes (1946), Quisno *et al.* (1946a.

b; 1947a, b), Hucker *et al.* (1947), Quisno and Foter (1947), and Lind (1948). Brewer (1944) reported that inaccuracies in the F. D. A. method were due mainly to lack of uniformity in the composition of the subculture medium, particularly in respect to peptone; mutation of the test organism; and variations in the numbers of microorganisms transferred from the medication tube to the subculture tube in the standard loop, caused by differences in surface tension. Others have experienced difficulties in transferring small amounts of antiseptics because of differences in them. Tobie and Orr (1944, 1945) substituted the use of a graduated 0.2-ml. Kahn pipette for the standard loop in carrying out the F. D. A. phenol coefficient test with germicides containing small amounts of added synthetic detergents, because of variations which they found in using the standard loop. They subcultured 0.02-ml. amounts of the medication mixture as the equivalent of the average standard loop. Dunn (1937) experienced difficulties of a similar nature in carrying out the F. D. A. cup-plate test and resorted to the use of graduated 1.0-ml. pipettes to plant a constant volume of germicide in the cup, instead of the six drops called for in the standard procedure.

One of the disadvantages of the F. D. A. phenol coefficient method has been the difficulty of distinguishing between a true bactericidal and a bacteriostatic end point in the case of some compounds of high bacteriostatic properties. The use of specific neutralizing agents in the subculture media, such as those developed by Quisno *et al.* (1946) and Armbruster and Ridenour (1947), should do much towards the elimination of this particular problem. The neutralizing medium of Quisno *et al.* (1946a) contains as special ingredients lecithin and Tween 80 (a polyethylene derivative of sorbitan monooleate manufactured by the Atlas Powder Co.). The lecithin acts as the neutralizing agent, while the Tween 80 helps to solubilize and disperse it. These ingredients are added to the standard F. D. A. broth, or to any other suitable subculture medium. In the case of the F. D. A. broth, 0.7 g. of lecithin and 5.0 g. of Tween 80 are used for each liter of medium. Lecithin and Tween 80 do not inhibit the growth of the test organisms in the concentrations employed, according to the authors. The medium developed by Armbruster and Ridenour (1947), which largely eliminates bacteriostasis, contains 29.5 g. of thioglycollate fluid medium (Difco), 30.0 ml. of Tween 20 (Atlas Powder Co., Wilmington, Del.), 2.0 g. of asolectin (Associated Concentrates, Inc., Atlanta, Ga.), 0.50 g. of agar and 1,000 ml. of distilled water. The pH is adjusted to 7.0 ± 0.1. Tween 20 (polyoxyalkylene sorbitan monolaurate) is used chiefly as a dispersing agent for the asolectin, but it also accomplishes some neutralizing action. Asolectin is a purified phosphatide which is used as the principal neutral-

izing agent of the quaternary ammonium compound. The agar aids in the clarification of the medium and in permitting the growth of discrete colonies. The Tween-Asolectin-Thioglycollate (TAT) broth has given good results with both small and large numbers of bacteria and with high quaternary-organism ratios.

Weber and Black of the U. S. Public Health Service (1948b, c) have carried out considerable research concerning the relative efficiency of quaternary inhibitors. They found that lecithin ("Asolectin" or "Yelkin B. T. S.") in "Tween 80," in a ratio of 20 parts of lecithin to 1 part of quaternary, demonstrated satisfactory inhibitory action towards *Escherichia coli*. A ratio of lecithin to quaternary of 100:1 was satisfactory for *Staphylococcus aureus* and *Micrococcus caseolyticus*. In tests to determine the germicidal efficiency of quaternaries, the use of 9 ml. blanks which contained enough lecithin to provide a ratio of lecithin to quaternary of 100:1 and of tryptone glucose extract agar which contained lecithin in "Tween 80" (1 g. "Asolectin" to 7 ml. "Tween 80") was found to be suitable for *E. coli*, *Staph. aureus*, and *M. caseolyticus*. In protecting *E. coli* 20 parts of sodium naphuride to 1 part of quaternary was also satisfactory.

Klarmann and Wright (1946a) concluded, as a result of their experiments with glass strips and filter papers, that the bacteria in a medication mixture enter into a combination with the quaternary ammonium compound which precipitates them from suspension and causes them to become attached to the carriers, such as glass or paper. The organisms thus become practically unavailable for transfer in the standard loop. However, Quisno *et al.* (1947b) found that approximately the same dilution of a quaternary ammonium salt was lethal to *S. aureus* at 37°C. (98.6°F.) regardless of whether the medication tube was composed of glass, paraffin, lead, aluminum or certain plastics.

Klarmann and Wright (1948) have described a "semi-micro" method for testing quaternary ammonium disinfectants. According to this technic, 0.05 ml. portions of a 24-hour F. D. A. broth of the test organism are placed in the bottoms of sterile 25 x 150 mm. test tubes, using care that the pipette does not touch the tube walls. The tubes are placed in a water bath maintained at 20°C. (68°F.). A 0.5 ml. portion of diluted disinfectant at 20°C. (68°F.) is added to each tube and the contents thoroughly mixed. Exactly 10 minutes after adding the disinfectant, 20 ml. of Bacto-Oxgall broth is transferred into each tube, using aseptic technic. The tubes are incubated at 37°C. (98.6°F.) for 48 hours. This procedure eliminates the necessity of transferring a representative portion of the medication mixture to a subculture tube and makes use

of Bacto-Oxgall as a neutralizer of the bacteriostatic action of the quaternary ammonium compound.

Klarmann and Wright (1946a), who pointed out the shortcomings of the phenol coefficient test as a means of evaluating the quaternary ammonium compounds, suggested the use of the F. D. A. filter paper test as one for producing more accurate measurements. The filter paper test is described by Ruehle and Brewer (1931). It is normally used for testing substances not soluble or completely miscible with water or substances such as soaps or tooth pastes. Klarmann and Wright (1946a) obtained considerably lower phenol coefficients for the quaternary ammonium compounds when tested by the filter-paper method.

The agar cup-plate test, described by Ruehle and Brewer (1931), is customarily used for the evaluation of the antiseptic and germicidal properties of such substances as salves, ointments, and creams. It has been employed by a number of investigators for the study of quaternary ammonium salt preparations. However, Tobie and Ayres (1944) found a lack of correlation between the results obtained with two quaternary ammonium compounds when using the phenol coefficient test and the agar cup-plate test. It was their opinion that the discrepancy was due to the unequal diffusion rates of the compounds. Hoogerheide (1945) discovered that the zones of inhibition on agar plates did not correlate with the results secured in broth. Quisno *et al.* (1946), while investigating the death rates of bacteria caused by quaternary ammonium salts, observed that results obtained with liquid media and those obtained on agar plates did not agree. They therefore undertook tests to determine the reason for this. The addition of 0.2% of dissolved agar, and likewise of the same amount of undissolved agar, to dilutions of four popular market preparations of quaternaries before the addition of *S. aureus* resulted in much lower germicidal dilutions than in the controls which contained no agar. Quisno and his associates suggested that the physical adsorption of the quaternary ammonium salts by the agar was responsible for the reduction in their potency and concluded that the agar cup-plate test was inappropriate for the evaluation of the germicidal activities of preparations containing these compounds.

Baker *et al.* (1941a) have evaluated germicidal cationic and anionic detergents on their ability to inhibit the metabolism (respiration and glycolysis) of various microorganisms.

Weber and Levine (1944) devised a method for determining the time required for a germicide to destroy 99% of the spores of *Bacillus metiens*. The organism is grown on an agar slant for 20 days at 30°C. (86°F.). The growth is then washed off into Butterfield Formula C water. The suspension thus obtained is filtered through Whatman No.

2 filter paper to remove any clumps of bacteria and then heated at 80°C. (176°F.) for 10 minutes in order to destroy the vegetative cells.

The oval tube method described by Barker *et al.* (1947) has been used satisfactorily to determine the bactericidal effectiveness of hypochlorites and a number of quaternary ammonium compounds for such test organisms as *S. aureus* (Gov. 209) and *Escherichia coli*. The time required to effect destruction of 99.9% of the bacteria is determined.

A glass slide method for assessing the germicidal effectiveness of compounds has been developed and described by Johns (1947). The advantages claimed for this method are the avoidance of the "skips" and "misses" of the phenol coefficient method, a closer approximation of the conditions under which sanitizing agents are used, and flexibility. The technique consists of the preparation of the slide, the treatment of the slide with the dilution of the germicide, and the plating of the slide after rinsing to remove the germicide. A 20- to 24-hour growth of the test organism on agar is washed off into sterile distilled water and filtered through a No. 2 Whatman filter paper. The turbidity of the suspension is adjusted to match that of a suspension of *S. aureus* showing a plate count of 200,000,000 per ml. One milliliter of the suspension is mixed with 60 ml. of a 1/10 dilution of sterile skim milk in a container of such size that the liquid is 1½-inches deep. The lower half of a sterile glass slide is infected by immersing it in the liquid. The slide is drained for 10 seconds on the rim of the container and then on sterile filter paper. Enough slides are prepared in this manner for the test, which is carried out before the films have dried over more than one fourth of the area. The slides are dipped for the designated lengths of time, usually 1 to 20 seconds, in 100 ml. of the germicidal solution at the temperature of the test, removed, agitated in tap water for 5 seconds, rinsed, shaken sharply, placed in a sterile petri dish and covered with the desired type of agar. Control slides are used. The end point is reached when 99.9% of the bacteria have been destroyed.

Mallmann (1945), Mallmann *et al.* (1946b) and Mallmann and Leavitt (1948) have described a use-dilution technique, which is essentially as follows: Glass cylinders are infected with a suspension of *S. aureus* and dried on filter paper for at least 30 minutes. The cylinders are then placed in medication tubes containing various dilutions of the quaternary ammonium compound and the bacteria thereon are exposed to the sanitizer for periods of 1, 5, 10 and 30 minutes at 20°C. (68°F.). After suitable exposure, the cylinder is dropped into a 10-ml. portion of standard broth (Difco disinfectant). The tube containing the cylinder is agitated and then 1 ml. and 0.1 ml. portions are plated in tryptose

agar. The tubes and plates are incubated and then observed for the presence or absence of growth and for bacterial counts, respectively.

Mallmann *et al.* (1946b) have described a speed-reaction test. One milliliter portions of the saline washings from slant cultures of each of three test organisms (*S. aureus, E. coli* and *Micrococcus caseolyticus*) are added to different concentrations of the disinfectant. At 5-, 10- and 15-second intervals, 1-ml. portions are removed from each medication tube and placed in 9-ml. portions of cold sterile saline containing soap (or other neutralizing agent). The number of surviving bacteria are ascertained by plating in tryptose glucose agar suitable dilutions of the neutralized saline suspensions. Mallmann *et al.* found that this test showed the applicability of cationics for sanitizing beverage glasses, whereas the phenol coefficient, use-dilution technique and wet filter paper technique did not.

Mueller *et al.* (1947) have described a method for testing quaternary ammonium compounds which they believe produces a truer picture of germicidal efficacy (under actual working conditions) than the phenol coefficient test. The procedure is as follows: The vegetative cells or the spores of the test organism are washed into phosphate buffer solution. The suspension is then put through a hand homogenizer. The turbidity of this suspension is determined with a spectrophotometer. It is then standardized to the desired number of microorganisms per ml. (by reference to standard curves). One milliliter of the suspension is added to 99 ml. of the desired dilution of the germicide, previously placed in a flask and brought to the temperature of the test and agitated with a mechanical stirrer. At specified time intervals, 1-ml. portions of the medication mixture are withdrawn from the flask and transferred to a solution containing an inactivator. The numbers of bacteria are then determined by standard plating procedures. Results are reported in terms of the percentage destroyed or surviving. Mueller and his associates found sodium naphuride a satisfactory inactivator although there were indications that it was not effective for all organisms. A concentration of 400 p.p.m. of sodium naphuride was found to be necessary to inactivate 200 p.p.m. of the quaternary ammonium salt for 48 hours.

A laboratory procedure for evaluating the practical performance of quaternary ammonium compounds and other germicides proposed for sanitizing food utensils has been described by Weber and Black (1948a, d) of the U. S. Public Health Service. The basis upon which the germicides are compared is the amount of time required to destroy 100% of the cells of the test organism [usually *Escherichia coli* or *Staphylococcus aureus* (S-209)] in a suspension containing about 100

million cells per ml. Very briefly the test is as follows: A 5 ml. portion of the germicide at 25°C. (77°F.) is released rapidly into 5 ml. of bacterial suspension (at 25°C.) in a medication tube. The contents of the tube are mixed by a swirling motion. At the end of 15, 30, 60, 120 and 300 seconds, 1-ml. portions are removed with 1.1-ml. pipettes and placed in tubes, each containing 9 ml. of sterile inhibitor solution [lecithin (asolectin), Tween 80, and phosphate buffer]. The latter mixture is agitated by swirling. One milliliter and 0.1 ml. portions of the mixture are plated out using tryptone glucose extract agar which contains added lecithin (asolectin) and Tween 80 and which is adjusted to pH 7.0. The plates are incubated at 35 to 37°C. (94.8 to 98.6°F.) for 24 hours when *E. coli* is used and for 48 hours or longer when *Staphylococcus aureus* is employed. The plates are observed and the colonies counted. (The reader is referred to the original papers for further details of the method.)

The method of Nungester and Kempf (1942) is concerned with evaluating infection prevention. An area of the tail of a mouse is infected with the test organism, which is capable of producing an infection unless destroyed or inhibited. The area is then treated with the disinfectant, and placed in the peritoneal cavity of the mouse. If the germicide destroys the test organism, there is no infection. The *in vivo* method of Sarber (1942) is similar in many respects to this procedure. An area of the skin of a white mouse is slightly abraded and then infected with an organism, such as an hemolytic *Streptococcus*, which will cause a fatal peritonitis unless destroyed. The germicide is applied to the skin area for 3 minutes. The skin thus treated is then placed in the peritoneal cavity. No harmful results are obtained if the germicide destroys the test organism.

Another *in vivo* method for evaluating the germicidal efficacy of quaternary ammonium compounds has been reported by Kenner *et al.* (1946). In this method, white mice are injected intraperitoneally with cultures of *Salmonella typhimurium* which have been exposed for 5 or 10 minutes to various dilutions of the germicide. Each mouse receives an intraperitoneal injection of 0.25 ml. of a mixture of bacteria and germicide and 0.25 ml. of sterile 3% mucin. After 5 hours, each mouse is anesthetized and specimens of heart blood and peritoneal fluid are removed aseptically. These are placed in the broth medium described in *Circ.* 198 of the U. S. Department of Agriculture. After incubating for 1 day the tubes are examined. Bacto S S agar plates and bismuth sulfite agar plates are streaked with material from each tube showing growth. The identity of the organisms as species of the genus *Salmonella* is established by means of Kligler's iron agar slants. The

recovery of an organism indicates that the germicide in the dilution employed has failed to destroy all of the cells. Kenner and his associates found that results obtained by this method correlated well with those obtained by the F. D. A. test.

In the chick embryo method of Green and Birkeland (1942), the chorioallantoic membrane is inoculated with 0.02 ml. of a 1/10 dilution of a 23- to 25-hour old culture of *S. aureus* (Gov. 209). After 18 hours and once daily during the next 5 days, 0.2-ml. amounts of the germicide are dropped gently onto the membrane. On the sixth day, the surface of the membrane is stroked with a sterile moist cotton swab and the material plated.

Salle *et al.* (1937, 1938) developed a method for determining the relative toxicity of a germicide towards living embryonic tissue and bacteria. A toxicity index, based on the results of the test, represents the ratio of the highest dilution of the germicide which prevents growth of the embryonic tissue to the highest dilution of the germicide required to destroy the test organism in 10 minutes. The lower the toxicity index, the more desirable the germicide. An index of less than 1 is indicated.

A method for comparing the relative toxicity of a germicide or antiseptic towards tissue and microorganisms has been developed by Welch and Hunter (1940). It is based upon the complete inhibition of the phagocytosis of artificially opsonized staphylococci. The tissue used is either guinea pig or human blood. The toxicity index is obtained by dividing the highest dilution of the antiseptic which is toxic for the tissues by the highest of the antiseptic which is capable of destroying the test organism. Toxicity indices obtained by Welch and Hunter for a group of antiseptics were comparable to those obtained by Salle and his associates when using the chick embryo technique. Welch and Brewer (1942) described some of the results secured with quaternary ammonium salts, which demonstrated low indices.

Hirsch and Novak (1942) also devised a method for determining the relative toxicity of the antiseptic towards phagocytes and microorganisms.

A method in which the time required for the concentration of an antiseptic nonirritating to the eyes of an albino rabbit to destroy *S. aureus* was employed by Whitehill (1945) for evaluating liquid antiseptics. Each dilution of the antiseptic, made in *M*/15 phosphate buffer at a pH of 7.4, was tested in the conjunctivae of a 2-kg. albino rabbit and checked on the eyes of a 350-g. guinea pig. Irritation of the eye was indicated by photophobia, discharge, inflammation, edema or cloudy cornea. Of a group of antiseptics sold for the purpose of disinfecting abraded skin, mucous membranes and eyes, the quaternary ammonium

compounds, with the exception of one product of technical grade, were superior to the organic mercury compounds tested.

A method for estimating the toxicity of a compound by measuring the gain made by growing white rats has been described by Harshbarger (1942). The agent being evaluated is added to the diet in measured amount. The gain made by the rats eating the disinfectant is compared with that made by the control rats.

In evaluating germicides of the quaternary ammonium type, it is often necessary to neutralize or inactivate the bactericidal action. The inactivating agent must not possess germicidal or antiseptic properties and it must be able to stop all germicidal and antiseptic action of the quaternary ammonium compound and maintain this condition for a suitable length of time. Already a number of reports have been published concerning inactivating agents. Reference has been made earlier to the media prepared by Quisno *et al.* (1946) and by Armbruster and Ridenour (1947), which contained neutralizing agents. Weber and Black (1947) reported that Duponol WA, lecithin and Tween 80, sodium naphthuride, Tergitol WA 7, and Triton X200 were effective as inhibitors of alkyldimethylbenzylammonium chlorides. They also found that Duponol WA effectively inactivated octadecenyldimethylethylammonium bromide, alkenyldimethylethylammonium bromide, alkylarylpyridinium chloride and cetylpyridinium chloride. Lawrence (1947) found that the sodium salt of symmetric bis (*meta*-amino-benzoyl-*meta*-amino-*para* methylbenzoyl-l-naphthyl-amino-4,6, 8-trisulfonic acid) carbamide more closely satisfied the requirements of the ideal neutralizing agent than any of a series of compounds which precipitated quaternary ammonium solutions.

Mueller *et al.* (1947) found that sodium naphthuride was the most satisfactory inactivator of those examined. It was not germicidal; it was readily soluble in water; it was not affected adversely by the usual sterilizing processes; and it was effective when stored for 3 weeks or longer at 40°F. The amount required to inactivate 200 p.p.m. of a quaternary ammonium salt for a period of 48 hours was 400 p.p.m. Mueller and his co-workers reported that activated charcoal, bentonite, Duponol PC, tincture of green soap, turkey red oil, and castile soap (U. S. P.) were inadequate as inactivators for the quaternary ammonium compounds.

Eckfeldt and James (1947) described the use of sodium oleate as a neutralizing agent. They concluded that sodium oleate neutralized most of the germicidal action of the quaternary ammonium compounds immediately, did not retard the multiplication of the undestroyed bacteria in the culture medium, aided in the removal of the undestroyed bacteria

adherent to the glass walls of the medication tube, and did not interfere with the observation of results.

V. Methods for Estimating Quaternary Ammonium Compounds

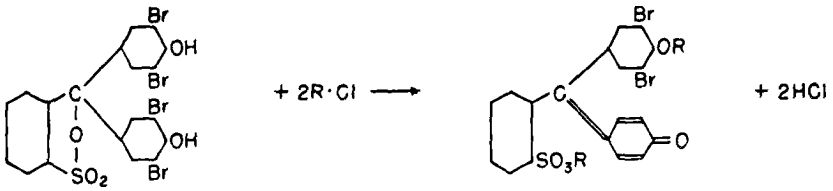
Means for estimating the concentration of quaternary ammonium compounds and for detecting their presence are essential for health authorities, research workers and users. DuBois (1945; 1946) has reviewed the methods used for estimating the quaternary ammonium compounds; these usually involve procedures for titrating the cation. However, a few methods are concerned with the estimation of the anion when this is a halide.

The method of Hartley and Runnicles (1938) depends on the change of color of bromphenol blue from purple to pure blue in the presence of cations of the high molecular quaternary ammonium compounds in a slightly alkaline medium. Hartley (1934) showed that long-chain salts in high dilution may exert a large effect on the color of acidimetric indicators. The effect obtained is correlated with the charges on the indicator ions and micelles. An indicator neutral in form is ordinarily displaced to the acid side by negative micelles and to the alkaline side by positive micelles. DuBois (1945) found most satisfactory results were obtained by the Hartley-Runnicles method when a 1:2,500 solution of a pure anion active agent, such as the alcohol sulfates (Aurinol, Duponol, etc.) or sulfonates (Aerosol OT, etc.), was added gradually to 2 ml. of a 1:2,500 solution of the quaternary ammonium compound containing 0.1 ml. of a 0.01% solution of bromphenol blue made slightly alkaline by the addition of ammonia until the color of the indicator changed from true blue to purple when observed under artificial light. The method is said to be accurate within 1% for concentrations of 1:1,000.

Krog and Marshall (1940) used the following test to determine the presence and/or concentration of alkyldimethylbenzylammonium chlorides in rinse solutions. According to the authors, ammonia, soap, sodium phosphates, ethanolamines and temperature do not interfere with this test. One milliliter of the solution to be tested is placed in a standard colorimetric tube. To this are added 5 ml. of $N/1$ sodium hydroxide solution and 5 ml. of ethylene dichloride. The tube is closed with a rubber stopper and the contents are agitated. For qualitative determination 1 ml. of 0.04% bromthymol blue (Clark's indicator) is added and the tube shaken carefully. Ethylene dichloride, which forms the lower layer, becomes colored blue when the quaternary ammonium compound is present, but is colorless when none is in the sample. For a quantitative determination, the bromthymol solution is added drop by

drop, shaking the contents of the tube after each addition. The blue color is removed from the aqueous layer by the ethylene dichloride as long as there is any of the quaternary ammonium compound present. The end point is reached when the aqueous layer retains the blue color. The concentration of the compound is calculated from the relation that 1 ml. of 0.04% bromthymol blue is equivalent to 0.47 mg. of alkyldimethylbenzylammonium chlorides.

A method for estimating the concentration of dilute solutions of quaternary ammonium cations of the general formula $(R^1R^2R^3R^4N)^+$, in which R^1, R^2 and R^3 are methyl or longer chained alkyl groups and R^4 is CH_2, C_4H_9, or a longer chained aryl-alkyl group, has been developed by Auerbach (1943). In this method bromthymol blue (dibromothymol sulfonphthalein) and bromphenol blue (tetrabromophenolsulfonphthalein) form colored salts with the quaternary ammonium compounds, which may be extracted from aqueous alkaline solutions by solvents, especially the chlorinated ones. In practice, the salt formed in a carbonate solution by union of the quaternary with the dye is extracted with ethylene dichloride and the intensity of the color is estimated by means of a photoelectric colorimeter. The reaction is as follows, where R represents the cationic portion of the quaternary ammonium compound:



For reaction with 357.5 mg. of alkyldimethylbenzylammonium chlorides, it requires 335 mg. of bromthymol blue.

In later modifications, Auerbach (1944) used benzene as the solvent instead of ethylene dichloride, since drying the latter affected the intensity of the color. The size of the sample was reduced to 10 micrograms of the salt and the color intensity was determined photocolorimetrically using a special light filter. The error of this method is reported to be ± 2%, occasionally ± 5%.

A method developed by Brooks and Hucker [see DuBois (1945)] which lends itself readily to field use, is essentially as follows. Into a suitable container are placed 1 ml. of the quaternary ammonium compound and 1 ml. of ethylene dichloride. The quaternary is then titrated drop by drop with a 0.04% solution of bromphenol blue buffered to a

pH of 4.5 to 4.8 [0.4 g. of bromphenol blue dissolved in a solution containing 532.5 ml. of citric acid solution (19.2 g. per liter) and 467.5 ml. of a disodium phosphate solution (71.6 g. per liter)], and shaken after each addition until the end point is reached where the color of the organic layer changes from blue to a yellowish green.

Botwright's method (1946), a modification of the Brooks-Hucker method, is suitable for estimating concentrations of the quaternary ammonium compounds as small as 2.5 p.p.m. One milliliter of the indicator solution (16.5 g. of disodium phosphate, 9.8 g. of citric acid, and 0.04 g. of bromphenol blue made up to 1 liter with distilled water) mixed with 1 ml. of ethylene dichloride is titrated with the quaternary ammonium compound. The mixture is shaken after each addition and the end point is evidenced when the greenish blue color of the ethylene dichloride layer changes to pure blue. DuBois (1946) found that the average deviation in this method was never larger than 0.02 ml.

Flanagan *et al.* (1947) have described a method for determining the amount of quaternary ammonium compound in a sanitizing solution. A sample is placed in a test tube, and the sodium salt of a condensed aryl sulfonic acid (reagent) is then added. A precipitate forms at the critical concentration of the quaternary ammonium salt. The visibility of the precipitate is increased by adding a dye. Estimation of many quaternaries in concentrations higher than one part in 5,000 parts may be made by this method.

A test in which sodium naphthalene sulfonate is added drop by drop to the sanitizing solution of a quaternary ammonium compound has also been described by Flanagan *et al.* (1947).

Harper *et al.* (1948) have devised a method for determining low concentrations of quaternary ammonium compounds, which they state is simple, rapid, sensitive and accurate. Their method is as follows: One milliliter of the solution containing the quaternary ammonium compound is placed in a test tube and 0.1 ml. of a buffer solution (5% solution of analytical grade citric acid adjusted to a pH of 3.5 with $N/10$ sodium hydroxide, and containing 1% of tetrachlorethane as a mold growth preventative) is added. One milliliter of indicator solution is added next; this results in the formation of two distinct layers. The tube is shaken until the lower layer becomes pink. The upper layer should be colorless. The contents of the test tube are then titrated slowly with an anionic solution, shaking after each addition until the color of the lower layer is lost. The amount of the anionic solution used is an indication of the quantity of the quaternary ammonium compound present. Standards may be prepared from solutions of known value and a standard reference curve constructed from data obtained from the

determinations. The indicator solution mentioned above is prepared by dissolving eosin yellowish dye (about 90% concentration) in acetone of analytical grade in the proportion of 0.1 mg. of dye to 1 ml. of acetone, then by adding the acetone-eosin solution to tetrachlorethane in the proportion of 1 ml. of acetone-eosin solution to 9 ml. of tetrachlorethane, and by removing the reddish color from the solution by adding analytical grade citric acid crystals at the rate of 5 mg. of crystals to each milliliter of indicator solution. The solution is filtered through a Whatman No. 1 (or equivalent) grade of filter paper. The anionic solution is prepared in 0.01% concentration by making a 1:1,000 dilution of 10% dioctyl sodium sulfosuccinate (Fisher Laboratory Aerosol).

An argentimetric titration method has been described by DuBois (1945), which is an application of Fajan's method for estimating organic halides, using absorption indicators such as eosin and dichlorofluorescein to determine the end point. The sample of quaternary ammonium salt should contain about 0.4 g. of the anhydrous substance. It is diluted in 25 to 50% aqueous isopropyl alcohol in order that the end point (which is due to the adsorption of the indicator ions on the precipitate), may be more readily discerned. The solution is titrated with 0.0985*N* silver nitrate. DuBois found that eosin and dichlorofluorescein were good indicators; potassium chromate gave molecular weights which were smaller than the true ones.

DuBois (1945) has also devised an iodine-iodide titration method for estimating high molecular quaternary ammonium compounds. As described by him, the procedure is essentially as follows. About 0.05 g. of the quaternary ammonium compound is dissolved in 500 ml. of water. Five milliliters of 20% potassium iodide and 10 ml. of 1% starch solution are added to this. Titration is accomplished by adding *N*/10 iodine solution, drop by drop, until the color of the solution changes from yellowish brown to green and finally to blue. The end points for different compounds vary somewhat. The average error may be as low as plus or minus 1½% or as high as plus and minus 10%.

VI. Applications

1. Basic Principles

Like other chemical germicides and disinfectants the quaternary ammonium compounds are reduced in efficiency in the presence of organic matter. It is therefore essential to clean thoroughly the surfaces to be sanitized with hot or cold water and suitable detergents before the application of the quaternary ammonium materials. In this connection, it is important to bear in mind the incompatibilities of these compounds

with soap, certain anionic compounds and with some detergents. Therefore, after the surface has been treated with chemical cleaners, it is well to rinse it with potable water.

The concentration of the quaternary ammonium compound used for sanitizing will depend on a number of factors, among which are the following: the nature of the surface being treated, the amount and kind of organic matter present, the amount of moisture, the temperature, and the time available for treatment. In general, it is well to follow the manufacturer's directions regarding the concentration to employ. However, as a result of experience, the use of a greater or lesser concentration may be indicated.

The quaternary ammonium compounds may be applied as aqueous solutions to the cleaned surface by spraying, mopping, wiping, or immersing the object to be treated. Spraying is an effective method for treating walls, floors, tanks or other large areas. Mopping or wiping procedures may be used for treating the exterior surfaces of small objects. Rinsing may be used for cans, jugs or other containers. In the case of pipes, pumps, or other equipment where the surfaces are difficult to reach, the sanitizing solution may be pumped or allowed to flow through by gravity. Glasses and many other small objects may be sanitized simply by immersing them in the germicidal solution.

Sanitizing solutions used repeatedly should be checked for concentration frequently. Kits for this purpose may generally be purchased from the suppliers of the quaternary ammonium compounds, or the user may prepare his own reagents. Test papers are now being developed for quick determinations. (Refer to the section on methods for estimating the quaternary ammonium compounds.)

2. General Applications

Quaternary ammonium compounds have practical applications in the baking, brewing, dairy, frozen-food, drying and other food industries; in food storage plants; in butcher shops and fish markets; in eating and drinking establishments; in the medical field; in barber and manicure shops and beauty parlors; and in other fields. Solutions of the quaternary ammonium salts may be used to sanitize or disinfect the floors, walls, ceilings, moving belts, conveyers, trimming tables and other equipment in the canning, quick-freezing and dehydration industries; bread slicers, dishes, glasses, silverware, meat choppers, ice cream scoops, coffee urns, show cases, counters, steam tables, and refrigerators in eating and drinking establishments; cans, pails, milking machines, churns, cows' udders, cooling tanks and garbage cans on the farm; foot baths, locker rooms and showers in the gymnasium; and surgical instruments,

TABLE VIII

Some Applications of the Quaternary Ammonium Compounds

Field	Applications	References
Baking industry	General	Lehn and Vignolo (1946b)
Brewing industry	General	Lehn and Vignolo (1946a)
Dairy industry	Dairy industry	Scalcs and Kemp (1941) Levowitz (1944) Lawrence (1946a, b) Vignolo (1946) Mueller *et al.* (1947) Portley (1948)
	Dairy pasteurization plant	Krog and Marshall (1942)
	Dairy plant disinfectants	Jacobsen (1946)
	Dairy utensils	Frayer (1943, 1944)
	Ice cream sanitation	Krog (1942)
	Milking machines	Mallmann *et al.* (1946), Mueller *et al.* (1947)
	Milk shipping can sanitation	Jamieson and Chen (1944a, b)
Eating utensils	Beverage glasses	Walter (1942) Walter and Hucker (1942)
	Eating utensils	MacPherson (1944)
	Bactericidal detergent	Guiteras and Shapiro (1946)
	Rinse in dishwashing	Mallmann and Zaikowski (1947)
	Sanitation in food establishments	Macnab (1947)
	Sanitizing dishes	Mallmann *et al.* (1946)
	Sterilization of dishes and utensils	Weber (1948)
Dried and frozen egg industries	Washing dirty eggs	Penniston and Hedrick (1944, 1945, 1947) The Emulsol Corp. (1947)
	Sanitation	McFarlane *et al.* (1945)

Fisheries	General	Dunn (1947)
Frozen foods industry	Sanitation in frozen food industry	Obold and Hutchings (1947)
General food field	General	Lawrence (1946b) Varley (1947)
	Sterilization agents for food ind.	Scales and Kemp (1940)
Refrigerated storage	Cold storage plants	Dunn (1946)
	Food storage rooms	Mallmann and Churchill (1946)
Medicine	Bactericide in operating room	Walter (1938)
	Bronchial lavage in non-tuberculous	Stitt (1943)
	Clinical uses	White *et al.* (1938)
	Cutaneous germicide in major surgery	Hagan *et al.* (1946)
	Disinfection of clean, unwashed skin	Gardner and Seddon (1946)
	Hand disinfection	Wetzel (1935) Jötten and Schön (1936)
	Injury	Wright and Wilkinson (1939)
	Ocular application	Thompson *et al.* (1937)
	Prevention of impetigo neonatorum	Fisher (1944)
	Preservation of biological materials	Maier (1939)
	Skin antiseptic	Brown *et al.* (1944)
	Skin disinfection	Helmsworth and Hoxworth (1945)
	Skin sterilization	Clarke (1942)
	Sterilization of surgical instruments	Spalding (1939)
	Surgery	Williams *et al.* (1943)
	Surgical procedure for thyroidectomy	Cutler and Zollinger (1938)
	Treatment of skin infections	Forman (1943)
	Treatment of trichomonas infections	Rodecurt (1935)

TABLE VIII (cont.)

Field	Applications	References
Veterinary medicine	Treatment of chronic mastitis in cows	Scales and Kemp (1941) Bryan *et al.* (1944)
	Treatment of ringworm in calves	Bryan and Young (1945)
Miscellaneous	Disinfectant	Hauser and Cutter (1944) Caesar (1935) Domagk (1935, 1938) Hornung (1935, 1936) Kliewe and Maier (1936) Lübben (1935) Maier and Müller (1936) Schmidt (1935) Schneider (1935)
	Destruction of water-borne cysts of *Entamoeba histolyticus*	Fair *et al.* (1945)
	Fungicide	Dunn (1937) Howard and Keil (1943) Lawrence (1947)
	Preservation of gelatin and sucrose solutions	Tice and Moore (1946)
	Isolation of coli phage	Kalter *et al.* (1946)
	Mildewproofing of cellulosic fibers	Borghetty (1945)
	Mordant in cell wall stain	Dyar (1947)
	Control of gill disease in salmonid fishes	Rucker and Ordal (1947)
	Washing cucumbers	Bernstein and Epstein (1948)

wounds, skin, wash basins, bed pans and toilets in hospitals. There are many other miscellaneous uses, such as the disinfection of animal cages, vegetable counters and goggles and their use as a fungicide in agriculture. Table VIII presents information concerning applications which have been studied in some detail.

3. Brewing Industry

Uses of quaternary ammonium compounds, particularly the 10% solution of 9-octadecenyldimethylethylammonium bromide (Thoral), in the brewing industry have been described by Lehn and Vignolo (1946). One ounce of the compound is mixed with 4 gallons of water and applied hot or cold by spraying, immersion, flushing or wiping to the surfaces of the equipment previously cleaned with suitable detergents. Lauter tubs, brewing kettles, hop strainers, fermentation tanks and surface coolers may be treated by spraying. Plate coolers, beer or wort lines, filtering devices, pumps and fittings are best handled by circulating the sanitizing agent through them. Floors and walls may be sprayed or mopped.

4. Dairy Industry

Applications of the quaternary ammonium compounds in the dairy industry have been studied by Scales and Kemp (1940, 1941), Krog and Marshall (1942), Frayer (1943, 1944), Jamieson and Chen (1944a, b), Levowitz (1944), Rahn (1945), Botwright (1946), DuBois and Dibblee (1946b), Hughes and Edwards (1946), Jacobsen (1946), Mallmann *et al.* (1946a), Mueller *et al.* (1946, 1947), Vignolo (1946), Hucker *et al.* (1947), Mull and Fouts (1947) and others.

The effect of the cationic germicides on metals and rubber, such as are found in the dairy industry, have been studied by several investigators. Krog and Marshall (1942) stated that Roccal was no more corrosive to metal and rubber than ordinary water. Mueller *et al.* (1946) reported that six quaternary ammonium compounds examined by him were practically noncorrosive to metals. Hucker *et al.* (1947) concluded that quaternary ammonium compounds, as represented by alkyldimethyl 3,4-dichlorobenzylammonium chloride and diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride, probably have little, if any, corrosive effect on the metals and alloys commonly employed in the manufacture of dairy and food equipment.

Quaternary ammonium compounds have been used successfully to wash the udders of cows in this country, according to reports from Scales and Kemp (1940), Frayer (1944) and Mueller *et al.* (1947). It was stated by Mueller and his associates that a solution containing 200 p.p.m. (1:5,000) of a quaternary ammonium compound was used as an

udder wash for a period of over 4 weeks without the observation of chapping or cracking of udders, teats, or the hands of the milkers. On the other hand, Hughes and Edwards (1946) reported that the application of 1% of cetyltrimethylammonium bromide in a lanette, wax-oil base, twice daily over a period of 3 months in an effort to reduce the transmission of an infection caused by *Streptococcus agalactiae,* resulted in the appearance of lesions of the teats.

Mallmann *et al.* (1946a) carried out extensive studies, covering a period of approximately a year, to determine the effect of the method of sanitizing mil' ' -- -hin-s on the bacterial content of the milk. At the beginning of , the milk producers were divided into five groups for study, each one containing good, medium and poor producers. Each milking machine was supplied with new rubber parts. Cleaners and sanitizers were supplied to all except the control group, which was permitted to use items of their own selection. In Table IX are shown some data on the cleaners and sanitizers used and their application. Over the period of May 17 to October 17, the average total bacteria and thermoduric bacteria counts were considerably lower for milk produced by group 4 than for milk produced by the other four groups. This group used Solvay 600 as the cleaner (in common with the other groups, excepting the control group), stored the tubes and cups of the milking machine in 1:6,400 alkyldimethylbenzylammonium chlorides and rinsed the equipment in the same concentration of sanitizer before use. Additional studies on selected farms confirmed the results obtained by the field groups.

Mueller *et al.* (1947) have reported that the metal parts of milking machines may be sanitized effectively by immersing them for a period of 3 minutes in a solution containing 200 p.p.m. of the quaternary ammonium agent. However, it was not possible to sanitize the rubber inflation tubes of the teat cups effectively by just dipping them into a solution containing 400 p.p.m. of the sanitizer when going from one cow to another.

Quaternary ammonium compounds have a number of applications on the dairy farm, in addition to their uses to sanitize milking machines and wash the udders of cows. Krog and Marshall (1942) found that Roccal exerted definite germicidal action on the bacteria found on properly cleaned milk handling equipment. Jamieson and Chen (1944a, b) advocated the treatment of clean milk and cream cans with a spray containing a quaternary ammonium salt or sodium hypochlorite at least 15 minutes before the use of the containers, followed by their inversion and drainage. Mueller *et al.* (1947) reported that milk pails may be sanitized effectively by immersing them for a period of 3 minutes in a

TABLE IX

Data Concerning Comparative Study of Methods of Sanitizing Milking Machines [a]

Group	Cleaner	Sanitizer	Method of storage of tubes and cups	Rinse before use
1 [b]	Own selection	Own selection	Optional [e]	Optional [e]
2	Solvay 600	Hypochlorite [c]	In hypochlorite solution	In hypochlorite solution
3	Solvay 600	Hypochlorite [c]	In alkali solution [f]	In hypochlorite solution
4	Solvay 600	Quaternary ammonium compound [d]	In 1:6,400 quaternary ammonium solution	In 1:6,400 quaternary ammonium solution
5	Cleaner-sanitizer	.....	None	None

[a] Compiled from information contained in the article by Mallmann *et al.* (1946a).
[b] Control group.
[c] BK powder.
[d] Alkyldimethylbenzylammonium chloride (BTC).
[e] No instructions given and not necessarily required.
[f] Rubberkleen.

1:5,000 dilution (200 p.p.m.) of a quaternary. Jacobsen (1946) stated that quaternary ammonium compounds are used extensively for dairy farm sanitation. Vignolo (1946) has provided directions for the application of quaternaries in dairy plants. Levowitz (1944) has discussed general cleaning and sanitizing procedures for dairy and other food plants.

The use of quaternary ammonium compounds in farm dairies and other milk plants has not been universally accepted by public health agencies. Apparently one of the reasons has been the fear that milk producers of unscrupulous nature might be tempted to reduce the bacterial content of milk by the inclusion of the compound. It is not the intent of the present author to state the pros and cons of this argument. However, reference to certain reports now to be mentioned should help to clarify the situation.

DuBois and Dibblee (1946b) carried out tests to determine the influence of certain quaternary ammonium compounds on the bacterial content of milk. They reported that a 1:500 dilution (2,000 p.p.m.) of alkyldimethylbenzylammonium chloride reduced the initial count of milk immediately after its addition, but that it had no appreciable effect on the bacterial content of the milk after the latter had been incubated for 24 to 48 hours. The compound in 1:500 and in 1:5,000 dilutions checked the growth of a Gram-positive species of *Streptococcus* but not of *E. coli* when these organisms were inoculated into sterile milk. Otherwise, the quaternary lacked influence on the bacterial content of raw and pasteurized milks when used in concentrations of 1:500 to 1:25,000. Therefore DuBois and Dibblee concluded that rinses containing the quaternary ammonium compounds in concentrations of about 1:5,000 would have no effect on the bacterial content of milk due to the large dilution factor provided by the milk. DuBois and Dibblee (1946b) found the Hartley-Runnicles method for estimating quaternary ammonium compounds to be satisfactory and the Brooks-Hucker method to be unsatisfactory in the presence of milk.

Mull and Fouts (1947) studied the effects of Roccal on the bacterial contents of raw and pasteurized milk and on flavor. They concluded that Roccal, when added directly to milk, reduced the bacterial content, particularly of milk of low initial bacterial content, and that 200 to 250 p.p.m. (1:5,000 to 1:4,000 concentrations, respectively) of the sanitizer would have to be added to milk of low quality to accomplish significant decreases in the numbers of bacteria. When the concentration of the quaternary ammonium compound in milk was 10 p.p.m. (1:100,000) it could be detected by taste; at 20 p.p.m., the milk was slightly bitter; and at 40 p.p.m., the milk was very bitter in taste.

It may thus be concluded that the amount of quaternary ammonium compound used for rinsing purposes is insufficient under normal circumstances to reduce the bacterial content of milk and may cause flavor defects.

5. Eating and Drinking Establishments

Among the reports concerned with use of quaternary ammonium compounds for sanitizing dishes and other eating utensils may be mentioned those of Krog and Marshall (1940), Walter (1942), Walter and Hucker (1942), Powney (1943), MacPherson (1944), Botwright (1946), Mallmann *et al.* (1946b), and Mallmann and Zaikowski (1947).

Krog and Marshall (1940) suggested the following method for sanitizing dishes in restaurants and soda fountains where the manual operation is employed. Remove the organic debris with the aid of running water. Wash the dishes by hand in water at 120°F. which contains soap or some other detergent. Rinse the dishes in a solution of alkyldimethylbenzylammonium chlorides for at least 1 minute. Remove the dishes and allow them to dry in the air. They found that a 1:5,000 dilution of the quaternary ammonium salt demonstrated a marked germicidal action against the bacteria associated with eating and drinking utensils. Application of the germicide for a period of 1 minute reduced the bacteria to less than 100 per tumbler rim. In dishwashing machines containing two or three compartments, the detergent or soap used had little or no effect upon the efficacy of the germicide (Powney, 1943). Krog and Marshall (*loc. cit.*) recommended a cold water rinse of dishes followed by their exposure for 1 minute to a 1:5,000 concentration of alkyldimethylbenzylammonium chlorides in those taverns where the dishes were not washed in soap and hot water.

Walter (1942) and Walter and Hucker (1942) reported on the use of alkyldimethylbenzylammonium chlorides for the sanitization of beverage glasses in taverns. They found that a 1:5,000 aqueous solution of the compound generally markedly decreased the numbers of organisms and eliminated the coliform bacteria from the rims of the glasses treated. They concluded that the agent demonstrated promise as a sanitizer in taverns which serve carbonated beverages.

MacPherson (1944), as a result of tests carried out in food-dispensing establishments and hotels, concluded that hand-washed eating and drinking utensils may be effectively sanitized by rinsing them in a 1 : 5,000 aqueous solution of alkyldimethylbenzylammonium chlorides. He also found that ice cream scoops may be sanitized effectively by keeping them in a solution of the same concentration.

Botwright (1946) carried out studies on sanitization of drinking glasses in some restaurants and taverns in and about St. Louis. More than

50,000 organisms were frequently found on drinking glasses before they were exposed to sanitizing agents. Botwright compared Amerse(9-octadecenyldimethylethylammonium bromide), other quaternary ammonium compounds and chlorine as sanitizers for glasses and concluded that the quaternary ammonium compounds were superior as sanitizers; they are more stable and are free of objectionable odors and tastes in the concentrations used. He mentioned that glasses sanitized with the quaternaries should be rinsed in cool, running water before use for beer, since these agents tend to decrease foam stability.

Mallmann *et al.* (1946) reported that 800 glasses could be sanitized effectively by exposure for about 30 seconds in 5 gallons of hypochlorite solution, containing 180 p.p.m. of available chlorine, or in the same quantity of a solution of quaternary ammonium compound (Roccal, BTC, and Hyamine 1622), containing 1 ounce of 10% solution (1:6,400). They found that the presence of neutralizing agents in the cleaners (wetting agents and polyphosphates) had no adverse effect on the sanitizing value of the quaternary ammonium compounds when not more than 800 glasses were sanitized by the two and three tank systems of washing and sanitizing.

Mallmann and Zaikowski (1947) described a new process for sanitizing machine-washed dishes at temperatures below the 170°F. normally employed when sanitizing is accomplished by heat. In this process, the dishes are exposed to 1:6,400 to 1:10,000 concentrations of the quaternary ammonium compounds at a temperature of 120°F. for 10 seconds. This exposure destroys Gram-positive and Gram-negative bacteria in 5 to 10 seconds in the absence of organic matter.

6. Frozen and Dried Egg Industry

Certain germicides may be used effectively to reduce the bacterial contents of egg shells immediately before the eggs are broken for freezing or for drying, according to Penniston and Hedrick (1944, 1945, 1947). This is a particularly significant fact, since dirty eggs are used extensively in the frozen and dried egg industries on account of their lower cost. Although washing eggs with water removes much of the soil and many of the bacteria, Penniston and Hedrick (1944) found that from 3 to 6 million bacteria still remained on the shell surface. Although most eggs are sterile at the time of laying, they become contaminated and the number of bacteria in the egg pulp may average 1,500,000 per egg. As a result of washing the eggs with a 0.04% solution of Emulsept, or with water containing 100 p.p.m. of chlorine, the bacterial content was reduced to 3,000 or less per egg. The solution containing the Emulsept [N(higher acyl esters of colamino formyl-

methyl) pyridinium chloride] could be used for washing six to ten times as many eggs as the chlorine solution while effecting a similar reduction in the numbers of bacteria present on the shell surfaces and in the wash water. Since susceptibility to bacterial infection is increased by washing eggs before storage, they should be thus treated only when they are to be broken immediately for processing.

7. Frozen Food Industry

The use of sanitizing techniques in the frozen food industry has been described by Obold and Hutchings (1947). They reported that inspection belts, metal conveyors, blanchers, cleaning reels and rotary washers should be cleaned with detergents, rinsed to remove the cleaners, and then treated with 200 p.p.m. of chlorine solution or with a 1:500 solution of a quaternary ammonium compound. Containers, crates, hampers and lug boxes should be washed thoroughly with a 2% solution of trisodium phosphate, or steamed, and then treated with a 1:500 solution of a quaternary ammonium compound or with 200 p.p.m. of chlorine solution.

8. Medical Applications

Some of the more important medical applications of the quaternary ammonium compounds have been referred to in Table VIII. Mention of some uses has also been made in other sections of this paper. The following paragraphs give some additional details.

Kramer and Sedwitz (1944) reported on the uses of a 1:200 tincture and a 1:1,000 aqueous solution of Ceepryn (cetylpyridinium chloride) in a hospital at Youngstown, Ohio. The tincture was used routinely for 3 years on operative cases (about 18,000), during which time there were apparently no cases of irritation or infection. A 1:1,000 aqueous solution of Ceepryn was used on all patients requiring wet dressings or germicidal irrigants over a period of 21 months.

Stitt (1943) used Ceepryn, diluted with Bledsoe-Fischer hypertonic saline solution to yield a 1:5,000 concentration, for the bronchial lavage of nontuberculous infections, such as chronic purulent bronchitis. The quaternary ammonium compound was nonirritating in the concentration used and was the most valuable germicide employed.

The favorable use of a baby lotion containing cetyltrimethylammonium bromide for the prevention of impetigo in newborn babies has been described by Fisher (1944). The lotion contained 0.16% of cetyltrimethylammonium bromide, boric acid, lanolin and mineral oil. The babies were cleaned with the lotion on the fifth day following birth and daily thereafter (as well as after each diaper change) until dis-

charged from the hospital. No cases of impetigo developed in the 3,520 infants treated, as compared with 17 cases out of 2,009 babies treated during a previous 2-year period when this particular lotion was not used.

Solutions of cetyltrimethylammonium bromide have been used for various other purposes. Barnes (1942) found that a 1% solution greatly reduced the numbers of bacteria on the skin, and particularly on the hands; it was effective for treating wounds and burns. Forman (1943) used the compound for removing scabs and crusts from impetiginized areas and for cleansing skin areas which had been covered with ointment. One patient with impetigo of the face developed a dermititis of moderate severity after using Cetavlon twice daily for 6 days for the purpose of removing crusts. Two patients complained of dryness of the skin after its use. Williams *et al.* (1943) recommended the use of a 1% solution of CTAB for cleaning and "sterilizing" utensils and instruments and for cleaning wounds and the skin surrounding them. Skin reactions followed in a low percentage of cases when the compound was applied to the hands of sensitive individuals. Continued use resulted in chapped hands in some instances.

9. Veterinary Applications

As a treatment for chronic mastitis, Scales and Kemp (1941) suggested that a mixture of Zephiran, Triton 720 (a dispersing agent) and 33% milk might be infused into the udder at body temperature after the cow had been milked.

Bryan *et al.* (1944) treated with Phemerol 171 cows which had chronic streptococcic mastitis, but which did not demonstrate marked induration of the udder. During treatment each quarter of the udder was infused with 75 ml. of Phemerol (1:1,000 aqueous solution). In one lot of 160 cows, 90 recovered after one treatment; 40, after two treatments; and 17, after three treatments. Out of the 171 cows treated, 86% were freed of the infection. The mucous linings of the teats and milk cisterns of most of the cows underwent a transitory thickening and abnormal milk was produced for varying periods after the treatment. The greatest decrease in milk production occurred in the animals which were in the early period of lactation at the time of treatment. The reduction in the bacterial content of milk from treated and recovered cows varied from 20 to 99%.

Ringworm in calves, of the type caused by *Trichophyton tonsurans*, has been cured by the application of a 1:1,000 aqueous solution of Phemerol, according to Bryan and Young (1945). The solution was

applied to the lesions twice a week for 3 weeks to a month. The ringworm lesions disappeared within 1 month.

10. Miscellaneous Applications

The use of cetyltrimethylammonium bromide for cleaning and sterilizing has been reported by a number of workers. Barnes (1942) found a 1% solution of this compound to be effective for cleaning closet bowls and bath tubs. Forman (1943) used a 2% solution of Cetavlon for removing dirt, grease and microorganisms from wash basins and bath tubs located in wards containing soldiers who were being treated for staphylococcal and streptococcal skin infections. Williams *et al.* (1943) recommended the use of a 1% solution of CTAB for cleaning and "sterilizing" utensils and instruments.

For the isolation of the gamma bacteriophage of *E. coli* from sewage, Kalter *et al.* (1946) recommended the use of a final dilution of 1:5,000 of Emulsol-607, Zephiran or cetylpyridinium chloride.

Fair *et al.* (1945) reported that the water-borne cysts of *Entamoeba histolytica* can be destroyed by a number of quaternary ammonium compounds. They found Fixanol, Sapamine KW, Nopco-QCL, and Ceepryn to be efficient cysticides. For the disinfection of water, they recommended the use of 30 p.p.m. of one of these compounds for a period of 10 minutes, or 10 p.p.m. for a contact period of 2 hours. Some other quaternary ammonium compounds and neutral detergents were not so effective as these. Three anionic detergents showed little promise. The presence of suspended organic matter and the pH had little effect upon cysticidal activity.

Sotier and Ward (1947) reported that Hyamine 1622 (diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride) was very effective as a germicide when used with a modified soda. Polymine D, a mixture of 5% of the quaternary and 95% of modified soda (Na_2CO_3: $NaHCO_3$, 1 : 1.39), was shown to be a good cleaner for jute-packed water mains, for which purpose chlorine-liberating compounds did not yield successful results.

According to Krueger *et al.* (1942), the influenza virus (types A and B) contained in throat washings, egg fluids or ground mouse lung menstrua may be freed from bacterial contaminants by treating with a 1:20,000 dilution of Zephiran for 20 minutes at 20°C. (68°F.).

A 1:2,000 solution of alkyldimethylbenzylammonium chlorides inactivates tetanus toxin, but a 1:20,000 solution will not (Neter, 1942a).

A 1:10,000 solution of alkyldimethylbenzylammonium chlorides inhibits the clotting of oxalated human blood plasma by staphylococcus cultures and dilutions up to 1:1,000,000 delay such clotting (Neter,

1942b). Likewise, dilutions of Zephiran up to 1:100,000 inhibit fibrinolysis by hemolytic streptococci.

Dyar (1947) advocated the use of 0.34% cetylpyridinium chloride as a mordant in a cell wall stain. The cell becomes positively charged by the cationic agent.

A procedure for reducing the microorganisms on cucumbers and thus decreasing the spoilage and improving the quality of pasteurized pickles made from them has been described by Bernstein and Epstein (1948). A germicidal soaking solution is prepared by adding a quaternary ammonium compound (Emulsept) to a modified (by extension) tank of a tomato-washing unit in the proportions of 1 ounce of the quaternary to 5 gallons of water. The cucumbers are submerged in the germicidal soak at 100 to 110°F. for about 3 minutes, after which they are washed with high-pressure water sprays to remove the germicide and any loosely adherent soil. There is a substantial decrease in the numbers of the microorganisms on the cucumbers as a result of this treatment.

VII. Summary

Hundreds of different quaternary ammonium compounds have been prepared and screened to obtain some information concerning their germicidal value. Many of the compounds investigated possess high germicidal activity; some, however, are low or lacking in bactericidal efficacy. Potentially there are many unexplored combinations possible, some of which may be even more powerful or of lower toxicity than the best compounds now known.

Quaternary ammonium compounds differ amongst themselves in respect to structure, germicidal activity, toxicity, solubility, surface activity, and response to temperature, pH and in other ways. Most of the compounds now used commercially as germicides and sanitizers are active in high dilutions against both Gram-positive and Gram-negative microorganisms. Their action is rapid. Against bacterial spores they are of lower efficiency, but most of them destroy a high percentage of the resistant forms rather quickly. The majority of the quaternaries demonstrate highest germicidal activity in alkaline solutions, but at least one is as active in acid as in alkaline solution.

In the dilutions used, the quaternary ammonium compounds are relatively free of color, flavor, odor and toxicity, and for this reason are especially desirable for use in the food industries. The compounds are quite stable at room temperatures and may be stored for several years without appreciable loss in activity. They may be used in hot or cold solutions. They are nonirritating to the skin, noncorrosive and do not injure rubber or plastics in the dilutions employed.

The quaternaries are incompatible with soaps, phospholipides, anionic detergents and certain polyphosphates. Therefore they should not be used with these compounds.

The quaternaries have many fields of usefulness, particularly in the food industries. A number of these have been referred to. Experimental work, some on a large scale, has been carried out to determine the value of these compounds as ingredients of vegetable and fruit washes, tooth powders, etc., with promising results.

The use of quaternary ammonium compounds as sanitizers in the food industries, including eating and drinking establishments, has been accepted by many cities and municipal authorities but not by all. Therefore, where there is any question concerning the use of a compound, the matter should be referred to the local health department.

Standardization of testing procedures would be of benefit to all concerned. At the present time many different methods are being used, or have been proposed for use, for the microbiological evaluation of the compounds. The Food and Drug Administration phenol coefficient test has been employed by many workers, but its use has been subject to criticism. Emphasis is now being placed on use-dilution methods for evaluation. There are likewise a number of procedures available for determining the quaternaries chemically. Recently, the Hartley-Runnicles method (as described by DuBois) was approved by the Chemical Analysis Committee, Disinfectant Section, National Association of Insecticide and Disinfectant Manufacturers, New York (*Soap Sanit. Chemicals* 23 (No. 9), 169, 1947).

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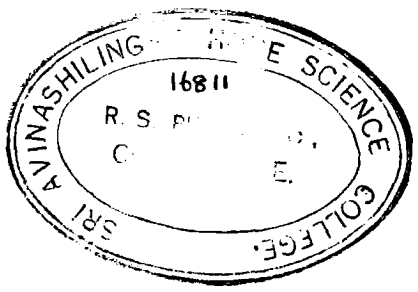

The Pharmacology of DDT

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I. Introduction

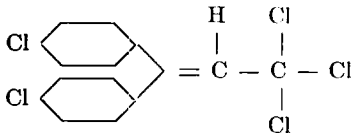
The control of insect pests is an age-old problem. Old records indicate that sulfur was recommended for controlling pests as long ago as 1000 B.C. Although many of the insecticides which are in use today have been known for centuries it is only during the last few decades that chemical means have been used to any extent for the control of insect pests. Probably the greatest impetus to employing insecticides has been the spread of the Colorado potato beetle eastward and the importation of foreign insects that found conditions favorable in the United States and rapidly became obnoxious pests. The currant worm and the Japanese beetle may be cited as examples. The health hazards involved in the introduction of disease-carrying insects from all parts of the globe, "hitch-hiking" to the United States by airplane, have also stimulated the search for more efficient methods of control.

About 1925, Lauger *et al.* (1944) began a series of investigations on some of the older insecticides in an attempt to improve the mothproofing

properties of rotenone and pyrethrin. Among the many compounds studied was *p,p'*-dichlorodiphenyl sulfone. During the course of the work it was found that by substituting a trichloroethane for the sulfone grouping the resulting compound was an excellent contact insecticide. Thus DDT was rediscovered as a chemical entity after a lapse of some seventy years from the time it was first described by Zeidler in 1874.

II. Chemistry

The common or popularized expression DDT is a contraction for dichlorodiphenyltrichloroethane. The name assigned to it in the 1946 Chemical Abstracts is 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane, and is represented by the following formula:



The compound is also known by a variety of trade names, such as Gesarol, Neocid, Neocidols, Gesapon, Gesarex, GNB, and GNB-A-DDT. Theoretically, there are forty-five possible dichlorodiphenyltricholoroethanes. In the usual commercial process for manufacturing DDT the original procedure of Zeidler is followed, that of condensation of 1 mole of chloral with 2 moles of chlorobenzene in the presence of sulfuric acid. At least three isomers are the products of this reaction, the *p,p'*-isomer (DDT) which constitutes about 70%, the *o,p'*-isomer representing approximately 18%, with the *o,o'*-isomer representing about 6%, making an over-all yield of approximately 95%. Only the *p,p'*-isomer is highly insecticidal, but the other isomers contribute to the toxicity in mammals.

Commercial DDT is a gray-colored powder with a mild fruity odor and a setting point of 88°C. (190°F.). By recrystallization from ethyl alcohol pure DDT may be obtained; a white crystalline powder with a melting point of 108.5-109.0°C. (226-228°F.) (corrected). Purified DDT is quite stable and will withstand boiling in water for several hours, or the dry substance can be heated to 115-120°C. (239-248°F.) without decomposition (Cristol and Haller, 1945). No appreciable loss occurs after prolonged exposure to air. Irradiation of the solid for hours with a mercury-vapor lamp has only a slight effect as shown by a small depression in the melting point; alcoholic solutions have been exposed to sunlight for a year without showing changes. A number of materials, notably anhydrous chlorides of iron, chromium, and aluminum, cause catalytic decomposition of pure DDT (Fleck and Haller, 1945). When DDT is

mixed with other insecticides, fertilizers, and fungicides, little or no decomposition is noted. Technical grades of DDT are less stable and this is probably due to the catalytic action of small amounts of iron impurities.

DDT is readily dehydrochlorinated by alcoholic potash yielding 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene through the loss of one mole of HCl. This reaction is made use of in some of the analytical procedures.

The solubility of DDT is of considerable practical importance in view of its use as solutions in sprays and emulsions and the necessity for stripping residues from sprayed materials. DDT is soluble in water at about one part in ten million. Other solvents are listed below and the solubility values represent the quantity in grams of the substance soluble in 100 g. of solvent. The data were obtained from several sources (Cox, 1945; Gunther, 1945a, 1945b). The observations were made at what may be considered as "room temperature" (24-30°C.) (75-86°F.).

TABLE I

The Solubility of DDT

	Grams per 100 g. solvent
1. Cyclohexanone	100–120
2. Benzene	77–106
3. Dioxane	46–100
4. Chloroform	31–96
5. Methylene chloride	84–91
6. *o*-Dichlorobenzene	63–71
7. Tetrahydronaphthalene	52–71
8. Ethyl acetate	68
9. Xylene	56–62
10. Tetrachloroethane	56
11. Acetone	40–55
12. Tetralin	52
13. Pyridine	51
14. Carbon tetrachloride	18–48
15. Toluene	48
16. Ether	27–45
17. Benzyl benzoate	39–41
18. Dimethyl phthalate	31–33
19. Indalone	29
20. Triton	20
21. Cottonseed oil	9–20
22. Tung oil	10–14
23. Kerosene (vaporizing)	11
24. Fuel oil No. 2	10
25. Sesame oil	10
26. Petroleum ether (100–106°C.) (212–222°F.)	10

TABLE I (cont.)

27.	Fuel oil No. 1	8
28.	Cyclohexanol	8
29.	*N*-butyl alcohol	8
30.	Kerosene (crude)	5–8
31.	Isoamyl alcohol	7
32.	Petroleum ether (30–60°C.) (86–140°F.)	4–6
33.	Isopropyl alcohol	5
34.	Methyl alcohol	5
35.	Spray oil	5
36.	Ethyl alcohol absolute	4
37.	Tertiary butyl alcohol	4
38.	Liquid paraffin	4
39.	Kerosene (purified)	2–4
40.	Ethyl alcohol, 95%	2
41.	Freon (12)	2

III. ANALYTICAL PROCEDURES

The widespread use of DDT makes it mandatory that analytical procedures be developed that will meet the various conditions of determining small quantities in spray residues and biological materials. A sensitive colorimetric method for DDT and isomers is described by Schechter *et al.* (1945). An intense blue color develops when the tetranitro derivative, obtained by nitrating the sample with fuming nitric acid in the presence of concentrated sulfuric acid, is dissolved in benzene and treated with sodium methoxide in methyl alcohol. The maximum absorption spectrum is at 600 mμ for the *p,p'*-derivative. The major impurity, the *o,p'*-isomer, develops a violet-red color with maximum absorptions at 590 and 511 mμ, thus offering a means for determining *p,p'*- and *o,p'*-isomers in mixtures of the two. Another method for determining mixtures of the two isomers is based on freezing-point depressions and setting points (Haller *et al.* 1945; Fleck and Preston, 1945).

The determination of DDT as spray residue presents a more difficult problem and the choice of a method depends on what complicating factors are involved. Wichmann *et al.* (1946) have compared three independent methods on sprayed apples. The sample preparation is common to all three and consists of washing the fresh fruit with benzene, dehydrating the washings with anhydrous sodium sulfate and then determining the DDT by one of the following procedures: (a) by the labile and total chlorine determinations (Gunther, 1945c; Stepanow, 1906); (b) by the colorimetric procedure of Schechter already mentioned; and (c) by the dinitrophenyl hydrazine method which involves the conversion of the

o,p'- and *p,p'*-isomers to the corresponding ethylenes, oxidation of the ethylenes to dichlorophenones, and the conversion of the latter to the 2, 4-dinitrophenylhydrazones (Haller *et al.* 1945; Grummitt *et al.* 1945).

The introduction of analogs of DDT into commerce, especially DDD (1,1-dichloro-2,2-bis(chlorophenyl) ethane) has made it necessary to distinguish between closely related compounds. The method described by Stiff and Castillo (1945) is specific for DDT even in the presence of large amounts of DDD. A red color is produced with a xanthydrol-pyridine-potassium hydroxide reagent in the presence of DDT, whereas DDD does not react with the reagent. The reaction has been adapted for qualitative and quantitative determinations of DDT in apple spray residues by Claborn (1946).

The determination of DDT in body fat of experimental animals and milk requires certain modifications of the Schechter procedure before satisfactory recoveries can be obtained. Samples containing large amounts of fats must be treated with pancreatic lipase and after removal of the hydrolytic products, nitration of the isolated DDT can be carried out in the usual manner (Clifford, 1947).

A biological method has been developed which makes use of the toxic response of the house fly to DDT. The method has been applied to determine DDT in animal tissue and excreta (Laug, 1946a).

IV. Stability of DDT

It is common knowledge that repeated spraying of DDT is necessary to control some insect pests. This suggests that the insecticide loses its efficiency, and various reasons have been advanced for this inactivation. Volatilization, the effect of weathering, occlusion or attenuation on developing fruit are a few that may be mentioned. Wichmann *et al.* (1946) studied the fate of DDT spray residues by observing the effects of artificial irradiation, daylight, and temperature on DDT activity. When crystalline DDT was moistened with benzene and then exposed to irradiation, decomposition could be initiated in the few minutes required for the solvent to evaporate. As exposure was continued at least half of the DDT was lost in 5 or more hours. Exposure to sunlight for periods of 2 to 93 days resulted in the loss of about one-third in activity. Heating the compound to 54°C. (130°F.) for more than 2 weeks failed to produce any significant reduction in weighted samples of DDT. From these observations it was concluded that any loss of DDT was due to an oxidative process as one of the mechanisms involved and not due to volatilization.

V. Pharmacology

1. Systemic Actions

DDT is a central nervous system poison, and although insects and mammals show the same general responses, some differences in individual species have been noted. When insects come in contact with the poison there is a delay of about 20 minutes before symptoms of nervousness and agitation appear. These are followed by bursts of erratic behavior, loss of equilibrium and eventual paralysis. Death may be delayed for as long as 24 hours.

The symptoms of acute poisoning in mammals usually begin as tremors of the muscles of the head and neck. The onset after oral administration of DDT may be delayed for several hours but can appear in 1 hour. The tremors progress caudally and increase in intensity with time so that eventually purposeful movements are difficult or cannot be accomplished. Frequent episodes of tonic or clonic convulsive seizures manifest themselves within 30 to 40 minutes after the onset of the tremors. As intoxication progresses the convulsive episodes occur with increasing frequency, becoming almost continuous, and the animal enters into a stage of depression which gradually deepens, terminating in respiratory failure and death in 2 to 24 hours after the onset of the initial tremors (Philips and Gilman, 1946).

It is probable that the symptoms of DDT poisoning in man may show the same pattern as observed in lower animals. An uncomplicated case of poisoning has not been reported, but from the meager information available it appears that giddiness, nervous tension and involuntary muscular tremors are some of the symptoms.

2. Mechanism of Convulsive Action

It has been shown by Lewis and Richards (1945) that DDT is not toxic to tissue cultures. Various cells from chick embryos and one-day-old rats have shown no alterations in growth in the presence of DDT, and the authors could offer no explanation of the apparent paradox of a substance being toxic to an intact animal and yet nontoxic to isolated cells in cultures. Yeager and Munson (1945) have presented evidence to show that the tremors produced by DDT are the result of a direct action on the myoneural junctions. Motor nerves appear to be more susceptible than sensory nerves. Carey *et al.* (1946) have given further support to this thesis by their observations of changes induced by DDT in the motor end plates of rats poisoned with the insecticide. Degenerative changes leading to disappearance of the motor end plate and acute

atrophy of many muscle fibers are believed to account for the severe neuromuscular symptoms of DDT poisoning with other reactions probably contributing to the cause of death.

3. Cardiac Effects

It is known that halogenated hydrocarbons produce an excess excitability of the heart muscle so that any coincident increase in activity of the sympathetic nervous system can result in ventricular fibrillation. Philips *et al.* (1946) have shown that ventricular fibrillation can be induced in dogs poisoned with DDT by the injection of a challenge dose of epinephrine. Sympathetic stimulation that accompanies the emotional disturbance induced by DDT convulsions can act in the same way. The sensitized myocardium can be thrown into fibrillation under such conditions and may account for the sudden death observed in animals where the severity of the convulsions was not sufficient to cause lethal effects.

4. Percutaneous Absorption, Irritation and Sensitization

Since methods of use afford ample opportunity for exposure to DDT in dry powder and in solution, skin absorption, irritation, and sensitization assume considerable importance. Draize *et al.* (1944) studied percutaneous absorption of DDT in simple mixtures with bland powders, in combination with lousicides, insect repellents, surface active agents, and various solvents. DDT even as a micronized powder did not penetrate intact skin of the rabbit sufficiently to elicit symptoms of poisoning. No irritation was noted. It was found that in acute exposure DDT in dimethyl phthalate solution was absorbed from intact and abraded skin and caused severe poisoning. No deaths were observed with doses as high as 2.82 g./kg. but all animals exhibited severe intoxication. In subacute experiments doses as low as 150 mg./kg. applied by inunction produced symptoms in guinea pigs, rats, and rabbits after thirty daily applications. Dogs completed a 90-day inunction period at levels of 1200 mg./kg. daily without showing toxicity, which emphasizes wide species variations. Cameron and Burgess (1945) point out that the solvent is a matter of great importance in influencing absorption of DDT through the skin, and they present data to show that daily applications of 10 mg./kg. as a 1% solution in ethyl alcohol is fatal to rabbits after nine to twelve inunctions. DDT as a solid or in solution is not appreciably irritating to the skin. Patch tests and the daily contact of hands with 30% solutions in dimethyl phthalate produced no evidence of irritation in humans. Draize also reported sensitization to DDT in guinea pigs, but Dunn *et al.* (1946) were unable to confirm this and felt that con-

taminating substances in older preparations of DDT may have been responsible for the observed skin sensitivity.

5. Acute Toxicity

Table II is a compilation of acutely fatal doses of DDT in a number of animal species and insects by several routes of administration. The

TABLE II

The Acute Toxicity of DDT

Animal	Route of administration	Approximate LD_{50} mg./kg.	Source
Rat	Oral	200	Philips and Gilman, 1946
Rat	Oral	150	Smith and Stohlman, 1944
Rat	Oral	800	Cameron and Burgess, 1945
Rat	Oral	180	Woodard *et al.*, 1944
Rabbits	Oral	300	Smith and Stohlman, 1944
Rabbits	Oral	300	Cameron and Burgess, 1945
Rabbits	Oral	> 400	Woodard *et al.*, 1944
Mice	Oral	< 448	Woodard *et al.*, 1944
Mice	Oral	200	von Oettingen and Sharpless, 1946
Cats	Oral	500	Philips and Gilman, 1946
Cats	Oral	300	Smith and Stohlman, 1944
Dogs	Oral	< 500	Philips and Gilman, 1946
Guinea pig	Oral	> 562	Woodard *et al.*, 1944
Guinea pig	Oral	400	Cameron and Burgess, 1945
Chicks	Oral	> 300	Woodard *et al.*, 1944
Goat	Oral	> 1000	Spicer *et al.*, 1947
Rat	Intravenous	< 50	Philips and Gilman, 1946
Rabbit	Intravenous	< 50	Philips and Gilman, 1946
Cat	Intravenous	50–60	Philips and Gilman, 1946
Dog	Intravenous	75	Philips and Gilman, 1946
Monkey	Intravenous	60	Philips and Gilman, 1946
Mice (young)	Aerosol	26.4 mg./liter	Neal *et al.*, 1945
Rats (young)	Aerosol	32.9 mg./liter	Neal *et al.*, 1945
Rat	Subcutaneous	1500	Cameron and Burgess, 1945
Rabbit	Subcutaneous	250	Cameron and Burgess, 1945
Guinea pig	Subcutaneous	900	Cameron and Burgess, 1945
Rat	Percutaneous	3000	Cameron and Burgess, 1945
Rabbit	Percutaneous	300	Cameron and Burgess, 1945
Guinea pig	Percutaneous	1000	Cameron and Burgess, 1945
Cockroach	Surface	10	Tobias *et al.*, 1946
Bluebottle fly	Surface	9–28	Tobias *et al.*, 1946
Housefly (adult)	Surface	8–21	Tobias *et al.*, 1946
Housefly (adult)	Surface	2.23	Laug, 1946a
Housefly (newly emerged)	Surface	2	Tobias *et al.*, 1946
Cockroach	Intra-abdominal	5–8	Tobias *et al.*, 1946

insolubility of the insecticide in water (body fluids), which in turn influences absorbability, is probably responsible for the inconsistent responses to single acute doses in any given species. However, once the substance enters the blood stream and absorbability no longer is a factor, the wide variations become less distinct and the contrast between mammals and insects becomes less sharp.

The acute fatal dose of DDT for man is not known. Biden-Steele and Stuckey (1946) report a fatal case of poisoning in which it was estimated that 500 mg./kg. had been swallowed.

6. Chronic Toxicity

Although a substance may cause only slight manifestations of toxicity following the ingestion of a large single dose, long-term ingestion of small amounts of this same substance may reveal deleterious effects which would have otherwise escaped attention. Since DDT may be taken in

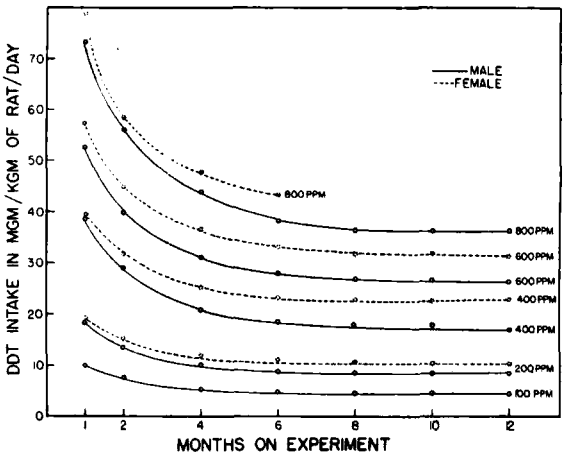


Fig. 1. Correlation of the daily intake of DDT at various feeding levels with body weight of rats.

During the rapid growing period of the rat, the intake of DDT is quite large, especially for those animals on the two highest dietary levels. The slope of the curves indicates that there is a rapid decline in DDT intake as the rats approach the plateau growth period. At the same time there is no significant change in total daily food consumption, which accounts for the fairly constant level of DDT ingestion after the sixth month on the diets. It may be pointed out that the sex difference in susceptibility to DDT poisoning is probably due in part to the greater average food consumption on a body-weight basis of females over males. (Fitzhugh and Nelson, 1947). The chronic oral toxicity of DDT (2,2-bis *p*-chlorophenyl-1,1,1-trichloroethane), *J. Pharmacol. Exptl. Therap.* **89**, 18, 1947.

with food as spray residue in small amounts for long periods of time, Fitzhugh and Nelson (1947) determined the chronic effects of feeding DDT to rats during the life span of the animal. Feeding was conducted at levels of 100 to 800 p.p.m. of DDT mixed with a basic diet and continued for 2 years. Chronic effects were observed in rats in all concentrations. Those animals on 400 and 800 p.p.m. showed the characteristic early symptoms of DDT poisoning as manifested by muscular tremors appearing from time to time throughout the course of the experiment. Animals on lower doses showed increased irritability. Female animals were more susceptible to the action of DDT than males. Retardation of growth was noted in the female rats on the 400 p.p.m. level, males at 800 p.p.m. Increased mortality was evident in the females at all levels fed. Food consumption was not affected by the presence of DDT in the diet. Fitzhugh (1948) also studied the influence of DDT on reproduction. He found that the number of young rats that live through the nursing period is reduced when the mothers are on a diet containing 50 p.p.m. of DDT. At 600 p.p.m. of DDT in the diet the number of living young was not influenced in the first generation. In the second generation there were very few living young at birth and none survived the nursing period. No effect was noted on reproduction with 10 p.p.m. DDT in the diet.

7. Absorption and Distribution

Balance studies on absorption and excretion of DDT have not been reported. That DDT is irregularly absorbed is amply demonstrated by the extreme variability of animals in their toxic response to oral doses. Unquestionably this variation is due to the insoluble nature of the compound since oil solutions are more toxic than water suspensions.

Distribution studies have been carried out on rabbits and rats. Laug (1946b) fed 200 to 400 mg./kg. of DDT in oil solution to rabbits for a period of 2 to 4 days. Ether extracts were made from various tissues and the DDT determined by the bioassay method already mentioned. The analyses revealed that DDT was always present in the kidney and blood, in the majority of instances in the liver, occasionally in the bile, seldom in the brain, and never in the urine. The amounts ranged from 2 to 22 γ/g. of liver and kidney, from 1 to 8 γ/g. of blood and irregular in the bile. Laug and Fitzhugh (1946) determined the distribution of DDT in chronically poisoned rats. One group of animals received 800 to 1200 p.p.m. in their diet for 6 months. Measurable amounts of DDT were found in the liver, kidney, spleen, muscle, brain, and perirenal fat. The perirenal fat contained fifty to one hundred times an amount of DDT as that of the other tissues. Tissues other than fat ranged from 4 to

34 γ/g. of fresh material. Rats fed DDT in concentrations of 100 to 800 p.p.m. for 2 years showed a definite correlation between tissue level and diet level. This was not observed in the 6 months' study. The correlation was most evident in the case of the perirenal fat where 100 p.p.m. level animals deposited 100 γ and the 800 p.p.m. animals deposited 4200 γ/g. of fat. The kidneys revealed values which were four to five times higher in the long-term experiments than in the short-term experiments. Storage of DDT, especially in the body fat of animals ingesting small amounts of the insecticide, has been demonstrated in cows, monkeys, dogs, and poultry.

The amount of DDT stored in the fat is dependent on the dietary level and the length of duration of intake. Woodard and Ofner (1946) showed that with a low level of intake the maximum concentration appeared to be reached in about 2 months. With higher levels there was a tendency to continual additional storage of DDT. An animal may store the equivalent of several times the acute intravenous dose in long-term ingestion experiments. The DDT disappears from the body fat after discontinuation of administration. Storage in rats is reduced to about one-half the original values in 3 months. Also, the reparative processes in the liver appear to be complete in 8 to 10 weeks. Dogs likewise show a gradual disappearance of DDT after cessation of exposure.

8. Fate and Excretion

DDT does not appear in the urine of animals which have been fed the substance. The observations of Laug (1946b) indicated that most of the chemical is excreted unchanged in the feces. White and Sweeney (1945) were the first to show that DDT is metabolized to the corresponding substituted acid, di-(*p*-chlorophenyl) acetic acid. They employed rabbits in their experiments. Neal *et al.* (1946) established the fact that the acid was also found in the urine of man, and Woodard *et al.* (1948) observed a similar phenomenon in dogs. These latter investigators also observed that rabbits excrete a conjugate which yields the acid on hydrolysis. Neal found that following the administration of 11 mg./kg. to an adult man, di-(*p*-chlorophenyl) acetic acid excretion shows a sharp rise reaching a maximum on the second day, decreasing rapidly on the third and fourth days, and then decreasing gradually for the next ten or more days. The fraction of a given dose which is metabolized in this way is not known.

DDT is secreted in the milk of cows, goats, dogs, and rats (Spicer *et al.* 1947). Toxic symptoms have developed in other animals fed the milk, indicating that considerable quantities may be eliminated in this way.

All of the DDT in milk appears to be concentrated in the butterfat, and butter made from such milk may show significant amounts of DDT.

VI. Toxicity to Man

Uncomplicated cases of human poisoning are extremely rare because most of the reported poisonings are concerned with solutions of DDT and the toxic manifestations of the solvent cannot be ruled out with certainty. Biden-Steele and Stuckey (1946) report a fatal case of alleged DDT poisoning of an individual who drank an emulsion containing 20% DDT dissolved in methyl cyclohexanone. The estimated total dose of each ingredient was 34 and 75 g., or 500 and 1000 mg./kg. of body weight, respectively. The authors suggest that the solvent contributed to the toxicity. Hill and Robinson (1945) report the death of a child aged one year and seven months who drank about 30 cc. of 5% DDT in kerosene. The lethal dose of DDT was estimated as being approximately 150 mg./kg. The solvent may have been a contributing factor. In another case (Wigglesworth, 1945) a laboratory worker who allowed an acetone solution of DDT to come in contact with his hands showed symptoms resembling those seen in animals. These symptoms developed 10 days after exposure. The exact dose was not determined. Velbinger (1947) experimenting on himself took single doses of 0.5, 0.75, 1, and 1.5 g. of DDT dissolved in olive oil at widely spaced intervals. He reported that the 1.5-g. dose produced some nervous system disturbances and some changes occurred in the formed elements of the blood. Neal and von Oettingen (1946) exposed two human subjects to a dispersion of DDT in a sealed chamber. They dispensed 10.4 g. of an aerosol containing 5% DDT in cyclohexanone and Freon into the chamber every 15 minutes for 1 hour and for 6 consecutive days. No subjective or objective manifestations of poisoning were observed. They also report that single total doses of 475 and 770 mg. dissolved in olive oil and administered gastrically to one individual on separate occasions failed to give any evidence of toxicity.

Probably the most informative observations on the effect of DDT in man were those reported by Garrett (1947). He describes the symptomatology as observed in twenty-eight individuals who inadvertently ate biscuits made from flour containing 10% DDT. The biscuits were not well cooked; consequently, any decomposition of DDT due to heat in the mixture must have been quite small. The estimated quantity of DDT which was ingested varied from 4.1 to 20 g. per individual. The symptoms consisted of vomiting, numbness and weakness of the extremities, mild convulsions, loss of proprioceptive and vibratory sensations, and hyperactive reflexes. Most of the individuals recovered in 24 hours

with a few still showing some effects 48 hours later. Three of the poisoned individuals had not recovered full use of the hands after 5 weeks. Blood and urine examinations revealed nothing abnormal at any time.

VII. Pathology

The gross pathologic changes induced by DDT are not significant except in chronically poisoned animals. Fitzhugh and Nelson (1947) report that the livers, and to a lesser extent the kidneys, of chronically exposed rats were larger than those of the controls. Histopathological examination of organs from at least ten species of animals in various grades of chronic poisoning and by a number of investigators reveals that the outstanding lesions are found in the liver. This ranged from a moderate degree of centrolobular necrosis of the hepatic cells in animals on the higher dosage levels that survived a week or more, to a combination of central necrosis and reparative hypertrophy in some cases of longer-standing intoxication. There was a tendency to hepatic cell tumors in rats after 18 months of DDT ingestion. The dog appears to be the more suspectible to liver damage than other species, frequently showing jaundice, and hemorrhages in several locations. The pathologic changes in other organs are of a minor nature (Nelson *et al.* 1944).

It would appear that the nervous manifestation of DDT poisoning would point to an impairment of the central nervous system. Careful studies of dog brains by Haymaker *et al.* (1946) revealed little of note. Some degenerative changes were found in the cerebellum of animals given relatively large doses for prolonged periods of time. The changes were interpreted as being slowly progressive and probably irreparable.

VIII. Health Hazards

Generally speaking if an insecticide is not acutely toxic in single oral doses, difficulties arise when attempts are made to assess the risks associated with the widespread use of such a substance. DDT belongs in this category. Two types of hazards are involved in the use of DDT. The first has to do with the industrial aspects such as manufacturing, handling, and spraying procedures. Various governmental agencies have defined the precautions necessary in the commercial handling of DDT products. That these precautions are adequate appears to be confirmed by lack of definite evidence of injury from occupational exposure. The second type, which has to do with the protection of the public against consumption of injurious amounts of DDT, is a more difficult problem. There is probably little danger incident to the use of household sprays. As already mentioned, humans have been exposed to concentrations of DDT in the form of an aerosol considerably in excess of amounts usually

employed for such purposes without showing definite evidence of injurious effects. However, the additive effect of the solvent must not be overlooked. DDT as a dry powder is not absorbed through the skin unless oils and greases have been previously applied to the exposed areas (lotions, cold creams, etc.). Oil solutions of DDT are rapidly absorbed and hence added precautions are required when such products come in contact with the skin or are spilled on clothing.

The greatest hazard of DDT lies in its use on food and forage crops. The fact that DDT is but slowly detoxified in the animal body leads to an accumulation of concentrations considerably higher than that which obtained in the food ingested. This becomes of paramount importance with respect to dairy products. Pharmacological investigations have shown that DDT concentrates in the fatty tissue and can be excreted in milk. All of the DDT appears to be concentrated in the butterfat, so that cream, butter, and ice cream made from such milk can contain significant amounts of DDT. Schechter *et al.* (1947) have reported that milk from dairy cattle fed DDT-sprayed forage crops contained as much as 26 p.p.m. of DDT. Butter made from this milk contained as high as 534 p.p.m. and animal fat 179 p.p.m. It seems clear, therefore, that the presence of small amounts of DDT in animal food may be as significant as larger amounts present on fruits and vegetables consumed directly by man. It is generally agreed that any amount of DDT that is detectable is undesirable in milk. By and large man is more susceptible to the majority of toxic agents than other animals. The human population is quite variable and although it is possible to predict in a general way the potential dangers of a poison by transference of animal data to man, an accurate appraisal of the potentialities for harm is difficult.

IX. Treatment and Antidotes

In the treatment of acute poisoning measures should be directed toward removal of the DDT from stomach and intestinal tract. The stomach should be emptied by lavage or by an emetic regardless of whether vomiting has already occurred. Elimination of the poison from the intestinal tract can be hastened by the use of saline cathartics. Castor oil should not be administered. Because of the chemical stability of DDT, chemical antidotes are probably useless. If neurological manifestations of DDT intoxication become evident an anticonvulsant drug may be given. Philips and Gilman (1946) have found that the best physiological antagonist is phenobarbital. Enough of the barbital should be given to control the tremors and convulsions. Experiments indicate that this dose is well below the anesthetic level.

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Analysis of Foods by Sensory Difference Tests

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I. Introduction

In food research, selected judges are used to measure and characterize differences in odor, flavor, texture, and other qualities of experimental samples. The differences can be estimated with a small panel of judges —generally five to ten persons. However, small differences that may indicate the beginning of important changes can be estimated reliably only if the judges are selected for their acuity and consistency in recognizing differences between the samples under consideration. A small, trained, selected panel obviously would not be used to determine individual preference or to appraise a food in terms of consumer acceptance.

The selected panel test should be supplemented generally by physical and chemical tests. Use of all three tests should lead to a better knowledge of the chemistry of processing and storage changes and should help to detect important changes that might be measured by only one of the methods. Frequently, however, chemical and physical tests are unsatisfactory. The results of Pearce (1945) illustrate this problem:

> The suitability of a number of objective tests of milk-powder quality was assessed against subjective scores of palatability. The objective tests investigated were: oxygen and water sorption of the powders; chlorophyll and peroxide oxygen values of the fat; "browning" of the powder; fluorescence values; changes in peroxidase, trimethylamine, volatile sulphur compounds, and diacetyl content; solubility by centrifuging and a potassium chloride solution method; titratable acidity; pH; congo rubin and iron numbers; foaming volume; coagulation by acid, alcohol, and rennet; dielectric constant; colour intensity and colour quality; refractive index; viscosity and surface tension. The subjective measurement of palatability was finally adopted as the most precise measure of milk powder quality.

While it is recognized that reliable and valuable results can be obtained by the use of laboratory panels, certain limitations in addition to those usually encountered in biological studies are recognized. Individuals are subject to variation in response due to a large number of unknown factors; results usually are expressed in relative, not absolute, terms; much time and material are required to obtain valid results; and small laboratories may not have sufficient personnel from which to select satisfactory panels. A serious limitation of the entire field of selected panel testing is the lack of established techniques for carrying out the tests.

The purpose of the present discussion is to bring together material that is of interest to persons engaged in food research. Desirable and undesirable features of the various judging methods will be surveyed. The methods that may be used to increase the accuracy of tests will be discussed as they apply to the experimental plan, the judges, and the condi-

tions of testing. (Finally, it will be shown how chemical and physical measurements supplement the panel tests. The relation of physiology and psychology of sensory reaction to foods as well as threshold tests will be omitted, except where they apply directly to the subject under consideration.

One difficulty encountered in this field is the confusion in nomenclature. Many of the terms are used differently by different authors and many of them lack specificity. For example, some use "subjective" to refer only to consumer preference tests, while others use it to include all tests that depend on sensory reaction. Some use "objective" to refer only to the type of tests described in this paper; others use it to mean chemical, physical, and similar measurements and exclude any tests dependent primarily on the sensory reactions of individuals. "Analytical" has been used to differentiate the type of test described here from consumer preference tests (Hicks, 1948). (The terms "organoleptic," "palatability," "eating quality," and "taste tests" have been used for many years before there was full appreciation of the several types of tests dependent on human reaction to foods. The terms "palatability" and "quality" seem to the authors to imply high quality or acceptability, a concept which is not considered in the difference tests.)

The methods considered in this paper employ the senses as the primary measuring device. They are, therefore, sensory methods of analysis as distinguished from physical, chemical, and microbiological methods. The consumer acceptance or preference tests are sensory tests also, but their object is to determine what a representative population prefers whereas the test discussed here, like the chemical, physical and microbiological tests, is used to measure differences in samples regardless of preference. Although consumers in various parts of the country may prefer different samples of a food, sensory tests for differences should yield the same results (within experimental error) regardless of regional preferences. It seems preferable to use "subjective" to designate tests that measure the preference of individuals and "objective" to designate tests that measure differences in samples, whether measured by human senses or by other means. This paper is therefore concerned with objective, sensory tests. They might also be called sensory difference tests. It is hoped that these few comments will aid the reader in the interpretation of terms used in this paper. We have attempted to avoid the use of ambiguous terminology except in a few cases in which it was necessary to follow the usage of an original paper. It would be useful to have clarification of the terminology in this field of research.

II. Methods of Expressing and Analyzing Differences

Judges express their sensory reaction to flavor, texture or other factors in terms of scores, ranks, or the numbers of samples that are alike or different. Because of the amount and variability of the data derived from an evaluation test, interpretation usually cannot be made by direct examination. Therefore, the data are summarized and tested for reliability by statistical methods. Such methods express the probability that the differences obtained in the experiment could occur by chance. The probability is commonly expressed in degrees of significance. Thus, the term *significant* indicates that the difference would be expected by chance five times in a hundred ($P = 0.05$ or probability at the 5% level); *highly significant* indicates that the difference would be expected by chance once in one hundred times ($P = 0.01$); *very highly significant* indicates that the difference would be expected by chance once in one thousand times ($P = 0.001$).

1. Paired and Triangle Difference Tests

In these tests judges indicate whether there is a difference in a particular characteristic (e.g., flavor, tenderness) or any difference of any kind between samples. The information obtained is the number of judges who indicate the presence or absence of the difference. A paired test (Cover, 1936; 1940) and a triangle test (Helm and Trolle, 1946; Gray *et al.*, 1947) have been described.

In the paired test two samples are submitted to judges. A typical question asked of the judges is, "Which is the more tender sample of meat?" Sometimes a standard sample is presented first, and the judges are asked which of two unknowns is the same as the standard.

In the triangle test three samples are examined, two of which are duplicates. Judges are asked to select the identical samples. Often judges are also asked to indicate whether the odd or duplicate samples have the distinguishing characteristic to the more pronounced degree. The latter data are then analyzed by the paired procedure.

Chi-square is used to analyze the data of the paired and triangle tests. Chi-square analyses evaluate the probability that the number of judges who indicated a difference between samples is no greater than might be obtained by chance. Cover (1940) describes in detail the modification of the Chi-square analysis she used with her paired-difference data.

Helm and Trolle (1946) report the numbers of correct answers necessary to establish significance in the triangle test for given numbers of judges. In order that this method might be used for any number of judg-

ments, Snedecor (1947) very kindly supplied the present authors with the following formula for applying Chi-square to triangle data:

$$\text{Chi-square} = \frac{(a - rb)^2}{r\,(a+b)}$$

a = the number failing to identify duplicates; b = the number identifying duplicates; r = the "chance" ratio of a to b. In the triangle test the value of r is 2, since it is possible to make three different selections as the duplicate samples, only one of which is correct. In routine work it is simpler to use the formulas:

1) $b' = \frac{1}{3}\ (N + \sqrt{7.682N})$ for significance at the 5% level, or
2) $b' = \frac{1}{3}\ (N + \sqrt{13.270N})$ for significance at the 1% level

where b' = the number of identified duplicates needed for significance at the levels indicated, and N = total number of persons in the trial. The numbers 7.682 and 13.270 are derived from the values of Chi-square at the 5% and 1% levels of significance, respectively.)

Although the Chi-square analysis does not indicate the *degree* of difference between samples, both Cover, with the paired data, and Gray *et al.* (1947), with triangle data, discuss a procedure for interpreting degrees of difference between two different sets of samples. Both are based on interpreting levels of probability; that is, if a higher percentage of the judges detect a difference between one set of samples than between another set, there probably is a larger difference between the first set of

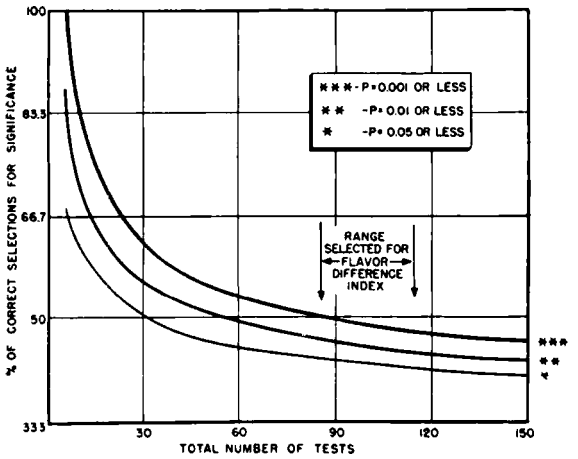

Fig. 1. Per cent correct selections for significant results in the triangle test as a function of test replications (Gray *et al.*, 1947).

samples. Figure 1 illustrates the effect of the number of judgments or tests in this procedure.

It may be seen that for a small number of judgments (for instance, five) it is necessary for every selection to be correct before the difference between two samples can be regarded as very highly significant ($P = 0.001$). When the total number of tests is eighty-five or more, the correct answers required for such significance is about 50%, and the curve becomes almost level beyond eighty-five. Increasing the total number of tests beyond this number has little effect in decreasing error. Thus in this region of the curve, interpretation of significance between two tests is not much influenced by number of judgments.

2. Dilution Test

The dilution test determines the smallest amount of unknown that can be detected when it is mixed with a standard material. To the authors' knowledge dried egg is the only product to which the method has been applied, and fresh egg was the standard used. The method has been used only for flavor but probably could be used for odor; it applies only to homogeneous substances, but many foods can be made homogeneous without effect on the quality factor under consideration.

Bohren and Jordan (1946; 1948) developed this method and it will be described rather completely here because the details have not been previously published. Preliminary to judging unknowns, it was determined that judges could consistently distinguish between samples when each successive sample contained 20% less dried egg (the remainder of each sample being fresh egg). Thus, there were twenty possible variations, ranging from 100% to 1.4% dried egg, which were assigned scores from 1 to 20. Much less dried egg would be required for detecting very poor quality dried egg than for good quality; therefore, all twenty dilutions are not used for any one dried-egg sample.

The shell eggs used in these investigations were from specially-fed hens; eggs laid in the afternoon were held in cool, clean storage and used the next morning. Dye was added to the egg mixtures before they were scrambled to eliminate color difference. The cooked eggs were cooled to room temperature before testing.

Judges were supplied with a labeled fresh egg sample. Some of the unknowns were duplicates of the fresh egg and others were mixtures of the fresh and dried egg. Judges were asked to indicate samples which they thought contained dried egg.

During one judging period, each of six judges made ninety-six judgments regarding varying numbers of replicates of six different consecutive dilutions of one dried egg sample and the coded fresh eggs in order to

establish the score for the dried egg. In preliminary trials the investigator selected the six dilutions (from the twenty possible) that were expected to bracket the sample. The goal in bracketing was to obtain answers of which 75% were correct for one of the dilutions near the center of the regression line for percentage of dried egg in samples and percentage of correct answers by judges.

The scrambled eggs were presented in dishes on trays. Each tray contained a labeled fresh egg and eight unknowns. The unknowns might be seven fresh egg samples and one containing dried egg or any combination of the two kinds of unknowns of which the total was eight. The distribution of the various possible combinations was randomized. Only one concentration of dried egg was used on any one tray. An example of the material used in one test is shown below, although, of course, the tray numbers listed do not represent the order of presentation of samples to judges.

Tray number	Dilution or per cent dried egg in the sample	Score corresponding to the dilution
1 and 7	21.0	8
2 and 8	16.8	9
3 and 9	13.4	10
4 and 10	10.8	11
5 and 11	8.6	12
6 and 12	6.9	13

Thus there were six different trays, each containing only one concentration of dried egg and each of the six trays was duplicated as regards dilution so that there were twelve trays in all.

The judges' data were converted to percentage of correct answers and graphed against the score or percentage of dried egg in the samples. The regression line and error were calculated. The score for the dried egg was read from the line at the point where 75% of the answers were correct.

3. Scoring Tests

Numerical rating or scoring tests are used more frequently than any other of the objective sensory tests. Unlike the others reported here there is considerable latitude in the design of scoring tests.

Frequently many factors are judged. It is obvious, as Platt (1933) has pointed out, that the factors to be scored should be placed on the record in logical order; first the factors the judge estimates by sight, then odor, and finally the factors which cannot be scored until the food is taken into the mouth. Any form which simplifies recording and leaves the judge free to concentrate on decisions is, of course, an advantage.

A large number of scoring forms are recorded in the literature. The

grading chart for rating mealiness of potatoes is a good example of a scoring form for rating one factor in several ways (Sweetman, 1936); and the record prepared by Alexander *et al.* (1933) for the Committee on Cooking and Palatability Methods for Meat is an illustration of a comprehensive form.

There is considerable variation in the range of scores used by different investigators, but scales ranging from 1 to 5, 7 or 10 are most common. Of course, failure to use the entire range of scores or using fractional scores changes the number of intervals.

Baten (1946) reported an elastic system as regards number of intervals. The scoring form consisted of a horizontal line 6-inches long with "excellent" written at one end, "poor" at the other. The judge recorded his rating by placing a vertical line across the horizontal one at the point that indicated his opinion, and the distance to the vertical line for each sample equaled the score for that sample. The products scored by the line method were also scored numerically by the same judges. The judges did not like the line as well as the numerical form, but statistical analysis showed that they graded more accurately with it.

The basis for selecting a given number of intervals is the number that judges can distinguish, but it may be necessary to make allowance for the fact that judges may not use the highest and lowest scores. Also the investigator probably does not know how many distinguishable intervals will be encountered in a given experiment. A large number of intervals, of course, would increase the variability of scores and thus the experimental error. Probably ten intervals would be sufficient in most experiments.

Statistical significance of results obtained in the numerical rating procedure usually is tested by the "t" test when two treatments are compared or by analysis of variance when two or more treatments are compared. Both procedures test the hypothesis that samples were drawn from the same population and that the difference between the samples is no larger than would be expected in random sampling. Thus significance is based on a ratio, the numerator of which is the difference between mean scores of different samples and the denominator is an expression of the experimental error.

When replicate panel means rather than individuals' scores are analyzed, the variation in level of scoring of different judges has less effect on results than is sometimes realized. For example, when three individuals give one sample scores of 6, 8, and 10 in each of four judging periods, the mean is 8 each day. Thus there is no experimental error due to varying level of scoring of different judges on one day.

4. Ranking Tests

In the ranking test judges are asked to rank samples in decreasing or increasing order of some characteristic, such as amount of rancidity in fat. The results can be summarized by enumerating the number of judges who give each rank to a sample, or by averaging the ranks. Bliss *et al.* (1943) converted ranks to scores by referring to Table XX in Fisher and Yates, *Statistical Tables for Biological, Agricultural, and Medical Research.* The ranks were transformed to scores because the distribution of simple ranking departs more from the normal form than one would prefer for direct use in analysis of variance, the statistical procedure used here.

White *et al.* (1944) used the Bliss procedure with smoked meat and Handschumaker (1948) used it with reverted hydrogenated soybean oil shortening. The latter paper is of special interest because five of the samples in the series of six were controls. The controls were made from mixtures of cottonseed oil and soybean oil of appropriate hardness. The five samples contained 100% cottonseed oil, 25, 50 and 75% soybean oil (the remainder being cottonseed oil), and 100% soybean oil. The controls were prepared each test day by a standard procedure including heating the samples at 140°C. (284°F.) for 4 hours. All samples were coded and the judges were asked to place them in the order of intensity of reversion.

A number of factors that relate to accuracy of all of the methods are discussed in the next section.

III. Factors Related to Accuracy of Tests

1. Experimental Plan

a. Characteristics Evaluated. The large number of food characteristics that can be evaluated by individuals is illustrated by the meat record developed over a period of years by Alexander *et al.* (1933). They scored "aroma and aroma smokiness" of the entire sample, "translucence, firmness, waxiness and flavor" of fat, "texture, cohesion, firmness, flavor, saltiness, tenderness and quantity of juice" of the lean. Aroma was described as "pungent, flat, cheesy, stale, spicy, sour, sweet, spoiled, fresh, briny, musty, rancid or other." Flavor of lean and of fat were described by similar terms.

If the sensory difference tests are to serve research to the fullest extent possible, it is important that specific terms be developed for each product evaluated, so that all terms mean the same thing to all persons who use them. This is partly a matter of special investigations for the purpose of

isolating and thus identifying the characteristics and partly a matter of training the judges. The latter will be discussed in a later section. One of the first steps in obtaining more exact terminology is to break down each factor into its simplest parts so that judges do not need to weight several factors in reaching a decision. An example is the separate scoring of texture of skins and cotyledons of peas. It is obvious that terms such as "excellent-poor" or "desirable-undesirable" do not meet the requirement of specificity. It is very difficult to develop a scoring record without using general terms and those expressing desirability; considerable knowledge about the food and the effects of treatments on it is required to distinguish the characteristics that are important.

Although a large number of characteristics can be judged, there are advantages in limiting the number to one or two. If only one factor is evaluated the investigator can select conditions that accentuate this one factor. Cooking methods that accentuate flavor differences might be used even though they are not best for all factors. Samples might be blended or subdivided to mask texture differences, then dyed to mask color differences. After some experience with a food it is usually possible to select one factor that changes first or is most important. Time spent on secondary factors might better be spent on the important characteristic. Also it may be desirable to test only a portion of a sample. For example, yolks rather than whites of eggs are commonly scored because off-flavors are more pronounced in yolks (Slocum *et al.*, 1933; Sharp *et al.*, 1936; Mallmann and Carr, 1941).

There seems to be no good reason for determining a composite score for total quality of samples rather than scores for the separate factors. If total score is ***the only one judged,*** the cause of a low or high score cannot be known. Since each judge weights the various factors by his own standards, total score is of limited usefulness. If the total score is obtained by ***adding the scores for color, texture, flavor and other factors,*** a sample with very poor flavor but good qualities in other respects might receive a medium score. This is misleading, since for all practical purposes the food might be inedible. It is also possible that a desirable characteristic of a product may be associated with an undesirable one, such as mealiness and sloughing of potatoes. If the ***separate scores are weighted*** by the planner of the investigation or only a few persons, the total score has limited value because the system of weighting is not commonly accepted. Total score is somewhat like averaging the peroxide, reducing, and free fatty acid values of a sample of fat. Before deciding to obtain a total score by any method, one might do well to ask himself, "What information does the total score give that cannot be given better by scores for the separate factors?"

b. Cooking the Samples. The method of cooking the samples almost certainly will greatly influence the conclusions of any experiment, yet very little information is available regarding the effect of variation in methods. Because of the number of different foods, possible cooking methods for each, and limited evidence for any one method, the authors can do little more than indicate the problems that exist.

One of the first decisions that must be made in planning a project for a cooked food is to select a cooking method or a standard product to be obtained by the method. Often this decision is arbitrary. Even when the method or product has been selected, there is difficulty in reproducing it, especially in determining the end point for doneness. Frequently, a standard method does not serve the purposes of the experiment. For example, the purpose may be to develop a method of using dried eggs to make sponge cakes that judges cannot distinguish from a sponge cake made with fresh eggs. Development of such methods is usually time-consuming.

A few procedures that are rather uniformly used by investigators will be described. Considerable work has been done in standardizing roasting methods used for meat and poultry. Since the same principles apply to both, examples have been selected from poultry only. When flavor, aroma, tenderness, and juiciness are scored, birds are usually roasted in an uncovered pan. The range of oven temperatures used has been limited in the different experiments to approximately 150 to 160°C. (302 to 320°F.); and a constant oven temperature has been used. The end point of cooking is determined by the internal temperature of the meat; various investigators have used different final temperatures to indicate doneness (85-88°C.; 185-190°F.) (Stewart *et al.*, 1945; Koonz and Trelease, 1946; Hanson *et al.*, 1942; Trelease and Koonz, 1945; Schreiber *et al.*, 1947). The internal temperature is determined by a short thermometer inserted into the center of the thigh muscle. Training is necessary in the proper placing of the thermometer, since if the bulb is not in the center of the muscle, the bird may be removed from the oven before it is completely done.

Cooking of fresh eggs for test purposes is relatively easy; they are cooked in the shell for a constant time in boiling water (Pennington, 1932; Slocum *et al.*, 1933; Sharp *et al.*, 1936). Part of the procedure with dried eggs has been standardized. The powder is reconstituted with distilled water by stirring, beating or shaking the material for a constant number of revolutions. One part of dried egg and three or four parts of water by weight generally are used (Wilson and Slosberg, 1942; Stewart *et al.*, 1943; Boggs and Fevold, 1946). They are usually served as scrambled eggs, and they are cooked in a water bath with constant stirring

(Bate-Smith *et al.*, 1943; Stewart *et al.*, 1943; Boggs and Fevold, 1946). The water bath method was selected because of ease of controlling the temperature during cooking. Shortening ordinarily is not used because it interferes with the estimation of flavor (Wilson and Slosberg, 1942). The method of determining the end point of cooking scrambled eggs is not satisfactory; a constant time of cooking does not give uniform doneness. The only method to date that has been used for determining the end point is to judge the appearance of the cooked eggs.

The need for standardized methods for cooking foods is very great. More basic information about the foods themselves would aid in the development of standardized cooking methods. In their absence it is essential that cooking methods be carefully described if results derived from various laboratories are to be of the greatest value.

c. Effect of Quality of the Food. The quality of the foods used in a test has been shown to affect panel accuracy in two ways. Variability of scores is much greater on low than on high quality material; judges tend to score a given sample differently depending on the quality of the other material present at the same time.

Thistle *et al.* (1943b) report a standard error of 0.65 palatability unit (10-point scale) for poor-quality dried eggs but 0.28 unit at higher quality levels. Hopkins (1946) tabulates the standard deviations for average scores of 0.0 to 1.0, 1.1 to 2.0 and so on to 9.1 to 10.0 for four products: butter, dried eggs, dried milk, and ration biscuits. The panels for the four products varied from six to seventeen judges. Several hundred samples of each product were graded. This worker concluded that the scores became clearly less consistent as quality decreased and were possibly most discrepant between scores of 2 and 5, but owing to the infrequency of samples of the lowest quality, the zone of maximum uncertainty was not well established. Sometimes it is possible to adjust the treatments and the storage periods to avoid the lowest quality material and thus improve accuracy.

Dove (1943) in an investigation of sweet corn pointed out a tendency of judges to evaluate a given sample differently when scored with poorer or better samples. The judges were asked to rank samples in order of preference and to give their reasons for the placement in terms of juiciness, maturity, sweetness, tenderness, and so on. Eighteen varieties of corn were first separated into three groups of good, medium, and poor quality. Then, without knowledge of the judges, a new variety was introduced, first with the poor varieties, then with the medium, and finally with the best varieties. The order of placement of the new variety when presented with the different groups was logical, but the reasons given for placement disclosed that judges considered the new variety "sweet and

tender" when it was compared with the poor varieties, but "tasteless and flat" with the best varieties. Investigators should recognize that the quality of material scored at one time affects panel accuracy, and samples should not be scored at different times with samples of differing quality unless such a comparison is the purpose of the experiment.

d. Standards. Because judges can give only comparative, not absolute, values to samples, a reference point is often found useful. A standard may be labeled or it may be coded and presented with the other unknowns. When labeled it is meant to serve as a guide to the judges.

Coded standards are used in interpreting data. Slocum *et al.* (1933) and Sharp *et al.* (1936) adjusted the scores for the unknowns to the shifting scores for the standard. Most investigators have not adjusted the scores but have used the standard only to check whether the scores of the entire panel had shifted. The very presence of a standard may stabilize the judges' scores. Sometimes a varying number of coded standards are included in the series; Sharp *et al.* (1936) did this in judging eggs.

A special value of a standard is that it relates unknowns to a sample whose quality is likely to be recognized. The standard may be strictly fresh material, material processed under the best-known conditions, a well-known variety, or any other meaningful material.

Punnett and Eddy (1930) described a procedure for coffee judging in which an unknown sample was matched with one of several standards. The method is based on the assumption that there are two kinds of flavor deterioration: weakening of the natural flavor and development of a stale flavor. The unknown coffees were stored under varying conditions following roasting and grinding. The standards consisted of two series of samples of varying concentrations of fresh and stale coffees of the same blend as the unknown. The coffee for the fresh-flavor standards was roasted and ground on the day it was judged. The authors did not describe the handling of the "thoroughly stale" coffee. The unknown was first matched with one of the fresh-flavor standards and if it also exhibited stale flavor it was then matched with the stale series. The complete designation of a cup of unknown coffee would be expressed as a percentage of fresh flavor and a percentage of stale flavor.

The Handschumaker (1948) ranking method for oils previously described (see p. 227), in which five of the six samples in any one test are standards, is similar to that described above for coffee.

Standards are especially useful in storage studies, which are difficult under any conditions, because of the time interval between analysis [illegible] hen standards are used it may not be possible to compare samples judged months apart. Storage investigations

must always be interpreted with caution, especially if the differences are small. One may have to be satisfied to compare only treatments at a given storage period.

Although standards are very useful, one must consider whether the time and material spent on them gives as great a return as the same amount invested in additional treatment comparisons. Many investigators feel that the number of samples judged at one time should be limited to three or four or even fewer samples; and if one of these is a standard, it constitutes a large percentage of the total investment.

Of course, a standard is very misleading if its quality is not practically constant. This is especially true when the standard is labeled and its score is arbitrarily fixed, because changes in this fixed standard may cause a shift in the scores for the experimental samples. Specialists for particular foods are usually qualified to decide whether a given procedure is likely to yield relatively constant quality.

e. Replications. The number of replications needed in a particular experiment depends on the variability of the samples, the variability of the judges' results, the magnitude of the difference between samples and the completeness of information desired in one experiment.

Variability of the sample may be reduced by selecting uniform material and in some instances making the food homogeneous before submitting it to the judges. In the case of animals, the raw material would be selected to include only one age, sex, breed, and so on. For example, Mackintosh *et al.* (1936) found that in a group of beef cattle, including yearlings and mature cattle, there was a significant correlation ($r = -.60$) between scores for tenderness and shear-force readings. However, when they limited their experimental groups to yearling steers, the correlation was $-.986$. Further selection can be made from within each sample. Cover (1936, 1940) in roasting studies with beef used paired samples (from same location in a single muscle) from paired slices (same slice within the roast) from paired cuts (same ribs) from the right and left sides of the same animal.

Sweetman (1936) has indicated the problem of within-sample variation of potatoes. She found that usually two and occasionally all four of the classes of mealiness which they judged were represented in a ten-tuber sample. However, the majority of the tubers included in such a sample usually fell within a single class. Variability of fruits and vegetables can be reduced by selecting samples to include only one variety, maturity, size, soil condition, holding period after harvest, and so on. In many instances the best solution to the problem is to grind, chop, or otherwise subdivide the food, as was done by Batchelder *et al.* (19[illegible] with broccoli.

One of the most important factors determining the number of replications required is the accuracy of the judges. Hopkins (1946) pointed out that the size of panel required to produce an average assessment of specified accuracy may be calculated if the magnitude of the discrepancies between individuals is known. Thus, because of the greater variability in grading of poor-quality dried egg than in the grading of good samples, with his data nearly eight times as many judges would be needed in the low-quality range as in the high-quality range in order to equalize the variance of the scores. Also, a considerably larger panel would be needed if the required sensitivity for mediocre-quality eggs were decreased from 1.0 to 0.5 unit. Hopkins (1947) showed that, because of individual variations, more judges are needed for a given degree of reproducibility in interpanel than in intrapanel comparisons.

Several workers have reported better results when they decreased the panel size to include only the best judges. Sharp *et al.* (1936) found that better results were obtained when the five most consistent judges scored a series of egg samples twice than when ten members of the panel graded the samples once. Likewise, Marcuse (1947) reported better differentiation between egg samples by the use of results of only two of the seven members of the panel. King *et al.* (1936) reported that equally good results were obtained with a panel of sixteen experienced persons as were obtained with an untrained panel of ninety-six. It is obvious that the number of judges should be large enough to counteract unusual variability due to illness or any of the many other factors that may influence judges from day to day.

Another factor influencing the number of replications is the completeness of information desired from one experiment. Often it is possible to get replications of raw material and treatments without increasing the number of judgments required to show significance for one lot of food. An example of such a plan follows:

		Treatment			
		A	B	C	D
Raw material lot 1	Scoring period 1				
Raw material lot 2	Scoring period 2				
Raw material lot 3	Scoring period 3	(Average Scores)			
Raw material lot 4	Scoring period 4				

Whether such a plan should be used is dependent on knowledge of the uniformity of raw material and of ability to reproduce the treatment under consideration. If the completed experiment shows that raw material lots did respond similarly to a treatment, much information has been obtained from one experiment. Of course, if the raw material lots did not respond similarly it is not possible to distinguish raw material variability

from panel variability. However, if a conclusion on one lot cannot be repeated, the information is not very useful.

2. Judges

It has been mentioned that one of the serious limitations of all objective sensory tests is the variability of individuals' response to a given stimulus and of one individual's response at different times. Individuals vary in their threshold, in degrees of difference they can distinguish, in ability to reproduce scores at different times, and in ability to identify one flavor in the presence of others. These variations can be decreased by training and selecting judges, checking their performance on tests, maintaining judges' interest in the work, and minimizing the effects of prejudice and fatigue.

Solutions of pure chemicals have been used for some years to demonstrate the differences in sensitivity of individuals to sweet, salty, sour, and bitter sensations. However, except in extreme cases, the results of such tests have not been of much value in predicting sensitivity to foods (King, 1937; Hopkins, 1946). Examples of the variations in threshold of individuals to certain substances in foods include differences in reaction to saltiness of bacon (Hopkins, 1947), differences in ability to detect fishiness in turkeys (Asmundson *et al.*, 1938), and insensitivity to mustiness in eggs (Sharp *et al.*, 1936).

Evidence of variation in threshold levels and of ability to detect one flavor in the presence of others was given by Hopkins (1946). He found considerable variation in the ability of an unselected group of thirty persons to detect sucrose, caffeine, glutamic acid, tartaric acid, and sodium chloride in scrambled eggs. Using a scoring range of 0-10, he found that some individuals scored samples as low as 5.4 that were scored as high as 10 by others. One subject gave a score of 10 to every sample, indicating that he did not detect the added substances. In another study on butter, dried milk and ration biscuits, similar evidence of individual variation was obtained. Dawson *et al.* (1947) found that when carbohydrates had been added to dried eggs being studied for development of storage flavor, certain of the panel members were unable to differentiate between the storage flavor, and the sweet or other flavor of the added substance. The better judges, however, were able to distinguish between the flavors.

Health, smoking, psychological factors and age have been considered possible causes for individual variation in ability to distinguish differences in food. Good health is an obvious factor, since even mild infections of nose or mouth are known to affect flavor perception. Strong emotional stimulus affects the ability of individuals to concentrate and

undoubtedly reduces their accuracy. Although there appears to be no proof that smoking affects the ability of judges, some workers request smokers to refrain from smoking for one to two hours before serving on a panel. Age has also been thought to have an effect on sensory perception. However, the evidence of the superiority of any age group is incomplete and contradictory (Harding and Wadley, 1948; King, 1937; Helm and Trolle, 1946).

a. Training. The need for preliminary training has been mentioned by many workers. This need was illustrated recently by the results of Dawson *et al.* (1945) who found in judging the flavor of scrambled eggs that the panel error was highest the first week and lowest during the last part of a year's study. Practice periods were used by Sharp *et al.* (1936) and Mallmann and Carr (1941) in studies on egg flavor. Moser *et al.* (1947) trained a panel to test soybean oil with a series of samples rated previously by industrial experts. Handschumaker (1948), in other experiments with soybean oil, presented samples of reverted oil to the panel so that the judges could become acquainted with the characteristic odor. Sharp (1941) placed mental concentration and training first among the factors important in good judging; natural ability was thought to be of minor importance. He recommended training judges by presenting them with samples in which a single flavor characteristic is prominent. After the individual has learned to recognize the flavor and estimate its intensity, the flavor is diluted and the training process repeated. The next step proposed is for judges to learn to place in order a series of samples differing by easily recognizable degrees; then the interval is narrowed. Training over a period of days or even months is recommended for each flavor.

Training should include the presentation of series of samples differing in all of the characteristics of importance in the investigation. The amount of training required will vary for each study. Judging tenderness of meat, for example, might require little training; on the other hand, evaluating the flavor of milk that has many off flavors is more difficult and requires more training.

b. Selecting. Since it has been shown that individuals vary considerably in sensitivity to sensory stimuli, it appears only logical to select persons demonstrating acuity and consistency in detecting differences in the product being tested. As Handschumaker (1948) has pointed out, the advantage of using only the best judges lies in the fact that the experimental error will be reduced; smaller differences between experimental samples will be noted. When unreliable judges are included in a small panel, significant differences cannot be distinguished between any except widely differing samples (because of the large error variance).

The material used for selecting judges might be samples for which the treatment is known to affect quality, such as dried eggs stored at 15.6 and 37.8°C. (60 and 100°F.) or known mixtures of the two eggs or any high and low-quality material.

An example of panel selection has been described by Helm and Trolle (1946). They had accurate physical and chemical tests for beer but no reliable method that could establish "taste" differences between experimental samples. They therefore decided to select a panel of experts. The tests were planned to determine the ability of subjects to distinguish between two beers, using the "triangular test" previously described (p. 222). Samples of beer differing in odor, fullness or bitterness were used. The difference between samples in each series was of such magnitude as to differentiate sensitive from insensitive judges. Each series was tested six times, the order of samples differing at each period. Before testing, the subjects were informed of the nature of the difference in the particular beers being examined. The performance of individuals was analyzed by a modification of the Chi-square method. Persons whose results indicated that their choice could have been made by chance alone only one time in a thousand ($P=0.001$) were classified as "expert" tasters. Of the fifty-one persons who completed all series, only six had an "expert" rating in all of them. For their panel, Helm and Trolle chose the twenty persons having the highest percentage of correct answers. This screened group averaged 76% identification of duplicates and was a more suitable panel than the entire group for distinguishing differences in beer.

Dove and Farrell (1945) described a method of choosing panels in which judges were tested for ability to recognize deterioration in test foods. Paired samples, which had been held at different storage temperatures, were presented to prospective judges to determine whether they could distinguish a difference between them. Dove (1947) reported that judges were also chosen for their ability to differentiate between pairs of samples which had been subjected to "greater concentration or dilution, or exhibit such differences as exist between varieties or between different recipes, or show the effect of differing methods of harvesting, preparation, cooking, seasoning, canning or storage." To qualify as a good judge by this method, one had to be able to recognize differences in eighteen or more of the twenty pairs of samples presented.

A method used by Trout and Sharp (1937) in determining the effect of a large number of factors, such as temperature of sample, on accuracy is well adapted for use in selecting judges. Samples were diluted to give ten concentrations of flavor, and judges were asked to rank them in order of their concentrations.

Accuracy of placement of samples was estimated by calculating the

coefficient of correlation between the known rank and that given by the judges. These authors pointed out the importance of including an adequate series of placements. The probability of perfect order by random selection for series consisting of 1, 2, 3, 4, 5 or 6 samples is 1 chance in 1, 1 in 2, 1 in 6, 1 in 24, 1 in 120, or 1 in 720, respectively. They point out also that in judging a series of samples it is often easy to select the extremes. This end selection reduces the number of unknowns of the series and increases the probability of correct order placement. Prior to 1937 the summation-of-difference-in-rank method was used for summarizing this type of data, instead of calculating the correlation coefficient as described above. Trout and Sharp (1937) and also Baten and Trout (1946) concluded that the summation-of-difference-in-rank method is of little value because there are so many ways of making large scores as compared to those for making small scores.

c. Checking Performance on Tests. Selection of a judge for use on a panel is usually made on the basis of relatively few trials. For this reason and also because there is no assurance that acuity and consistency will continue, it is advisable to provide for some means of continually checking the judge's performance. If his performance is not satisfactory, his results should be discarded. This procedure seems to the authors to be as justifiable as discarding results obtained with a pH meter that is found to be faulty. It is, of course, necessary that all of the individual's data for the period be discarded. The method of checking depends on the design of the experiment.

Procedures for checking the ability of a judge to reproduce his own scores have been suggested. Dawson *et al.* (1945) used average deviations between each individual's duplicate scores for a long series of samples, and also scores given a control sample presented at each scoring period. Lowe and Stewart (1947) suggested the use of deviations of an individual's scores from his own mean score for each sample. Sharp *et al.* (1936) used deviations in scores occurring between the first and second testing of the ***same samples*** presented coded in different order. All of these methods measure reproducibility of an individual with himself but do not relate reproducibility on one sample to differences between unlike samples. An individual who consistently uses less than the total scoring range will appear to score samples more consistently than individuals who use the total range available.

To evaluate consistency Bliss *et al.* (1943) used the correlation coefficient for the individual's first score with his own duplicate score for a series of samples of varying quality. This method relates reproducibility to the magnitude of differences shown for unlike samples and is, there-

fore, a better criterion of an individual's ability than reproducibility alone.

In the triangle-difference test, the duplicates serve as a check. In the ranking method as used by Handschumaker (1948) the five controls with known order serve as a check on the individuals.

With numerical scoring, when replicate samples are not used and when conclusions are based on the variability of individuals' scores for one series, good reproducibility is required of an individual's scores both with himself and with other members of the panel. Sharp *et al.* (1936) used deviations of the individual's scores from the mean score for the panel. Marcuse (1945, 1947) described a control chart method and applied it to data for dried eggs. The reader is referred to the two papers for description of the method. The method measures agreement of an individual with others, since the data for individuals who failed to score the control sample within the pre-established limits were discarded. Moser *et al.* (1947) applied the control chart method in their work with oil. They calculated the correlation coefficient, the regression coefficient or slope, and the standard error of regression for individual's scores with average scores for the remainder of the panel. These statistics together give considerable information regarding the individual's agreement with the panel and relate it to differences for unlike samples.

Consistency of trained selected judges in scoring of egg samples is illustrated in the following examples. In an investigation reported by Sharp *et al.* (1936) scoring was based on a 1-5 point system with 0.25 intervals permitted between points. Four judges scored a series of eighteen eggs. After the eggs were scored once, they were shuffled and lined up anew on numbered spots on the table. The average difference in score between the first and second testing of the eggs was 0.25, 0.17, 0.24 and 0.26 for each of the judges. In an experiment covering a period of

TABLE I

Averages of Daily Deviations of Judges' Scores *

Judge	Standard deviation	
	Between the first and the second score	From the mean score of all the judges
1	0.352	0.387
2	0.401	0.386
3	0.461	0.387
4	0.454	0.408
5	0.345	0.413

* Sharp *et al.*, 1936.

one year, and including 1,928 opinions expressed by each judge, the averages of the daily deviations of the judges' scores were as given in Table I. In this series the judges gave the same score to the eggs on both tastings 30 to 45% of the time. One instance is reported in which a judge, scoring eggs with a good range of flavor, gave the same score to 15 of the 18 eggs both times he judged them.

The ability of the judges to score consistently the unknown fresh eggs in a series is shown in Table II. Scores ranged from 1 to 5, with 1 the best possible score. As has been noted in other experiments, the standard deviations for high quality eggs (Table II) were less than for those of poor quality (Table I).

TABLE II

Mean-Flavor Scores of the Unknown Fresh Eggs *
(averaged in groups of 8 eggs each)

Group	Number of opinions	Mean-flavor score	Standard deviation
1	78	1.035 ± 0.007	0.094
2	80	1.072 ± 0.010	0.136
3	79	1.117 ± 0.011	0.146
4	79	1.047 ± 0.007	0.096
5	79	1.079 ± 0.009	0.127
6	55	1.177 ± 0.014	0.155

* Sharp *et al.*, 1936.

Levin (1943) conducted an experiment to determine how consistent the individual panel members were in judging flavor of replicate samples of dried whole eggs and how well they agreed with each other in their rating of the samples. Twenty-four composite samples of dried eggs were prepared and scored under similar conditions at four different periods. Analysis of variance was used to determine the significance of the results. The individuals showed consistency with themselves and others in scoring the same samples at different times and showed differences between samples of different quality.

McCammon *et al.* (1934), using a scoring range of 1 to 5 points for the flavor and odor of eggs, found that the judges were able to reproduce their results with a high degree of accuracy. The average deviation for individuals' scores ranged from 0.12 to 0.28, with an average for all subjects of 0.20.

d. Attitude. It is generally agreed among those who direct research using difference tests that the attitude of the judges is of great importance to the success of the experiment. The problem may be separated into two parts: one is concerned with the creation and maintenance of interest and

confidence of individuals on the panel; the other is concerned with the avoidance of prejudice which may result where there is too much knowledge about the problem under investigation. As is true of other analytical methods, the judge, who in reality is an analyst, should be a careful observer who is conscientious and willing to concentrate on the job at hand.

Interest in tests may be created and maintained by informal conferences where results are presented (Weaver, 1939; Moser *et al.*, 1947) and by the use of dilution tests that give the judges confidence in their own ability (Trout and Sharp, 1937). In the opinion of the present authors, the triangle test (p. 222) challenges the judge to do his best. If he commits himself to the decision that there is a difference between samples, he dislikes failing to identify the duplicates.

One problem that faces the person directing research is that of giving the panel enough information to sustain interest but not so much as to prejudice it. Bengtsson and Helm (1946) state that in most instances it is advisable to acquaint the panel with the objects of investigations; however, information should be withheld if it would lead to preconceived opinions about samples. Some have withheld all information (e.g., Dove, 1947); others feel that requests to report off-flavors influence the judges to "find" off-flavors (Asmundson *et al.*, 1938). A tendency of judges to be influenced by factors other than those under consideration has been mentioned. Scott-Blair *et al.* (1941) found that in judging cheese, the experts (cheese-makers) had difficulty in judging firmness, probably because they were influenced by other characteristics that they knew were important. The scores of the nonexperts correlated well with a mechanical test of firmness. Vail and Conrad (1948) preferred scoring of odor rather than flavor because, among other reasons, juciness and tenderness of the meat were thought to influence the scores for flavor.

It is obviously necessary to code any samples presented to judges. The problem of coding was handled in a rather elaborate fashion by Cover (1940) in the paired-eating method on meat. The order of presenting samples was randomized, and the samples were presented in different order to different judges. Since the judges scored their samples while sitting around a table, and since they could talk freely about the samples, such a method was necessary to prevent identification of the products. Even though the samples were coded, free discussion while samples are being scored seems inadvisable, since judges can be influenced by the comments of others.

Masking all characteristics except the one under consideration is another means of minimizing prejudice. In the case of color this can be done by adding dyes to the food (Bohren and Jordan, 1948), by serving

the food in dark or opaque utensils (Gray *et al.*, 1947), and by using special lights. The Quartermaster Food and Container Institute for the Armed Forces in Chicago is equipped with such lights (Dove, 1947). Bengtsson and Helm (1946) discuss some of the disadvantages of masking differences in appearance of samples:

> Investigations on beverages served in black glasses show that it is appreciably more difficult to detect differences in flavor when the vessels are opaque. Even if the beverages do not differ in color, it seems that the visual impression reinforces the sense of taste in some way. The disturbing influence of black glasses is presumably an illustration of the previously mentioned fact that all conditions of an irritating nature reduce the capacity to detect differences in flavor. However, if drinks of different color are to be compared, it is impossible to avoid the use of dark glasses.

e. Fatigue. The problem of fatigue includes not only the question of the actual tiring of the sense organs but also the possible mental or psychologic fatigue that results if the panel is presented with too many samples at one time or too often during a single day. Some workers recommend judging odor first and then tasting the samples in order of increasingly strong odor (Moser *et al.*, 1947; Bengtsson and Helm, 1946; Platt, 1933). It has been the experience of the present authors that, after tasting one sample of sulfited apples, it is difficult to distinguish between the flavors of succeeding samples. Because sulfite flavor is not noted until it has been in the mouth for some time, judges should allow a longer interval than usual between tasting of sulfited samples. When judging spices which have a strong flavor, Cartwright and Nanz (1948) incorporated them in a basic bland formula, such as white sauce.

Bengtsson and Helm (1946) ably describe the factors involved in fatigue as follows:

> The gustatory nerves, and especially the olfactory nerves, become fatigued very rapidly and cease to react when subjected to prolonged stimuli. For this reason the number of samples to be examined on the same occasion must be very limited. The stronger the taste and odor of a substance, the smaller the number of samples an individual can test before he must rest. In some cases the normal capacity to react can be rapidly restored by rinsing the mouth with water or by eating white bread. In other cases it is necessary to allow about an hour to elapse between tests.

These authors also noted that some substances affect the gustatory nerves in such a way that subsequent taste sensation is abnormal.

It has frequently been observed that the first sample presented makes a more pronounced impression on the judges than subsequent samples; retasting is not always a successful method of overcoming the first impression. One way of attempting to minimize this effect is to serve a sample, not a part of the experimental series, before the series is scored. Such a plan was used in the present authors' laboratories in experiments

on grapefruit juice, since the first sample judged always seemed more sour than others.

There is evidently a considerable range in the number of samples that can be successfully evaluated without fatigue, the number depending upon the product being studied and the judge. Sharp *et al.* (1936) found that, of four judges, only one showed evidence of fatigue with 160 judgments of egg flavors within a period of 2 hours; there was no increase in deviations in the scores of duplicates of any of the four judges with 120 judgments. These particular judges felt that unreliability was caused by "mental fatigue, lassitude or weakening of the power of concentration" rather than by dulling of the sensory organs. The unusual interest of the judges in this study was undoubtedly of help in maintaining such consistent results over a large number of samples; such a situation could not be expected with every panel.

McCammon *et al.* (1934) in a study on eggs found that judges became unreliable when more than ten or fifteen samples were presented at one time. Asmundson *et al.* (1938) presented only three or four samples of turkey at one time in order to prevent fatigue. Moser *et al.* (1947) used the paired method of comparison for oil samples, because they felt that sensitivity could be better maintained with only two samples. Bengtsson and Helm (1946) say that the number of samples to be graded at one time should be severely limited and should not exceed two or three. They state that in the dairy industry one hundred samples of butter are tasted at one time, but "with such a high number it is inconceivable that small differences in flavor can be detected. This practice cannot, therefore, be recommended for experimental work."

To avoid fatigue Handschumaker (1948) recommended smelling a series of reverted soybean oils rather than tasting them. He claimed that the discriminating powers of persons who tasted five or six oils were usually lost before a series was completed; whereas, sensitivity to odor could usually be retained throughout the series, or, if it were lost, could usually be restored by a few breaths of fresh air. In tests conducted by the Bureau of Human Nutrition and Home Economics (Anon., 1943) solutions were discarded rather than swallowed, since it was thought that such a practice might prevent fatigue. In the work of Moser *et al.* (1947) no samples of the soybean oil were swallowed, regardless of their nature.

It is obvious that if the judge is required to score several qualities of each product, mental fatigue will occur sooner than if only one factor is scored.

The question of the most suitable time of day for evaluation of food products is also concerned with the problem of fatigue of judges. In most cases the judging is scheduled for the middle of the forenoon or the

middle of the afternoon, but Helm and Trolle (1946) permitted beer judges to come at any time that was most convenient. Baten (1946), in an effort to find the time of day most suitable for testing of apples, presented judges with slices from the same apples before and after a meal, and at every hour of the day. The results of this one study showed no advantage for any time of the day.

Goetzl's (1948) investigations on individuals' thresholds to coffee odor are a guide to time of day best for odor testing. He reports as follows:

> We measure the amount of air carrying the odor of coffee which is necessary to produce the sensation of coffee odor in our subjects. The smallest volume of odorous air, which upon injection into the nostrils produces the sensation 3 times in succession is recorded.
>
> Threshold determinations are performed between 9:30 and 10 and between 11:30 and 12 o'clock in the morning and between 12:30 and 1, 2:45 and 3:15 and between 4:30 and 5 o'clock in the afternoon. Our subjects eat breakfast before 9 A.M. and dinner after 5 P.M.; lunch is eaten between 11:30 A.M. and 12:30 P.M.
>
> Meals are preceded by a period of increased olfactory acuity and followed by a period of decreased acuity. When food is ingested between meals, the precibal increase fails to occur; when a meal is omitted the postcibal decrease does not develop. Between 50 and 100 persons have been tested in the last 2 years; test periods vary from a few weeks to more than 1½ years.

This report is the only one that the present authors have found giving evidence that odor (and presumably flavor) can be judged more successfully at one time than another.

f. Judging Odor. Aspects of flavor may be judged by sniffing a food as well as taking it into the mouth, because flavor is considered a combination of odor and taste. A number of authors have reported better results with judgments of odor than with flavor. Gray *et al.* (1947) found this to be true with beer; McCammon *et al.* (1934) and Gaebe (1940) found similar results with eggs. Handschumaker (1948), with reverted soybean oil, found that the less acute individuals on their panel often found it necessary to taste the samples because of the increased concentration of the factor obtained in this manner; however, these individuals usually lost their discriminating powers before reaching the end of a series of samples. Most of the discriminating judges succeeded in making their decisions after smelling the samples rather than tasting them. Vail and Conrad (1948) found better correlation of aldehyde and peroxide values of stored, frozen chickens with aroma scores than with scores for "flavor plus odor." They pointed out that within-sample variation was eliminated in odor scoring because all judges sniffed the entire casserole of chicken. Learning to judge flavor was considered more difficult, because

variations in juiciness, tenderness and perhaps other factors might influence flavor scores.

3. *Conditions of Testing*

a. Environment. Since the detection of small differences in food quality demands close attention to details, it is desirable to conduct tests in an environment conducive to concentration. Of primary importance is prevention of interruptions or distractions while a panel is judging samples (Trout and Sharp, 1937; Bengtsson and Helm, 1946; Lowe and Stewart, 1947). Disturbing factors decreased accuracy of the judgment of concentration of solutions, and distractions had a greater influence when a difficult series was being judged than when the series was relatively easy (Trout and Sharp, 1937). The use of individual booths where judges may be seated comfortably while they are examining the samples has been recommended by several workers (Moser *et al.*, 1947; Dove, 1947). Such a plan has the advantages of eliminating distractions and of avoiding collaboration in judging. It also prevents the influencing of judges by remarks or facial expressions of other members of the panel.

Distraction can be reduced further by the use of air-conditioned rooms to provide constant temperature and humidity and an atmosphere free from odors that might influence the panel (Moser *et al.*, 1947; Dove, 1947; Bengtsson and Helm, 1946).

In order to judge color satisfactorily, suitable lights and background for them are necessary. Eastmond (1948), on the basis of wide experience with color measurements of foods at the Western Regional Research Laboratory, makes the following recommendations: "Light of intensity from 30 to 50 foot-candles at the table surface should be diffused uniformly over a large enough area to permit comparison of several samples with allowance for free movement of samples. Light similar in spectral character to a moderately overcast north sky is preferred, although the specifications need not be as exacting as recommended by Nickerson (1946) for standard grading work. Satisfactory illumination can be secured with incandescent lamps and special blue glass filters. It is desirable that the table and surrounding wall areas be painted neutral gray of Munsell value about N 7/, *i.e.*, a gray which reflects about 40-45 percent of the light that falls on it."

b. Utensils. The selection of utensils to be used in serving experimental samples is usually governed by two considerations; the requirement of uniformity and the requirement that the utensil shall impart no flavor to the food being judged. The need of supplying a uniform background for the samples is obvious, since the appearance of the product may be affected by the color, texture, size and shape of the utensil. The utensils

used should be completely odorless and tasteless. Plates, knives, forks, spoons, or glasses used in tests must be carefully washed, and precautions should be taken that they do not acquire flavors during storage in cupboards or elsewhere. It is well known that paper utensils often impart flavor to products. McCammon *et al.* (1934) felt that glass rods were superior to wooden or metal spoons because the former did not impart any taste to the egg samples being judged.

c. Sample Size. Generally equal amounts of each sample are presented to all members of a panel; however, the question has been raised whether the judges should be limited in the amount of sample. Most investigators who have discussed this matter state that the judges should be allowed as much of the sample as they need in order to come to a decision. Of course there are often limitations in amount available because of limitations of supply of raw material or because of limitations of comparable parts in experimental samples, as in judging specific muscles in meat and poultry. Weaver (1939) in studies on milk flavor permitted judges to take as much of the samples as they desired. He found that they usually used 8 to 12 ml., but they used less when the odor of a sample indicated that it was rancid.

Gray *et al.* (1947), because of limitations in amount available, served judges 30-ml. samples of beer. Trout and Sharp (1937) found that in placing a series of solutions in order of concentration, it was necessary for judges to recheck their judgments a number of times. When allowed free access to solutions, they used approximately 6 ml. per taste. The number of times the solutions were retasted depended upon concentrations involved, the differences in concentration in the series, and the number of samples in the series. The larger the number of samples and the more difficult the series, the more times retasting was necessary. The amount of solution likewise varied with the difficulty of the series, and the authors felt that limiting the amount of solution available would have been a handicap in the effort to achieve the greatest accuracy. The amount of material available to judges should be sufficient to allow for comparison of various samples with one another and for rechecking judgments. When a large number of comparisons is required, a greater amount of material should be provided.

Although the number of bites, sips, or sniffs of one sample usually is not limited when quantity of material available permits repetitions, it may be desirable to use a uniform quantity of material per taste or odor trial. Presentation of samples of uniform size has been achieved by various means. Burettes of the automatic delivery type may be used for control of size of samples. McCammon *et al.* (1934) attempted to present samples of egg yolk of uniform size by using a glass rod for sam-

pling, but they found that the size of the sample adhering to the rod was affected by the viscosity of the yolk and the depth of the yolk in the glass. For sampling oil, spatulas have been constructed by flattening and corrugating the ends of glass rods. The spatulas are dipped in oil according to a definite procedure and weighed. Only the spatulas found to pick up a uniform amount of oil are used in experimental work. The importance of presenting samples of uniform size will readily be appreciated by investigators who have watched the amount of a very desirable sample disappear more quickly than others in a series being judged by an appreciative panel. If several judges follow one another in judging the same set of samples, differences in amounts of samples may be interpreted by the last judge as an indication of preference of those preceding him. Trout and Sharp (1937) in studies on various solutions emphasized that "care was taken to see that the beakers carried a uniform level of solution at the beginning of each placement trial."

d. Sample Temperature. The matter of choosing a satisfactory temperature for a food or beverage being tested involves several factors whose relative importance must be weighed according to the purposes and limitations of each problem. One question is whether the product should be presented at the temperature at which it is normally served or at a temperature that will bring out maximum differences in samples. Other aspects to consider are facilities for maintenance of desired temperature and possible difficulties from lack of sensibility at extremes of temperature. It is obvious that samples to be compared should be at the same temperature.

Much of the early literature on temperatures used in tests of pure solutions has been reviewed by Trout and Sharp (1937). They themselves conducted such studies. Using temperatures of 2, 21 and 35°C. (35.6, 69.8, and 95.0°F.) for solutions of different concentrations of sodium chloride, sucrose, lactose, lactic acid, and quinine sulfate, they asked a panel to place the solutions in order of concentration. They found that each of four judges was able to place the sodium chloride solutions with a higher degree of correlation at 21°C. (69.8°F.) than at either of the other temperatures. With sucrose and lactose solutions higher correlations were obtained at 35°C. (95.0°F.) than at either of the other temperatures. With lactic acid solutions, both of the higher temperatures were favored over 2°C. (35.6°F.) but there was no difference at either of the higher temperatures. The results with quinine sulfate seemed to point to 21°C. (69.8°F.) as being the best temperature. The authors felt that "distraction due to a slight pain" might have influenced results at the temperature extremes and that temporary anesthesia was important at the low temperature. A temperature of 21°C. (69.8°F.) was

believed to provide fewest "distracting influences." In further studies of the temperature effect in detecting oxidized flavor in milk, these authors indicated that a temperature of approximately 21°C. (69.8°F.) was most satisfactory.

Bengtsson and Helm (1946) comment as follows:

> The optimum temperature for the perception of taste is generally considered to be 20°C. or somewhat higher; some workers give figures as high as 37°C. When 50°C. is reached, the gustatory nerves cease to function. Thus an investigation of coffee, for example, should not be performed with excessively hot samples. On the other hand, when the temperature is below 15°C., the taste effect is also strongly reduced. In selecting the experimental temperature, it must be decided whether the object is to detect differences in flavor under optimum conditions or to determine the reaction to consumption in the normal manner.

Punnett and Eddy (1930) disagreed with the above statement regarding the temperature at which coffee is judged. In discussing the two flavors of coffee, freshness and staleness, they state that "these flavors can readily be distinguished by using different parts of the mouth for tasting—the back of the tongue for the coffee freshness and the sides of the tongue near the front for the staleness. Even if both are present at the same time, which may be the case in a coffee which is only slightly stale, the coffee freshness can be observed as described, especially when the beverage is as hot as bearable in the mouth. The staleness is better noted when the beverage has been cooled off considerably."

Dove (1947) also mentions that "different temperatures of the foods bring out different tastes and different responses to texture, juiciness, etc." The Vail and Conrad (1948) plan for scoring odor of cooked chicken included scoring at three intervals after removal from the oven, one immediately after cooking, one about 15 minutes after removal from the oven, and one after the chicken had stood for 5 additional minutes with the cover removed from the container. The latter period was believed to correspond most nearly to conditions as they would ordinarily exist at the time of eating. Trelease and Koonz (1945) felt that aroma differences of cooked poultry were most pronounced immediately after the birds were removed from the oven. Marble *et al.* (1938) found a close correlation in scores when turkey was scored while it was still hot and after it had been allowed to cool.

Various temperatures have been found to be satisfactory for the testing of different commodities. In their experiments on beer, Helm and Trolle (1946) served samples at 12°C. (53.6°F.) and Gray *et al.* (1947) used a temperature of 11°C. (51.8°F.). Weaver (1939) in studies on factors affecting milk flavors conducted a test on duplicate samples of milk at 21 and 35°C. (70 and 95°F.). He found that in most cases the warmer

sample received the lower score, probably indicating that the warmer temperature intensified the odors present. In serving halves of apples at different temperatures, Baten (1946) found that most of the judges considered the cold samples (0.6°C.) (33°F.) to be more juicy than those at room temperature (22.2°C.) (72°F.). The same was true with pears. He also found that a poor quality dessert apple served cold was considered equal to a good quality dessert apple served at room temperature. Moser *et al.* (1947) state, "the odors and flavors of oils are more readily detected if the oils are warmed." In order to maintain a constant temperature in the oils under examination, they had a special table constructed with a top consisting of an electrically heated aluminum plate.

e. Removing Flavors from the Mouth. Rinses of various kinds have been proposed from time to time as an aid in increasing accuracy of flavor judgments. Their use has been advocated particularly in cases in which strongly flavored substances are under consideration, and where it was felt that the flavor of one sample would influence the reaction to the succeeding samples. The most universally recommended rinse is water, usually served at body temperature. Some have suggested white bread, acetic acid, apple slices, crackers. No reports have been found regarding the superiority of any of these materials for use with particular products. Crocker (1945), after considering the use of such materials, states that "something may be gained by these artifices, especially the rinsing with water, to remove adherent material, but the best general technique is to allow the saliva to lave the taste buds in a natural manner and thereby to prepare them for the next tasting."

Trout and Sharp (1937) studied the amount of sodium chloride solution retained after judges had held 10 ml. of a 1% solution in their mouths for a period of 15 seconds and then rejected it. Approximately 10% of the salt solution was retained in the mouth after the solution was rejected. The first rinsing with 10 ml. of distilled water removed approximately 69% of that retained, and after two rinsings approximately 96% was removed.

When substances are used to remove flavors from the mouth, it probably is necessary to wait for the effect of rinses to disappear before judging the next sample. The usefulness of rinses is open to question, since no report of an experiment in which samples were judged with and without rinses was found.

f. Time Allowed for Judging. Very little information has been published on the amount of time permitted for judging samples; the conclusion therefore appears justified that in most cases no time limit is placed on judging. Helm and Trolle (1946) permitted a maximum of 15 minutes for any one set of three beer samples. Trout and Sharp (1937) measured

the amount of time required for reaching a decision on a single sample of a pure solution and found that it usually averaged between 5 and 10 seconds. When judges were asked, as part of the study, to hold a sample in their mouths for 15 seconds, they felt the period to be unusually long. In most of these studies the judges were able to place a series of ten samples in order in from 3 to 6 minutes. These authors reviewed the early literature on the reaction time to taste stimuli and on the time required for making judgments.

IV. Chemical and Physical Tests as Supplements to Sensory Difference Tests

Chemical and physical tests on foods are valuable supplements to panel tests, but few are available that even partly replace objective sensory tests at present. It is probable that the chemical and physical tests are more reproducible and less time-consuming than the difference tests. However, the latter may not be true, since many different tests would be required to measure the myriad changes that occur during processing and storage. Usually it is desirable to show that a chemical or physical test measures a characteristic that correlates with something detected by panels. Furthermore, with any change in experimental conditions, the relationship must again be established. Yet it is desirable to replace panel tests with chemical or physical tests whenever possible.

Some examples of the status of chemical and physical tests as they are related to sensory difference tests will be discussed. When most dried eggs on the market contained about 5% moisture, salt-water fluorescence was widely used as a measure of palatability of stored dried eggs (Pearce and Thistle, 1942; Thistle *et al.*, 1943a). Then there developed a growing appreciation of the fact that this measurement by itself was not a reliable criterion of the flavor of the powder (Stewart *et al.*, 1943; Fryd and Hanson, 1945). Boggs *et al.* (1946) then found that salt-water fluorescence values correlated well with palatability scores for powders containing 4 to 5% moisture ($r = +.97$) but correlation was lower for powders containing less than 2% moisture ($r = +.71$). They also showed that lipide fluorescence values, on the other hand, correlated well with palatability scores for powders of either high or low moisture content. Later Fevold *et al.* (1946) obtained evidence that salt-water fluorescing substances arise in the white of the egg, which do not contribute materially to loss of palatability of whole egg powders during storage. The yolk, however, does deteriorate markedly and imparts the characteristic "stored" flavor in the dried whole egg. Furthermore, it was indicated that the phospholipide fraction of the lipides of the yolk is the source of substances imparting off-flavors and off-odors to stored egg powders.

In the field of meat research, considerable work has been done in an effort to correlate physical measurements of tenderness, texture and juiciness with the panel scores for the same qualities. Many devices for measuring tenderness have been proposed, but the one which has shown the most successful results is the modified Warner-Bratzler shearing apparatus (Warner, 1928; Bratzler, 1933). This device measures the force required to cut across a cylinder of meat of known diameter or, in the case of poultry, across certain muscles. High correlations were found between judges' scores for tenderness and shear force values for many meats (Mackintosh *et al.*, 1936; Brady, 1937; Satorius and Child, 1938; Stewart *et al.*, 1941; Shrewsbury *et al.*, 1942; Paul *et al.*, 1944; Ramsbottom *et al.*, 1945).

Other physical tests that have been used to explain differences in tenderness of meat found by panels are determination of size and number of fibers in muscle bundles, estimations of the proportion of elastic and collagenous tissue, and histological changes, nodes, internodes, and breaks in muscle fibers (Ramsbottom *et al.*, 1945; Hanson *et al.*, 1942; Lowe, 1948; Paul *et al.*, 1944).

Although most workers have found no relation between press fluid and juiciness of meat, as evaluated by judges, Tannor *et al.* (1943) found high correlation under certain experimental conditions. They reported that the method can be used for beef, lamb, and pork, at least in cases where the variation in juiciness is due to animal production factors or to differences in the internal temperature of the cooked meat.

Sweetman (1936) reviewed the literature and her own work on chemical and physical tests related to mealiness of potatoes. The tests discussed were cell-bursting, soluble and insoluble pectin content of uncooked potatoes, total nitrogen, protein nitrogen, starch and dry matter content, hydrophilic quality of potato starch when gelatinized in distilled water and under the conditions within the tuber, size of starch grain, external appearance, and internal anatomy. She found that although there was a general tendency for some of the tests to correlate with mealiness, this relationship was too irregular for predictive value.

A similar situation exists for oils. The literature is too voluminous to summarize here, but a statement by Beadle (1945) indicates the status of the problem. In discussing the induction period for oxidative rancidity he states that there is an implication "that there is a reliable test for the condition of rancidity." He further states, "Such is not the case. Without stopping here to summarize the available literature, attention is called to the fact that in the final analysis, rancidity must be detected through organoleptic observation. . . . No one chemical test has been devised which can accurately measure and correlate all of the factors

which act simultaneously to produce the odors and flavors called rancidity."

Similar illustrations, in most cases less comprehensive than those mentioned above, could be cited for most foods.

VI. Discussion

1. Comparison of the Difference Tests

The intrinsic differences between scoring, ranking, dilution, paired, and triangle difference tests are not large. Any of them can lead to accurate estimates of differences in foods or can fail depending on the way they are applied. The paired and triangle difference tests have in general been well conducted. In these tests no more than three samples are evaluated in any one judging period; only one factor is judged or all but one are masked; sufficient replications are used as shown by statistical analysis of their data. In the few papers published on the ranking method, the number of samples and charcteristics judged in one period have been limited.

The dilution method as it has been applied probably has given the most accurate results of any of the methods. Judgments have been based on the difference between samples, both of which were present at the time of grading; the decision requested was simple; the samples had rather good flavor, were not strong or objectionable; there was no important difference between the samples except flavor; the score was determined from a line established from a trend of the results. The slope established in preliminary trials and the standard error of regression further checked the line from which the score was read. Still greater accuracy probably would have been obtained had the planners of this dilution experiment selected and trained judges and decreased the number of samples scored in one judging period.

The scoring method has been used more than any of the difference tests, and practically every type of experimental plan has been reported. There has been a tendency to have too many variables and to attempt to get too much information about each sample with too little regard for the factors of accuracy. Many scoring procedures would be improved by incorporating more of the good features of the other methods, namely, limiting the number of samples and characteristics evaluated in one judging period, presenting at one time all samples for which comparative data are desired, and scoring sufficient replications.

The principle of dilution offers considerable promise as a research tool for flavor and odor testing. It could be used in any of the methods. It is especially well adapted to the difficult problem of storage studies for

which all other approaches have shortcomings. However, the lack of suitable standard material will prevent its use with many foods.

The very important question of how much time and material are used in the various sensory difference tests is primarily a matter of the number of treatments to be compared in an experiment, the number of replications, and the number of samples compared at one time. The number of treatments about which comparative data are desired is dependent on the purpose of the experiment and will not be discussed further. The number of replications has been discussed (p. 232). Assuming a constant number of treatments and replications, the time and materials used will depend upon the experimental design as it relates to the number of samples compared at one time. In the paired and triangle tests the design limits the number to two; and the triangle test, of course, uses 50% more material than the paired test. In the scoring method, the number of samples could be limited to two. For any large number of treatments, however, a comparison of only two at a time is very costly. For example, if information regarding the comparison of each sample with each other sample in a series of five treatments is desired, ten judging periods are required. Accuracy is believed to be increased by this procedure. More research is needed on the effect of number of samples on accuracy.

2. Status of Difference Tests

It is of interest in a survey of a field of work to weigh its contributions and weaknesses and to consider trends and needs. The authors will, therefore, give their opinions regarding these subjects as they are related to difference tests.

It is obvious that this type of investigation can contribute useful information about foods. Considerable knowledge about the causes of changes in food qualities that can be judged by sensory methods has been obtained; the difference tests are the only independent methods that could have produced this information.

The principal weakness in this work is that too many investigations are carried out without even moderate precautions to obtain accurate results. One reason for frequent failure to obtain the results desired is that the panel results are often considered as only minor parts of investigations primarily concerned with chemical, bacteriological or other aspects of foods.

Accurate difference tests are costly both in time and materials. Some investigators simply have failed to pay the necessary price to secure accurate results. Many practical evaluation plans must be compromises between the amount of work that can be done and the amount of information desired. As a matter of principle, it is better to proceed from a few

well-established facts to general knowledge than to conjecture on the basis of results of poorly conducted, numerous experiments.

The working time of the judges can be decreased in several ways. The judges might be asked in some investigations to evaluate only key quality factors. Treatments which protect flavor, for example, often protect color and other qualities too. One person who is familiar with the product under consideration could attend judging sessions in which a key factor is evaluated and make observations that may be of interest in later work. This person might also eliminate obviously poor samples in preliminary trials. Chemical and physical measurements may be used to decrease the work of judges. A physical measurement of color, for example, could be made on all samples; judges would be used to determine the magnitude of color differences they can detect with selected samples.

Difference tests usually require the knowledge and skills of workers in several fields. Often one or more of the fields is not represented when the plan is made, thus weakening the experiment. To develop a good investigational plan, usually the following are required: knowledge of (1) raw material, its composition, and the effect of various cultural and handling practices on it, (2) commercial processing procedures and the cost and equipment involved in changes of procedure, (3) principles of food preparation, and (4) designs of plans suitable for the judges available and for statistical analysis of the data obtained.

A conspicuous recent trend in this field of work is the tremendous increase of interest in it. This trend is probably due, in part at least, to the urgent need during World War II for more and better information about foods. As a result of the increasing interest in the subject, differentiation in the purposes and methods of consumer preference and objective sensory tests is being clarified.

The entire field is seriously handicapped by the lack of knowledge of the effect of different procedures used in conducting the tests. A few recent papers have shown the increase in information and accuracy that can be obtained by carefully applied methods. These should stimulate the development of better techniques and plans.

VII. Summary

The literature on the testing of differences in foods by selected judges is reviewed and evaluated for the purpose of bringing together information of value to individuals engaged in food research. In these tests judges express differences in flavor, texture or other components of quality in terms of scores, ranks, or the numbers of samples that are alike or different. Procedures applying specifically to each method of expressing differences are discussed.

To obtain high accuracy, experiments should be designed to: (1) minimize within-sample variation, (2) limit the number of samples and characteristics of each that are judged in one period, (3) submit at one time all the samples for which comparative data are desired, (4) relate experimental samples to control samples, (5) mask all characteristics except the one under consideration, (6) eliminate samples of strong odor or flavor when possible, (7) judge sufficient replicates to show that trends can be repeated or replicate sufficiently that data can be analyzed statistically. It is also important in judging any characteristics that the terms used mean the same to all persons.

To obtain accurate judging it is important to: (1) train judges to distinguish differences in specific characteristics, (2) select judges for acuity and consistency, (3) check performance to determine whether judges continue to be acute and consistent, (4) maintain interest and confidence while avoiding prejudice, and (5) avoid fatigue by limiting the number and kind of samples and factors scored.

The environment and other conditions of testing are recognized as important for accurate results.

Few well-established facts regarding techniques and plans for sensory difference tests are available.

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The Chemistry of Fruit and Vegetable Flavors

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I. Introduction

Flavor is important. A food may be nourishing and have many vitamins, but if flavor is missing all the pleasure and enjoyment obtained from eating it is eliminated.

Flavor stimulates the flow of gastric juices. The importance of this was illustrated by Pusey (1940) in the report of his own case. He had lost twenty pounds in four months' time due to the loss of his sense of taste. He did not lose his appetite, but the loss of taste prevented him from eating. Finally he realized that the symptoms coincided with his acquisition of a new dental plate, which covered most of the hard palate. On removing the plate while eating a steak which appeared to be devoid of flavor, he was instantly rewarded by the return of his taste. The condition was removed only by substituting an upper plate which left most of the hard palate uncovered. This phenomenon is difficut to explain as there are no taste buds located on the hard palate.

The popular conception of flavor is synonomous with taste, perhaps because we have no separate verb to describe the act of determining the flavor of a substance. When we taste we are actually judging the flavor, for flavor is an elusive blend of sensations. It includes not only taste but also the sense of smell and touch.

Taste itself comprises the four main components of salty, bitter, sweet, and sour. The salt and sour tastes differ from the bitter and sweet in that the latter are usually nonionic, especially in the more commonly occurring cases of sweetness and bitterness.

Saltiness is best exemplified by sodium chloride. Although this salty taste is an ionic effect, it is not quite so simple as it would first appear. If saltiness were caused by the chloride, the addition of hydrochloric acid to a solution of this salt should increase the saltiness by reason of the increase of the chloride ion. Fabian and Blum (1943), however, showed that hydrochloric acid had no effect, whereas citric, acetic, lactic, malic, and tartaric acids increased the saltiness.

On the other hand if saltiness in this case were due to the sodium ion, all sodium compounds would taste alike, which of course is not the case. Actually both the cation and the anion affect the taste.

The sour taste is associated with the hydrogen ion although it depends not only on the ion concentration but also on the total acid present (Harvey, 1920). The anion also appears to play a part because very dilute solutions of acetic acid are more sour than they would be if based only on hydrogen ion and total acid concentration.

Bitterness is shown by the alkaloids, the glycosides, and the tannins. Quinine and brucine are examples of the alkaloids; hesperidin in oranges and naringin in grapefruit illustrate the glycosides. The astringent taste of unripe apples and persimmons is due to tannins as is the bitterness of tea that has been steeped too long.

Sweetness, like bitterness, is exhibited by compounds which have little in common. For example, the polyhydroxy sugars, saccharin (*o*-sulfo-

benzimide), and the α-amino acids all have a sweet taste. Even dealing with the same type of compound we find variations in effects. For example, in the formation of maltose from two molecules of glucose there is a decrease in sweetness (Täufel, 1925), but by joining fructose and glucose to form sucrose, sweetness is increased.

It has been suggested at times that there are two additional taste senses; those of pungency and of meat. Pungency, however, is the result of stimulation of the heat receptors by such things as pepper and ginger. In a like manner *l*-menthol produces a sensation of coolness. Therefore, pungency cannot be rightfully considered as one of the "tastes," but is an accessory factor. Moncrieff (1946a) ascribes pungency to a separate sense called the chemical sense.

The meat taste is more a question of odor rather than taste, since eliminating the odor factor leaves the various meats indistinquishable from one another.

The sense of touch helps to differentiate a crisp, juicy apple from a tomato or a bite of cheese.

The exact nature of the taste mechanism is unknown, although a great deal of work has been done on the relation of taste and odor to chemical constitution. This is a subject in itself and will not be treated in this paper except for a brief mention of one or two facts to illustrate the complexities of the problem. For example, some stereoisomeric substances have entirely different tastes. Barral and Rane (1918) point out that although *d*-phenylamine and *d*-histidine are sweet, the corresponding *l*-compounds have a bitter taste. *d*-Tryptophane is tasteless, *l*-tryptophane is slightly bitter, and the racemic mixture is sweet. The chemical properties of optical isomers are identical; it would appear, therefore, that taste is not entirely chemical in nature.

Druce (1929) found that in a homologous series of organic compounds, bitterness increased and sweetness decreased with rising molecular weight. He also found *m*-nitroanaline to be sweet, but the *o*- and *p*- compounds were bitter.

These few examples will serve to illustrate the problems involved in attempting to correlate taste with chemical constitution; although there are some general relations that can be given for associating odor and taste to constitution, no hard and fast rules can be formulated. For a further discussion along these lines, the reader is referred to the excellent work of Moncrieff (1946a and 1946b).

There is also the question of individual variations in judging flavor. An unfamiliar substance given to a group for classification of its flavor will elicit a variety of opinions as to what its taste resembles. It is this

factor which requires the careful selection of the members of a tasting panel.

Most of the fruits and vegetables depend for their flavor on volatile components. The work on the identification of these is rather meager, and it is hoped that this presentation will serve to emphasize the need for more knowledge in this field.

Inasmuch as coffee, tea, and cocca may be considered as products of fruits or vegetables, they are included in this survey. Several miscellaneous products whose flavoring components have been investigated but slightly, are also discussed here in order to bring the information together in one place.

II. Discussion

1. Fruits

a. Apples. Thomae (1911; 1913) steam distilled apple peelings to obtain a watery distillate with a few drops of oil on the surface. Extraction with ether and subsequent treatment with alcohol yielded a crystalline material and a yellow oil that possessed the odor of apples. No attempt was made to isolate and identify any of the constituents.

Since most of the flavor and odor seemed to be in the peel, Power and Chestnut (1920) steam distilled 161 kg. of peel from 805 kg. of Ben Davis apples, obtaining 163 liters of distillate. A solid precipitated out which was not affected by concentrated nitric or sulfuric acids and which had a melting point of 63°C. (145.4°F.). They postulated this as being slightly impure triacontane, $C_{30}H_{62}$ with a melting point of 65.6°C. (150.1°F.).

The aqueous distillate was concentrated by repeated steam distillation to a small volume. Without further separation this material was saponified and from the alcohol portion an amyl alcohol was identified by oxdidation to a valeric acid, this in turn being identified by analysis of the silver salt. A trace of methyl alcohol was also present. There was no evidence to indicate whether or not any of these alcohols were present in the free state.

The acid fraction from the saponification contained formic, acetic, caproic, and small amounts of caprylic acids, the latter three being identified by analysis of their silver salts. Again there was no evidence as to whether or not these acids existed in the free state as esters.

In the parings from Springdale apples, the aforementioned workers, after hydrolyzing an extract, found ethyl, amyl, and a trace of methyl alcohol present. Ethyl alcohol was identified by the iodoform test so that the possibility of isopropyl alcohol being present was not eliminated.

In the acid fraction of the saponified product formic, acetic, and caproic acids were found.

In the steam distillate from crab apples Power and Chestnut found furfuraldehyde and acetaldehyde. The acetaldehyde was present to the extent of about 0.001% of the weight of the peelings. From the saponified products they identified methyl and an amyl alcohol, formic, acetic, caproic, and possibly a slight amount of caprylic acid; the latter's presence being based on the analysis of a slightly impure silver caproate.

The oil yields were 0.0035% of the weight of the peel for the Ben Davis apple and 0.0043% for the more odorous crab apple.

Power and Chestnut (1922) also claim to have found geraniol in the distillate from McIntosh apple peelings. On hydrolysis the oil had a rose as well as an amyl alcohol odor. On oxidation this was converted to a mixed lemon and valeric odor. A positive iodoform test indicated the possibility of acetone. Evaporation of the oxidized oil to remove volatile material enabled them to detect levulinic acid by the iodoform test.

As Power and Chestnut (1921a) point out, the title of the paper by Kodama (1920) "On Odor of Apples, Ethereal Oils Obtained from Leucic Acid" is rather misleading since none of the compounds prepared by this author have been shown to be present in apples.

Milleville and Eskew (1944; 1946) have reported that the volatile flavoring concentrates from fresh apples can be used to advantage commercially. In their process apple juice is flash distilled to remove 10% of the liquid. This portion contains most of the volatile flavoring constituents and is further concentrated on a fractionating column to give a water solution of the flavor constituents concentrated 100 to 150 fold. The flavoring concentrate can then be added to various food products such as jellies, sherberts, and beverages.

In the production of this volatile flavoring material, alcohol present in the juice is concentrated to a point where it exceeds 0.5%, which makes the product taxable and brings it under the jurisdiction of the Bureau of Internal Revenue. Because of this, workers at the Eastern Regional Research Laboratory are attempting to reduce the alcohol content below 0.5% (Wells, 1948). The addition of this volatile flavor concentrate to apple jelly is considered an adulteration by the Food & Drug Administration.

Howard (1947; 1948) lists the following compounds as being present in the apple essence: acetaldehyde, *n*-hexaldehyde, methanol, ethanol, *n*-butanol, *n*-hexanol, *n*-propanol, ethyl acetate, ethyl formate, ethyl propionate, isopropanol, isobutanol, *d*-2-methylbutanol, *n*-caproaldehyde, 2-hexeneal, methyl butyrate, ethyl caproate, and acetone. Alcohols com-

prised 92%, aldehydes 6%, and esters 2% of the total volume of volatile oils.

b. Cherries. Nelson and Curl (1939) distilled 94 liters of Montmorency cherry juice *in vacuo* and then concentrated the distillate to a volume of 500 ml. They isolated and identified benzaldehyde from the distillate by means of its semicarbazone. On this basis they found 2.8 mg. of aldehyde per liter of juice. A fraction boiling at 73-76°C. (163.4-168.8°F.) consisted of 35% methyl and 65% ethyl alcohol. A trace of geraniol was indicated by the roselike odor which, on oxidation, was converted to a faint lemon odor. Phenols were not present.

c. Oranges. Hall and Wilson (1925) crushed whole California Valencia oranges in obtaining the juice for examination of the volatile constituents. Most of the peel oil was removed by centrifugation, and the juice then distilled *in vacuo*. Any oil that separated during the distillation was considered as peel oil. The distillate was then concentrated by repeated redistillation. Any oil that separated on the first redistillation was also added to the peel oil. The remaining oil recovered during the further concentration was classified as juice oil. By redistilling several times, 182 g. of oil exclusive of the peel oil were obtained from 39,085 liters of juice. Acetaldehyde, acetone and ethyl alcohol were found in the water layer of the distillate. The juice contained 0.018% alcohol.

Citral was identified in the oil; however, the odor of the aldehyde fraction also indicated the presence of an unidentified aldehyde. The aldehyde-free oil was saponified and from the acid fraction caprylic and acetic acids were identified.

The alcohol fraction of the saponified material was found to contain geraniol and amyl alcohol. Phenylethyl alcohol was claimed to be present, although the claim was based solely on the production of acetic acid by chromic acid oxidation of one of the fractionation residues. The refractive index, boiling point, and density of the bulk of the alcohol fraction indicated the presence of linalool. However, no derivatives could be isolated and all tests for linalool were negative. It is unfortunate that the whole fruit was used in this work since, with the large volumes used, some of the peel oil constituents probably remained dissolved in the distillates.

For comparison, the constituents of the orange peel oil are given here in brief form. Approximately 90% of the oil is *d*-limonene unaccompanied by any other hydrocarbon (Wallach, 1884). The remaining 10% consists of oxygen-containing compounds. Semmler (1891) found citral to be a component. From the fractionation of 42 kg. of oil, Stephan (1900) found *n*-decylic aldehyde, but no citral. This lack of citral is probably due, as pointed out by Guenther and Grimm (1938), to the use

of sodium bisulfite for the isolation of the aldehyde. Citral has a tendency to form the soluble dihydrosulfonic acid. In addition, Stephan isolated *d*-linalool, *n*-nonylic alcohol, and *d*-terpineol, which were present in part as esters of *n*-caprylic acid. Nelson (1934) found citral and decyl aldehyde in Florida Valencia orange oil, and Guenther and Grimm (1938) found a mixture of α- and β-citral in California orange oil.

Little has been done on the cause of "cooked flavor" and of the "off flavors" which develop during processing and storage of canned orange juice. Nolte and von Loesecke (1940) associated part of the "off-flavor" with the oxidative changes in the fatty material. Boyd and Peterson (1945) observed a direct relationship between the volatile oil content and the "off-flavor" development. Canned juice with volatile oil content below 0.007% did not develop off-flavors even after 18 months storage at room temperature. On the other hand, samples containing 0.01% oil developed objectionable flavors. Elimination of all the oil, however, is not the solution to the problem, since juice prepared free of any peel oil was "flat." Henry and Clifcorn (1948) supported the contention that the volatile oil was largely responsible for the development of off-flavor. Although no evidence was presented, they claimed the responsible constituents to be unsaturated hydrocarbons or alcohols and specifically they believed that *d*-limonene was associated with the flavor deterioration. They found the "cooked flavor" to be unrelated to the "off-flavor" brought about by storage.

d. Peaches. Power and Chestnut (1921b) found the oil in peach pulp to range from 0.00074% to 0.00082% of the weight of the pulp. The oil was obtained by distillation, and was found to contain acetaldehyde, furfural, and possibly cadinene. Although a color test for cadinene was given with sulfuric acid, the sample was too small to prepare a derivative. After hydrolysis of the oil, methyl alcohol, formic, acetic, valeric, and caprylic acids were identified. The alcohol fraction from the saponification had an odor of linalool. On oxidation this gave a lemon odor and reduced Schiff's reagent, indicating the presence of citral originating from oxidized linalool. Citral was apparently present as one or more esters.

e. Pineapple. Haagen-Smit *et al.* (1945a and b), investigated the volatile flavor and odor constituents of pineapple. The winter and summer crops were studied separately, because the latter is much sweeter and has a more fruity flavor. Volatile material for the work was obtained by vacuum distillation of large quantities of fresh fruit. A total of 4150 kg. (9130 lb.) of fruit was used. The still charge of trimmed fruit amounted to 2896 kg. (6371 lb.). To prevent decomposition of any of the flavoring compounds, the temperature of the still contents was maintained below

40°C. (104°F.). The vapors were first condensed in a water-cooled condenser and then in a series of traps ranging in temperature from −20 (−4°F.) to −180°C. (−292°F.). By redistilling the condensate from the condenser, the more volatile material was concentrated in alcohol-carbon-dioxide cooled traps. Saturation of the distillates with ammonium sulfate gave oil and aqueous layers. Acetaldehyde was found in the aqueous phase and identified by means of its 2,4-dinitrophenylhydrazone. The oily layer was dried and then fractionated, partly at atmospheric pressure and partly in a vacuum.

During distillation of the fruit, all of the aromatic material came over in the distillate. The residue had only a sweet and a sour taste arising, presumably, from the sugars and nonvolatile acids. Addition of the volatile oil to a solution of the residue gave the typical pineapple flavor.

As might be expected from the difference in flavor of the fruits, the summer fruit contained more volatile oil than the winter fruit; 190 mg./kg. and 15.6 mg./kg. respectively. Quantitatively, this difference was mainly in the ethyl acetate and ethyl alcohol content. The summer fruit had 119.6 mg. of ethyl acetate and 60.5 mg. of ethyl alcohol per kg.

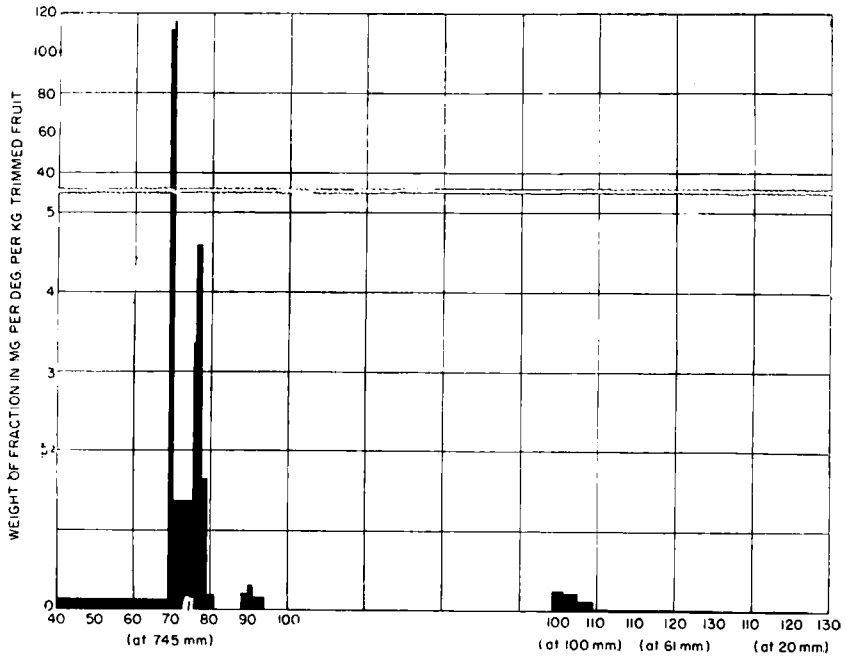

Fig. 1. Fractionation of volatile oil from pineapple, summer fruit (1940). From "Flavor Studies on Pineapple" by A. J. Haagen-Smit, *Am. Perfumer Essent. Oil Rev.* April (1946). Reprinted by permission of the copyright owners.

of trimmed fruit, compared to 2.91 mg. of ethyl acetate per kg. for the winter fruit. No alcohol was found in the winter fruit. Figs. 1 and 2 graphically illustrate the differences in the lower boiling fractions of summer and winter fruits. Figs. 3 and 4 show the corresponding higher boiling fractions.

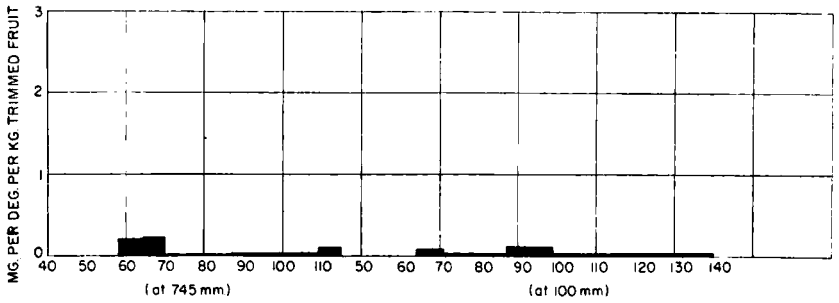

Fig. 2. Fractionation of the volatile oil from pineapple, winter fruit (1939–40). From "Flavor Studies on Pineapple" by A. J. Haagen-Smit, *Am. Perfumer Essent. Oil Rev.* April (1946). Reprinted by permission of the copyright owners.

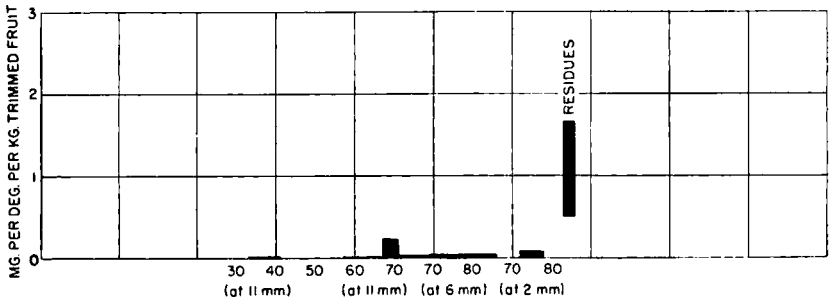

Fig. 3. Fractionation of the higher boiling volatile oil from pineapple, winter fruit (1939–40). Plotted from data taken from "Chemical studies of pineapple (*Ananas sativus* Lindl), I. The volatile flavor and odor constituents of pineapple" by A. J. Haagen-Smit, J. G. Kirchner, A. N. Prater, and C. L. Deasy, *J. Am. Chem. Soc.* **67**, 1646 (1945).

Table I gives a summary of various components that have been identified. As can be seen from this table, most of the flavor and odor constituents are esters. These were identified by hydrolyzing and preparing the 3,5-dinitrobenzoates and *p*-phenylphenacyl esters of the component alcohols and acids. One difference to be noted is the fact that all of the esters in the winter fruit were methyl esters except for the ethyl acetate, whereas the summer fruit contained mostly ethyl esters.

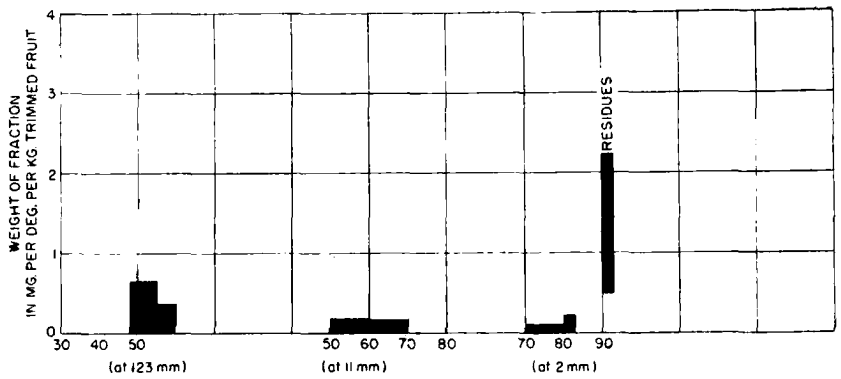

Fig. 4. Fractionation of the higher boiling volatile oil from pineapple, summer fruit. Plotted from data taken from "Chemical studies of pineapple (*Ananas sativus* Lindl), I. The volatile flavor and odor constituents of pineapple" by A. J. Haagen-Smit, J. G. Kirchner, A. N. Prater, and C. L. Deasy, *J. Am. Chem. Soc.* 67, 1646 (1945).

TABLE I

Summary of Components of the Volatile Oil of Pineapple [a]

Winter fruit (1939–1940)	*Mg./kg. fruit*
Ethyl acetate	2.91
Acetaldehyde	0.61
Methyl isocaproate	1.4
Methyl isovalerate	0.6
Methyl *n*-valerate	.49
Methyl caprylate	.75
Methyl ester of five carbon hydroxy acid	Trace
Sulfur-containing compounds [b]	1.07
Summer fruit (1940)	
Ethyl acetate	119.6
Ethyl alcohol	60.5
Acetaldehyde	1.35
Ethyl isovalerate	0.39
Methyl *n*-propyl ketone	Trace
Ethyl acrylate	0.77
Ethyl *n*-caproate	.77
Summer fruit (1942) boiling above 100° at 745 mm.	
Acetic acid	0.49
Ethyl ester of a C_5 unsaturated acid	1.08
Methyl ester of a C_5 unsaturated acid	0.68
Methyl ester of a C_6 keto acid	Trace
Sulfur-containing compound (b)	0.88

[a] Haagen-Smit *et al.*, *J. Am. Chem. Soc.*, 67, 1647 (1945) Reprinted by permission of the American Chemical Society.

[b] Identified as methyl β-methylthiolpropionate. Cf. Haagen-Smit *et al.*, *J. Am. Chem. Soc.*, 67, 1651 (1945)

A sulfur-containing compound was isolated and identified as methyl β-methylsulfonylproprionate, which has a formula $CH_3SCH_2CH_2COOCH_3$. This component is a good illustration of the effect of very small quantities of a substance on the flavor and aroma of food. Although present in minute amounts it was found to make a definite contribution in reproducing the pineapple flavor from the various fractions.

f. Raspberries. Coppens and Hoejenbos (1939a) investigated the volatile constituents of the raspberry. Using 510 kg. of fresh raspberries they obtained 480 kg. of juice which was extracted with ether. The extract was separated into an acid and a neutral fraction by washing with 10% soda solution.

From the acid fraction a small amount of oil was obtained which was split into three fractions. The *p*-toluidides of *n*-caproic and benzoic acids were isolated. Other fatty acids were known to be present but could not be identified because of the small quantities of material available.

The 92 g. comprising the neutral fraction was roughly divided into four fractions by distillation. In the lower fractions diacetyl was identified. A 70-80°C. (158-176°F.) fraction was shown to contain ethyl alcohol by the iodoform reaction and by oxidation to an aldehyde; a derivative was not prepared. Methyl alcohol could not be found.

Three grams of a constant boiling liquid isolated from the alcohol fraction was shown to contain ethyl acetate. This fraction was not washed free of alcohol as shown by an ester number (the number of milligrams of potassium hydroxide required to saponify 1 g. of ester) of 564 as compared with the calculated value of 633. The saponified fraction yielded a *p*-toluidide of acetic acid. The positive iodoform test was, of course, valueless since alcohol was present before the saponification. However, all facts considered, the ester was undoubtedly ethyl acetate. Isoamyl alcohol and benzaldehyde were also identified by appropriate derivatives.

From the residues of the original distillation a fraction was obtained which appeared to contain a quantity of menthone, although its identity was not definitely established. In the same fraction, after saponification, phenylethyl alcohol was identified. An additional compound was isolated which had a coumarinlike odor. Although the melting point was low, it did not give a melting point depression when mixed with an authentic sample of coumarin.

Naves (1947) found β-ionone in the marc of raspberries. This, however, might very well have been a decomposition product.

g. Strawberries. Coppens and Hoejenbos (1939b) also investigated the volatile constituents of strawberry juice. In this work they used 590 kg. of fruit from which they obtained 475 kg. of juice. As in their work on

raspberries they extracted the juice with ether, which was then concentrated and separated into acid and neutral fractions. Acetic and *n*-caproic acids were identified in the acid fraction by means of their *p*-toluidide derivatives. Cinnamic acid was also found to be present.

The neutral fraction, amounting to 86 g. was fractionated roughly into four parts. A fifth part was obtained by steam distillation of the residue from the first fractionation. For identification these fractions were then separated into somewhat narrower boiling regions. The first fraction gave the iodoform reaction for ethyl alcohol. Acetone was shown to be absent. After removing the alcohol from this fraction, the presence of ethyl acetate was ascertained by saponifying and then identifying the acetic acid as acet-*p*-toluidide and the alcohol by use of the iodoform test. Methyl alcohol was not present; but there was an indication of diacetyl.

The second fraction contained a carbonyl compound in an amount too small to identify. Butyric and acetic esters of either ethyl or isopropyl alcohol were present. Isoamyl *n*-caproate and possibly some free isoamyl alcohol were shown to be present. There was also evidence to indicate that *n*-hexanol was present as an acetate.

Except for the first fraction there appeared to be no attempt to determine whether or not there was any free acid or alcohol in the various fractions prior to saponification. Thus, in most cases it is not certain that all of the acid or alcohol was produced by the hydrolysis of an ester.

The third fraction contained the terpene compounds *dl*-terpineol, *l*-borneol, and *dl*-isofenchyl alcohol. These were probably present as acetates. A semicarbazone derivative appeared to be that of acetophenone. The fourth fraction contained terpin, a fatty acid having the formula $C_{18}H_{36}O_2$ (possibly lanostearic acid) and a keto compound with the formula $C_{10}H_{18}O_3$. Benzoic acid was isolated from the fifth fraction after it had been split into two parts by distillation.

h. Grapes. Although there is little information concerning the volatile flavoring constituents of grapes, methyl anthranilate has been known to be a constituent for some time. Power and Chestnut (1921c) found from 0.2 to 2.0 mg. of the ester per liter of juice. Not all grapes contain this ester (Power and Chestnut, 1923; Sale and Wilson, 1926) as none could be found in *Vitis vinifera* (European grapes). Sale and Wilson (1926) ran analyses on a considerable number of varieties and found the methyl anthranilate content to vary from 0.0 to 3.8 mg./kg. of fruit. At the same time they found the volatile ester content to vary from 6 to 366 mg./kg. and the volatile acid content from 3 to 121 mg./kg. The volatile flavoring is found throughout the pulp and is not concentrated wholly in the skins.

i. Lemons. Francesconi (1929) claims to have identified isovaleric acid

in lemon juice. This claim, however, was based solely on the smell of the acid and its ethyl ester. On the basis of a reaction with ferric alum, he also indicated the presence of isoamyl alcohol in lemon juice.

j. Bananas. Rothenbach and Eberlein (1905) found amyl isovalerate, and probably ethyl and amyl acetate in bananas. Kleber (1913), who is usually credited with the first work on fruit flavors, isolated a volatile oil by steam distillation from bananas which he identified as amyl acetate.

2. Vegetables

a. Carrots. The oil constituents of the carrot root are unknown and relatively little is known concerning those of the leaf. Pictet and Court (1907) found pyrrolidin and daucin $C_{11}H_{18}N_2$, an alkaloid, in the leaf.

A little more is known concerning carrot-seed oil, because of its commercial importance. Pictet and Court (1907) found that the seed oil contains an alkaloid which is not the same as that found in the leaf. Landsberg (1890) thought pinene was present and also a compound $C_{10}H_{18}O$ but was not able to identify either of them with certainty. Richter (1909) detected the occurrence of butyric acid, palmitic acid, probably esters of acetic and formic acids, pinene, *l*-limonene, and daucol $C_{15}H_{26}O_2$, which was probably a diatomic sesquiterpene alcohol. The percentage of these constituents in the oil was as follows: pinene and *l*-limonene 14%, sesquiterpenes 35%, esters 9%, and palmitic and butyric acids 0.84%.

Asahina and Tsukamoto (1925; 1926) found in the Japanese carrot-seed oil, asaron ($C_{12}H_{16}O_3$), a sesquiterpene named carotol ($C_{15}H_{26}O$), and bisabolen.

b. Celery. Volatile oil is present in all parts of the celery plant, but to the greatest extent in the seeds. Practically nothing is known concerning the flavoring constituents of the oil from the roots and the herb, although the glycoside appiin has been shown to be present. The seed oil, however, has been investigated more extensively. Approximately 70% of this oil consists of hydrocarbons, one of which is *d*-limonene. Ciamician and Silber (1897a) found in the higher boiling oil, which remains after the fractionation of the celery oil, palmitic acid, a phenolic substance, a phenol ($C_{16}H_{20}O_3$), a sesquiterpene selinene ($C_{15}H_{24}$), a lactone sedanolid ($C_{12}H_{18}O_2$), and sedanonic acid anhydride. They reported sedanolid and sedanonic acid anhydride as the characteristic odorants of celery-seed oil. Schimmel & Co. (1909; 1910) found 60% *d*-limonene, 10% *d*-selinen, 2.5% sedanoloid, 0.5% sedanonic acid anhydride, and 2.5-3% alcohol. Semmler and Risse (1912a,b; 1913) later

showed the natural selinene to be a mixture of pseudo-(β)-selinene and a little ortho-(α)-selinene.

c. Garlic. Like its close relative the onion, garlic owes its pungent odor to a sulfur-containing oil. Wertheim (1844) examined garlic oil and reported it to consist chiefly of allyl sulfide $(C_3H_5)_2S$. Later, Semmler (1892a) reported that there was no allyl sulfide in garlic oil. Semmler did find a disulfide $C_6H_{10}S_2$, however, which constituted 60% of the oil and was mainly responsible for the typical garlic odor. It boils at 79 to 81°C. (174.2-177.8°F.) and 16 mm. pressure. Another disulfide was present to the extent of 6% of the oil. Boiling at 66-69°C. (150.8-156.2°F.) under 16 mm., its reactions indicated the presence of allyl-propyl disulfide. A trisulfide with the formula $C_3H_5 \cdot S \cdot S \cdot S \cdot C_3H_5$ formed another 20% of the oil, leaving a residue which was shown to contain a compound having the composition $C_6H_{10}S_4$.

Cavallito and Bailey (1944) and Cavallito *et al.* (1944) extracted ground garlic cloves with alcohol. From this extract they isolated a colorless liquid (6 g. from 4 kg. of garlic) having a more characteristic garlic odor than any of the previously described sulfides. This compound, having an empirical formula of $C_6H_{11}OS_2$ and containing either an -S-(SO)- or an -S-O-S- grouping, was called allicin. It is relatively unstable in the pure form and decomposes on dry distillation or even on standing at room temperature. Alkaline hydrolysis produces mainly allyl disulfide and sulfur dioxide. Later, Cavallito *et al.* (1945) discarded the term allicin for the antibacterial principle in garlic, to prevent confusion with established medical products. These authors could not find any oil in the whole garlic since it appears only after crushing. They found evidence of a precursor which, when acted on by an enzyme present in garlic, gave allicin. This in turn gave the various sulfides present in garlic oil. The closely related onion (*Allium cepa*) did not contain either allicin or its precursor; however, certain varieties contained an enzyme capable of acting on the precursor. The white onion did not contain this enzyme, whereas the red and yellow varieties did.

Stoll and Seebeck (1947; 1948) isolated the precursor of allicin which they designated as alliin. Alliin is nonbactericidal but on treatment with the enzyme present in garlic the bactericidal compound allicin is formed. They ascribe the following formula to alliin: $CH_2{:}CH \cdot CH_2 \cdot SO \cdot CH_2 \cdot CH(NH_2) \cdot COOH \cdot \frac{1}{2}H_2O$. Allicin was shown to possess the -S-SO- group, but the mechanism of the conversion of alliin to allicin is unknown.

d. Onion. The common onion owes its pungent odor to a volatile oil which is decomposed by distillation at ordinary temperatures. It can, however, be distilled at reduced pressures. Kooper (1910) found thio-

cyanic acid and allylthiocyanate in the freshly expressed oil. He could not detect the presence of formaldehyde, acetaldehyde, or acrolein.

Semmler (1892b) isolated a disulfide of the formula $C_6H_{12}S_2$ which boiled at 75-83°C. (167.0-181.4°F.) and 10 mm. pressure. This oil had a distinct but not unpleasant onion odor, and was probably either $CH_2{:}CH{\cdot}CH_2{\cdot}S{\cdot}S{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3$ or $CH_3{\cdot}CH{:}CH{\cdot}S{\cdot}S{\cdot}CH_2{\cdot}CH_2{\cdot}CH_3$. It could be hydrogenated to $C_6H_{14}S_2$. A polysulfide was also present that probably contained the same carbon radicals since on reduction with zinc dust a compound of the formula $C_6H_{12}S$ was formed. A third fraction appeared to be identical with one found in Asafetida oil and possessed the formula $C_{10}H_{18}S_2$ or $C_{11}H_{20}S_2$. Semmler could find no terpenes nor any allyl sulfide in onion oil. From 5000 kg. of onions he obtained 233 g. of oil, a yield of 0.046%.

More recently Kohman (1947) has shown the presence of propionaldehyde to the extent of 52 mg./kg. of raw onion. He also obtained evidence to suggest that the lachrymator substance is a thioaldehyde present in a concentration of approximately 100 mg./kg. of onion.

e. Allium scorodoprasum L. This is a species closely related to the common onion. Kaku (1926) identified three sulfur compounds; a propylallyl disulfide $C_6H_{12}S_2$, diallyl disulfide $C_6H_{10}S_2$, and diallyl trisulfide $C_6H_{10}S_3$. He could not find $C_6H_{10}S_4$ which Semmler (1892a) found in garlic. He did, however, isolate a small quantity of diallyl sulfide, $C_6H_{10}S$, and a crystalline organic acid whose molecular weight was 362. It melted at 76.5-77°C. (169.7-170.6°F.).

Abe and Inakivi (1938) investigated the same plant and found propylallyl sulfide, $C_6H_{12}S$, but were unable to isolate the propylallyl disulfide found by Kaku in this plant and by Semmler in garlic.

f. Parsley. Very little is known concerning the oil of the herb itself. The oil has an odor of fresh parsley and contains 0.058% of organic sulfur compounds that have not been identified (Dahlen, 1875). The herb also contains some apiol (Schimmel and Co., 1909). Pictet and Court (1907) isolated a crystalline hydrochloride, the base giving a pyrrole reaction on heating with zinc dust. On treatment with alkali it gave an odor of an alkyl amine.

Since the oil of the seed is used in commerce it has been investigated more intensively than the herb. It is viscid with only a slight odor of fresh parsley. The oil is present to the extent of about 2 to 7% in the seed. The main fraction of the oil is apiol, a compound closely related to myristicin, except that the former has an additional methoxyl group. Ciamicin and Silber (1888b; 1889; 1890) and Thoms (1903a) established the structural formula as that of 4-allyl 1-3,6-dimethoxy-1,2-methylenedioxybenzene.

Bignami and Testoni (1900) isolated the oxidation products of two compounds, $C_{11}H_{12}O_3$ and $C_{13}H_{18}O_4$ from parsley-seed oil. Later Thoms (1903b; 1908) identified these compounds as 1-ally-2,3,4,5-tetramethoxybenzene and myristicin. He also found small amounts of phenols, aldehydes, and ketones that were not otherwise identified. A trace of palmitic acid was also present. From the hydrocarbon fraction he obtained a nitrosochloride of α-pinene.

g. Parsnip. The root of the parsnip yields an oil on distillation, the composition of which has not been studied.

The seeds of this plant, on the other hand, have been investigated to a limited extent. Van Renesse (1873) identified the major fraction of the oil as an octyl ester of *n*-butyric acid. The fraction below this yielded a mixture of acids which van Renesse assumed to be a mixture of esters of propionic and butyric acids.

Gutzeit (1875) identified ethyl and methyl alcohol in the aqueous distillate of the oil (free and as esters); Haensel (1907) found heptoic and apparently butyric acid. Schimmel and Co. (1908) also reported a large amount of caproic acid.

h. Radish. The first work on this product by Pless (1846) yielded a small quantity of colorless, sulfur-containing oil which had the taste but not the odor of radishes. Betram and Walbaum (1894) steam distilled 75 kg. of finely ground radish and obtained an aqueous distillate of unpleasant odor. From this a few grams of oil were obtained that would not react with ammonia. Gadamer (1899a) repeated this work with similar results. He decided that the radish oil decomposed on distillation and so extracted the ground radish with ether. After removal of the ether he had an oil with the taste and odor of the radish. After standing a long time crystals precipitated out that were probably those of "raphanol." Raphanol was actually isolated by Moreigne (1896) from the root of *Raphanus niger*. It had the properties of a lactone and was assigned the formula $C_{29}H_{58}O_4$. Furthermore, Moreigne found sulfur, but no nitrogen in radish oil.

Heiduschka and Zwergal (1931) steam distilled ground radish in order to isolate the compound responsible for the characteristic radish taste. Their analysis indicated the presence of a butyl crotonyl mustard oil sulfide ($CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot S \cdot CH_2 : CH \cdot CH_2 \cdot CH_2 \cdot N : C : S$) or butyl butyl mustard oil sulfide ($CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot S \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot N : C : S$). They also isolated a compound oil with the properties of an aromatic mustard oil similar to that found in horseradish, but the quantity obtained was unfortunately too small to enable determination of its structure. They did not obtain the raphanol described by Moreigne. Since a crotonyl sulfide had already been found in the seed oil of *Brassica*

napus (rape), also a cruciferaceae, they assigned this designation to the substance isolated.

i. Water Cress. (Roripa nasturtium-aquaticum). This is a native of the Old World but has become widely distributed in America. It has a sharp mustardlike flavor, and since it belongs to the Cruciferaceae (mustard) family, one would expect it to contain a mustard oil.

Hofmann (1874b) used the technique described for garden cress (see below) and obtained an oil that did not have the cresslike odor. On fractionation the major part of the oil was identified as phenylpropionic acid nitrile, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CN$. Also obtained was a small amount of crystalline material which could not be identified.

Gadamer (1899c) showed that by grinding the herb so as to liberate enzyme(s) a different oil was obtained. He isolated an oil with a strong mustard-oil odor that was identified by means of thiourea as phenylethyl isothiocyanate, $C_6H_5 \cdot CH_2 \cdot CH_2 : NCS$.

j. Garden cress or Peppergrass (Lepidium sativum L.). Garden cress has an agreeable spicy flavor and is used for garnishing. The oil from it was first prepared by Pless (1846) who observed that, on distillation, a sulfur-containing oil was produced that was heavier than water. The compound exists as a glycoside in the plant; it is released by enzymatic action when the plant is crushed.

Hofmann (1874a) steam distilled the whole herb and found that the oil remained in solution. Extraction of the distillate yielded a light yellow oil which, on fractionation, gave a colorless product boiling at 231.5°C. (448.7°F.). This oil was identified as benzyl cyanide; it comprised three-fourths of the total fraction. A sulfur-containing compound was noted in some of the lesser fractions; its identity was not established.

Gadamer (1899b) believed that the oil obtained by Hofmann was a decomposition product since the crushed herb has a mustard oil odor. The enzyme must be brought into contact with the glycoside by grinding, otherwise hydrolysis cannot take place. Instead, by action of the hot water, the benzylcyanide is formed. Gadamer subjected the ground seeds of garden cress to the action of the myrosin in white mustard seeds, and on distillation obtained the expected benzyl mustard oil.

k. Potatoes. The substances responsible for the odor and taste of potatoes are volatilized by steam. Kroener and Wegner (1942) and Kroener (1944) obtained 0.6-1.0 g. of oil from 100 kg. of potatoes. The fatty acid fraction was removed by chilling; the oil thus obtained was fractionated under vacuum to yield a pentanol. Sulfur compounds were present in the higher boiling fraction.

l. Cucumbers. Takei and Ono (1939) obtained a light yellow oil by steam distilling fresh cucumbers and extracting the distillate with ether.

The neutral oil had a cucumber fragrance; on fractionation it yielded 2,6-nonadienal identified by its 2,4-dinitrophenylhydrazone and its semicarbazone. From the fractions boiling at 80-100°C. (176-212°F.) and 4 mm., and 100-135°C. (212-275°F.) and 2.5 mm., the allophanate and 4'-iodobiphenylurethan of a, 6-nonadien-1-ol were isolated. They called the latter, cucumber alcohol.

m. Miscellaneous. Griebel (1925) lists a large number of fruits, nuts, tubers, leaves, petals, and fungi that contain acetaldehyde, thus indicating that this compound may be an intermediate product in metabolism.

Methyl acetylcarbinol has been mentioned quite often as the aroma-producing factor in bread. Biacetyl is also present in bread. Tóth (1941) found that 80-90% of the biacetyl was located in the crust. He found average values of 0.3 mg. per 100 g. for biacetyl and 2.0 mg. per 100 g. for methyl acetylcarbinol. The actual quantities vary according to the quality and age of the bread. Antoniani *et al.* (1940) found no relation between the content of acetyl methylcarbinol or its derivative, biacetyl, and the flavor of bread. However, this does not mean that they are not contributing factors.

Komm and Lehmann (1940) found that steam distillation was unsatisfactory in investigating the aromatic principles in rye bread because of the high resistance of the bread pulp. They used a highly flavored, 2-3-day-old regimental bread. The finely crumbled bread was extracted for 18 hours with boiling methyl alcohol and obtained a brownish, viscous material with a strong bread odor. By fractionating and refractionating at varicus pressures this material was split into numerous fractions. From the fraction that contained all the volatile material an acid residue was obtained by extraction with ether. Upon saponification and after acidifying, it yielded a small amount of oil that was then divided into three parts. One of these possessed the strongest odor of the original bread. All the fractions were examined for various characteristic groups by means of micro tests. Carboxylic and oxy acids were detected in twenty-four fractions. One fraction gave a positive test for acetic acid and another for lactic acid. A positive test for phosphoric acid was obtained in seven fractions. Nearly all of the fractions gave positive tests for aldehydes, furfuraldehydes, and for α, β-unsaturated acids. Thirty of the thirty-seven fractions reduced Fehling's solution. None of them gave a test for phenols but most contained traces of higher alcohols. Five fractions gave positive tests for hydroxyfurfuraldehyde. The one with the most pronounced odor of the original bread appeared to be a substituted furfuraldehyde. No compounds were positively identified by means of derivatives.

Freise (1935) investigated the essential oils of certain mushrooms.

The oil exists mainly in glycosidal combination in the stems and cap; a small portion is combined in the waxlike outer surface of the cap. The glycosides can be hydrolyzed by enzymes extracted from the fungi with glycerol; they are extractable with water. The chief constituent of three of the oils was found to be benzyl isothiocyanate ($C_6H_5CH_2 \cdot NCS$). Two of the oils were mainly phenylethyl thiocyanate, $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot SCN$ although $C_6H_5 \cdot CH(OH)CN$ was also detected in two of them.

Nelson (1928) is of the opinion that the flavor of maple syrup is due to unstable phenolic compounds. He was able to isolate a crystalline substance with a melting point of 74-76°C. (165.2-168.8°F.), which was thought to be an aldehyde.

Takei and Imaki (1936b) found acetic and propionic acids, stigmasterol, syringic acid, and benzaldehyde in molasses.

3. Non-Alcoholic Fruit and Vegetable Beverage Products

a. Coffee. A great deal of work has been done on coffee; however, much of it does not throw any light on the nature of the chemical components responsible for its flavor. The development of the off-flavor in coffee that has been standing for some time is probably due to a combination of factors. No doubt a rancidity is produced by the oxidation of some of the fatty components, but the staleness seems to arise from several causes: (1) oxidation of nonfatty, unstable components, (2) loss of volatile aroma factors, and possibly (3) inter-reaction of unstable components. It is well known that coffee does not keep as well when ground as when stored as the whole bean, so it appears that oxidation does play an important role in the development of stale flavor. However, this is not necessarily due to fatty acid oxidation. Bengis (1935) showed that the oil extracted from stale coffee absorbed much less oxygen than that extracted from freshly roasted coffee, but as shown by the excellent work of Elder (1937; 1940), the fatty acids may be stabilized toward oxidation, and yet, staleness will develop.

The tendency for fats to become rancid has been judged by various tests that measure the products of fatty decomposition. However, as pointed out by Elder (1937), when applied to coffee oil these tests are apt to produce unreliable results because of the unstable nature of the flavor constituents. For this reason, he used the method in which the oxygen absorption induction period is measured and which has been described by Holm and Greenbank (1924a; 1925), Holm *et al.* (1927), and French *et al.* (1935).

Freshly-ground coffee was divided into two batches, the control samples being vacuum sealed and the test samples being kept in a slip-covered can. The oil was extracted with Skelly-Solve B (b.p. 60-70°C.;

140-158°F.) and the solvent subsequently removed in a vacuum. Organoleptic tests were made on each sample in order to check for staleness. No difference was detected between the vacuum-packed control and the test samples until 2 weeks had elapsed. At this time a staleness was apparent in the sample kept in contact with air. On the other hand, no significant difference was noticeable in the length of the induction period throughout the test period of 91 days (Table II), thus proving that the staleness was not due to rancidity of the fats.

TABLE II

Oxidation-Induction Periods of the Fat Extracts from Roasted Coffee [a]

Days of storage prior to fat extraction	Induction period, hrs.	
	Vacuum packed coffee [b]	Coffee packed in slip-covered can [c]
3	30	37
14	37	33
21	30	33
35	30	
56	33	32
91	33	33

[a] Lucius Elder, Jr., *J. Ind. Eng. Chem.*, 29, 267 (1937). Reprinted by permission of the American Chemical Society.

[b] Roasted and vacuum-packed 9 days prior to beginning of storage test

[c] Same lot of coffee, removed from the vacuum pack and stored as indicated

Further proof that staleness is independent of fat rancidity was demonstrated by Elder (1940) in stabilizing the fats by means of antioxidants. By comparing the induction period of oil extracted from coffee, before and after roasting, he found that the roasting process increased the stability of the oil fraction as measured by the oxidative induction period. The oil from green coffee could not in itself be stabilized by roasting, but by adding certain compounds that are formed in the coffee bean by roasting, the green oil could be stabilized. Numerous compounds were tried; pyrrole and the aliphatic mercaptans greatly increased the oil stability to fatty oxidation. Because pyrrole gave the greatest stability, a series of tests were run using 0.1 and 0.05% pyrrole as the stabilizer. In addition to the two best batches (which were packed in slip-covered cans), two control batches were prepared, one packed in a slip-covered can and one vacuum packed. The testing periods were 3, 14, 21, 35, 56, 91, 193, 381, and 728 days. After 2 weeks the test samples were more resistant to oxidation than the vacuum packed sample, but the latter was fresher by the organoleptic test. It was also shown that oxidation induction periods of the two control batches were nearly identical over the 2-year

storage period, although the taste tests indicated a difference after 2 weeks.

Coe and LeClerc (1934) showed that color tests for rancidity and the peroxide test for decomposition products do not necessarily show that an oil was rancid. Oils that were protected from light of wavelength 4900 to 5800 A. did not become rancid even though the peroxide value was equal to or higher than a corresponding unprotected oil that was rancid.

Prescott *et al.* (1937a) investigated the cause of staleness and found that a moist atmosphere contributed greatly to a loss of coffee flavor. They could find no evidence to indicate that staleness was related to rancidification of the fats.

Johnston (1938) investigated the effect of moisture on flavor of roasted coffee, and found that at 100% humidity a sample acquired a stale flavor in 3-4 days, at 50% humidity in 7-8 days, and at 0% only a slight staleness after 1 month. Coffee stored for 31 days in the 100% humid atmosphere could not be recognized as coffee by taste. Johnston suggests using the measurement of oxygen absorption as a method of determining degree of roasting since preliminary data seems to indicate that the oxidizable material in coffee increases with degree of roasting (darkness).

It would seem from these results that the cause of staleness must be sought for in the odor and flavor constituents. From a study of the various constituents that have been isolated from coffee it is easy to see how staleness could arise through changes in these rather than through an oxidation of the fatty material.

The earlier work on coffee was on the volatile material arising from the roasting of the coffee. Bernheimer (1880) found acetic and palmitic acids, methylamine, pyrrole, hydroquinone, caffeine, and acetone. He also obtained a compound distilling at 195-198°C. (383-388.4°F.), which he believed to be a methyl ether of saligenin, and to which he attributed the aroma of coffee. However, as first pointed out by Erdmann (1902), the properties of Bernheimer's "kaffeol" do not coincide with either of the two possible methyl derivatives of saligenin, *o*-methoxybenzyl alcohol [$C_6H_4(OCH_3)CH_2OH$] or *o*-hydroxylbenzylmethylether [$C_6H_4(OH)CH_2OCH_3$]. Bötsch (1880) indicated that the former compound could not be "kaffeol" because of the great difference in boiling points, and also because the synthetic product did not possess a coffee odor. The remaining possibility was eliminated by Thiele and Dimroth (1899) when they synthesized the compound and found a guaiol-like odor instead of a coffee odor. In addition, the synthetic compound was unstable to distillation at atmospheric pressure, whereas "kaffeol" was stable.

Monari and Scoccianti (1895) examined the gases from the roasting of coffee and found pyridine to be present. Jaeckle (1898) also found

pyridine, and in addition furfurylaldehyde, trimethylamine, ammonia, formic acid, as well as the previously discovered compounds: caffeine, acetic acid, and acetone.

Erdmann (1902) steam distilled coffee and then extracted the distillate with ether. He obtained 83.5 g. of oil from 150 kg. of coffee or .0557%, whereas by a simple water distillation he only obtained 11.1 g. from 50 kg. Separating the oil into an acidic and a neutral fraction he found a small amount of acetic acid and a large amount of valeric acid. The acid fraction represented 42% of the oil. The neutral fraction was fractionated under reduced pressure. He found furfuryl alcohol, some phenols, and a small amount of a nitrogen containing compound boiling at 93°C. (199.4°F.) at 12 mm. On decomposition the latter compound gave a product with a pyridine-like odor. Erdmann believed that the aroma of roasted coffee was not due to a phenol, as Bernheimer stated, but rather to a nitrogen compound.

It is interesting to note that Erdmann roasted equal amounts of the so-called coffee tannic acid, raw sugar, and caffeine to obtain a coffee odor. Raw sugar and coffee tannic acid gave a burnt odor reminding one of coffee, but it was only by adding the caffeine that the fine odor of coffee was obtained.

Mention should be made of chlorogenic acid and trigonelline. Chlorogenic acid is a depside of quinic acid and caffeic acid, and according to Freudenberg (1920) it was first isolated by Payen (1849) in an impure state. A good deal of research has been done on this astringent compound which may contribute to the taste of the coffee. Slotta and Neisser (1939) analyzing fourteen different coffees, found the chlorogenic acid content to vary from 4.5 to 7.5% in the raw bean and 3.3 to 4.9% in the roasted coffee.

Heiduschka and Brüchner (1931) report that trigonelline was first isolated from coffee by Palladino (1894), who called it "coffearin." Later it was recognized as being identical with trigonelline ($C_7H_7NO_2$) the betaine of pyridine-β-carboxylic acid. Trigonelline is present to the extent of 0.8 to 1.2% in raw coffee and 0.3 to 0.6% in roasted coffee (Slotta and Neisser, 1939).

Other compounds have been reported by different workers. Grafe (1912) found acetic and valeric acids, phenols, furfuryl alcohol, furfurane derivatives, and a pyridine compound in coffee. Bertrand and Weisweiller (1913) also found pyridine in coffee oil. Schmalfuss and Barthmyer (1929) reported diacetyl as a component of coffee.

Staudinger and Reichstein (1926; 1928) also isolated and identified a number of the constituents of natural coffee oil. In order to isolate the oil, they subjected coffee to vacuum distillation at 2 mm. pressure and a

temperature of 100-110°C. (212-230°F.) after adding 2% water. The volatile material was condensed in three traps, maintained at −20, −80, and −180°C. (−4, −112, and −292°F.), respectively. This procedure yielded a considerable number and variety of compounds. These authors reported a number of sulfur-containing compounds which previously had not been reported in coffee. Among these were methyl, furyl, and thienyl mercaptans, hydrogen, and dimethyl sulfide. Minute traces of mercaptans (especially furyl and thienyl mercaptans) were considered to contribute greatly to the formation of coffee aroma.

A series of nitrogen-containing compounds also identified were: pyridine, pyrazine, methylpyrazine, *N*-methylpyrrole and 1-furfurylpyrrole. The carbonyl compounds included acetaldehyde, α-methyl-*n*-butyraldehyde, acetone, furyl ketone, diacetyl, and acetyl propionyl. The alcohols found were methyl, furfural and certain higher ones. The acids identified were acetic, isovaleric, and palmitic. Staudinger and Reichstein also identified phenol, pyrocatechol, guaiacol, vinylguaiacol and 2:3-dihydroxyacetophenone.

These workers maintain that the aroma and flavor of coffee are due to the mercaptans and to mercaptols. The latter are formed by the condensation of aldehydes and ketones with mercaptans. In support of this view they identified methyl-α-oxysulfide, benzyl-α-oxysulfide, methylethyl (furyl)-α-oxysulfide, and nonylmethyl (methyl)-α-oxysulfide.

Bengis and Anderson (1932; 1934) isolated a new compound in coffee oil which they called kahweol. No mention is made of its taste and aromatic properties, but its great instability might easily lead to the production of other compounds within the oil, and for this reason some of its characteristics are presented here. It was isolated from the unsaponifiable fraction of a petroleum ether extract of freshly roasted coffee, and represents a major part of the unsaponifiable material.

Kahweol was shown to be extremely sensitive to acids, decomposing in a very short time with the production of a purple color. It is also sensitive to oxygen, light, and heat, but appears to be stable to alkali. Even when kept in a sealed tube in an atmosphere of carbon dioxide, the compound became deeply colored (yellow) within 24 hours. The tentative formula for kahweol is given as $C_{19}H_{26}O_3$. It contains an hydroxyl group and possibly a carbonyl group.

Prescott *et al.* (1937a), because of their results on the effects of moisture on coffee, used an extraction method to obtain the essential oils. Extraction with methanol yielded a material which, on concentration, proved to be too viscous to distil. However, by repeated solution and precipitation, a good deal of tarlike material was eliminated. The remaining volatile oil was fractionated under vacuum. A fraction boiling

at 77°C. (170.6°F.) and 40 mm. pressure, was shown to consist mainly of furfuraldehyde. The fraction boiling at 88-94°C. (190.4-201.2°F.) and 40 mm. pressure contained furfuryl alcohol, as shown by its urethane derivative. The fraction boiling at 92-100°C. (197.6-212°F.), and 4.5 mm. pressure, gave a *p*-nitrobenzyl ester of acetic acid. The total amount of the volatile material obtained in this manner was 0.55 ml.

These same workers extracted a coffee brew by passing it through tubes of ether and then ethyl acetate. Separating the resulting oil into four fractions, they found diacetyl, ethyl alcohol, acetic acid, furfuryl alcohol, a phenolic substance, furfuraldehyde and furfuryl acetate.

The work of Prescott *et al.*, in contrast to that of Erdmann, indicates that the chief acid in coffee oil is acetic rather than valeric. In both cases Santos coffee was used but the methods of obtaining the coffee oil were different. As shown by the work of Haagen-Smit *et al.* (1945a,b) on the differences in summer and winter pineapple, it is possible that variations in climate, fertilizer, etc., can affect both the quantity and the type of flavoring constituents in coffee.

Prescott *et al.* (1937b) repeated some of their previous work and, in addition, examined decaffeinated coffee by the same general procedures. This latter material was easier to work with because of the smaller quantity of fatty material present. They were the first workers to report the occurrence of terpenes, sylvestrene and eugenol in coffee. They also reported for the first time the occurrence in coffee of diethyl ketone, *n*-heptacosane (a long chain aliphatic hydrocarbon) and a hydrocarbon with a melting point of 117-118°C. (242.6-244.4°F.). They confirmed the presence of kahweol, *p*-vinyl guaiacol, and guaiacol. Guaiacol and a vinylguaiacol had been previously reported by Staudinger and Reichstein (1926; 1928). Vanillone and *p*-vinyl catechol were probably present in the materials isolated by Prescott *et al.*, if we are to judge by the data.

The work of Johnston and Frey (1938) should be mentioned, because they obtained an oil from coffee by dry distillation under a vacuum of approximately 0.005 mm. The distillation vessel was maintained at a temperature of 100°C. (212°F.) and the distillate collected in dry ice and liquid air traps. Acetaldehyde was found as one of the constituents, in addition to other carbonyl compounds that could not be separated for identification. They identified diacetyl by its semicarbazone and methylacetylcarbinol indirectly by its boiling point and by oxidation to diacetyl. This latter finding is in keeping with the results of Schmalfuss and Barthmyer (1929) in which they vacuum distilled the diacetyl from coffee in a carbon dioxide atmosphere. With subsequent addition of ferric

chloride solution they were able to distill off an additional quantity of the same substance.

Johnston and Frey confirmed the presence of hydrogen sulfide and organic sulfur compounds, but had insufficient material to identify the latter. They isolated a few drops of oil from their distillate which, on exposure to air, darkened immediately and deposited a resin or tarlike material. An unidentified ketone was obtained that gave a 2,4-dinitrophenylhydrazone which melted at 258-259°C. (496.4-498.2°F.). These authors agreed with Staudinger and Reichstein concerning the importance of the sulfur compounds in the aroma and flavor of coffee.

Bauer and Neu (1942) isolated a compound of formula $C_{20}H_{28}O_3$, which they called coffeol. This should not be confused with the kaffeol of Bernheimer (1902). There seems to be some question as to the actual existence of the latter compound since Sethness (1924) is the only worker who has been able to isolate a compound with the same boiling point as kaffeol.

b. Cocoa. Originally cocoa flavor and odor were assumed to be connected with cocoa-red, the coloring matter of fermented cocoa. However, Bainbridge and Davies (1912) showed that the aroma and flavor was due to an essential oil. The shelled cocoa beans were pressed to remove as much cocoa butter as possible and then steam distilled. The distillate on extraction with petroleum ether gave 24 g. of oil from 2,000 kg. of cocoa.

From the acid fraction of the oil, octoic, valeric, and *n*-nonoic acids were identified as the silver salts. Hexoic acid was obtained from the original residue of the cocoa distillation by treatment with 1% sulfuric acid and superheated steam. The purified oil was fractionated under vacuum. One fraction contained amyl acetate as proved by examination of the saponified products, and an oil with a lemon-grass odor. Another fraction, on saponification, yielded octoic, acetic, and propionic acids, and an alcohol which appeared to be amyl alcohol. Thus this fraction was apparently a mixture of amyl esters and the substance with the lemon odor. A third fraction appeared to contain some amyl butyrate, with perhaps a little esterified *n*-nonoic acid. The major part of the oil appeared to be linalool. A nitrogen constituent was isolated which could not be identified.

Schmalfuss and Barthmyer (1929) reported the presence of diacetyl in cocoa. More recently Fincke (1932; 1938) worked on the aroma-producing compounds in cocoa, but unfortunately these papers were not available for reviewing. In contrast to Schmalfuss and Barthmyer, Fincke (1933) does not believe diacetyl to be of importance to cocoa odor.

Adam *et al.* (1931) identified one of the catechins in cocoa as *l*-acacatechin. However, Freudenberg *et al.* (1932) have shown that this catechin is *l*-epicatechin.

c. Tea. In ancient times tea was used medicinally as a nerve stimulant. Today its use is similar, but not strictly by prescription. Two general types of tea are available as beverage bases: black tea and green tea. Black tea is made from the leaf buds and young leaves which are first withered and then rolled. They are then "fermented" by enzymes. The "fermentation" is stopped by "firing" the tea with hot air, and after drying it is ready for the market. Green tea is manufactured without withering the green leaf. Oolong tea is an intermediate type of tea made by partial fermentation of the green leaf.

Although the use of tea as a drink dates back thousands of years, the flavor constituents have been investigated in some detail only in the last twenty to thirty years. The greater part of this work has been done in Japan.

Flavor in tea is determined by two classes of organic compounds: the tannins, consisting of polyphenols and certain volatile organic compounds consisting of alcohols, aldehydes, ketones, and esters. A number of nonvolatile unidentified compounds have been mentioned.

The two flavanols, quercetin (Duess, 1923) and kaempferol (Oshima and Ka, 1936), have been isolated from green tea. Tsujimura (1941a) isolated 0.1-0.18% quercetin and 0.02-0.06% kaempferol from tea leaves. Tsujimura (1941b) also found 0.03% ellagic acid.

Tsujimura (1929) first isolated *l*-epicatechin from green tea as colorless prisms (Fig. 5, I). He prepared the pentaacetyl and tetramethyl derivatives. The original formula for catechin was suggested by Perkin and Yoshitake (1902) mainly on the close comparison between *l*-epicatechin and quercetin (Fig. 5, II). From the latter, Freudenberg and Purrmann (1924) made *l*-epicatechin.

Later Tsujimura (1930) isolated catechin-galloate (Fig. 5, III). On boiling with 5% sulfuric acid, gallic acid was split off. Deijs (1939) confirmed this work. Tsujimura (1931) synthesized the heptamethyl derivative of catechin-galloate directly. The absorption spectra of the derivative is identical to that of the natural product. Neither of the reactants, however, had similar absorption spectra. Tsujimura (1934) isolated crystalline gallocatechin (Fig. 5, IV) from a green tea of superior quality. Inferior quality green tea gave no crystals. All the catechins isolated by Tsujimura had an astringent taste. Oshima (1936) and Oshima and Goma (1933) also found epicatechin and gallocatechin in Formosan tea.

More recent studies on both green and black teas have been made by

Bradfield *et al.* (1946). Partition chromatography was used on Ceylon tea. About 80% of the total polyphenols were soluble in moist ether; from these, eight polyphenols were isolated, with six of these definitely catechin compounds and their derivatives. These workers isolated *dl*-gallocatechin (or the epimer), *l*-gallocatechin, *l*-epicatechin, a catechin, *l*-gallactocatechin gallate, and *l*-epicatechin gallate. The remainder consisted of a galloyl ester, an unknown polyphenol, traces of gallic acid, a phenolic substance, and green and yellow pigments. The polyphenol fraction, insoluble in moist ether, was not examined. Although no or-

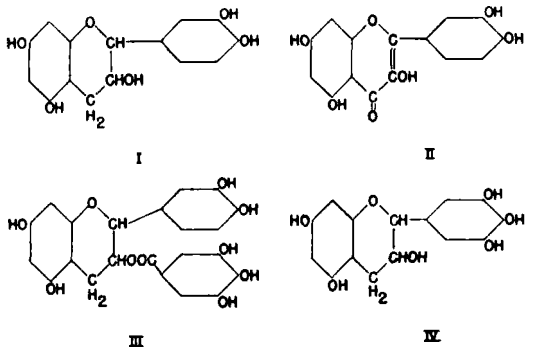

Fig. 5. Comparison of quercetin and catechins of tea.

ganoleptic observations were reported, it can be assumed, on the basis of other investigations, that most of these compounds were astringent to taste. Of the total polyphenols, 47.8% by weight were isolated. The major constituent was *l*-gallocatechin gallate (24.5%). Preliminary data on China Gunpowder tea and an unknown green tea indicated a similar composition. Therefore, as Bradfield points out, the variations in the quality of black tea are probably not due to variations in the green leaf catechins.

A great deal of work has been done by the Japanese on the volatile constituents of tea. Takei and Sakato (1932) obtained 0.014% oil from fresh, young tea leaves and 0.007% from summer leaves. They isolated and identified 3-hexen-1-ol as the naphthylurethan and the iodophenylurethan derivatives. This alcohol represents from 30-50% (Takei and Sakato, 1932) of the total oil. In addition, 5-6% of the corresponding aldehyde is present. Both of these compounds possess a green tea leaf odor.

Takei *et al.* (1934b) found a number of acids in the oil of fresh tea leaves: these included acetic, propionic, butyric, valeric, caproic, and

palmitic. Manufactured tea contained only caproic, palmitic, and heptoic acids in addition to *o*-hydroxy methyl benzoate. Later, the same workers (Takei *et al.*, 1935a; 1936; 1937a) found additional constituents: *n*-hexyl, benzyl, *n*-octyl, benzylethyl, and phenylethyl alcohols; another alcohol, $C_5H_{12}O$, was found, probably β-methylbutan-α-ol. They also found benzaldehyde as a constituent (Takei *et al.*, 1937b).

Geraniol was found as an ester and in the free state. The hydrolyzed ester fraction also yielded octoic acid and the $C_5H_{12}O$ alcohol.

A fraction boiling at 105°C. (221°F.) at 15 mm. pressure was found to be linalool which comprised approximately 13% of the total oil. Acetophenone was also found in the same fraction. Finally, they added phenol, cresol, and hexoic acid to the list of compounds known to occur in tea.

There have been a greater number of studies on green tea than on black tea. The work of Bradfield (1946) indicates the presence of a high molecular weight substance in black tea infusion. Dialysis of a black tea infusion for 14 days left 45-60% of the original color in the solution and an appreciable amount of dissolved material. By contrast, a green tea infusion dialyzed almost completely. The non-dialyzable material of black tea also seemed to be a measure of quality; an inferior quality tea contained more undialyzed substance than the superior quality tea. On being heated for 15 hours the superior quality tea became unpleasant in taste and was darker in color. During this period the undialyzed material increased from 16 to 34% of the original polyphenol content.

Bradfield (1946) and Bradfield and Penney (1944) showed that black tea extract contains caffeine, inorganic salts, a gum containing some carbohydrate, and two major groups of polyphenols. Of the latter, one group is extractable by ethyl acetate, while the other remains in aqueous solution. The ethyl acetate extract yielded an orange powder soluble in hot water and ethyl alcohol. This powder consists of polyphenols and most of the caffeine. After extraction with ethyl acetate the remaining aqueous solution yielded a brown powder.

Five different tea samples were examined: ordinary Sylhet, upper Assam, high-grown Ceylon, Dooars, and Nyasaland. The extractable fractions were examined for optical rotations. The two samples that differed most in quality of brew were examined for absorption spectra. The orange and brown liquors absorb strongly at the violet and thus contribute color to tea. Concentrated solutions of the extractable polyphenols gave a bright red color, while concentrated solutions of the nonextractable polyphenols gave a dull reddish brown. A comparison was made of the two samples that differed most on the ratio of extractable polyphenols to nonextractable polyphenols. The brighter red liquor

had 13.2% extractable and 11.5% nonextractable polyphenols whereas the duller liquor had 8.5% extractable and 10.8% nonextractable polyphenols. The relation between color and strength of tea liquor, however, is probably not so simple.

An aqueous solution of the orange powder has a powerful astringent taste, and gives a red color to the infusion. The brown powder solution has a weak taste. The caffeine, nonphenolic gum, and the inorganic ash add very little to the taste and color.

The Japanese have also investigated the volatile compounds of black tea oil. Yamamoto and Kato (1934) separated, by steam distillation, the odor constituents from tea leaves undergoing fermentation during the manufacture of black tea. The oil was separated into several fractions: the one boiling at 36°C. (96.8°F.) contained isovalderaldehyde, α, β-hexenal, and isobutyl aldehyde, and that boiling at 40-44°C. (104-111.2°F.) β, γ-hexenol. The higher boiling fractions contained methyl salicylate, phenylethyl alcohol, and citronellol.

Yamamoto and Kato (1935a), using Formosan black tea, isolated, in addition to the compounds mentioned above, salicylic and palmitic acids, traces of butyric, isovaleric, and phenylacetic acids, butyraldehyde, geraniol, an unknown phenol, an unsaturated terpene alcohol, and an acid melting at 128°C. (262.4°F.).

Yamamoto and Kato (1935b) steam distilled seisin-oolong tea. The acid fraction contained traces of phenylacetic and salicylic acids. Thirteen grams of neutral oil were obtained. They isolated practically the same compounds as they found in the black tea. They did not find palmitic, butyric, and isovaleric acids, the unsaturated terpene alcohol, or the acid melting at 128°C. (262.4°F.).

Takei *et al.* (1935b) prepared a series of synthetic compounds from the main constituents of green tea oil. They claim to have made the products that give black tea its characteristic odor. However, there is no evidence that these products are present in black tea oil, or that they are produced during the fermentation as the authors claim.

Yamamoto and Ito (1937) steam distilled Formosan black tea and extracted the distillate with ether. The acid fraction of the oil yielded propionic, isovaleric, caproic, hexenoic, caprylic, palmitic, salicylic and benzoic (a trace) acids and cresol. The basic fraction contained quinoline and a sulfur compound in an amount too small to permit identification. Caproaldehyde and benzaldehyde were identified as 2,4-dinitrophenylhydrazones and semicarbazones. Hexanol, hexanal, octanol, linalool, phenylethyl alcohol, citronellol, and geraniol were also identified.

Takei *et al.* (1937b) isolated methyl ethylacetaldehyde, isobutylaldehyde, isopropylaldehyde, propylaldehyde, benzaldehyde, a C_{5-8} ketone,

and a ketone with the formula $C_6H_{12}O$ from the dust of Formosan black tea. The same authors (1938) found hexyl, octyl, and phenylethyl alcohols, 2-hexen-1-ol, and geraniol all previously isolated from tea by Yamamoto and Ito (1937). Takei *et al.* also found butyl, isoamyl, and benzyl alcohols to be present in their samples.

Yamamoto *et al.* (1940) obtained the following neutral substances from the steam distillate of Formosan black tea: chiefly benzyl and phenylethyl alcohols with phenylpropyl alcohol, linalool, a secondary terpene alcohol, and geraniol. The oil also contained 2-acetylpyrrole, small amounts of methylmercaptan and probably methyl sulfide. The latter compounds are interesting since they and similar ones have been found to be essential to the coffee flavor.

III. Summary

The flavors of fruits and vegetables are highly dependent on the volatile materials they contain. For example, in radishes it is mostly mustard oils, in onions and garlic organic sulfur compounds, and in fruits mainly esters. In other cases it may be terpenes. If we compare the large number of fruits and vegetables whose flavors have been studied with those whose flavoring components have not been studied, the limited information available in this field becomes apparent. The flavors of little more than a dozen fruits and vegetables have been investigated and in some cases this work is quite incomplete.

This lack of knowledge is largely due to the very small quantities of flavoring materials present, and in some cases to their unstable nature. Usually it is necessary to handle tons of raw material to obtain a mere hundred grams of the flavoring oil. Furthermore, when this oil is split into its numerous parts, the yields of some components may be only a fraction of a gram. Small as these may be, they all add their part to complete the characteristic flavor of the product. Nevertheless, some workers have totally ignored these important constituents.

The fact that flavor is due to a mixture of different types of compounds is often ignored. Saponification of a mixture of alcohols, esters, and acids will permit an ultimate analysis of the component parts, but will provide no knowledge of how these are or are not combined.

Some of the earlier work was done before the development of appropriate identifying derivatives. The investigator was forced at times to identify by odor. Odor is often of value in indicating the direction that identifying work should take, but it is hardly a means of positive identification.

In some cases the determinations of flavoring constituents of fruits have been interrupted before the work was completed. In others only

those components present in large amounts have been identified, so that there is still much work to be done. Even with the more complete work such as with fresh pineapple, raspberry, and strawberry, there is still the interesting problem relating to characteristics of the flavor in the canned fruit.

The majority of the work on fruits and vegetables is incomplete. As indicated previously, the work on apples needs to be verified and completed. The individual esters should be separated before saponification so as to determine which ones are present. The same criticism can be applied to the work on peaches; that is, the esters should be separated prior to saponification.

The flavoring constituents of cherries, grapes, lemons, and bananas have only been touched upon; a more thorough investigation should be undertaken to complete the work. Similarly with carrots, celery (roots and herb), parsley (herb), parsnip, cress, and potatoes. The work on radishes needs repeating in order to clarify the conflicting reports pertaining to the nature of the flavoring constituents.

Scarcely any of the more common vegetables have been investigated for their flavoring components. Peas, beans, cabbage, beets, squash, and many others still await their turn in the laboratory. The little work that has been done on vegetables has concerned itself chiefly with portions of the plant not used for food. Although some of the components of the seed oil may exist in the edible portion of the plant, the flavoring components are not usually identical.

A great deal of work has been done on coffee flavor, but what happens when coffee becomes stale is still uncertain. Isolation of the components from stale coffee would enable a comparison with those of the fresh coffee, and a better understanding of how "staling" develops. A similar statement may be made for tea, although investigations indicate that the "off flavor" developing in tea is at least partially due to the reactive nature of the polyphenols. The effect of the volatile flavoring components is not known. It would be interesting to know the amounts of the various component parts essential to a good tea.

Fundamental research in food flavors is needed to assist producers and processors obtain and retain the original fresh flavor in food products. Information showing the relation of variety, harvesting, handling and processing technique and procedures to flavor losses and changes would be most welcome. Although difficult, this is truly a worthwhile field of investigation.

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Histological Changes Induced in Fruits and Vegetables by Processing *

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I. Introduction

Probably the first attempt to relate the characteristics of a foodstuff with histological properties was made by Anthony van Leeuwenhoek in April of 1676. His curiosity regarding the pungency of pepper had lead him to place some whole peppercorns in water and to let them stand thus for some three weeks. He hoped thereby to soften the peppercorns, thus making them easier to study with his microscope. The result was quite unexpected, for rather than discovering the peculiar pungency-producing structure of the the peppercorn, he saw bacteria for the first time.

The present summary is not a comprehensive survey of all reported observations on histological changes occurring in fruits and vegetables during ripening, storage and processing. With one exception (p. 334) only observations on histological changes during processing are considered; the majority of these are concerned with the action of moist heat on fruits and vegetables. We have tried mainly to point out that there is often a close relation between the cellular structure of the plant tissue and the quality of the finished product. Only examples which best show this relationship have been selected for review.

Food technologists, generally have a minimum of training in the fields of histology and plant breeding. Plant tissues are complex and may be considered as myriads of small individual colloidal systems. An understanding of the colloidal natures of these individual units and their interrelationships should increase the effectiveness of any food technologist. The concepts of plant structures found in articles and books pertaining to foods are sometimes appalling to the histologist. One example of this is given; it was selected from the excellent book of Tressler and Evers (1943).

It should be emphasized that a thorough knowledge of the normal structure of plant tissues is a prerequisite to any critical study on changes in tissues brought about by processing.

1. Histology of Normal Tissues

a. Cell Types. There are four general types of mature tissues in plants: (1) the storage or parenchyma tissues [Plate 1], (2) conducting tissues [Plate 2, Figs. 12, 13, 14 & 15], (3) supporting tissues [Plate 2, Figs. 9 & 10], and (4) protecting tissues [Plate 2, Figs. 9, 10, 11, 16, 17 & 18; Plate 12, Fig. 54a]. Most edible parts are largely parenchyma tissues, with the conducting tissues forming an anastomosing network throughout. The supporting tissues, such as collenchyma, fibers, and tracheids are usually undesirable in processed and even fresh foods. In some instances, however, when present in small amounts they give a desirable firmness to fruits and vegetables. The walls of the cells forming the

supporting tissues (except collenchyma) as well as certain types of conducting cells (tracheids, vessel elements) are lignified or infiltrated with lignin, a hard substance which is undesirable in most foods.

Parenchyma cells are roughly 600 μ in diameter. Although they are mostly isodiametric cells with twelve sides (Plate 1, Figs. 4 and 5), brick

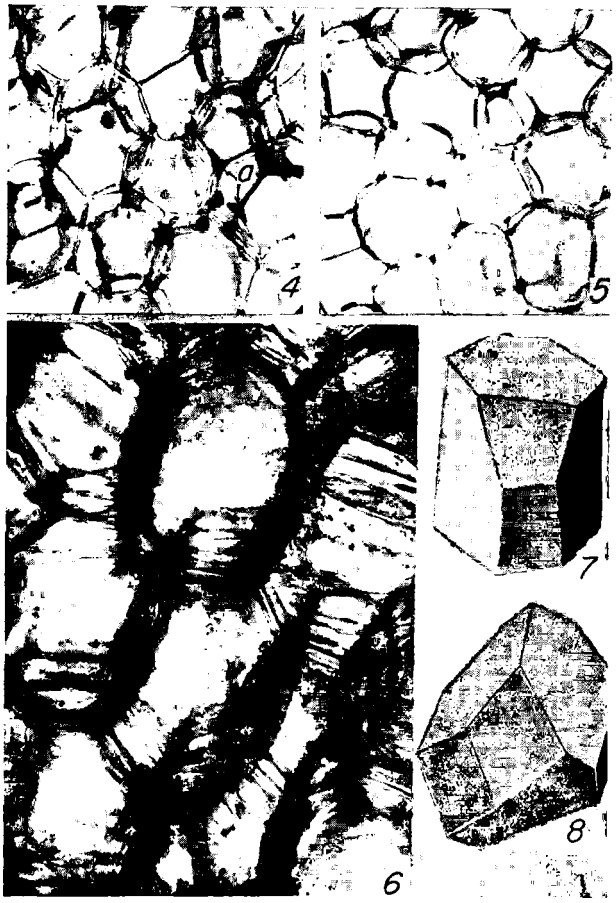

Plate 1

Fig. 4. Parenchyma cells from living onion. Dark regions (a) are intercellular spaces filled with air (Currier, 1946).

Fig. 5. Parenchyma cells from blanched onion. Intercellular spaces are filled with water (Currier, 1946).

Fig. 6. Nonliving parenchyma cells; with much intercellular air.*

Figs. 7 and 8. Diagrams of parenchyma cells showing three-dimensional shape (Marvin, 1939).

* All unacknowledged figures were prepared by Edward L. Proebsting especially for this review and are indicated by an asterisk after the legend.

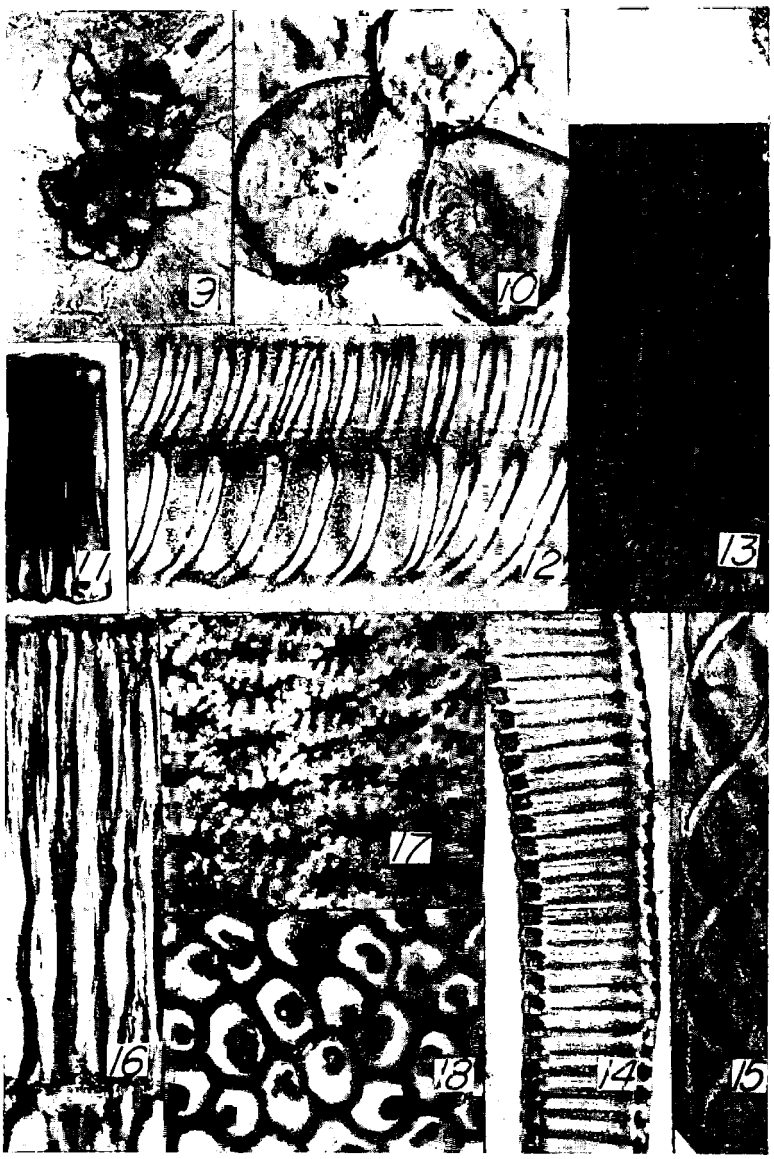

Plate 2

Fig. 9. Group of stone cells in pear, × 100 (Crafts, 1944).

Fig. 10. Several stone cells of pear, × 400 (Reeve, 1948).

Fig. 11 Protecting cells from seed coat of bean. From macerated tissue, ca. × 270.*

Figs. 12, 13, 14 and 15. Water conducting elements from leaves.*

Fig. 16. Longitudinal section through the seed coat of a pea showing the protecting cells (Reeve, 1947).

Fig. 17. Cross section through outer end of the protecting cells; note thick irregular cell walls (Reeve, 1947).

Fig. 18. Cross section through lower end of protecting cells. Note thin, more regular cell walls (Reeve, 1947).

shaped parenchyma cells with fewer sides also occur. Their location within the plant and their function has some influence on their shape. For instance in the upper surface of leaves, the photosynthesing cells are long and narrow; in a potato tuber the storage cells are isodiametric.

The cell walls of parenchyma cells are largely cellulose. While in general quite thin, most parenchyma walls are supplied with simple pits, circular or oblong regions of a very thin cellulose layer. The cells of parenchyma tissue are held together by pectic compounds and any processing method which reacts on either the pectic cementing substance or on the cellulose of the cell wall will have an effect on the finished product (see page 310).

The shape of parenchyma cells is such that they do not fit together perfectly. The intercellular spaces thus normally formed are filled with air (Plate 1, Fig. 4a and Plate 12, Fig. 55). During blanching the intercellular air may or may not be replaced by water (Plate 1, Fig 5) (see page 333). This simple fact has a rather profound influence on the appearance of and possibly the keeping qualities of finished products.

Parenchyma cells of important vegetable and fruit products are alive previous to processing and although seldom considered, the method and rate of killing these cells may have important influences on the quality of the product. The rapid changes taking place in green peas after harvest is a good example of the necessity of rapid killing.

The principal water conducting elements found in fruits and vegetables are composed of a linear series of dead cells, the contents of which have been displaced by an aqueous salt solution. The individual cells are called tracheids or vessel segments dependent upon whether their end walls are present or absent at maturity.

Vessels are long tubes composed of a number of cellular elements placed end to end. The terminal walls of the vessel elements are dissolved forming a continuous unobstructed pathway for water movement. The walls of vessels are not uniformly thickened. Several types of vessels are known: (1) annular vessels, in which the thicker lignified portions of the wall are in the form of rings (Plate 2, Fig. 12), (2) spiral vessels, the lignified portions of wall forms a spiral (Plate 2, Fig. 15), (3) pitted vessels, the thin nonlignified portions of the cell wall are pits. Since vessel elements have relatively thin lignified walls they may not be tough in processed foods.

Food conducting elements, the sieve tubes, are generally not lignified, at least while functioning as food conducting cells for the plant. They are normally present in the edible portions of plants. It is possible that in older tissues of some vegetables they may constitute an undesirable feature but since no studies relative to this question on these elements

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in vegetables or fruits have been made there is no information on this point. Fibers may occur in the phloem or plant food conducting tissue. When present in excess they are objectionable because of toughness.

Fibers are pointed elongated cells with thick lignified cell walls and a small lumen or cell cavity (Plate 3, Figs 19 to 22). In young tissue

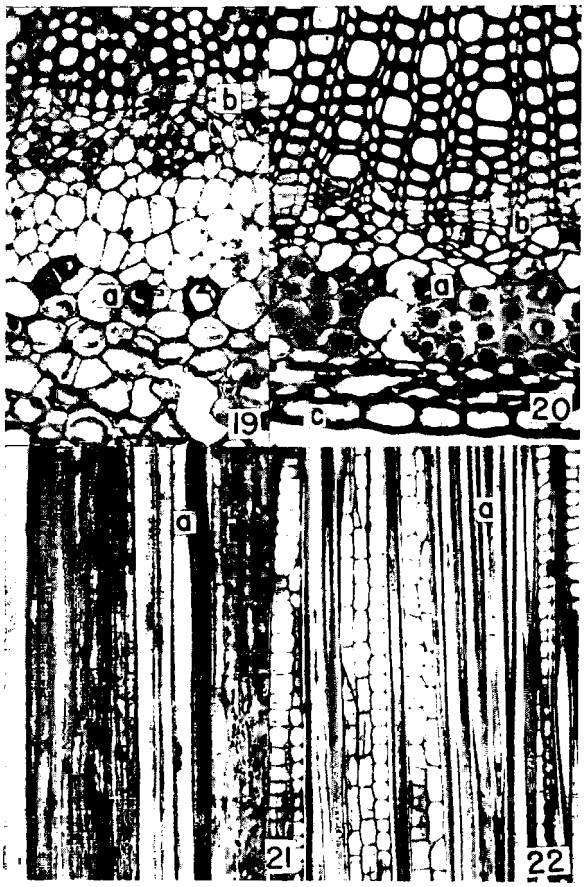

Plate 3

Sections of an herbaceous stem to show the development of fibers as the stem ages (Esau, 1943).

Fig. 19. Cross section of young stem. The darker, thick-walled cells at *a* are fibers. The cells at *b* are dividing cells forming the cambium region.

Fig. 20. Cross section of old stem. Note at *a* the great increase in numbers of heavy-walled fibers; the cambium at *b*.

Fig. 21. Longitudinal section of young stem; a few fibers at *a*.

Fig. 22. Longitudinal section of old stem; many fibers at *a*.

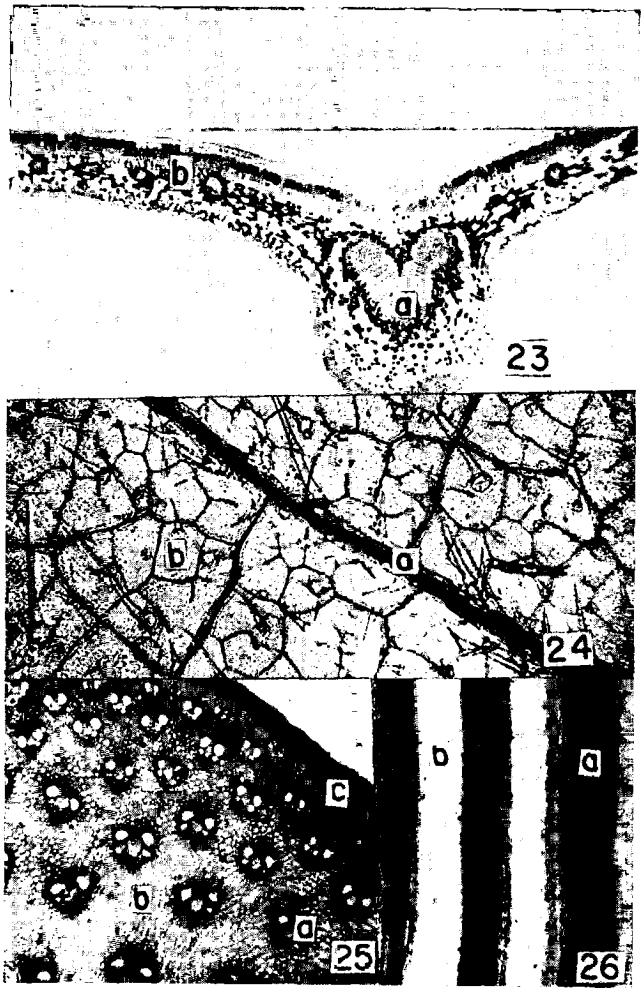

Plate 4

Fig. 23. Cross section of a leaf. Conducting tissue, with some strengthening tissue forms the main vein (*a*). The leaf blade (*b*) is formed by parenchyma cells and small veins.*

Fig. 24. Surface view of a whole leaf. Dark lines (*a*) are veins composed of conducting and strengthening cells. Parenchyma cells (*b*) form the main body of the leaf blade.*

Fig. 25. Cross section of an old (inedible) asparagus stem. Conducting and strengthening tissues (*a*) are grouped in discrete bundles and are surrounded by parenchyma cells (*b*). Fibers have formed just under the epidermis of the stem (*c*). In a young asparagus stem there would be no fibers and the bundles would be in a rudimentary stage of development. In many stems the conducting cells form a continuous cylinder within the stem.*

Fig. 26. Longitudinal section of old asparagus stem showing the conducting and strengthening bundles (*a*) embedded in parenchyma tissue (*b*).*

(Plate 3, Figs. 19a and 21a) they are not well developed, the lignin only being deposited as the plant organ (stem, root, or leaves) ages and additional supporting material is required (Plate 3, Figs. 20a and 22a). While the examples chosen to illustrate this point are not from food products, similar changes take place in all aging plant organs.

Another type of supporting cell known as collenchyma is sometimes of importance in young vegetables. It is an elongated, pointed cell with unevenly thickened walls composed only of cellulose and pectic substances.

Most edible fruits and vegetables consist largely of parenchyma tissue (Plate 1), (carrots, potatoes, cabbage, fruits, peas, grain endosperm) with minor amounts of anastomosing xylem and phloem elements (Plate 2, Fig. 13; Plate 4, Fig. 24). All vegetables and fruits are protected by cork or epidermal layer of some sort, usually of specialized parenchyma cells, either secreting cutin or impregnated with suberin (Plate 3, Fig. 20c; Plate 12, Fig. 54a). Frequently hair cells (peach) or sclereids (pea, Plate 2, Figs. 16, 17 and 18) form an important cell type in the epidermis. The epidermal layer is of considerable importance in processing, and its structure has much influence on the desirability of a given product.

Although the fruits and vegetables contain all of these cell types, the relative proportions of them differ greatly in response to genetic constitution, environmental conditions, age, and plant organ, whether root, stem, or leaf. Plate 4, Figs. 23 and 24, show a cross section and surface view of a typical leaf. The anastomosing vascular bundles of the veins (a) are clearly visible throughout the parenchyma tissue (b). Development and lignification of fibers in these vein regions (i.e., in cabbage) would lead to a tough, stringy, and unacceptable product.

Fibers are generally associated with vascular bundles or groups of cells which are specialized for support and conduction (Plate 4, Fig. 25a; Plate 5, Fig. 28), or in the peripheral regions (usually in old stems) of the stem (Plate 4, Fig. 25c). In some vegetables such as carrots and beets (Plate 5, Fig. 27), the lignified fibers may occur in the center or core of the root (xylem) or be fairly uniformly distributed.

Woodiness or stringiness in old vegetables (i.e., carrot roots) is due to the deposition of lignin in the walls of the fibers as well as in the vessels. Fibers are in general objectionable in processed foods because of the toughness and stringiness they impart. Furthermore, lignin is indigestible and does not add to the nutritive value of the food.

b. Summary of Cell Types. Parenchyma tissue is composed of thin-walled isodiametric cells with a living protoplast. They are storage cells, containing starches and sugars, or synthesizing cells containing the green

pigment, chlorophyll. This tissue forms the ground tissue of practically all edible vegetable and fruit products.

The conduction tissues form two groups: food conducting (phloem) and water conducting (xylem). The cells of the xylem (vessels and tracheids) become lignified, hence large amounts of xylem elements are

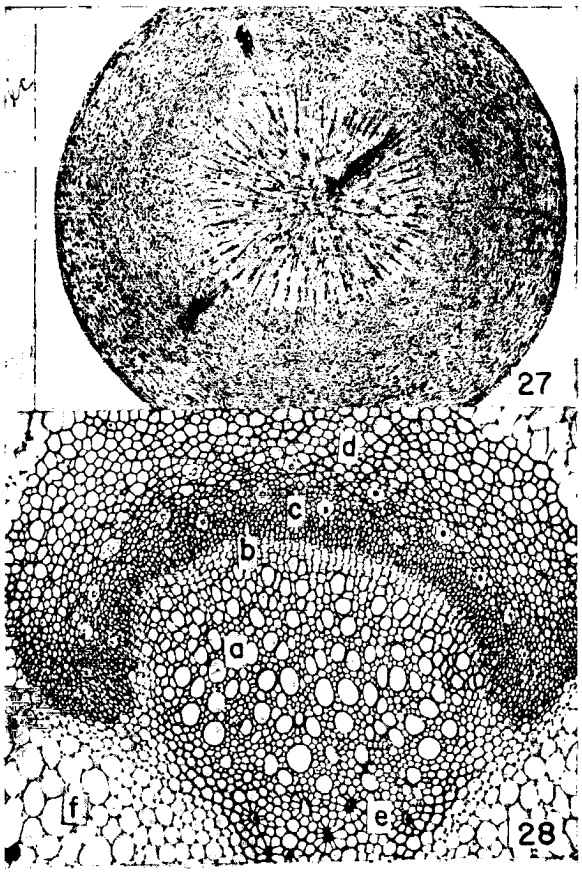

Plate 5

Fig. 27. Cross section of a young carrot root, composed largely of parenchyma tissue. The water-conducting elements are scattered throughout the center or core of the root; the food-conducting elements are scattered through a peripheral zone or the flesh. No fibers or other undesirable thick-walled cells have been formed (Esau, 1940).

Fig. 28. Cross section of conducting and strengthening bundle of a celery stalk. Water-conducting cells at *a*; cambium, *b*; food conducting, *c*; strengthening, *d*; oil ducts, *e*; and parenchyma, *f* (Esau, 1936).

objectionable. Phloem elements are generally not objectionable in food products. They are not usually present in large amounts.

Supporting tissues, whether composed of fibers or collenchyma cells, are usually objectionable, imparting a woodiness or stringiness to food products. This is especially true of fibers which have become lignified.

c. Contents of Normal Cells. The protoplasm in parenchyma cells is generally parietal, lying close to the cell wall, with thin strands traversing a relatively large central vacuole. This picture may be variously modified depending on the amount of storage starch present in the cell or on the size and number of the green pigmented chloroplasts. The protoplasm is a complex colloid with some characteristics of an emulsion.

Organized bodies of various types occur in parenchyma cells. Perhaps the plastids are the most important of such bodies from the viewpoint of food research. There are three general types of plastids; chloroplasts, leucoplasts and chromoplasts.

Green leaves, (spinach, celery, cabbage) contain chloroplasts, the centers of carbohydrate synthesis. Structurally the chloroplast may appear homogeneous or granular. The granules (grana) are thought to contain the chlorophyll, while the background material (stroma) is colorless. There is good evidence to indicate that the submicroscopic structure of the chloroplast is regular and complicated. The chloroplast is not simply a homogeneous mixture of various protein and lipoidal substances but is an organized body in which each substance has an exact spatial orientation.

Leucoplasts are colorless storage plastids. They occur in vegetables such as potatoes, beans, peas, etc., where they are associated with the formation of starch. In some fruits, in carrots, sweet potatoes, and other colored vegetables some of the plastids, lack chlorophyll but contain an excess of the carotenoid pigments (see page 328). They may or may not contain small amounts of starch. These are chromoplasts. Their structure varies greatly in different plants; their function in the plant is not known. In addition to these protoplasmic bodies concerned with food manufacture the protoplasm also contains a nucleus, mitochondria, and highly refractive oil droplets.

The vacuole of most parenchyma cells is a dilute aqueous solution of various inorganic and organic salts, pigments and food materials separated from the protoplasm by a thin cytoplasmic membrane.

This brief discussion of cellular structure will serve to point out that the cell is a complex, organized body. Its very existence is dependent upon a balance maintained by interfaces, between plastids, nucleus, vacuole and the cytoplasm, and molecular orientations such as that mentioned for the chloroplasts.

The importance of cellular organization in food processing has not been fully appreciated. The food chemist is not dealing with a homogeneous mixture of chemical substances. While we do not in any way underestimate the importance of investigations on homogenized tissues, tissue extracts and so forth, such investigations inevitably obscure certain relationships. The presence of substances in a cell, even a dead one, does not insure that a given reaction will occur. The spatial relationship of substances within cells must be of great importance in determining the course of changes in foods during processing and storage.

2. Physiology of Normal Tissues

a. Normal Cell Turgidity. The crisp firm texture of normal fresh plant tissue is, in addition to cellular cohesion and structure, chiefly due to cell turgidity which is a function of the water absorbing power of the cell and the availability of water. Many food processing techniques so alter the histochemical features of the tissue that a flabbiness (loss of turgor) results which may greatly decrease the acceptability of the finished product. A knowledge of the fundamentals of water retention by living and dead tissue are requisite to a lucid interpretation and profitable clarification of many food acceptability problems.

For example, it is possible to anticipate, in part at least the effect on the final cell turgidity of immersing a section of parenchyma tissue in strong brine solution, of blanching, of freezing and thawing, or of drying and rehydrating.

The living plant cell is an osmotic system which maintains its turgor by literally "sucking itself full of water." During imbibition the vacuole becomes large, pressing the water-rich protoplasm tightly against the partially elastic cell wall, which stretches to a maximum (at full turgidity). To say that the cell is an osmotic system implies a differentially permeable membrane enclosing a solution filled central cavity. Such a system is represented by the living protoplasm which, surrounding the solute-rich vacuole and enclosed within the permeable cellulose wall, acts as a differentially permeable membrane. This allows water to pass through fairly rapidly but prevents or greatly impedes the penetration of most dissolved substances.

Because of their metabolic activity plant cells are able to accumulate and retain quantities of inorganic solutes, greatly in excess of the concentration found in the external normal environment. In addition to these solutes, organic foods which are elaborated in various parts of the plant body, may be stored in high concentration within the vacuole and protoplasm of parenchyma cells. Consequently there exists within the

vacuole a high concentration of osmotically-active material which, because of its presence, increases the water retaining power of the cell.

Water will diffuse from regions where the activity of its molecules is high to regions of lower activity. This tendency to diffuse has been designated diffusion pressure (Meyer and Anderson, 1939). Under the same conditions of pressure and temperature pure water has a higher diffusion pressure than water in any other system. The diffusion pressure of water may be increased: (1) by increasing the external (hydrostatic) pressure upon it or (2) by increasing the temperature. It may be decreased by: (1) reducing the pressure upon it, (2) reducing the temperature, (3) the addition of solutes, and (4) the addition of colloidally active material which may become more or less hydrated. If a nonturgid living parenchyma cell is placed in water the diffusion pressure of water within it will be less than the pure water outside and water will diffuse into the cell, causing an increase in volume. Concomitant with this increase in volume there will be an increase in hydrostatic pressure within the cell as the wall approaches its elastic limit. This turgor or hydrostatic pressure will increase the diffusion pressure of water within the cell and equilibrium will be attained when the increase in diffusion pressure due to turgor is just counterbalanced by the decrease due to the presence of solutes within the cell.

Obviously then, at equilibrium any addition of solute to the bathing water would cause a reduction in diffusion pressure of that water and water will diffuse out of the cell resulting in loss in turgor. The more salt added to the external solution the more water will be lost from the cell and greater will be the loss of turgor.

Such a condition will result for instance when cucumbers are placed in brine solution or strawberries and fruits are frozen by the immersion method. We would expect the protoplasm to be drawn away from the cell walls as the cells lose water and the vacuoles become small. This condition is known as plasmolysis (Plate 12, Fig. 56).

b. Affect of Death on Cell Turgidity. Any food processing technique which alters the permeability of the protoplasm, the ability of solutes to be retained within the cell, the elasticity of the cell wall, or the colloidal nature of the cell contents will alter the water retaining power of the cell, and possibly the crispness of the final product. Death of the cell results in an increase in permeability of the protoplasm and if the cell is in a dilute aqueous medium there will be a rapid diffusion of solutes out of the cell to regions of lower concentration. Accompanying this loss of solutes water will be lost and cell turgidity reduced. If the stored food in the parenchyma cell is chiefly soluble there will a great loss of solutes and the flabbiness of the tissue will be apparent. On the other hand if much of

the reserve food is in the form of starch grains or other colloidally active material water loss will not be as great and cell turgidity correspondingly more. The killing treatment may change the colloidal nature of the cell contents in such a way that their inhibitional properties are increased, thus reducing the effect of solute loss on water retaining capacity. This might well be the effect of heat treatment of starchy products such as sweet potato where the starch is gelantinized. This may involve a partial hydrolysis of the starch grains (Sinoda and Kodera, 1932) an increase in possible points of hydration of the constituent carbohydrate and a consequent increased capacity for swelling. The resulting gelatinized grains swell and fill the cell often breaking through the walls (Plate 10). In this case not only is the texture a function of cell and tissue firmness due to hydrated starch, but the individual grains may retain sufficient rigidity to give an acceptable and desirable texture to the finished product.

In the normal parenchyma tissue the large intercellular spaces are filled with air. Upon death of the tissue in a humid atmosphere, many of these may become filled with solution pressed out of the cells by contraction of the cell wall (see page 333).

If the tissue is of a type having highly elastic walls full turgor will represent a highly distended condition and large water content. On death of this type of tissue water and solution will be forced out in large amounts due to the contraction of the distended walls. On the other hand should the tissue be composed of cells having walls of little elasticity and great rigidity, full turgor will be represented by a relatively small change in volume over a flaccid condition and consequently death will result in a smaller contraction of the wall and a smaller solution loss, although the solution will be held within the lumen of the cell in a much more loose state than where the cell is alive.

Colloidal materials that will not pass through the dead cytoplasm will retain water of imbibition through activity of their surfaces and consequently cells having large quantities of protoplasm, starch, etc., are likely to retain more of their water on death than cells of smaller colloidal content. Water may be held within tissue by capillarity of the intercellular air spaces.

In summary then there are five chief internal factors that control water retention of living and dead plant tissue:

1. The concentration of osmotically active materials within the vacuole and cytoplasm of the cell.
2. The permeability of the protoplasm.
3. The amount of colloidally active materials within the vacuole, cytoplasm, and cell wall.

4. The elasticity of the cell walls.
5. The presence of intercellular spaces in the tissue.

III. Histological Changes Induced by Processing Technique

1. Changes in Cellular Adhesion

a. Cellular Adhesion and Texture. In many products acceptable texture ranks high among the factors determining consumer acceptance. Of the various histochemical changes which occur during food processing, changes in cellular adhesion are of primary importance in determining the final texture of the product.

In the discussion of the general morphology of the cell, the presence of an intercellular substance of pectic nature was mentioned which binds the cellulose, pectic-cellulose or hemicellulose primary walls of two adjacent cells together. The qualitative and quantitative characteristics of this cementing material may vary, not only between varieties of the same species, but also within each variety, and between materials of different ages or grown under different conditions. For example, Reeve (1947) in studying the relationship of histological characteristics to texture of pea coats found that the middle lamella was composed largely of pectic material in the young peas but became "incrusted" with pentosans or hemicellulose upon the maturation of the pea.

Toughness of seed coats was also found to be a reflection of plant nutrition. Peas grown under conditions of high calcium and low potassium produced skins tougher than the control while low calcium-high potassium conditions produced skins more tender than the control.

That softening of plant tissue may be correlated with pectic changes has been demonstrated by numerous studies on physical and chemical changes during the maturation of fruit and vegetables. Simpson and Halliday (1941) give a general discussion of this subject in the introduction to their article. This work indicates that as ripening progresses protopectin is converted to soluble pectin. There is a decrease in total pectic substances with overripening. Accompanying these changes there may be a thinning of the walls and a separation of the cells as these changes represent a lessening of the cementing power of the intercellular material.

In contrast to these changes which are due to enzymes, changes in pectic materials and the corresponding histological changes which occur during processing, are usually independent of the presence of active pectase.

b. Effect of Heat. The two major processing techniques which affect cellular adhesion are heat and chemical treatment. Chemical and histo-

logical changes that occur during the cooking of carrots and parsnips have been followed in detail by Simpson and Halliday (1941). In similarity to changes known to occur during ripening of fruit, these authors found an increase in pectin and pectic acid (or pectates) and a decrease in protopectin and total pectic substances during cooking. Table I presents a summary of their data.

TABLE I

Effect of Steaming on Pectic Substances of Carrots and Parsnips *

Fraction of pectic substance	Raw Carrots	Raw Parsnips	Steamed 20 minutes Carrots	Steamed 20 minutes Parsnips	Steamed 45 minutes Carrots	Steamed 45 minutes Parsnips
	%	%	%	%	%	%
Pectin	3.7	4.7	6.0	6.1	8.8	7.9
Protopectin	14.1	10.2	9.0	7.7	3.6	5.7
Pectic acid or pectates	0.8	1.6	1.0	2.0	1.3	2.1
Total pectic substance	18.6	16.4	16.1	15.8	13.7	15.7

* Results represent the per cent of dry weight and are computed from the average of five weights of calcium pectate in each case (Simpson and Halliday, 1941).

Correlated with the hydrolysis of protopectin to form pectin was an increase in softness. In addition to these changes microchemical staining tests indicated that there was an initiation of cellulose hydrolysis on cooking. There was a reduction in thickness and continuity with steaming.

Plate 6 (figures 29 and 30) shows photomicrographs of carrot tissue stained to show cellulose before and after steaming. A reduced thickness and continuity of cell walls indicates a probable initiation of cellulose hydrolysis on cooking. Plate 6, (figures 31 and 32) shows a carrot slice stained to show pectin before and after cooking respectively. Unfortunately the nature of the material necessitated the use of the paraffin embedding technique for its preparation and consequently unavoidable changes in wall thickness accompanying dehydration must have occurred. Nevertheless, the fact that all samples were treated similarly during the preparation should make the comparative results significant.

Probably the product most frequently investigated from the point of view of textural changes induced by processing is the potato. Some of the differences between mealy and waxy potato will be discussed in connection with their starch content (page 324). Although cell separation during cooking has often been considered a primary factor in mealiness (Freeman, 1942), Personius and Sharp (1938) were unable to find any difference between the adhesion of cells of soggy and mealy potatoes in either the raw or cooked tissue (Plate 7).

The method of determining cellular adhesion was more objective than employed heretofore, enabling them to obtain measurements on tensile strength. Their results indicate that complete weakening of the intercellular substance of potato tuber tissue can be brought about by holding

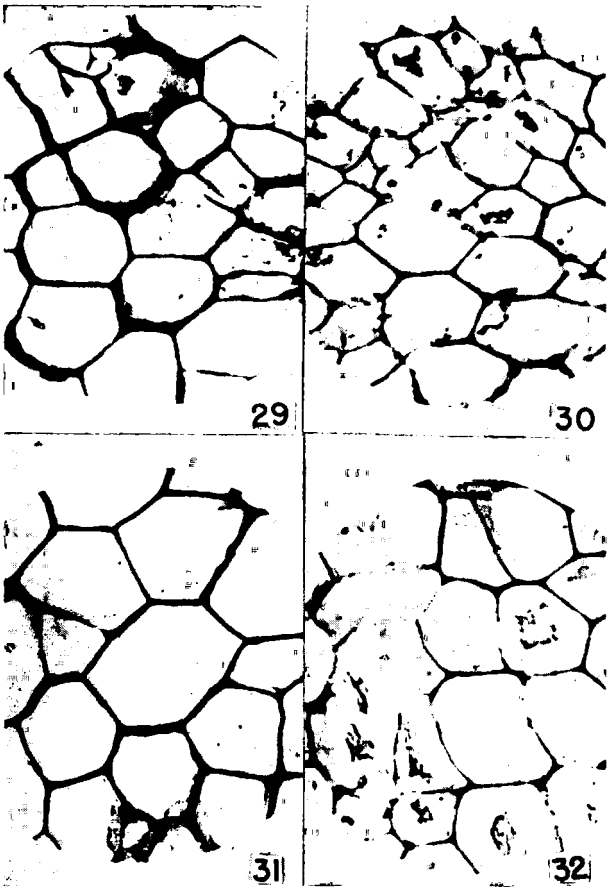

Plate 6

Fig. 29. Paraffin section of raw carrot tissue, cell walls stained to show presence of cellulose (Simpson and Halliday, 1941).

Fig. 30. Paraffin section of carrot tissue steamed for 45 minutes. Same staining as in Fig. 29. Compare thickness of cellulose walls (Simpson and Halliday, 1941).

Fig. 31. Paraffin section of raw carrot tissue stained to show the middle lamella of pectic material (Simpson and Halliday, 1941).

Fig. 32. Paraffin section of carrot tissue steamed for 45 minutes. Same staining as Fig. 31. Compare thickness of walls and note discontinuity of walls in Fig. 32 (Simpson and Halliday, 1941).

at 75°C. (167°F.). If the material is held at any temperature below this there is a weakening induced to a definite point beyond which further change will not occur unless the temperature is raised. This is shown by the graph in Fig. 1. This change in cellular adhesion was independent of the gelatinization of starch, which in potato occurs at approximately 67°C. (153°F.).

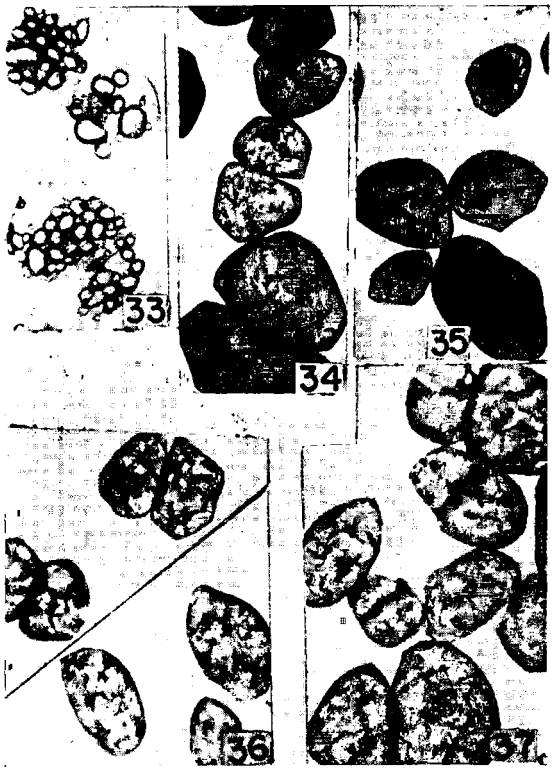

Plate 7

Fig. 33. Fresh potato cells showing intact starch grains (Personius and Sharp, 1939b).

Fig. 34. Cells from cooked mealy potatoes. Note that the cell walls are not broken, and that although gelatinization of the starch occurred the outlines of the starch grains are still apparent (Personius and Sharp, 1939b).

Fig. 35. Cells from cooked soggy potatoes. Appearance in general similar to Fig. 24. Outlines of starch grains somewhat less distinct (Personius and Sharp, 1939b).

Fig. 36. Chemically treated soggy potato. Note separated, but intact cells and gelatinization of the starch grains (Personius and Sharp, 1939b).

Fig. 37. Chemically treated mealy potato. Appearance similar to Fig. 36 (Personius and Sharp, 1939b).

An analysis of the water soluble pectin fractions of raw and cooked potatoes offers additional evidence that the solution or degredation of pectic materials is not the sole determining factor in mealiness in potatoes (Freeman and Ritchie, 1940).

In contrast to the effect of heat on potato tissue, boiling the seed coats of frozen peas for fifteen minutes did not reduce their toughness if they were originally tough (Boggs *et al.*, 1942). This is readily understand-

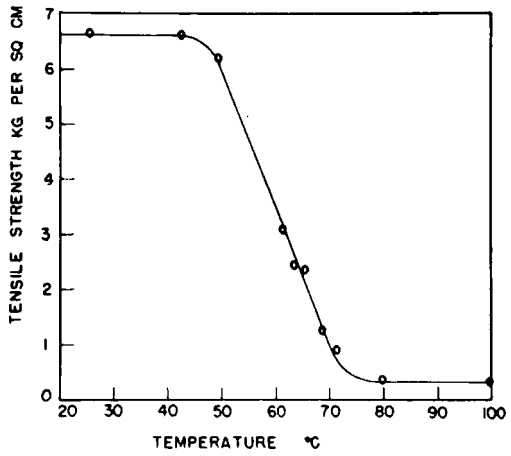

Fig. 1. Minimum values for the tensile strength of potato-tuber tissue obtained after holding at various temperatures (Personius and Sharp, 1938).

able when one considers the thick, pentosan-incrusted cell walls forming the tough seed coats of mature peas (Reeve, 1947, page 13).

Often associated with the preferable mealy texture of potatoes is the undesirable property of sloughing and splitting during boiling. Sweetman (1936) found a correlation of +0.47 and Barmore (1938) a correlation of +0.62 between mealiness and sloughing. Nevertheless, numerous exceptions have been observed. For instance the Red McClure variety of Colorado combines the preferable qualities of mealiness and high starch content with a tendency to remain intact after boiling.

Evidence has accumulated to indicate that sloughing and cracking of potatoes is accelerated by a turgid condition of the potato just prior to cooking (Barmore, 1938; Pyke and Johnson, 1940). The failure of the cementing materials of the cell wall during boiling would then be due to expansion of the potato on heating. Warm air storage prior to cooking induces water loss and hence reduces turgor. Potatoes so treated do not tend to slough as badly as the untreated potatoes.

c. Effect of Chemicals. A further factor which conditions the nature

of pectic materials in the cell walls and consequently the firmness of many processed foods is the quantity and quality of mineral salts in the processing liquid. It is well known that the degree of hardness or the mineral content of processing water may have a profound effect on the quality of canned foods (Huenink and Bartow, 1915). This is in reality a reflection of the histology of the tissue as represented by cellular adhesion. The addition of calcium chloride to the canning water of beans produced a range from tender (no calcium chloride added) to unmerchantable tough beans (calcium chloride 1000 p.p.m.). Conversely sodium carbonate causes a softening of the tissue probably due to the hydrolysis of hemicellulose.

The efficiency with which any particular chemical will alter the pectic nature of the cell walls and hence cellular adhesion is dependent upon the maturity of the tissue involved (at least as far as pea integuments are concerned). Reeve (1947) observed no effect of sodium hexametaphosphate, sulfites, oxalic acid, ammonium oxalate, or hydrochloric acid in concentrations up to 1% on the adhesive properties of the middle lamellae of well developed macrosclereids (protecting cells) of pea seed coats. Although some pectic materials were removed no effect on binding was observed. On the other hand the same tissue in a younger stage was weakened by these agents.

The two fundamental histological changes, i.e., starch gelatinization and decrease of adhesion, which occur during cooking potatoes can be simulated by chemical treatment (Personius and Sharp, 1939b). Starch gelatinization by chemical action is discussed on page 323. The removal of calcium from the calcium pectinate of the intercellular substance can be accomplished by treatment with ammonium oxalate. Microscopic observation of the tissue so treated showed a separation of cells similar to that induced by heating (Plate 7, Figs. 34, 35, 36 and 37). In this case also no differences were observed between the reaction of mealy and soggy potatoes to this compound.

Figure 2 shows the effect of various salts on the tensile strength of potato tuber tissue. It was concluded that the degree of cell adhesion change due to the treatment with salts is a result of the removal of calcium from calcium pectinate and the hydrolysis of insoluble protopectin to soluble pectin by such chemicals as ammonium oxalate and sodium nitrate. Hydrolysis is favored by heat and by a pH of 3 or lower. Similarly, part of the softening of pickles in strong acid is due to hydrolysis of the insoluble pectin of the cell wall to the soluble form (Fabian and Johnson, 1938). On the other hand, the presence of ions that would form insoluble pectates (barium, calcium, and magnesium) tends to

counteract the effect of hydrolysis and increases the toughness of the tissue.

Similarly Kertesz (1939a,b), Kertesz *et al.* (1940), and Loconti and Kertesz (1941) observed an increase in firmness due to calcium chloride treatment of tomatoes. This was found to be due to the formation of insoluble calcium pectate which could be counteracted with ammonium citrate (Loconti and Kertesz, 1941).

The phenomenon of sloughing of potatoes during boiling, which has previously been mentioned in connection with the turgor of the tissue,

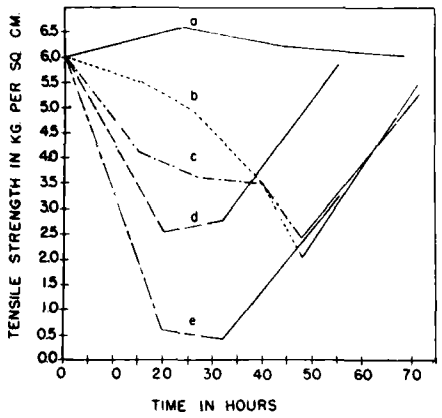

Fig. 2. Changes in tensile strength of potato tissue held 32 to 48 hours in water or in various salt solutions at 65°C. (149°F.), then transferred to calcium chloride at 20°C. (68°F.). (*a*, 1% $CaCl_2$; *b*, 0.2 *N* NaCl; *c*, 0.2 *N* KCl; *d*, H_2O; *e*, 0.5% $(NH_4)_2C_2O_4$.) Tensile strength increase, curves, *b*, *c*, *d*, *e* resulted from immersion in $CaCl_2$ (Personius and Sharp, 1938).

has been obviated by treatment of potatoes with calcium salts. During the cooking process substantial amounts of solutes including calcium are lost. Pyke and Johnson (1940) found that the addition of sufficient calcium ion to the water to maintain or raise the calcium content of the potatoes greatly reduced cracking and splitting of the potatoes and the associated sloughing was satisfactorily controlled.

2. Changes in the Starch Grain

a. Structure and Occurrence of Starch Grains. Starch grains are found in many types of plant cells, but they are particularly abundant in some parenchyma tissue. There are two general categories of starch grains, leaf starch and storage starch. The former occurs in chloroplasts of leaves and accumulates as a direct result of photosynthesis. Leaf starch

is not used commercially and is not as well known as storage starch. Spoehr and Milner (1935) have shown that it has somewhat different properties than storage starch.

Storage starch originates as a carbohydrate in the chloroplasts of leaves. From here the carbohydrate is translocated, as a sugar, to the leucoplasts in the storage organs of plants where it is converted into

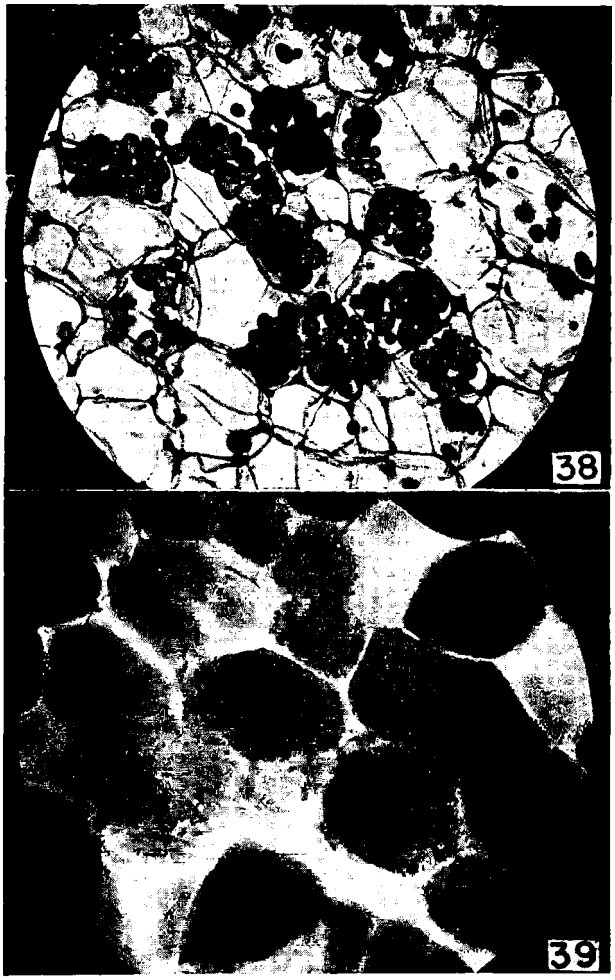

Plate 8

Fig. 38. Intact starch grains in cells of fresh potato. Stained with iodine (Crafts, 1944).

Fig. 39. Gelatinization of the starch in steamed potato. Stained with iodine (Crafts, 1944).

grains of storage starch. There appears to be a diurnal rhythm accompanying this carbohydrate movement, a fact which may account for the concentric rings observed in so many starch grains.

The general morphology of starch grains varies somewhat from plant to plant. Reichert (1913) has made an extensive survey of their appearance. Storage starch grains vary greatly in size. They may be spherical, oval, or somewhat irregular in shape; compound grains occur in many plants.

Most grains show a series of concentric layers, presumably formed by the deposition of starch about a central point or hilum. Since the layers of starch are not laid down uniformly about the hilum most grains have an eccentric form. Some grains typically have more than one hilum. Radial striations are visible in many grains under certain conditions.

The submicroscopic structure of starch grains has been investigated with x-rays, polarized light, and various other experimental procedures (Badenhuizen, 1937; 1938). According to Meyer and Bernfeld (1940) the starch grain is built up of radial crystalline micelles which are united along their edges by the principal valences of portions of carbohydrate chains. Haller (1940) believes that the grain is composed of two sorts of alternating lamellae; one of these is not soluble in alkaline reagents, the other is. Whether or not there are, in fact, one or two types of starch in the storage grain is a matter of dispute. The characteristic birefringence of the normal storage grain under polarized light is sufficient indication that the molecules have been deposited in some orderly arrangement (Schoch, 1941). Since this phase of the subject is rather more chemical than histological and is somewhat controversial, it is beyond the scope of this review.

b. Changes in Grains of Commercial Starches. Heat and various chemicals bring about a swelling and the visible disappearance of the individual starch grains (Plate 8). The grains are said to gelatinize and in so doing they form a colloidal gel. These gels show varying degrees of stiffness and fluidity depending upon the type of starch and the degree of gelatinization (Briant *et al.*, 1945). Rate of heat application and grain size do not appear to influence the final result (Beckford and Sandstedt, 1947).

Temperatures bringing about gelatinization are, however, variable, apparently being influenced by the size of the starch grain, degree of maturity of plant, source, history of starch grain, and the variety or strain of plant. According to Reichert (1913) swelling of the starch grain begins "between 45°C. (113°F.) and 55°C. (131°F.), sometimes higher, sometimes lower." Table II, taken from Reichert, gives an indication of the range of gelatinization temperatures. Other investigators have, how-

ever, obtained gelatinization temperatures which differ somewhat from those presented in Table II.

When a water suspension of starch grains is heated slowly the grains lose their birefringence and then swell (Schoch, 1941). This order of events may be ascribed to a partial hydration of the starch molecules and a disruption of their orderly arrangement. Once initiated, swelling continues until the individual grains are no longer visible under the micro-

TABLE II

Starch Gelatinization Temperatures *

Kind of starch	Swelling begins	Gelatinization begins	Gelatinization complete
	°C.	°C.	°C.
Rye	45.0	50.0	55.0
Corn	50.0	55.0	62.5
Horse chestnut (*Aesculus hippocastanum*)	52.5	56.25	58.75
Barley	37.5	57.5	62.5
Chestnut (*Castanea vesca*)	52.5	58.75	62.5
Potato	46.25	58.75	62.5
Rice	53.75	58.75	61.25
Arum maculatum	50.0	58.75	62.5
Arrowroot (*Maranta arund.*)	66.25	66.25	70.0
Tapioca (*Jatropha utilis*)	....	62.5	68.75
Arum esculentum	45.0	63.75	68.75
Sago (*Sagus rumphii*)	....	66.25	70.0
Buckwheat	55.0	68.75	71.25
Acorn	57.5	77.5	87.5
Wheat	50.0	65.0	67.5

* Reichert, 1913.

scope. The viscosity of the suspension does not increase until this latter stage is reached. The correlation between the temperature of a suspension of raw corn starch grains, its viscosity and the microscopic appearance of the starch grains is shown in Fig. 3 and Table III.

The starch grains undergo fairly distinctive histological changes as they swell during the early stages of gelatinization. Sjostrom (1936) has recorded, by means of a fine series of photomicrographs, early steps in the swelling of several commercially important types of starch grains. In the initial stages, grains from corn, tapioca and sweet potato show a rapid expansion of the inner lamellae. The surface layers, which appear to be more dense, are usually cracked by this internal pressure.

Wheat and rye starch grains in early stages of swelling may show well marked striations. At a temperature of about 67°C. (153°F.) the grains

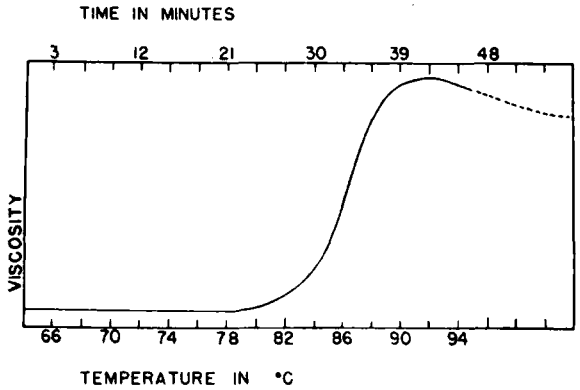

Fig. 3. Brabender amylograph viscosity curve for raw corn starch. (See Table III for related microscopic appearance of grains.) (Schoch, 1941.)

assume a characteristic baglike structure. Near the boiling point they take a curved shape, which, according to Sjostrom, is responsible for some of the specific physical properties of wheat starch paste. Grains of sago starch also swell to a baglike structure but they maintain this form near the boiling point. Generally a well defined craterlike opening is visible in one corner of the bag. This opening distinguishes sago starch grains from grains of arrowroot starch which also gelatinizes in the form of bags.

According to Sjostrom the characteristic feature in the gelatinization of potato starch is the tranformation of the grain into a ring. Upon the

TABLE III

Relation between Temperature and Microscopic Appearance of Starch Grains *

Temperature °C.	Microscopic appearance
64	No apparent effect
66	Slight swelling. Approximately 25% of granules have lost birefringence
68	Approximately 75% of granules have lost birefringence
70	Very few birefringent granules. Swollen approximately 2 ×
72	No birefringent granules
74	Swollen approximately 3 ×
76	Same
78	Slight additional swelling
80	Swollen approximately 4 ×. Granule outlines becoming vague
82	Same
84	Very vague

* Schoch, 1941.

application of heat the raw granule opens up along the median line, parts on both sides of the opening elongate in the course of gelatinization and the final result is a ring of more or less irregular shape. Some rings are smooth. In others the hydration develops peculiar units of rounded form, arranged in a more or less irregular manner and often showing individual concentric striations which are usually obliterated with increased swelling at higher temperatures. The present authors have not, however, observed these changes to take place while the starch is within potato cells (Plate 7, Fig. 33; Plate 8, Fig. 39).

The histological changes starch grains undergo during gelatinization may be variously modified by treating the grains with dilute acids at temperatures below that of gelatinization. This treatment results in pastes of greater fluidity. The grains themselves are brittle; they do not swell to the same extent as the untreated grains and they frequently retain much more structural detail, in the nature of striations, than do the untreated grains. These treated grains usually break apart along certain of the striations.

c. Effects of Milling. Intact starch grains (except in the living cell) are usually resistant to hydrolysis (Spoehr and Milner, 1939). This has some interesting and very practical implications. For instance, intact starch grains are hydrolyzed easily in living cells. If the leaf cells are killed in a manner which does not damage the starch grains or inactivate the enzymes, the grains resist hydrolysis even though brought under suitable conditions. That enzyme and starch are still capable of interaction is demonstrated by grinding the killed tissue (leaves) bringing enzyme and starch into intimate association and placing the mixture under favorable conditions for hydrolysis (Spoehr and Milner, 1939).

In wheat flours undamaged starch grains are resistant to diastatic action. However, injured grains develop under almost all types of milling and these injured grains (the so-called ghosts) do undergo autolysis (Jones, 1940). Intact wheat starch granules are lenticular in shape; they are from 30 to 35 μ in diameter and are quite elastic. Changes in their structure may be followed under the microscope. If the cover glass is pressed gently the grain will flatten to spring back into its normal shape when the pressure is released. If the pressure and release are repeated several times the granules finally crack or split in a characteristic radial fashion. If the grains are held under a continued firm pressure they will finally lose their elasticity and remain flattened and transparent. They then stain readily with Congo red. This type of injury to starch grains occurs characteristically in milling and it is only the injured grains (ghosts) which are susceptible to autolysis (Jones, 1940).

Ghosts appear in various cereal flours in different relative numbers.

They may be formed by compression of grains within particles of endosperm or on the surface of such particles. For instance, differences in the hardness of the wheat necessitates changes in the severity of milling, a factor which is likely to result in a varying number of ghosts. A direct relationship between the number of ghosts and the diastatic activity of wheat flour lead Jones (1940) to conclude that the "difference in diastatic activity between different types of wheat are thus not necessarily due to differences in amylase content or in starch susceptibility. They are at least partly to be attributed to differences in the physical hardness of the endosperm as affecting the extent of the damage to the starch during milling." Since mainly ghosts undergo diastatic conversion in ordinary flour doughs or suspensions, the presence of these damaged starch grains is a determining factor in the behavior of flours during fermentation. Jones believes that the varying content of ghosts in different flours fundamentally influences the fermentation and gassing power of the flour.

In the living cellular system starch is commonly thought to be deposited within small colorless leucoplasts. As the storage starch grain enlarges these small cytoplasmic starch depositories are stretched so thin that they become very difficult to demonstrate cytologically. Jones (1940) has shown that, in the case of the wheat grain, the amylase is distributed throughout the endosperm. Cytological details (Mann and Weier, 1944) further indicate that the enzyme is distributed throughout the living cell and is probably in intimate contact with the starch grain in leucoplasts (Spoehr and Milner, 1939).

If we accept the evidence indicating that storage starch grains in living cells are intimately associated with cytoplasm, it follows that dried storage starch grains, outside of the dead cell, must be surrounded by a thin, possibly even monomolecular layer or network of protein molecules derived from the living leucoplast. The fate of the enzyme, upon death of the cell, is not known but it is likely to be still associated with the dried grain. The presence of nitrogen, indicating protein in all starches and the autolysis of ghosts in wheat flour (Jones, 1940) suggests this possibility.

d. Effects of Blanching. Starch grains undergo typical changes within the cells of vegetables during processing. Since most processing techniques require heating of the raw product we shall pay more attention to the changes induced by heating, particularly blanching. In fact, very few observations have been reported on changes occurring in starch grains within cells and induced by factors other than heat.

There appear to be, in general, two kinds of storage starch in vegetables at least as characterized by differences in gelatinization temperatures in relation to the temperature of inactivation of the associated amylase.

Apparently the storage starch in roots (cabbage, carrot, sweet potato, parsnip, and turnip) gelatinizes at a lower temperature than that of enzyme inactivation, whereas the storage starch in stems (white potato) and seeds (beans and peas) does not gelatinize until after the enzyme has been inactivated. This observation was made by Mann and Weier (1944) and has not been sufficiently tested to be acceptable as an established generalization. The situation in leaf starch is not known.

The changes in the storage starch grains of the roots of carrots, sweet potatoes, and parsnips have been followed during blanching. In sweet potatoes the starch content is high, the cells being packed with starch grains. In carrots the starch content is low and variable, not only from root to root but between different regions of the same root. This variation may be roughly, but convincingly, demonstrated by treating cross sections of carrot roots with iodine-potassium iodide solution. The starch is generally found as a ring of irregular and varying width around the cambium, or layer of meristematic cells separating the xylem (core) from the phloem (flesh).

Carrot storage starch has a gelatinization temperature between 40°C. (104°F.) and 50°C. (122°F.). The amylase present in the carrot root is inactivated at approximately 75°C. (167°F.) (Mann and Weier, 1944). There is some indication that the inactivation temperature varies seasonally. Because of this low gelatinization temperature the rate of heating during blanching has a pronounced effect upon the relative amounts of starch and dextrin present in the blanched carrot. If the rate of heating be rapid, requiring less than 60 seconds to reach 75°C. (167°F.) (the enzyme inactivation temperature) no hydrolysis of the starch occurs and sections stain a deep blue with iodine. A slower rate of heating results in the formation of varying amounts of dextrins (as indicated by the purple, red, and pink staining of the carbohydrates when the sections are stained with iodine). It seems evident, furthermore, that slow rates of heating during blanching may greatly accelerate reactions before the enzymes are inactivated.

The conversion of starch in carrots to red and pink staining dextrins (iodine-potassium iodide test) may be brought about at low temperatures by treating sections with dilute solutions of KCNS, and other reagents, which rapidly gelatinize starch. It seems that, in general, gelatinization of the starch grains in carrots results in the immediate hydrolysis of the starch to dextrins, unless the amylase is inactivated.

Samples of commercially dehydrated carrots from ten dehydrating plants were found to be quite variable in starch and dextrin content. (Mann and Weier, 1944). An analysis of the blanched carrots for sucrose

content indicated that the rate of temperature increase during blanching does not significantly influence its content.

The behavior of starch in sweet potato (Sinoda and Kodera, 1932) and parsnips (Mann and Weier, 1944) is similar to that reported above for carrots. In both of these products the temperature of gelatinization of starch is lower than in the potato.

The parenchyma cells of the white potato are packed with storage starch (Plate 8, Fig. 38). Gelatinization occurs at 67°C. (153°F.) and may show varying degrees of completeness. It may also be brought about by various chemicals at lower temperatures (Plate 7, Figs. 36, and 37) (Personius and Sharp, 1939b). Weier (unpublished) was unable to obtain hydrolysis of gelatinized potato starch even when the gelatinization was brought about at low temperatures. Personius and Sharp (1939b) compared the gelatinization of potato starch in soggy and mealy potatoes. They concluded that there was no visible difference between the starches of these potatoes.

Briant *et al.* (1945) studied the physical properties of starch grains from a number of different varieties of potatoes and tried to correlate the differences observed with their culinary properties. The starch grains in the potato cells varied in diameter from 20 to 140 μ. In any given potato, however, the range was not this great. They found that potatoes containing a majority of small grains are likely to be soggy.

The first commercial attempts to prepare mashed dehydrated potatoes were not very successful. Recently more acceptable dehydrated mashed potatoes have been placed on the market. Freezing may play a part in the preparation of these potatoes. We (unpublished) have compared the histology of these dehydrated mashed potatoes (from frozen starch) with samples prepared for us by earlier techniques. Only the extreme variations will be discussed and for convenience the latter sample will be designated as I, and the new type commercial product as II. For microscopic examination, these samples were rehydrated according to the directions on the commercial package. For comparison an examination was also made of freshly prepared mashed potatoes. It should be remembered that the following description is histological and therefore is strictly qualitative.

In all cases gelatinization of the starch grain had occurred during processing; however the outlines of the swollen grains were apparent (Plate 9). With ordinary freshly prepared mashed potatoes (Plate 9, Fig. 40) and type II (Plate 9, Fig. 41) the cells were plump and intact. A small amount of free starch, (i.e., starch outside of the cells) could be occasionally observed. Type I (Plate 9, Fig. 42) presented a different picture. These preparations (twenty observations were made of each) contained

Plate 9

Fig. 40. Freshly mashed potato, unstained. Note intact cells, outline of gelatinized starch grains and lack of extracellular starch.*

Fig. 41. Commercially dehydrated mashed potatoes. Note intact cells, gelatinized starch and lack of extracellular starch.*

Fig. 42. Dehydrated potatoes of poor quality; note starch between cells.*

Fig. 43. Dehydrated potatoes, similar to Fig. 42. Note broken cells at *a*.*

many broken cells (Plate 9, Fig. 43a) with much free starch between the cells. In this latter case the rehydrated potatoes resembled a starch paste and would be considered to be very unsatisfactory.

When the rehydrated potatoes (all types) were allowed to soak for

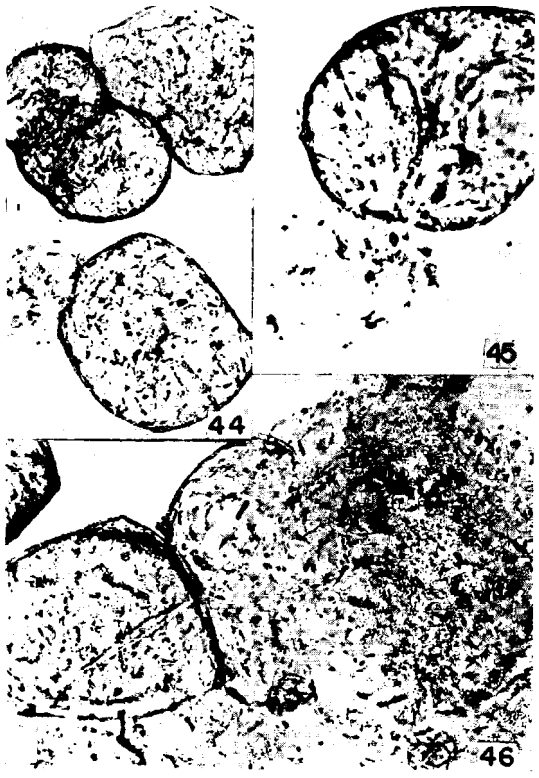

Plate 10

Figs. 44, 45 and 46. Freshly mashed potatoes allowed to stand. Series of photomicrographs showing states in extrusion of gelatinized starch from cells.*

some time the starch grains imbibed water, increasing in size, until they finally burst the cell at one point (Plate 10, Fig. 44). The starch then flowed out through this rupture, making a mass of starch many times the amount of the original starch grains within the original intact cell (Plate 10, Figs. 45 and 46). This would indicate that, upon standing, the quantity of free starch in a given batch of mashed potatoes increases, and probably decreases its palatibility.

These observations indicate that one factor influencing the quality of

mashed potatoes may be the amount of free starch present. It would seem that, other factors being equal, a preparation containing a great majority of intact cells and consequently only a small amount of free starch would be a superior product. Based upon these observations (from a histological viewpoint) fluffy mashed potatoes would be chiefly a mass of clean, unbroken potato parenchyma cells. Conversely poor quality mashed potatoes would contain a relatively larger number of broken cells with a quantity of free gelatinized potato starch.

The amount of moisture present in the plant tissue appears to influence the ease with which the starch grains gelatinize. For instance the starch grains in tissue dried without blanching contain intact starch grains even though final drying temperatures may be well above the usual gelatinization temperatures (Reeve, 1943; Mann and Weier, 1944). Gelatinization does not take place during the early stages of drying because of the low wet bulb temperature (Reeve, 1943). Mann and Weier report that gelatinization of carrot starch occurred at the normal temperature of 49°C. (120°F.) until approximately half of the moisture had been removed from the tissue. After this stage had been reached gelatinization did not take place even though the carrot dice were heated much higher.

e. Gelatinization and Nutritive Values. That there is a relationship between the nutritive value of intact and gelatinized starch grains has been shown by Hughes (1945) in some unpublished studies using swine. Raw potatoes, whether fresh or dehydrated are neither nutritious nor palatable; they must be cooked or blanched. One puzzling fact was the high nutritive value of certain batches of sun dried, unblanched potatoes from the San Joaquin Valley in California. Soil temperatures in this region normally reach 71°C. (160°F.); a temperature sufficiently high to bring about gelatinization of the potato starch grains. Even in Davis, California, on a hot summer day, potatoes allowed to remain on cement pavement will become hot enough to initiate gelatinization.

Cooked lima beans are fed in large quantities to swine. Hughes (1945) found that dry heat could not be substituted for steaming in accomplishing high nutritive value. Weier (unpublished) found that heating dry lima beans did not bring about the gelatinization of the starch grains as does steaming. It is interesting also to note that an increase in palatibility also accompanied the gelatinization of the starch grains in the lima beans.

Woodroof and Leahy (1940) reported that starch grains in peanuts will not gelatinize unless moisture is applied at some point during processing.

The existence of a heat labile fraction in the aqueous extract of raw

navy beans which retards the action of pancreatic amylase when tested with soluble starch has been reported by Bowman (1945).

It appears that two aspects of the influence of heat on the nutritive value and palatability of starches have been reported. The observations of Hughes and of Weier did not cover the activity of the amylase inhibitor while Bowman did not observe the relationship between temperature and gelatinization, nor did he test the activity of the pancreatic amylase on intact starch grains. It is possible that the enzyme does not digest the intact storage starch grain. The reason for the resistance of the intact grain to digestion may, of course, be due to the presence of an associated antiamylase. If this be true, the relationship between amylase and antiamylase in the living plastid must be explained. This is a problem that might well profit from a combination of histological and chemical techniques.

3. Changes Induced in Chromoplasts by Blanching

While sweet potatoes and most fruits contain carotinoid pigments localized in definite chromoplasts, the amount of pigment is small and the chromoplasts consequently few in number, small in size, and not always very intensively colored. The carotenoid content of carrots, on the other hand, may be proportionately high, particularly in older roots of certain varieties. Here the chromoplasts are numerous, large and well colored. The histlogical changes occurring in these chromoplasts during blanching and drying have been followed by Weier (1942, 1944) and Reeve (1943). The pigment content of tomatoes is also high but no observations on the changes induced in the tomato chromoplasts by processing have been reported.

Carrot chromoplasts are variously shaped. When starch is present the carotenoids may more or less surround some of the starch grains (Plate 11, Fig. 51). In general the pigment concentration is highest in the outer cells of the root and these cells do not contain starch. Here the pigment may be present in crystal-like needles or flakes or in tubes or spirals (Plate 11, Figs. 47a & 50). All carrot cells contain numerous highly refractive droplets that stain with fat dyes (Plate 11, Fig. 47b). When the cell is killed by a variety of methods, there is a great increase in the number of these fat droplets. Killing the cell by blanching, drying, or with reagents such as chloroform, ether, etc. results in a disintegration of the crystal-like chromoplasts. The pigment does not disappear but goes into solution in the oil droplets (Plate 11, Figs. 48 & 49). It is not possible to determine whether the oil dissolving the pigment comes from the oil droplets present in the living cell or from the protoplasm upon death of the cell. Possibly both sources of oil are involved. At any rate

Plate 11

Fig. 47. Chromoplasts *a* and oil droplets *b* in living parenchyma cell from carrot root (Weier, 1944).

Fig. 48. Destruction of chromoplasts upon death of cell (Weier, 1944).

Fig 49. Carotene in solution in oil droplets in dead cell from carrot root (Weier, 1944).

Fig. 50. Living carrot cell showing plate of carotene (Weier, 1944).

Fig. 51. Living carrot cell containing starch grain with associated carotene plates (Weier, 1944).

the pigment in dead carrot cells (killed by a variety of methods) is present mainly in oil droplets of varying diameters and not exclusively in the crystal-like chromoplasts of living tissue. The color of these oil droplets is a bright yellow· that of the chromoplast is a deep red or orange. While this difference is most likely due to a dilution of the pigment, other factors may be involved. This change in color may be responsible for much of the apparent color change occurring during blanching. Other factors such as intercellular air may also be involved.

After blanching and dehydration the carotenoids become dissolved in fat droplets. Upon standing the pigment disappears, leaving colorless droplets which stain deeply with Schiff's reagent (Weier, 1944). This observation suggested that the degradation of the pigment in stored dehydrated carrots might be associated with oxidative rancification of the fats in the droplets. Since the degradation of the pigment is very rapid in blanched but undried carrots (Weier, 1947) (small dice becoming white in less than 24 hours) a number of antioxidants were tested for their ability to protect the pigment. The results are shown in Table IV. The protective action of phosphate buffer (pH 7) was suggested; this has led to the discovery of a phosphatase that may have important implications in the processing of carrots (Mackinney, unpublished). Dried carrots, when ground to 48-16 mesh are also partially protected against pigment break-down by a few antioxidants (Weier and Stocking, 1946) (See Table IV). This protection is, however, not significant in diced dried carrots since the rate of pigment breakdown is normally slow in this product; it is not markedly retarded by the presence of antioxidants (Weier and Stocking, 1949).

Carrots dried without blanching are light in appearance due to the intercellular air and carotene degradation. This increased rate of pigment breakdown may be attributed, at least in part, to the intercellular air and to the absence of gelatinized starch which might be expected to retard the diffusion of oxygen into the carrot tissue (Reeve, 1943).

4. Influence of Blanching on Intercellular Air

The presence of large intercellular spaces filled with air is typical of most parenchyma tissue found in fresh fruits and vegetables (Plate 1, Fig. 4a, Plate 12, Fig. 55). Processing techniques may variously alter the proportion of air filled spaces in the tissue and consequently change the general appearance of the product as well as possibly its storage life. The extent of the air space in fresh fruits is variable and may range from as high as 15% of the total tissue volume in fresh peaches to small inconspicuous air spaces in prunes.

Plate 12

Fig. 52. Whole summer squash. Pitting injury due to 16-day storage at 0°C. (32°F.) and one day at 21°C. (70°F.) (Mann and Morris, 1947).

Fig. 53. Parenchyma cells from cold storage summer squash, mounted in dilute neutral red. Cells are dead and therefore do not accumulate dye (Mann and Morris, 1947).

Fig. 54. Section through injury pit of cold storage summer squash. *c*, epicermis, *b*, collapse of dead cells, forming the pits (Mann and Morris 1947).

Fig. 55. Living parenchyma cells, mounted in neutral red. Note accumulation of dye, *a* (Mann and Morris, 1947).

Fig. 56. Living parenchyma cells, mounted in neutral red and strong sugar solution. Note plasmolysed cells (Mann and Morris, 1947).

TABLE IV

Carotene Degradation in Blanched and Blanched-Dehydrated Carrot Tissue

Treatment[a] after Blanching	Storage condition	Storage interval	% Carotene remaining in tissue
None	Moist air at 60°C.	None	65[f]
Leach in running water	Moist air at 60°C.	None	55[f]
Phosphate buffer pH 8	Moist air at 60°C.	None	89[f]
0.12% $Na_2S_2O_5$	Moist air at 60°C.	None	89[f]
N.D.G.A.[b]	Moist air at 60°C.	None	97[f]
None	Dehydrated, ground to 20 mesh. 40°C.	7 days	75[g]
Leach	Dehydrated, ground to 20 mesh. 40°C.	7 days	45[g]
0.12% $Na_2S_2O_5$ + 0.3% pyro[c]	Dehydrated, ground to 20 mesh. 40°C.	7 days	96[g]
0.12% $Na_2S_2O_5$ + 0.3% pyro[d]	Dehydrated, ground to 20 mesh. 40°C.	7 days	85[g]
0.01% N.D.G.A. + 0.3% $Na_2S_2O_5$	Dehydrated, ground to 20 mesh. 40°C.	7 days	92[g]
None	Dehydrated,[g] ground to 20 mesh. Room temp.	2 months	21[g]
0.12% $Na_2S_2O_5$ + 0.3% pyro[c]	Dehydrated, ground to 20 mesh. Room temp.	2 months	100[g]
0.3% pyro	Dehydrated, ground to 20 mesh. Room temp.	2 months	69[g]
None	Dehydrated, dice[g] 40°C.	2 months	75[g]
0.12% $Na_2S_2O_5$ + 0.3% pyro[c]	Dehydrated, dice[g] 40°C.	2 months	80[g]

[a] Dice soaked after blanching for 5 minutes in designated solutions.
[b] N.D.G.A. = 0.01% nordihydroguaiaretic acid in 40% ethanol.
[c] This solution prepared from a 20-year-old sample of Merck photographic pyrogallic acid.
[d] This solution prepared from fresh C.P. pyrogallic acid.
[e] Data from Weier and Stocking, 1946.
[f] Data from Weier, 1947.
[g] Data from Weier and Stocking, 1949.

A white, chalky cast is imparted to the processed food by the presence of entrapped air. In some cases, such as dried apple, the appearance is acceptable to the consumer either because the normal unprocessed fruit has a similar appearance or because the consumer is accustomed to it. On the other hand such a chalkiness is often definitely not as acceptable as the more translucent appearance of the tissue containing no intercellular air (e.g., unblanched dehydrated peaches compared to those sun dried). In addition, the presence of entrapped air containing oxygen may affect the storage life of the product. No such problems are presented by the few fruits and vegetables which contain relatively little entrapped air, e.g., prunes.

The changes in intercellular air which occur upon heat processing have been described by Crafts (1944a, b). According to this investigator there are three general effects which account for the displacement of intercellular air from tissue during blanching: (1) Heating expands the air so that it escapes from the cut surfaces, (2) there is a certain amount of leakage of cell sap from the killed cells into the intercellular spaces, (3) heating softens the walls so that they bend and give. Thus when the heated tissue cools, sap fills the spaces from which the air had been expelled. Compare Figs. 4 and 5, Plate 1. Different effects may be observed by different heating procedures. During steam blanching the liquid available to fill the intercellular air spaces is the leakage of cell sap and a small amount of condensed steam. Often this does not cause a complete removal of intercellular air in the finished product, especially if the cell walls remain fairly firm. Such a condition may be found during the blanching of cabbage which, after steam blanching and subsequent drying, retains a chalky appearance. If, however, the product is treated during blanching (such as the sulfite spray used on certain vegetables prior to dehydration) sufficient excess liquid is present to fill the intercellular spaces so that the final product loses the typical chalky appearance. In this case the spray may also soften the cell walls somewhat.

When intercellular air has been completely replaced by liquid during processing, subsequent dehydration may occur without the repenetration of air into the tissue. This is especially true if the cell walls are not too rigid.

5. Low Temperatures and Food Processing

Low temperatures, 10°C. (50°F.) and below, are of importance, in a number of distinctly different ways in the processing of fruits and vegetables. Low temperature effects may be roughly divided into three categories; winter hardening, cold storage of fresh fruits and vegetables, and freezing and storage of frozen foods.

a. Winter Hardening. Low temperatures induce changes such as winter hardening in many types of vegetables while they are still in the field. Levitt (1941) has recently prepared a bibliography of the rather extensive literature on this subject. Some of these changes may be studied by histological methods; they undoubtedly have an influence on the quality of the finished product. However, few observations relating winter hardening to the finished product have been made and since the problem is concerned with factors which precede processing it will not be discussed further here.

b. Cold Storage. Temperatures ranging from 10°C. (50°F.) to freezing injure certain vegetables and fruit. This may subsequently influence the quality of the processed product. Since this too is properly a preprocessing problem no effort will be made to cover the literature on this subject. Since, however, the histological changes brought about by such treatment are produced by commercial procedures one example will be given (Mann and Morris, unpublished).

In general, vegetables belonging to tropical families and introduced into the agriculture of colder climates (melons, squash, peppers, etc.) suffer cold injury at relatively higher temperatures than do the native crops. Most usually this type of cold injury does not become apparent until the vegetable or fruit is removed from cold storage. For instance, summer squash after 16 days storage at 0°C. (32°F.) show few if any signs of injury. When the squash are removed from cold storage and placed at 21.°C. (70°F.) for one day, pits frequently appear on the surface of the fruit (Plate 12, Fig. 52). These pitted areas result from a collapse of the parenchyma and associated vascular tissue just below the epidermis (Plate 12, Fig. 54). At first it was thought that such pits resulted from the death of patches of cells while the cells in the nonpitted area remained viable. Obviously this hypothesis is subject to experiment. Living cells will accumulate certain nontoxic dyes (notably neutral red) in their vacuoles; dead cells will not (Plate 12, Figs. 55 & 53). Living protoplasts, as mentioned previously (page 308) may be plasmolyzed (Plate 12, Fig. 56); dead protoplasts do not shrink in strong salt or sugar solutions. Application of these tests to squash stored at 0°C. (32°F.) for 16 days showed that the majority of the cells beneath the epidermis were dead (Plate 12, Fig. 53). The subepidermal tissue is uniformly killed; death is not localized in the pitted areas. The pitting occurred only where the surface of the squash was injured, even very lightly, before storage.

Silver nitrate may be utilized as a general histological test for ascorbic acid (Giroud, 1938, Weier, 1938). Mann and Morris (1947) found that, by brushing a 1% solution of acid $AgNO_3$ over a section of squash tissue,

they could not only show a localization of ascorbic acid around the vascular tissues, but also follow its decrease on prolonged storage. These histochemical observations were checked by standard methods of analysis for ascorbic acid. Larger amounts of ascorbic acid were found in those regions that reduced the silver nitrate.

A great deal of work has been done on the physiology of apples during storage; the results show that a rapid change in texture occurs after they are removed from storage. The histological and cytological changes responsible for this deterioration are not well known.

c. Frozen Foods. The third low temperature process, freezing for prolonged storage, is of concern to this review. The literature dealing with histological changes brought about by freezing is not extensive, and in some respects, is not as critical as the importance of the subject warrants.

The most detailed studies on plant tissues in relation to histological changes during commercial freezing procedures have been carried out by Woodroof (1938). In many respects this work is excellent and the conclusions are of significance. On the other hand certain of his observations reveal a lack of acquaintance with cell structure and with some details of cell physiology, despite which Tressler and Evers (1943) quote some of his conclusions.

Judicious freezing probably results in less change in cells than any other method of killing. In addition, since chemical changes are greatly retarded in frozen vegetable products, such products should, and do retain fresh flavors better than other preserving procedures. At present little is known of the cytological changes taking place during commercial freezing procedures. When blanching precedes freezing, changes induced by heat killing must be considered also. As yet there has been no critical comparison of the histological changes brought about by freezing, by blanching, and by blanching and freezing.

For instance the firmness of frozen foods upon thawing is an important aspect of their quality. At least four factors are involved in the firmness of living fruits or vegetables used for freezing: (1) the turgidity of the living cell; (2) the type and amount of cell contents; (3) the nature of the cell wall, and (4) the middle lamella. Woodroof (1938) thoroughly appreciated the importance of these factors; two of his interpretations, however, need clarification. He comments upon the firmness of living tissues as follows: "The solidity, rigidity or firmness of tissues usually frozen (living parenchyma cells) seems to be due to the distribution and state of liquid in the cell, possibly in the form of surface films between granules, microsomes or droplets of various kinds found in living matter." As described on pages 307 to 310 the firmness of living parenchyma cells is largely due to turgidity. Woodroof further

describes a situation in strawberries which is of interest. Here, in the immersion method of freezing "there was a tendency for the cell contents to collect in the center of the cell, with the nucleus enveloped in the cytoplasm, in this position adding maximum strength to the cell wall." Living cells placed in strong brine or sugar solution will become plasmolyzed as described on page 308. In this stage the turgor pressure is reduced to zero and the tissue is flabby or wilted. Thus if strong solutions are used previous to freezing the tissue will be frozen in a wilted condition. Such a wilting might not, however, be expected to influence greatly the physical nature of the cell contents after processing and after thawing. Certainly the ball of plasmolyzed protoplasm in the center of the cell cannot support the cell wall.

The function of the middle lamella has been discussed at length on pages 310 to 316. The same facts apply to frozen products. The middle lamella holds the cells together, any action tearing the cells apart must involve the middle lamella. Since a separation of cells occurs during freezing, treatments designed to strengthen or weaken the middle lamella might be of considerable importance in processing frozen foods. We would emphasize the importance of this structure for the degree of firmness is certainly dependent, to a degree, upon the nature of the middle lamella.

In many cases vegetables destined to be frozen are first blanched. Histological changes during blanching have been discussed in detail above. Freezing imposes an additional and new physical condition. The most obvious visible factor is the formation of ice crystals. If there are differences in their formation between blanched frozen and fresh frozen products they have not been reported. Woodroof has carefully studied the patterns formed in plant tissues by ice crystals. His histological methods are excellent. There is no reason to suspect that killing the tissue as he did, with its subsequent embedding in paraffin and sectioning would introduce important histological errors of a type that might influence his conclusions. Woodroof finds that, in general, rapid freezing induces the formation of many small ice crystals both within the cell and in the intercellular spaces. Upon thawing rapidly frozen tissue there is thus a minimum of cell wall destruction and tissue alteration.

When the rate of freezing is slow, the ice crystals generally form in the intercellular spaces where they may become relatively large. During formation they frequently puncture the thin cellulose walls and push between the cells. Upon thawing, such tissue shows much damage. This leads to the loss of juice and a flabby condition of the tissue.

The amount of water reabsorbed by the cell contents upon thawing varies considerably. The number of intact cells and the cell contents are

determining factors in water reabsorption. The rate and degree of freezing apparently, in some way (other than cell breakage), influences the amount of water reabsorbed. Why this is so is not understood. It should be emphasized that dead cells cannot be expected to have the firmness of living cells.

The cell composition, particularly the amount of starch present, is an important factor in determining firmness. For example, peas contain much storage starch and are firm upon thawing. This is due mainly to the closely packed starch grains and their water absorbing properties.

The extent, location and size of the water conducting elements, particularly the spiral and annular elements, whether vessels or tracheids, may be of importance in determining the extent and location of ice crystal formation. Water can move relatively freely through these elements and it may be drawn some distance to centers of ice formation.

Some of the factors involved in freezing may be summarized as follows:

A. Ice crystal formation involves
   1. Location of crystals, whether intercellular or within the cell.
   2. Size of crystals.
   3. Shape of crystals.
   4. Extent and direction of water movement.
   5. Rate of freezing.
   6. Freezing temperature.

B. The living cells are killed by blanching or freezing.
   1. Cellular colloids are altered.
   2. Cell loses turgor; if a brine has been used to freeze living cells they are likely to be frozen in a plasmolyzed state.
   3. The activity of enzymes, state of starch and other such factors which may influence histological changes are not known.

C. Diffusion of water from or into dead cells is purely a physical phenomenon; for this reason thawed cells can never be as turgid as living cells.

D. The living parenchyma cell consists of:
   1. A thin cellulose cell wall.
   2. A living protoplast consisting of:
      a. Parietal layer of cytoplasm.
      b. Nucleus.
      c. Large central vacuole.
   3. By actively accumulating inorganic salts it maintains a state of turgor.

E. The middle lamella cements the cells together. (Because of this it

should be of great importance in maintaining a degree of firmness in frozen products.)

F. The firmness of thawed frozen foods involves:
   1. The cell contents (starchy vegetables are relatively firm upon thawing).
   2. The middle lamella.
   3. Intact cell walls.
   4. Nature of the cell walls.
   5. Formation of jagged intercellular spaces by large ice crystals.

6. Histology and Food Processing

Changes that take place during the processing and storage of foodstuffs have been studied mainly from the view point of the chemist. That much progress has been made in this field is obvious and the proficiency of the food chemist is high. There is no question, moreover, but that most problems of food processing must, in the last analysis, be solved upon a chemical basis. Histology can only be an aid to this end. Since, however, chemicals do not react over distance and foods are not homogeneous mixtures, a knowledge of the histology of normal and processed tissues should be of value in suggesting and in following chemical changes induced by processing and storage.

It should be emphasized that by histology we mean not only the details of cell wall arrangements but also the fine details of cell structure, including such things as the location and interrelations of intracellular pigments, proteins, carbohydrates, inorganic salts, enzymes, etc. The association of lipoid and pigment or of protein, carbohydrate, and enzyme must have a profound influence in a stored food product. Techniques for the detailed determination of the quantity and location of minute amounts of highly active and important cellular constituents have been devised (Society for Experimental Biology, Symposium #1 1947). These techniques are used at present, mainly in basic cytological studies by cytogenetists and cancer specialists. It is possible, for instance, to follow the hydrolysis of the purine bases within a single living cell. Caspersson (S. E. B. Symposium, 1947), using ultraviolet light, has been able to determine the nucleic acid content of extremely minute areas within a living cell. It seems possible that these techniques might be adapted to problems of food processing. For instance, it might be possible to follow, in detail, the chemical changes and the exact intracellular localization of the changes that occur in the darkening of dried fruit. The exact relationship of lipoid and protein could be determined in dried milk, poultry, and vegetable cells. Localization of enzymes and their relation to starch, plastids and other cellular constituents might possibly lead to

a better understanding, for instance, of the rapid changes taking place in freshly harvested corn and peas. While it would be presumptuous to predict that such information would lead to new and improved food processing procedures, the possibility of improvement is inherent in increased knowledge.

III. Summary

1. The edible portions of most fruits and vegetables are composed largely of parenchyma tissue. The cells of this tissue are generally thin walled, of cellulose and isodiametric. Intercellular air spaces are common. The cells are cemented together by pectic substances of the middle lamella. The rigidity of the tissue results from the turgor of the living protoplasts. In addition to the protoplasm and vacuole the protoplasts of parenchyma cells may contain either numerous large chloroplasts, and (or) grains of starch.

2. Changes in the chemical constitution of the middle lamella, which may be induced by heating or by various chemical treatments, can affect the adhesive properties of the middle lamella and thus influence the rigidity and texture of processed foods.

3. Death alters the permeability of the cell membranes to water and to solutes and hence cell turgidity. Thus, processed tissue cannot have the firmness of living tissue. The imbibitional properties of included substances, such as starch grains, influence the firmness of processed tissues.

4. Changes in starch grains generally occur during different types of processing; such grains may be injured during milling or gelatinized by moist heat during cooking or blanching. Such injury renders them less resistant to hydrolysis and may change their imbibitional properties. The gelatinization of starch grains may increase the nutritional value of a processed food and be a factor in the keeping quality of the product. This latter aspect of gelatinization may be applicable to dried fruits and vegetables. In starch and vegetables the gelatinization of the starch, with the possibility of the bursting of thin cell walls with the resulting accumulation of extracellular starch, may be an important factor in the quality of such products as freshly prepared or dehydrated, mashed potatoes.

5. Little is known of the changes occurring in the chloroplasts during processing. These bodies are complicated, both chemically and structurally. They are rich in proteins, lipoids, carbohydrates and pigments. In the living cell they are centers of active synthesis and hydrolysis. It seems likely that, in processed green vegetables such as spinach, the chloroplasts play a part in determining flavor, texture and keeping qualities. In some fruits and vegetables carotenoid pigments are present as

discrete bodies. Upon death the pigment goes into solution in oil droplets. This physical association of carotenoid pigments and oil might be expected to influence both keeping quality and flavor of the finished product.

6. The amount of intercellular air in processed parenchyma tissue has a pronounced influence on color, texture and keeping quality of the finished product. This is particularly important in dehydrated foods. When intercellular air is objectional, special procedures, such as blanching, must be resorted to in order to remove it.

7. The subepidermal cells of fruits of tropical families (squash, peppers, and egg plant) are killed at temperatures above, but close to, freezing. Injury during harvesting results in the formation of pits at injury points when such fruits are removed from cold storage. The ascorbic acid changes in such injured tissue may be followed by brushing acid $AgNO_3$ over the tissue.

8. Judicious freezing is probably the best method of preserving cellular detail. Since freezing also retards chemical reactions, it is one of the best preservatives of flavor. Such changes as may be induced in cellular details by freezing have not been thoroughly investigated. Gross changes in the structure of the parenchyma tissue may occur due to the formation of ice crystals; slow freezing results in large crystals and the formation of large ruptures in the frozen parenchyma tissue.

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The Spoilage of Fish and Its Preservation by Chilling

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I. Introduction

The annual World production of fish was estimated for representative prewar years to have been about 16.5 million tons (Sandberg, 1944). Fish is therefore a highly important source of food and research has clearly established its high nutritional quality.

Fish, however, is one of the most perishable of foods, so that its transport and wide distribution in acceptable condition presents a preservation problem of no small magnitude. Fishing grounds are frequently far distant from home ports and these in turn from the main centers of consumption. A number of countries, e.g., Canada, Iceland, Newfoundland, and Norway, in which fishing is one of the principal industries, produce much more fish than can be consumed at home, necessitating export over long distances. Many important species are caught in abundance during a relatively short season, and this large annual supply must be distributed more evenly over the year.

Man discovered in very early times, possibly by chance, how to preserve locally caught fish for long periods by salting, drying and smoking, or combinations of these; a major proportion of the world's catch is still preserved by these means In the more highly developed countries, however, where standards of living have been steadily rising during the last century the demand for such crude fish products has been steadily falling and increasing preference has been shown for fish that has retained to a much greater extent the flavor, odor, appearance, and texture of the freshly caught fish. Hence the growing demand for "wet," i.e., unprocessed, fish, even though frequently not of the freshest quality; or for very lightly cured fish; or for fish processed by modern methods of freezing and canning, which preserve the original characteristics of the fresh fish to a high degree. The primary problem of the industry in the more developed countries, therefore, is to retain the quality of freshly caught fish at sea or on land, in the "wet" or unprocessed condition, either for consumption in this condition or for processing by acceptable modern methods.

Historically, the problem presented itself acutely during the last quarter of the nineteenth century with the urge to tap richer, more distant fishing grounds in order to meet the needs of increasing industrial populations. The introduction of steam propulsion and of more efficient

catching methods and gear permitted more abundant catching and a wider radius of fishing; and the preservation of the catches in edible condition was accomplished by eviscerating the fish and chilling them in crushed ice—in natural ice to begin with, but later largely in artificial ice. By these methods an enormous increase in fish supplies has been brought about during the present century in the countries bordering the northern oceans. A wider distribution of "wet" fish inland has been helped by better, speedier transport. However, in recent years as nearer grounds have been overfished and still further ones exploited, the limitations of chilling as a preservative of quality have become increasingly apparent. Much thought is being given to the possibilities of improving this method of preservation, or of supplanting it by processing, e.g., freezing, or where appropriate, canning, at sea.

It is the purpose of this paper to review existing scientific and technical data concerning the various factors affecting the spoilage of "wet" fish and the retention of quality by chilling, and to indicate where our knowledge is insufficient and further research is required, and finally to suggest possibilities of improving current industrial practice in handling and storage.

II. General Description of the Spoilage of Fish

1. Introduction

The spoilage of fish, as of other foods, is usually stated to be effected by autolysis, oxidation, and bacterial activity. All the existing evidence goes to show that the last is apparently by far the most important factor in producing the more striking and undesirable alterations in the flavor, odor, and appearance of fish, although in fatty fish under certain conditions unacceptable oxidative rancidity may precede bacterial deterioration. It may well be that autolysis prepares the way for and assists the bacterial attack, but it has not been clearly demonstrated that it does so in whole fish under the normal conditions of handling and stowage, nor would it be easy to demonstrate. On the other hand, it seems very probable that the softening of the flesh as it spoils is largely autolytic in origin, although bacterial enzymes no doubt also contribute once the flesh has become grossly contaminated.

The present paper will be largely concerned with spoilage as effected by bacteria. In spoilage, there is interaction between bacteria on the one hand and the chemical and physical composition of the fish on the other. The kind and numbers of bacteria originally on the fish and subsequently picked up during handling and distribution are subject to a variety of ecological and seasonal factors and perhaps vary also with the species of

fish. On the other hand, the physical and chemical nature of fish may vary with species, season, state of maturity, age, nutrition, and environment. Moreover, the course of spoilage in any instance is subject to the influence of environmental factors, particularly temperature. These considerations serve to show how extremely complex the bacterial spoilage of fish is, and it should be said at once that research has gone only a short way to resolving the complexity.

Before attempting to discuss the bacteriology and biochemistry of spoilage in some detail, a broad account will be given of the spoilage of fish as it is normally perceived by the senses.

2. Types of Fish and Fishery

It is first of all advisable to note that marine fishes are conveniently and appropriately considered from many points of view as falling into two broadly different classes, "demersal" and "pelagic."

The demersal fishes (e.g., cod and allied species, the flat fishes, and the dogfish, skates, etc.), the flesh of which is relatively nonfatty (see review of the chemical composition of fish by Reay *et al.*, 1943), are generally caught by trawl net or line on or near the sea bottom, over a relatively wide geographical range in relation to the home port. These fish are in consequence gutted and washed at sea soon after catching and are stowed in ice for most of the time elapsing, at sea and on land, between catching and consumption or processing.

The pelagic fishes, e.g., herring, pilchard, and mackerel, the flesh of which serves as a main fat depot that fluctuates widely and seasonally in fat content according to food supply and sexual maturity, are caught by drift, ring or purse-seine nets as they shoal seasonally, as a rule within a comparatively short distance of the ports. These fish are not usually gutted or iced at sea. On being landed they are frequently transported and distributed to the consumer or processor still without being gutted, although ice is generally used for all but short distances.

For background information regarding the conduct of fisheries, and the characteristics and commercial treatment of the chief marine species, the reader is referred to Tressler's (1923) *Marine Products of Commerce.*

3. Organoleptic Characteristics of Fresh and Spoiling Fish

The freshly caught fish has a shining, iridescent surface, exhibiting bright characteristic colors and markings. The surface is covered with a nearly water-white, transparent, smooth, homogeneous, thinly spread slime. The eyes are bright, full and prominent with jet black pupil and transparent cornea. The gills are generally bright to brownish red, the tint depending upon species, and are free from any coating of slime. The

fish is soft and flabby, tending to retain finger indentations. Soon, however, when *rigor mortis* has set in, the flesh becomes hard, firm, and elastic, is not readily stripped from the backbone and does not readily yield juice under pressure. The odor of the fish externally and in the flesh is characteristically "marine" or "seaweedy," and in the case of the fatty, pelagic fish is also pleasantly oily.

The newly caught fish more often than not has food in its gut in process of being digested. The powerful digestive enzymes, even at ice temperature, rapidly attack and perforate the gut wall, and, along with bacteria from the gut, proceed to attack the belly wall and the viscera, which also have a high natural rate of autolysis that soon renders them pulpy and semi-fluid. If gutting and washing is unduly delayed, the belly walls or "flaps" rapidly become "jellied" at ordinary temperatures; even in crushed ice ungutted fish such as herrings, particularly when "feedy," i.e., very full of food, rapidly develop "torn bellies," at times within a day. Where possible, it is clearly advisable to gut and wash fish very soon after catching and, as already noted, this is usually done in the case of the demersal catches which normally are subjected to longer periods of transport and distribution.

In the newly caught, gutted, and washed fish, bacteria of normal marine types are present in the gut cavity and on all the outer surfaces including the gills. It has been clearly established by numerous workers that the flesh and body fluids of the newly caught fish are sterile; and it is generally agreed that little or no bacterial activity occurs until the period of *rigor mortis* has well passed its maximum—usually a period of one or two days in the case of fish stowed in ice. At the temperature of melting ice bacterial growth proceeds gradually on the external surfaces. Although the subject of some controversy, the commonly held view is that invasion of the flesh then takes place, proceeding from the gut cavity and gills via the vascular system, and through the skin.

Externally, as the fish spoils and finally becomes putrid, the surface loses its bright sheen and colors, and becomes covered with a thicker slime, which grows increasingly turbid and finally develops dirty, yellow or brown colors. The "flaps," thin and heavily infected tissue, soften and show reddish brown discoloration. The eyes gradually sink and shrink, the pupil becoming cloudy and milky, and the cornea opaque. The gills discolor first to a bleached, light pink, and finally to a greyish-yellow, and they become covered with a very thick slime. The flesh gradually becomes softer until it is very easily stripped from the backbone and exudes juice under the lightest pressure. The fish loses all elasticity and retains finger indentations. Along the backbone above the gut cavity and spreading back from the kidney towards the tail a reddish

brown discoloration develops in the flesh, due to haemoglobin and its oxidation products. Apart from this discoloration, the flesh loses its original, translucent sheen and becomes dull, milky, and opaque. If originally colored, the tint fades, often to a greyish-yellow.

Ungutted fish often emit odors of decomposition long before any spoilage of the flesh has taken place. This is due to decomposition of food in the gut. In the case of gutted and washed fish stowed in ice there is a gradual transition from the original fresh "seaweedy" odor to the final odor of putrefaction. The superficial slime and gills usually emit stronger odors of more advanced decomposition. In general, the succession of odors is "fresh," "sickly sweet," "stale," "ammoniacal," and finally, "putrid," the last characterized by substances such as hydrogen sulfide and indole. In the case of fatty fish stowed in ice, rancid odors may be detected.

Very fresh fish, when cooked, exhibit their delicate, pleasant, specific odors and flavors, and the texture is firm. As spoilage proceeds, the odors and flavor of the cooked fish generally become first "flat" and uninteresting, then stale or "fishy," and finally acrid, sour, and putrid. In the case of oily fish rancidity leads to a persistent bitter after-taste. When fish have proceeded beyond the "stale" stage the texture of the cooked flesh tends to become unpleasantly "sloppy."

For a more detailed description of the course of spoilage, reference should be made to Anderson's (1907) classic paper, entitled "On the Decomposition of Fish." Little has since been added to the descriptive information given therein, which is clearly based upon extremely thorough observation.

III. The Bacteriology of Fresh and Spoiling Fish

1. The Flora of Freshly Caught Fish

Early investigations were mainly concerned with the hygiene of fish handling, particularly in relation to food poisoning, and the methods and media employed were those that had been proved suitable for examination of meat for the presence of pathogens. Hunter (1920a; 1920b; 1922), Fellers (1926) and Harrison *et al.* (1926) were among the first to show that the majority of bacteria associated with fresh and spoiling fish belong to the water-soil types, which have a lower optimum temperature of generation than the normal pathogens (e.g., around 10 to 20°C. (50 to 68°F.) rather than 32 to 42°C. (89.6° to 107.6°F.)). Since the earlier investigations, however, there has been no decided change in the nature of the culture media used, the majority of which have been meat extract agars containing normal saline. Fish extract media have been and are

frequently used, but there has been no clear demonstration that quantitatively or qualitatively they support a different flora. ZoBell (1946), however, has persistently affirmed that a sea water supplement provides the optimum cultural requirements for marine microorganisms, and is in this respect superior to artificial sea water, or solutions of sodium chloride alone. The authors consider that whilst this claim is true, culture on normal saline meat or fish media supports the growth of by far the majority of organisms responsible for spoilage.

Table I summarizes the best existing data concerning the flora of freshly caught marine fish, indicating the frequency of occurrence of various groups.

It will be seen that in every analysis the aerobic flora is of the type normally regarded as autochthonous to soil, air, and water; and that the same groups are represented in practically every case, which indicates their wide geographical distribution. An explanation of the varying percentage distribution of the groups on the basis of geographical position, although tempting, would be an over simplification, since recent work by Shewan (1946a) has shown that the distribution may vary widely with season and possibly also with the species of fish and even with the individual fish.

Comparatively little work has been done on the strict anaerobes, which have been found almost exclusively in the gut contents of the fish. Experience indicates, however, that they play no important role in the spoilage of fish. *Clostridium botulinum* has been recorded for freshly caught fish only in sturgeon from the Caspian Sea, by Burova and Nasledisheva (1935), Burova *et al.* (1935) and Dobrowsky (1935). *Escherichia coli* has been found, but only when the fish were caught in polluted coastal or inland waters, (see the review by Griffiths (1937) and Wood (1940)).

As already noted, there is general agreement that in newly caught, healthy fish, the flesh and the body fluids are sterile. On the other hand, the external surfaces of the fish and the gut, when food is present, can carry very considerable bacterial loads. According to Lücke and Schwartz (1937) and Shewan (1944) the intestinal content of freshly caught haddock and codling may contain from 8 to 420×10^6 organisms per ml., (incubated at 20°C. (68°F.)). Aschehoug and Vesterhus (1947) report counts of 3 to 20×10^3/g. of gut contents of winter herring. Lücke and Schwartz (1937) and Shewan (1944) have found from 10^2 to 10^6 organisms (at 20°C. (68°F.)) per sq.cm. of skin with its adhering slime in the case of haddock, cod, red-barsch, saithe, herring and mackerel. The gills which, as Griffiths (1937) has remarked, have long been recognized as an important source of infection, have been little examined.

TABLE I

Aerobic Bacterial Flora of Fresh Fish
(Expressed as percentage of total number of organisms isolated)

a. Aerobes

Source of Data	Species	Source of Sample	*Achromobacter*	*Micrococcus*	*Flavobacter*	*Pseudomonas*	*Bacillus*	Miscellaneous
Reed and Spence (1929)	Canadian haddock	Slime	23	4	8	22	24	18
		Intestines	4.4	1	5.6	8.7	5.7	70 (mainly *Proteus*)
Stewart (1932)	North Sea haddock	Slime	57	22	11	5	—	5
		Intestines	80	—	4.5	—	4.5	11
Shewan (1938b)	Shetland herring	Slime	43	24	13	11	—	9
Thjotta and Sømme (1938)	Norwegian cod	Slime	48	14	25	5	—	8
		Intestines	55	11	—	—	—	33
Wood (1940)	Australian barracouta, whiting, mullet, etc.	Slime	19	48	17	7	9	—
		Gills	31	41	12	7	9	—
		Intestines	30	21	1	10	35	—
Aschehoug and Vesterhus (1943)	Norwegian winter herring	Slime	24.5	16.7	17.7	40.0	—	1.1
		Gills	33.4	3.9	13.7	47.0	—	3.0
		Intestines	72.5	3.4	—	24.1	—	—
Thjotta and Sømme (1943)	Cod, mackerel, plaice, whiting gurnard, etc.	Slime, Stomach, Intestines	21.2	4.0	6.0	4.0	—	64.8 (*Vibrios* chiefly)
Thjotta and Sømme (1943)	Red char, trout, perch, grayling, etc.	Slime, Stomach, Intestines	48.7	3.5	5.2	20.0	5.2	17.4
Dyer (1947)	Atlantic cod	Slime, Faeces	4.8	72.8	3.0	3.7	—	15.7

TABLE I (Contd.)

b. Anaerobes

Obst (1919)	Herring	Gills Intestines	Members of the *Clostridium* group isolated
Fellers (1926)	Pacific salmon	Slime	No anaerobes found
Reed and Spence (1929)	Canadian haddock	Intestines	Members of the *Clostridium* group present
Dobrowsky (1935)	Sturgeon	Gills Intestines Muscle, etc.	Members of *Clostridium* group isolated, e.g., *Cl. putrificum*, *Cl. botulinum*, etc.
Burova *et al.* (1935); Burova and Nasledisheva (1935)	Caspian Sea sturgeon	Intestines	Members of *Clostridium* group isolated, e.g., *Cl. botulinum*
Shewan (1938c)	North Sea haddock	Slime Intestines	No anaerobes found Members of the *Clostridium* group isolated, e.g., *Cl. sporogenes*, *Cl. capitovalis*, *Cl. tetani* (types V and X)
Shewan (1938b)	North Sea herring and mackerel	Slime Intestines	No anaerobes found Members of the *Clostridium* group present
Aschehoug and Vesterhus (1943)	Norwegian winter herring	Slime Gills Intestines	No anaerobes found

Shewan (1944) found 10^3 to 10^5 organisms (at 20°C. (68°F.)) per g. of gill tissue.

Extremely little is known of the factors which cause variation in the bacterial load of the surfaces of living fish. Slime has been thought to have bactericidal properties, but this has never been demonstrated. In the dead fish it appears to be a good medium for bacterial growth. It would seem reasonable to suppose that surface infection in the living fish is kept within bounds by the continuous secretion and sloughing of the slime. Shewan has begun to obtain data which suggest that there may be a seasonal variation in the bacterial load of the slime, which would no doubt reflect a similar variation in the bacterial population of the environment. Peak loads of $10^{4.5}$ to $10^{5.25}$ per sq. cm., mainly on haddock taken from the same ground in the North Sea from April to December over a period of five years, occurred in the period June to August. During the rest of the year counts ranged from $10^{3.5}$ to 10^4/sq.cm.

The figures of Aschehoug and Vesterhus (1947), for the bacterial population of the gut contents of herring are very much lower than those reported by Lücke and Schwartz (1937) and Shewan (1944) for haddock and codling. This difference may possibly be explained by the fact that the latter species normally feed on the sea bottom, the mud of which has been shown to carry loads of 9.3×10^5 to 3.1×10^7/g. [Shewan (1944) and ZoBell (1946)], whilst the former are pelagic fish feeding on plankton in clean ocean water. Lücke and Schwartz (1937) made the interesting observation that the infection of the skin of whiting and cod caught singly by line ($10^{1.5}$ to $10^{4.0}$/sq.cm.) was significantly lower than that for cod, saithe, red barsch, herring and mackerel caught by trawl net ($10^{3.5}$ to $10^{5.5}$/sq.cm.). It is suggested that the increased infection is due to the dragging of the fish over the bottom mud and to expression of the gut contents amongst the fish during the hoisting of the net.

2. The Flora of Spoiling Fish

Table II summarizes the best existing data concerning the flora of spoiling fish and its group distribution.

It is clear that whether the fish has been handled in the normal commercial manner or especially carefully treated, the same groups are present during spoilage as are found on the freshly caught fish. The percentage distribution, however, alters markedly during spoilage, the *Achromobacter* and the *Flavobacter* or *Pseudomonas* increasing relatively at the expense of the *Micrococci*. The general conclusion seems to be justified that the groups mainly responsible for the spoilage of fresh fish

TABLE II

Comparison of Aerobic Bacterial Floras of Fresh and Spoiling Fish
(Expressed as percentage of total number of organisms isolated)

Source of Data	Species	Description of sample	*Achromo-bacter*	*Micro-coccus*	*Flavo-bacter*	*Pseudo-monas*	*Bacillus*	Miscel-laneous
Stewart (1932)	North Sea haddock	Fresh—slime	57.0	22.0	11.0	5.0	—	5
Stewart (1934b)	North Sea haddock, codling, ling, etc.	7–8 days in ice—slime	66.0	11.0	20.0	3.0	—	—
Fellers (1926)	Salmon	24–72 hours (at ordinary temperature)	22.8	41.2	20.6	—	5.2	10.1
Fellers (1926)	Salmon	96–145 hours (at ordinary temperature)	61.4	13.8	11.7	—	2.8	10.3
Aschehoug and Vesterhus (1943)	Norwegian winter herring	Fresh—slime	24.5	16.7	17.7	40.0	—	1.1
Aschehoug and Vesterhus (1947)	Norwegian winter herring	Stored at 1–8°C. (33.8–46.4°F.) for 6–11 days —Flesh	58.5	0.8	7.0	33.7	—	—

TABLE II (cont.)

Shewan (1946a)	North Sea haddock	Fresh—slime	50.0 (Mainly *Achromobacter*)	34.3	—	—	15.7	—
Shewan (1946a)	North Sea haddock	12 days in ice—slime	94.0 (Mainly *Achromobacter*)	1.5	1.5	—	1.5	1.5
Shewan (1946a)	North Sea codling	Fresh—slime	60.0 (Mainly *Achromobacter*)	30.0	—	—	10.0	—
Shewan (1946a)	North Sea codling	12 days in ice—slime	94.0 (Mainly *Achromobacter*)	6.0	—	—	—	—
Shewan (1946a)	North Sea haddock	Fresh—slime	56.8 (Mainly *Achromobacter*)	35.2	6.0	—	2.0	—
Shewan (1946a)	North Sea haddock	12 days in ice—slime	86.0 (Mainly *Achromobacter*)	13.0	—	—	—	1.0
Shewan (1946a)	North Sea haddock	24 days in ice—slime	75.0 (Mainly *Achromobacter*)	23.0	—	—	2.0	—

during the first two weeks at chill temperatures are the *Achromobacter* and the *Flavobacter* or *Pseudomonas*.

3. The Comparative Biochemical Activity of the Bacterial Groups

Various attempts (e.g. Schönberg, 1938; Snow and Beard, 1939; Wood, 1940; and Dyer, 1947) have been made to assess the relative importance of the various groups in the spoilage process as it develops on the basis of biochemical properties, such as fermentation of carbohydrates, proteolysis (lysis of fish muscle suspensions or gelatine liquefaction), reduction of trimethylamine oxide (an important constituent of marine fish muscle discussed below), lipolysis, indole formation, etc.

From the data so far available, it is clear that all the bacterial groups contain members that exhibit proteolysis; trimethylamine oxide reduction coupled with the oxidation of lactic acid; lipolysis, which is important in the case of fatty fish; and degradation of carbohydrate, which is probably relatively unimportant in fish, owing to paucity of substrate. It is, however, extremely difficult as yet to reach general conclusions concerning the relative parts played in various biochemical ways by the different groups during the whole process of spoilage. It does seem, nevertheless, that the percentage of actively proteolytic types increases, the major contribution arising from the increased proportion and numbers of proteolytic *Achromobacter*. It also appears that, whilst their numbers increase, the average percentage of trimethylamine oxide reducing types

TABLE III

Number of Organisms Reducing Trimethylamine Oxide in Fresh, Staling, and Stale Fish

(Expressed as percentage of total number of organisms isolated)

Fish Type	State of freshness	% Reducing T.M.O. 20°C. (68°F.)	37°C. (98.6°F.)
Haddock	Fresh (less than 12 hours at 0°C.)	39.5	
	Stale (12 days in ice)	24.0	
Haddock	Fresh (less than 12 hours at 0°C.)	8.5	50
	Stale (12 days in ice)	9.0	22
Haddock	Fresh (less than 12 hours at 0°C.)	8	
	Stale (12 days in ice)	16	
	Stale (24 days in ice)	15	
Codling	Fresh (less than 12 hours at 0°C.)	13.0	5.0
	Stale (12 days in ice)	8	20.0
Codling	Fresh (less than 12 hours at 0°C.)	43	40
	Stale (15 days in ice)	19	30
Codling	Fresh (less than 12 hours at 0°C.)	32	55
	Stale (15 days in ice)	23	28

is broadly the same in fresh, spoiling, and spoiled fish, although there are both considerable increases and decreases recorded in individual instances. This is indicated by Shewan's data, hitherto unpublished, which are shown in Table III.

It is noteworthy that the proportion of trimethylamine oxide reducers varies as between different groups of fish of the same species. Variation between individual fish has also been observed. Moreover, some data have been obtained which suggest that there is also a seasonal variation. (Shewan, 1946b).

Lipolytic types, according to Snow and Beard (1939), appear to be widely distributed amongst the predominant groups found on fresh Pacific salmon.

4. The Increase in Bacterial Population during Spoilage

Only a few examples from the considerable amount of recorded data will be given. There have apparently been very few observations made on the increase in population that occurs in the slime of the fish during storage. Bedford (1933b) has reported counts of the order of 2.8 to 6.5×10^6 per g. of the dry skin of haddocks after storage for 30 to 40 hours at 25°C. (77°F.) as compared with "fresh" values of the order of 10^2 to 10^3. Fitzgerald and Conway (1937) have given bacterial counts ranging from 8.1×10^4 to 2.5×10^7 per g. of wet material for the slime of market fish, without indicating the species, conditions or duration of stowage. Fellers (1926) has recorded average counts per g. of gills in 138 Pacific salmon stored at 14.4 to 17.8°C. (58 to 64°F.) for 1 to 6 days. The average count rose from 3.7×10^2 to 2.4×10^8. In the same period the counts for the flesh of the belly and the back increased from zero to 9.3×10^5 and 4.4×10^5 respectively, the fish then being putrid.

Under chilling conditions, increase in bacterial population is, of course, much slower, but ultimately results in numbers of much the same magnitude in the completely spoiled fish. For example, Aschehoug and Vesterhus (1947) have reported bacterial counts for the flesh of ungutted winter herrings stored at 1°C. (33.5°F.) for periods up to 12 days. Starting from practically zero the population reached 1.6×10^6.

Over a period of 15 years at the Torry Research Station, Aberdeen, many counts have been made by Stewart and later by Shewan on the flesh of carefully gutted and washed haddocks during stowage in ice for periods up to 35 days. The population starting from zero in the fresh fish has been found on the average to reach 10^6 per g. in about 10 days, 10^7 per g. in about 25 days, and to increase only little more during a further 10 days, to about $10^{7.3}$ per g. After 10 days the fish were usually judged by organoleptic standards to be in a condition of incipient

spoilage, and to be completely spoiled and generally unacceptable soon after 15 days.

5. The Routes of Bacterial Attack

Anderson (1907) describes how by microscopic observation he found bacteria proceeding in gutted fish from the kidney, (which is not usually removed), along the cardinal vein, which lies beneath the backbone in the caudal region of the fish, and breaking up the corpuscles and finally entering the tail flesh, a process associated with the development of "red along the bone."

Since it became clear that in the freshly caught fish the flesh is sterile, whilst the external surfaces carry a bacterial load, it has been considered that the routes of attack must lie from the gills and kidney into the flesh via the vascular system, and directly through the skin and the peritoneal lining. Hildebrandt (1922) who, however, did not consider the vascular route, was of opinion that the skin formed a more effective barrier than the peritoneal lining.

Hunter (1920a) obtained bacterial counts for the belly walls of salmon two or three times as high as for the back muscle. A similar observation in the case of mackerel was made by Harrison *et al.* (1926) and for haddock by Birdseye (1929). Harrison *et al.* also found that in haddock the bacterial count for the subcutaneous back muscle increased somewhat earlier near the gills than at the caudal end. Stewart (1930) found that the bacterial count of the blood in ungutted fish was low, and inferred that the skin was a more important source of infection.

Beatty and Gibbons (1937) measuring chemical products of bacterial action found in cod only slight activity in a few days at 18°C. (64.4°F.) in the back muscle, and considerable activity in the peritoneum, whilst the gills spoiled very fast. They concluded that the main paths of infection of the flesh lay through the belly wall and the blood vessels. In the case of haddock they considered that bacteria also pass through the skin. Kiser and Beckwith (1944) also obtained higher counts for the belly walls of mackerel than for the back muscle. They also found that the blood of the heart was more highly infected than the muscle, suggesting invasion through the vascular system. Wood (1940) has made similar observations.

The fact that even in stale fish, particularly in large species such as cod, muscle samples aseptically removed from the centre of the flesh are frequently found to be sterile, prompted Lücke and Frercks (1940) to investigate in considerable detail the disposition of the bacterial load in market fish stowed in ice for several days. Sampling the flesh at various depths between the lateral skin and the backbone, they found that gen-

erally the count was highest just beneath the skin and decreased rapidly until the backbone was reached, when it frequently rose again, although never attaining the high subsurface level. These results suggest that the skin is penetrable with considerable ease and that infection can also proceed along the caudal vessel and probably along the dorsal vessels coming from the gills.

Dyer *et al.* (1946), however, studying iced, eviscerated cod in a somewhat similar manner, but sampling less frequently and at fewer positions between surface and backbone, have suggested that the skin and the peritoneal lining do in fact present quite an effective barrier to bacterial attack, the first layer of muscle under the skin remaining sterile for 10 days. Spoilage, they consider, occurs first in the gills, followed closely by the peritoneal lining, the surface slime, and the tissues ventral to the backbone (kidney and dorsal aorta); and they hold that it is the chemical products of this external bacterial spoilage rather than the bacteria themselves that penetrate into the flesh, rendering it stale and finally unacceptable.

All workers are agreed that bacterial growth commences externally with particular intensity at the gills and more rapidly reaches a high level there than in the interior. From all the evidence, excluding that of Dyer, *et al.*, it would seem that infection of the flesh can proceed along all the obvious routes, including penetration of the skin; and it is not clear that any one route is always more easily followed than another. It is difficult to reconcile the extremely slow or even nonexistent penetration to the superficial subcutaneous muscle noted by Dyer and his colleagues with the observations of Lücke and Frercks and of many other investigators, who, like them, have employed apparently unobjectionable techniques. It seems highly probable, however, that, as Dyer *et al.* have demonstrated, there is generally a diffusion of the chemical products of bacterial activity from the site of that activity into the flesh as well as a reverse diffusion of bacterial substrates. It is clear that the disposition of the attacking bacterial forces during spoilage and the concomitant movement of diffusible substances require much further investigation.

6. The Influence of Temperature and pH on the Bacteria of Fish

The influence of temperature upon the growth and biochemical action of marine bacteria has been well reviewed by ZoBell (1934). The work of Bedford (1933a), Hess (1932; 1934a; 1934b), and Kiser (1944), who have made special studies of the influence of temperature, and of others, who have had occasion to make routine counts of fish bacteria, usually at 20°C. (68°F.) and 37°C. (98.6°F.), has shown clearly that the great majority of the marine bacteria responsible for the spoilage of fish are

TABLE IV

The Influence of Temperature upon the Duration of the Lag Phase in the Growth of Marine Bacteria

Bacterial species	Duration of lag phase (hr.)									
	30°C. (86°F.)	25°C. (77°F.)	20°C. (68°F.)	12°C. (53.6°F.)	7°C. (44.6°F.)	6°C. (42.8°F.)	5°C. (41°F.)	0°C. (32°F.)	−3°C. (26.6°F.)	−4°C. (24.8°F.)
Pseudomonas fluorescens [a]	6	—	—	12–24	—	32	—	120	—	—
Pseudomonas fluorescens [b]	—	—	24	—	—	—	72	96	144	—
Achromobacter sp.[c]	—	3–4	—	—	12–28	—	—	—	—	125–193

[a] Müller (1903).
[b] Hess (1934b).
[c] Kiser (1944).

psychrophilic in type, growing at temperatures between about 30°C. (86°F.) and 0°C. (32°F.), some, however, growing at even lower temperatures down to −7.5°C. (18.5°F.). The optimum temperatures for growth for most of these types are in the range 10-20°C. (50-68°F.). Bedford (1933a), for example, working with 71 strains isolated from sea water found that all except six could grow at 0°C. (32°F.), whilst 22 could grow at −5°C. (23°F.), and 10 even at −7.5°C. (18.5°F.).

Table IV, summarizing observations by Hess (1934b), Müller (1903), and Kiser (1944), shows the extension of the lag phase, i.e., the whole period of adjustment preceding logarithmic growth, that results from lowering the temperature below that of maximum growth rate.

In the case of *Pseudomonas fluorescens* the lag phase is extended from about 1 day at the optimum growth temperature to about 4 to 5 days at 0°C. (32°F.), and to about 6 days at −3°C. (26.6°F.) Extension to as much as 8 days is obtained at −4°C. (24.8°F.) in the case of a species of *Achromobacter*.

Hess (1934b) has also made the important observation that although growth was most rapid at 20-25°C. (68-77°F.), the maximum total growth or crop was obtained at 5°C. (41°F.) using strains of *Pseudomonas fluorescens, Flavobacter deciduosum,* and *B. vulgatus*. Even at 0°C. (32°F.), and at −3°C. (26.6°F.) in some of these cases, greater crops were obtained than at 20°C. (68°F.) and 37°C. (98.6°F.).

Hess (1934b) and Kiser (1944) have calculated temperature coefficients (Q_{10}) of growth in the case of three species. The results are shown in Table V.

TABLE V

Temperature Coefficients of Growth of Marine Bacteria

Bacterial species	Temperature range		Temperature coefficient of growth (Q_{10})
	°C.	°F.	
Pseudomonas fluorescens [a]	20 to 5	68 to 41	3.7
	5 to 0	41 to 32	8.4
	0 to −3	32 to 26.6	9.3
Flavobacterium deciduosum [a]	37 to 20	98.6 to 68	1.2
	20 to 5	68 to 41	1.2
	0 to −3	32 to 26.6	11.2
Achromobacter sp.[b]	25 to 7	77 to 44.6	1.86 to 2.84
	7 to −4	44.6 to 24.8	4.58 to 5.82

[a] Hess (1934b).
[b] Kiser (1944).

The steep rise in the acceleration of the growth rate with rise of temperature, as the temperature is lowered into the "chilling" range, is noteworthy.

The same two workers, Hess (1934b) and Kiser (1944) have found that although most of the biochemical activities of representative marine bacteria persisted at or near 0°C. (32°F.), many could no longer be demonstrated at the minimum temperatures of growth, ranging down to −6.5°C. (21°F.). Sanborn (1930) has reported active proteolysis by some species on fish stored at −5°C. (23°F.). Horowitz-Wlassowa and Grinberg (1933) also found that proteolysis, as well as lipolysis, occurred at subzero temperatures; although fermentative activity was somewhat impaired. These workers have suggested that adaptation to low temperatures occurs with resultant increase of the biochemical activity over that initially exhibited in the new environment. Hess (1934a) found no real evidence for such adaptation, except in the case of nitrate reduction.

Schönberg and Debelic (1933), Coyne (1932; 1933b), and Kimata (1936) have studied the influence of pH upon the growth of members of all the important bacterial species found upon fish. Taking their results as a whole it appears that only a few strains were capable of growth below pH 6. These showed restricted growth, in some cases down to pH 5.2. Optimum growth occurred in the range pH 6.5 to 7.5. Some organisms failed to grow above pH 8. Hjorth-Hansen (1943) states that optimum growth occurs between pH 6.5 and pH 7.5. In the suceeding section it is shown that the pH of the flesh of fish caught under commercial conditions generally lies between pH 6.2 and 6.5 during full rigor mortis. Bacterial growth must on the average be considerably retarded under these conditions; which, however, do not fall far short of those that support optimum growth.

IV. The Biochemistry of Spoilage

1. Immediate Post-Mortem Changes

The flesh of the living fish is sterile and it is generally considered that bacterial spoilage of the flesh does not commence until rigor mortis has been resolved. Bate-Smith (1948) in a previous review has shown in the case of mammalian muscle how important the post mortem production of lactic acid and particularly the final pH of the tissue is in determining the onset of spoilage, and the physical behavior of the muscle in processing, as by curing and freezing. The minimum pH attained has been shown to depend upon the state of fatigue of the animal immediately prior to killing or more specifically to the stock of muscle glycogen available for degradation to lactic acid. Bate-Smith presents evidence of how

this stock can be beneficially increased by proper resting and, sometimes, feeding before killing.

The biochemistry of immediate post mortem change in fish muscle has not received nearly the exhaustive attention given to that in mammals, and data are therefore relatively sparse. There is, however, a striking contrast to be noted at once between the control that the meat and the fish industries can exercise over the condition of their respective raw materials. By far the majority of the fish caught come aboard the fishing vessel in nets or on lines in a state of fatigue following violent struggling, and nothing can be done about it. The few recorded observations on the glycogen and lactic acid content and pH of fish specially caught so as to reduce struggling to a minimum are from this point of view at any rate of somewhat academic interest.

a. Changes in the Glycogen and Lactic Acid Content and pH of Fish Muscle. The recorded values for glycogen in fish muscle immediately post mortem suggest that fish is generally poorer in this substance than meat. Sharp (1934) reports the highest values, viz., 0.61 to 0.85% in stunned and chilled, unexercised haddocks, 20 minutes after death. Macpherson (1932) reports values of 0.44 to 0.64% for haddock under similar conditions. Sharp also obtained a series of lower values, viz., 0.2 to 0.3%, which he explained reasonably by the fact that the fish had just spawned, and were therefore in poor condition. Macleod and Simpson (1927) found much lower initial values, about 0.2% on the average, for fish rapidly caught by hand line, a method that induces some struggling. Fish caught by commercial line trawl and examined probably 2¼ hours after hooking contained practically no glycogen.

The picture is fairly clear, as in the case of meat, that the amount of glycogen present in the muscles immediately post mortem diminishes greatly with the intensity of previous struggling and anoxemia, and that in commercially netted or trawled fish the glycogen content of the flesh must usually be at a very low level. Macpherson (1930) reported values for glycogen ranging from a trace to 0.015% in trawled haddocks taken from the market and estimated to have lain in ice for 75 hours. All workers are agreed that glycogenolysis sets in rapidly with death, the more rapidly the higher the temperature above 0°C. (32°F.), although Sharp (1934) notes arrest in this process at about 0.1 to 0.2% in muscle which originally had a high content of glycogen, e.g., 0.6 to 0.85%.

Macpherson (1930) and Sharp (1934) report values for lactic acid immediately post mortem, in unexercised haddocks of 0.03 to 0.15%. Sharp found "equilibrium" values during storage at 0°C. (32°F.) of 0.4 to 0.5% in the case of unexercised fish with initially high glycogen content. Values much higher than this have not been recorded for maximum lactic

acid contents, although a figure of 0.6% was obtained by Bate-Smith (private communication) in haddock muscle juice. Macpherson (1932) reports that trawled haddocks from the market, 75 hours on ice, had a lactic acid content ranging from 0.106 to 0.250%. Clearly the maximum values for lactic acid in fish muscle usually fall considerably below that which can be obtained for meat, viz., about 1%. The disappearance of lactic acid during spoilage is discussed later.

Reported values for the pH of fish muscle during the onset of rigor mortis are also few. Benson (1928) and Macpherson (1932) using the quinhydrone electrode, and Cutting (1939) using the glass electrode, have found that in general the pH of the flesh of unexercised haddocks lies between 7.0 and 7.3. Hjorth-Hansen (1943) reports pH values of 7.05 to 7.33 for newly killed cod. Both Benson and Cutting have found that the pH of the flesh of trawled or line trawled haddocks immediately on being caught is usually lower than this, viz., 6.5 to 6.9, although Benson records values above pH 7 for hake and skate, and Hjorth-Hansen values of 7.1 to 7.2 for newly caught halibut. Ultimate pH values during rigor mortis reported, or found at the Torry Research Station, for haddocks, whitings, and related species usually lie in the range pH 6.2 to 6.6. Hjorth-Hansen has noted, as others have found, that the fall to the ultimate pH value may occur over a period of 1 to 2 days at 0°C. (32°F.). Again, the comparison with meat is interesting. In the latter case ultimate pH values are on the whole distinctly lower, reaching even pH 5.5 in rested animals with high glycogen content. From unpublished work at the Torry Research Station on the titration of haddock muscle there appears to be no evidence that the buffering power of the muscle of such species as cod and haddock over the pH range in question is any higher than that of meat (Bate-Smith, 1938). The main factor influencing the high ultimate pH of fish muscle as compared with meat would therefore appear to be the amount of lactic acid produced. The comparatively high ultimate pH occurring in fish is probably one factor contributing to its acknowledged high perishability. As already noted the pH at which marine bacteria begin to exhibit strong growth lies very little above the range of the pH in rigor mortis. In this connection it is of interest to quote Hjorth-Hansen's (1943) values for the ultimate pH found in halibut and isuroid shark, which are 5.57 and 5.70 respectively, and to note that he correlates the well known, high keeping quality of halibut with its high acidity in rigor mortis. There appear to be no experimental data for fish showing direct correlation between ultimate pH in rigor mortis and initial glycogen content at catching, as affected by species, method of catching, nutrition, etc.

A number of workers have reported phosphate changes in fish muscle.

All agree that organic phosphates rapidly break down after death, the total inorganic P reaching values between 0.10 and 0.15%. This field requires detailed investigation in view of modern theories linking phosphates with the glycolytic cycle, muscle contraction and the development of rigor mortis.

b. Rigor Mortis. There are comparatively few published observations made under actual commercial fishing conditions upon the speed with which rigor mortis sets in and its duration in relation to condition of the fish at hauling, temperature, species, etc., and as demonstrably affecting subsequent keeping quality.

Anderson (1907) examined many lots of fish taken from the trawl net after being a certain number of hours at work and treated under different conditions. Some were killed when taken on board, some not killed, some gutted, and some not gutted. When such fish were compared under similar conditions with fish caught quickly by hand line, it was generally found that rigor mortis set in earlier and disappeared earlier in the trawled fish. He attributed the speedier onset of stiffening in the latter to the struggling, crushing and anoxemia undergone. He noted, however, that in any haul of trawled fish there were many that passed into rigor mortis in a manner similar to that of line caught fish and assumed that these had been the more recently entrapped. From the examination of some thousands of fish Anderson concluded that rigor mortis is later in appearing and lasts longer, when fish are in season, in a healthy and vigorous condition, killed and pithed or gutted at once on capture, handled as little as possible, and kept at a low temperature as in ice. The more conditions approximate to those mentioned, the later will rigor mortis set in—sometimes not for 10 to 30 hours and it may persist for 1 to 3 days. Anderson considered that in practice the most important factor is the maintenance of low temperature. Ewart (1887) had previously noted the effect of exhaustion upon the development of rigor mortis. He found that hand-line caught haddocks, killed and pithed immediately, remained stiff for 30 hours at 8°C. (46.4°F.) whilst fish caught in two hour hauls by trawl net passed through rigor mortis within 10 hours on the average. Considerable variation was noted in the case of the trawled fish. Ewart found that immediate gutting resulted in longer persistence of rigor mortis.

In discussing experiments on board commercial fishing vessels in the North Sea, Schlie (1934) records some interesting observations on the onset and duration of rigor mortis in sole, cod, haddock, ling, mackerel, and dogfish, which he noted by observing the mobility of jaw muscles and the gill covers as well as the rigidity of the body as a whole. Fish were examined at 30°C. (86°F.), 18°C. (64.4°F.), and 3.5°C. (38.3°F.), and

the marked influence of chilling in prolonging the final resolution of rigor mortis was confirmed. Schlie noted that, in trawling from depths of 100 to 150 meters, an average fishing depth, about 25, 45, 55, 75 and 85% of the cod, coalfish, ling, mackerel, and haddock respectively were dead when emptied from the net on deck, and that the remainder died within half an hour. He comments on the fact that such fish go into rigor mortis some hours before fish freshly hooked, immediately hauled, and killed. Approximate times after death observed for the beginning of rigor mortis at 2-3°C. (35.6-37.4°F.) were as follows: mackerel—a few minutes; herring—2 hours; haddock, cod, and coalfish—5 hours; flat fish—10 hours. Schlie contends, probably correctly, although his data are few, that rigor mortis endures for a shorter period, and the pH increases sooner from its minimum, as the amount of handling and agitation is increased.

Cutting (1939) has given an account of observations made on a research vessel upon fish of various species trawled on a shallow inshore ground. The hauls were only of 1 hour's duration so as to avoid great disparity in the condition of the fish arising from confinement for various times. The fish were not stunned, but were allowed to struggle on deck without interference. Various sizes and species were kept, gutted and ungutted, in ice and in air at deck temperature (11-16°C.) (51.8-60.8°F.). Rigor mortis was noted by observing the sag of the tail of the fish when it was held by the head in a horizontal position. Table VI summarizes Cutting's observations.

Even allowing for variation among fish of the same species, average specific differences were apparent in the onset and duration of rigor mortis. Thus, whitings passed into rigor mortis sooner and remained in that condition for a significantly shorter time than the other round fish studied. Plaice, although remaining stiff for about the same time as haddocks, actually emerged from rigor mortis later, since onset was delayed until about 10 hours after catching. In this connection it is perhaps significant that whitings are reckoned in the trade to keep less well than haddocks and codlings. It might have been expected that the flat fish, plaice and lemon soles, which are regarded as keeping somewhat better than cod and haddocks, would have emerged from rigor mortis significantly later than the latter. But it should be noted that whilst the onset of rigor mortis could be fairly easily recognized, the point of complete resolution was very difficult to determine. Later experiments by Cutting, however, at a different season of the year, have confirmed that whitings emerge from rigor mortis sooner than haddocks, and flat fish appreciably later. The size of the fish within a species had little apparent effect upon either the rate of onset, or the total time spent in rigor mortis, 10-lb. cod

TABLE VI

The Influence of Species, Treatment and Temperature on the Course of Rigor Mortis in Trawled Fish

(summarized from Cutting, 1939)

Species	Conditions of stowage	Time between hauling and commence-ment of rigor (hrs)	Time between commence-ment and full rigor (hrs)	Time in full rigor (hrs)	Time taken to come out of full rigor (hrs)	Time between hauling to end of rigor (hrs)	Number of fish observed (number of experiments)
Haddock	Ice	2	3	28	6	39	27(5)
Haddock	Air at 12°C. (53.6°F.)	2	3	16	5	26	24(5)
Whiting	Ice	1	1	16	3	21	11(1)
Whiting	Air at 16°C. (60.8°F.)	1	1	6	4	12	25(2)
Cod	Ice	3	4	28	5	40	25(3)
Cod	Air at 11°C. (51.8°F.)	1	3	20	6	30	2(2)
Megrim	Ice	2	3	28	6	39	2(1)
Megrim	Air at 12°C. (53.6°F.)	1	3	18	6	28	2(1)
Witch	Air at 16°C. (60.8°F.)	2	1	18	7	28	3(1)
Plaice	Air at 16°C. (60.8°F.)	10*	4	24	6	44	11(2)
Lemon sole	Ice	2	2	34	6	44	2(1)
Lemon sole	Air at 16°C. (60.8°F.)	3	4	28	3	38	3(2)

* Including an average of 6 hours after hauling during which response to stimulation was very marked.

behaving in the same manner as ½-lb. codlings. Gutting appeared to have little effect upon the total duration of the process. Chilling in ice did not affect the rate of onset, compared with storage at air temperature, but it delayed resolution by about 10 hours.

From all the data available it appears to be clearly established that chilling prolongs the final resolution of rigor mortis, and hence delays the onset of bacterial attack; that there is considerable variation among species, the flat fishes in particular passing out of rigor mortis somewhat later than the common round fishes; that rigor mortis sets in more rapidly and lasts a shorter time depending upon the extent of previous struggling and exhaustion and the amount of handling after death. Whilst there is considerable variation in the amount of "exercise" prior to hauling and in the proportion captured alive, the bulk of commercially caught fish go into rigor mortis much sooner than if the circumstances preceding slaughter could be controlled as in the case of domesticated land animals. It would also appear that in the case of most commerically caught marine fish, stowed in ice, a "rigor" period of about 2 days must elapse before the development of the bacterial attack, although this time is probably shorter in the case of certain species, e.g., whitings, and somewhat longer in the case of others, e.g., certain flat fishes, notably the halibut.

Clearly the whole field of investigation of the biochemistry of the immediate post mortem changes in the muscles of fish caught under commercial conditions requires much further integrated work, guided by the more recent biochemical conceptions of these changes.

2. Biochemical Spoilage Changes

a. Introduction. It has frequently been stated in chemistry text books that trimethylamine has a "fishy" odor. Actually, to be correct, the description should have been " 'stale fishy' odor," for the odor of perfectly fresh fish is not in the least like that of trimethylamine, and the misstatement probably reflects the all too frequent reception by the consumer of fish that is past its best. Bacterial spoilage, which had usually been thought of as being chiefly a degradation of protein to malodorous and ill-flavored products, was, as far as marine fish is concerned, first clearly shown just prior to World War II to occur broadly in two stages; firstly the bacterial reduction of trimethylamine oxide to the amine base coupled with the oxidation of lactic acid and sugar, and secondly, the degradation —largely bacterial—of the proteins. This is, of course, an over-simplification, but it represents a great advance in our knowledge of an extremely complex process, bringing two elements of major importance into clearer view.

b. Trimethylamine Oxide. In interesting contrast to urea, trimethyla-

mine oxide was synthesized in the laboratory by Dunstan and Goulding (1896) before it was first isolated as a naturally occurring substance from the flesh of dogfish, by Suwa (1909a; 1909b), who showed that bacteria reduce the oxide to the amine, to which he attributed the odor of staling fish. This was confirmed by Poller and Linneweh (1926) who further showed that the oxide can act as a hydrogen acceptor, being reduced by glutathione in the muscle system. Kutscher and Ackermann (1933) reviewed a considerable amount of work, mainly German, concerned chiefly with the distribution of trimethylamine oxide and other trimethylated and related nitrogenous bases in animal tissues, and with the possible explanation of their occurrence and their physiological significance.

From this early work it became clear that trimethylamine oxide is absent or present only in traces in the muscles of fresh-water fishes, but present in comparatively large amounts in those of marine fishes, and generally in greater concentration in the selachians or "urea" fishes, such as skates, rays and dogfish. Contributions to our knowledge of the distribution of the oxide in fishes have been made since 1933 by Reay (1938), Beatty (1939), Lintzel *et al.* (1939), Norris and Benoit (1945) and by Ronold and Jakobsen (1947). These have confirmed the earlier findings regarding variation with fresh-water or marine habitat and with teleost or selachian type. Reay *et al.* (1943) summarizes the figures recorded up to 1938 for the oxide content of aquatic species. The muscles of marine elasmobranchs have been found to have an oxide content ranging from 1000 to 1500 mg.% whilst the figures for marine teleosts range from 120 to 980 mg.%, the majority of the latter lying between 200 and 400 mg.%. There are interesting problems in the comparative biochemical and physiological aspects of the occurrence of the oxide in plants and animals from marine and fresh-water habitats which cannot be discussed here. Some of the recorded results, particularly those of Ronold and Jakobsen (1947), suggest that there is a seasonal variation in the trimethylamine oxide content; and it may well be that there are other factors such as age, environment, abundance of food, etc., which influence variation.

c. The Trimethylamine Oxide Reduction Phase of Spoilage. Beatty (1938) showed that about 95%, at least, of the trimethylamine found in spoiling cod muscle arises from trimethylamine oxide and from no other source. However, Ronold and Jakobsen (1947) found that somewhat more amine was produced in spoiling fish flesh (herring, brisling, etc.) than could be accounted for by the oxide present, and Beatty and Gibbons (1937) and Brocklesby and Riddell (1937), respectively, showed that sterile muscle press juice and sterile muscle do not reduce the oxide. Beatty and Gibbons (1937) and Watson (1939a) demonstrated that the

period of rapid trimethylamine increase in cod muscle and in the muscle press juice corresponded with the period of rapid bacterial multiplication.

Reference has already been made to the fact that only a fraction of the total bacterial population on fresh and spoiling fish are trimethylamine oxide reducers. These are mainly facultative anaerobic *Achromobacter,* which are capable of growth in the interior or on the surface of the fish. Watson (1939a) showed that, while molecular oxygen has some trimethylamine oxide sparing effect, conditions at the cell surfaces of organisms growing on the surface of the fish are actually mainly anaerobic, owing to the intense local demand for oxygen by the population as a whole, which includes obligate aerobes, and also owing to the low solubility of oxygen. Bacterial attack commences at the surface of the fish and trimethylamine oxide reduction commences there also.

Tarr (1939) first showed that the reduction of the trimethylamine oxide is due to a bacterial enzyme, which activates the oxide, rendering it susceptible to reduction by many of the dehydrogenases of the cell. Later Tarr (1940) established that the enzyme, which he called "triamine-oxidase," and which he studied experimentally in six species, comprising five genera (*Micrococcus, Achromobacterium, Escherichia, Aerobacter,* and *Pseudomonas*) isolated from spoiling fish, well water, and surface taint butter, specifically activates trialkyl oxides of the general type $R_3 = N = O$ with liberation of the corresponding base.

Tarr (1939) also showed that a variety of oxidisible substrates (lactate, succinate, acetate, formate, glucose, fructose, natural hexose monophosphate, phospohexonate, glycine, and alanine) accelerated the endogenous reduction of trimethylamine oxide in the presence of organisms containing the oxidase. At about the same time Watson (1939b), using a reducing *Achromobacter,* derived and tested the general equation $AH_2 + (CH_3)_3NO \rightarrow A + (CH_3)_3N + H_2O$, when AH_2 is a hydrogen donator and A the oxidized substrate. The reduction of the oxide as hydrogen acceptor with evolution of the base was found to be a linear function of time in the presence of cell suspensions and the donators, glucose, glycogen, lactate, and pyruvate. All strains of *Achromobacter* were not able to reduce the oxide, although shown to contain the same dehydrogenases, as indicated by the methylene blue technique. The work of Tarr and Watson thus fits well together.

In the same paper Watson (1939b) postulated the main reaction actually occurring in muscle or muscle juice as: $CH_3CHOH \cdot COOH + 2\ (CH_3)_3NO + H_2O \rightarrow CH_3COOH + 2\ (CH_3)_3N + CO_2 + 2\ H_2O$, and demonstrated the practically theoretical disappearance of lactic acid and appearance of carbon dioxide and trimethylamine in cell suspensions

incubated with lactic acid and the oxide. Acetic acid, however, was not determined.

Beatty and Collins (1939) and Collins (1941) following this work up in studies of the bacterial spoilage of prerigor cod muscle juice demonstrated clearly that during early spoilage the greater part of the bacterial action even in intimate contact with air is anaerobic in character. Later, when spoilage is well advanced, aerobic oxidation assumes the major role, if air is available. Spoilage in such an expressate was shown always to occur in two stages irrespective of the availability of air, first, oxidation of lactic acid and sugar, and to some small extent an unknown precursor of lactic acid, coupled with reduction of trimethylamine oxide to the volatile base; and, second, oxidation and hydrolysis of proteins; this latter stage representing advanced spoilage. In muscle juice treated with toluene it was shown that autolytic changes were negligible in proportion to bacterial spoilage. The juice used, which was expressed at 0-5°C. (32-41°F.) from prerigor cod caught by commercial line trawl and carried to the laboratory in sea water tanks, was considered by the authors, reasonably, it is thought, to be very similar in composition to the fluid present *in situ* in the flesh of commercially caught fresh fish, just in rigor mortis. Collins (1941) using this medium instead of artificial substrates, showed that the amounts of lactic acid and oxide disappearing and those of carbon dioxide, acetic acid, and amine appearing during spoilage, were sufficiently consistent with the theory of oxide reduction already outlined to permit the conclusion that bacterial spoilage in the first stage in fact proceeds substantially according to this theory, in both expressed juice and in whole fish emerging from rigor mortis.

The present authors and numerous other workers have confirmed this general two-stage conception of fish spoilage for many lean and fatty species of commercial importance, by measurement of the production of trimethylamine and ammonia and, in some cases, the disappearance of the oxide.

Sigurdsson (1947), in an excellent paper comparing chemical tests for the quality of herrings found that during storage at temperatures ranging from −2-27°C. (28.4-80.6°F.) the production of volatile fatty acids in the flesh followed that of trimethylamine very closely, and that up to the point at which the oxide was completely reduced, the acids and the amine were formed in amounts according reasonably well with Watson's equation. After this point had been reached, production of acids continued. There is apparently no evidence to show that bacteria bring about any appreciable volatile acid formation from fat, and presumably the source of the extra acid is protein or, to some extent perhaps, remaining percursors of a carbohydrate origin. Incidentally, Hillig and Clark

(1938) have shown for canned salmon and tuna that the higher volatile acids, propionic, butyric and isobutyric, increasingly predominate over acetic and formic acids as decomposition (presumably of proteins) proceeds to an advanced stage.

d. The Formation of Dimethylamine. Shewan (1937; 1938a) using a method of estimation specific for secondary aliphatic amines, has shown that dimethylamine, which is completely absent in newly caught fish, in iced haddocks increases in amount in almost linear fashion as storage proceeds, although formed in extremely small amounts as compared with trimethylamine. The dimethylamine begins to form almost immediately, while the fish are still in rigor mortis, thus definitely preceding the production of trimethylamine and any really substantial rise in bacterial population. Shewan (1938a) has suggested that the precursor may also in this case be trimethylamine oxide, but later he tested, with negative results, the capacity of a few bacterial strains, known to produce dimethylamine in fish juice, to form this amine from a trimethylamine oxide medium. Beatty and Collins (1940), who have confirmed Shewan's earlier results, found that dimethylamine was not produced in sterile muscle, and they suggest that it has a precursor, which acts as a hydrogen acceptor in oxidation-reduction reactions that occur during the proliferation of certain bacteria. The precursor is still undetermined. Shewan has found that while the diamine is produced in haddock, cod, and whiting, it is not formed at all in fresh-water perch and dogfish, and only in variable and small quantities in herring.

e. Proteolysis. Beatty and Collins (1939) showed that following the phase of trimethylamine oxide reduction, during which there was no appreciable change in amino-nitrogen, there was a marked increase in deamination with a corresponding formation of ammonia. Later, at higher temperatures, 25°C. (77°F.), there was an increase in amino-nitrogen, presumably as hydrolysis of the proteins increased.

In order to study the course of autolytic and bacterial proteolysis, Bradley and Bailey (1940) developed what they call the "tyrosine" color test. The phosphomolybdic reagent used develops a blue color in the presence of tyrosine (either in the free or peptide form), tryptophane, and cysteine, and should therefore be a measure of protein cleavage. It also reacts, however, with most phenols, sulfhydryl compounds (including hydrogen sulfide) and other reducing agents. The test, therefore, is by no means, on the face of it, a specific one for proteolysis; but the "tyrosine" value has been regarded by Bradley and Bailey (1940), by Tarr and Bailey (1939), Wood *et al.* (1942), and by Sigurdsson (1947) as affording a broad index of autolytic and bacterial degradation of protein. These workers have applied the test in following the spoilage of carp, herring,

pink salmon, cod and halibut at a variety of temperatures. On the whole the "tyrosine" value was found to rise fairly regularly over the whole period of storage from rigor mortis to the completely spoiled condition.

Sigurdsson (1947) studying the spoilage of herrings at various temperatures found that the amino nitrogen and "tyrosine" values in most cases ran fairly closely parallel during storage. Bacterial fermentation of lactic acid and carbohydrate was also followed by estimating volatile acids and trimethylamine, and fat oxidation by obtaining peroxide values. The combined data showed very significant differences in the relative rates of degradation of the various constituents of the flesh, depending upon the temperature. Thus, at 25°C. (77°F.) the development of volatile acids and trimethylamine was followed very closely by proteolysis, whilst at 10°C. (50°F.) the latter lagged considerably behind. At 0°C. (32°F.) volatile acid and trimethylamine production by bacteria was inhibited to such an extent that it did not get under way until proteolysis also became appreciable, when the two types of change proceeded at rather similar rates. At 0°C. (32°F.) the oxidation of the fat also became a signficant factor in the spoilage, whilst at higher temperatures it was negligible, until putrefaction had set in. At −2°C. (28.4°F.) (fish not frozen) it appeared that, as compared with 0°C. (32°F.), proteolysis—mainly enzymic rather than bacterial, it is presumed—proceeded at a relatively greater rate than the bacterial production of volatile acid.

These experiments illustrate perhaps more clearly than any so far published concerning fish, the effect of altering the temperature upon the complex of reactions that are responsible for spoilage and the need for much further similar investigation, simultaneously employing as many methods of analysis as possible. There is also a clear need, however, for more satisfactory methods, particularly in following proteolysis, more especially in its earlier autolytic stages. Estimation of ammonia, unfortunately not used by Sigurdsson in the experiments described, appears from the literature to be perhaps the most reliable method of determining approximately the beginning of bacterial proteolysis and of following its course. Confirmatory evidence can often be obtained by measuring the production of hydrogen sulfide, indole, and skatole.

f. Changes in pH. It is clear from the results of the many investigators of fish spoilage that, in general, as fish pass out of rigor mortis and as bacterial spoilage then develops, the pH of the flesh of lean fish, such as cod, rises from the rigor minimum to neutrality and then beyond to 7.5 to 8, or even somewhat higher, as real putrefaction proceeds. Hjorth-Hansen (1943) has noted that in halibut, however, which exhibit an abnormally low rigor pH of 5.5, the pH in the completely spoiled fish did

not exceed 7.04. Sigurdsson (1947) records pH values not exceeding 6.9 for completely spoiled herrings, which suggests that reduction of acidity proceeds more slowly during the spoilage of fatty fish, probably owing to hydrolysis of fat. Strohecker *et al.* (1937) have noted that the rise in pH during spoilage is greater in fish than in meat.

A general rise in pH in the flesh of fish during spoilage is to be expected, from the preponderance of base formation, but the coincident alteration in concentration of "buffering" substances over the pH range in question, and its relation to the speed and extent of the pH change has not been investigated in more than preliminary fashion. Reference should be made to Cutting (1937; 1938), Collins *et al.* (1941), and to Hjorth-Hansen (1943).

g. The Spoilage of Fat. As the results of Stansby and Lemon (1941) and Sigurdsson (1947) illustrate, there is considerable hydrolysis of fat during the spoilage of fatty fish such as herrings, mackerel, and salmon. The long chain free acids produced—to a concentration usually of 2 to 4% in spoiled fish—can have little or no influence, however, upon flavor or odor. On the other hand, fish oils, being highly unsaturated, are prone to oxidation and develop rancid flavors in consequence, and as the experiments of Stansby and Lemon (1941) and Sigurdsson (1947) illustrate, develop peroxides. It is probable that rancid flavor is not directly attributable to the fat peroxides as such, but rather to further oxidation products, such as aldehydes. At chilling and freezing temperatures, which depress bacterial spoilage, the fatty fishes may become unacceptable or at least unpalatable, mainly because of the development of oxidative rancidity. Banks (1935; 1937; 1938; 1939) has shown that in herrings—and presumably therefore in similar fatty fish—the atmospheric oxidation of the superficial fat is catalyzed strongly by a "lipoxidase" system, in which hematin pigments such as cytochrome are the chief elements. The latter occurs in peculiar abundance in the lateral brown band of the fatty fishes, which is the site of the most intense development of rancidity.

V. The Estimation of the Quality of Fish

1. General Consideration of Organoleptic and Objective Testing

There seems to be no doubt that wherever the opportunity exists for becoming thoroughly familiar with fish in all conditions from "freshly caught" to "very stale," the consumer as a rule prefers the freshest fish. Except in a few odd instances, e.g., halibut, where perhaps the high initial acidity makes it possible, there appears to be no advantage gained in the case of fish by delaying consumption on the analogy of conditioning meat

or game. Fish as a class are not so tough in texture as meat, so that "tenderizing" is unnecessary; and they are considerably more perishable, both from the point of view of bacterial attack, and, in the case of fatty fish, of oxidation of the fat.

A general description has been given earlier of the course of spoilage, of the marine bacteria mainly responsible and of the biochemistry of spoilage. It has been shown that chilling, i.e., reduction of temperature to a point short of that which causes freezing, has a marked effect in inhibiting, although not eliminating, the growth and spoiling activity of the microorganisms. For this reason, of course, chilling, as by stowage in crushed ice, is the most universally employed method of preserving "wet" fish. Since, however, spoilage continues to proceed even at 0°C. (32°F.), and since the fishing voyage and the distribution of the fish may together occupy some weeks, it is now necessary to indicate in more detail and on a time basis, the stages in the change from freshness to putridity in relation to consumer acceptability, and to discuss correlation between organoleptic judgments and objective (bacteriological, chemical, and physical) tests of quality.

Organoleptic methods are universally applied in estimating quality on the market, in the retail shop, and at the table. There is very considerable variation, however, in the sensitivity and tastes of judges, in the sensitivity of any one judge from time to time, and in the circumstances under which examinations are carried out, so that when a demarcatory decision has to be made between "fresh" and "stale" fish or between "stale" and "unacceptable" fish, there is apt to be some difference of opinion as to where the line should be drawn. As far as the establishment and conduct of routine inspection panels is concerned, experience has nevertheless shown that specially chosen, suitably sensitive persons can be trained to achieve a degree of uniformity in judgment sufficient for many practical purposes.

This variability in organoleptic estimation of quality has led to a search which is still continuing, for satisfactory objective measures of quality. An objective test must be simple and speedy in operation if it is to compare in convenience with the organolpetic method in the routine examination of large numbers of fish. Although not affording such convenience, an objective test, otherwise good, may still be valuable in assisting difficult organoleptic judgments. The method used should be capable of sensitively and accurately estimating the product or products of spoilage in question, which should be either absent or present in constant concentration in the unspoiled fish, and increase rapidly and regularly in amount once the spoilage reaction has commenced. The results of a good objective test must finally be closely correlated with the chang-

ing sensory estimate of the general quality of the fish, as it deteriorates.

The organoleptic method must clearly continue to be the most frequently used, if only because of the enormous quantity of fish that must be inspected. Where fish are presented to the inspector, as they should be, in more or less homogeneous lots, arranged, for example, in series from first to last caught in the cargo, the indubitably good and bad parts of the catch can be passed by rapid organoleptic examination, and the doubtful lots of fish could then be sampled and checked by an objective method. It hardly seems feasible at a first sale on the market to do more than sample doubtful lots of fish, even with a very speedy method, although at a later stage, as in a processing factory, it might be possible to combine organoleptic inspection of fish on a slowly moving belt with an objective test of all doubtful fish, if a very rapid, suitable one can be devised, and thus to reject fish below an agreed standard and to use them for some purpose not requiring such a high grade of quality.

There will always be some degree of variation between the opinions of the best judges regarding the quality of a sample of fish, and provided an objective test can be found that indicates change in general quality with at least the sensitivity of the organoleptic method, it should not be impossible to secure agreement concerning the point upon the objective scale beyond which it is not permissible to go and then to apply the test rigidly in all doubtful cases.

2. Objective Tests of Quality

a. Chemical Tests. The possibility of basing an objective test for quality upon the estimation of the products of spoilage, either as individuals or groups, has been much investigated in recent years, and considerable progress has been made. Excellent recent reviews are those by Notevarp *et al.* (1942) and Sigurdsson (1947) following a comprehensive earlier one by Boury and Schvinte (1935).

(1) *The trimethylamine and dimethylamine tests.* Boury and Schvinte (1935) in a critical study of methods of detecting incipient spoilage in fish concluded that the estimation of volatile nitrogen, amine or ammoniacal, was the best. These substances, present in only small amounts in the fresh fish, increased regularly with bacterial spoilage.

Beatty and Gibbons (1937) showed that autolysis played only a very small part in the production of volatile bases, the concentration of which increased in fish with the rise in bacterial population. They also showed that odors of incipient spoilage occurred at approximately the same volatile base content in the flesh, and that during the period from catching to the beginning of spoilage the volatile base figure rose on the average by 6 mg. nitrogen per 100 g. flesh. They found, however, with Reay

(1935) that the original values for total volatile base varied from 5 to 12 mg.% of nitrogen. The concentration of total volatile base, therefore, cannot serve as a useful index of incipient spoilage, since the original "fresh" value cannot be known. It was found that the original volatile base present in the flesh was almost wholly ammonia.

Beatty and Gibbons (1937) then showed that trimethylamine, which is practically absent in the fresh muscle, is produced by bacteria at a relatively greater rate than ammonia in the early stages of spoilage. The concentration of trimethylamine promised therefore, to be a more effective index of freshness. There was considerable disagreement among observers as to the first appearance of spoilage odors, but the general opinion was that these were first detected when trimethylamine reached a concentration of 4 to 6 mg. nitrogen per 100 ml. of press juice. At 10 mg.% the odors were definite and at 20 to 30 mg.% they were strong. In more than one hundred individual fish (cod, haddock, herring, hake, and pollock) the lowest value found for trimethylamine was 0.06 mg., the average being 0.17 mg. Odors were first detected at about 4 mg.% and were definite at about 10 mg. Hence the rise occurring up to the first appearance of odors was about twenty times the maximum variation among individuals and about twenty times the average original value. In the case of fish fillets stored at 0°C. (32°F.) there was found to be a latent period, varying from 5 to 12 days, during which no measurable amount of trimethylamine was produced, and then a measurable rise occurred about 2 days before spoilage odors were definitely detected. Beatty and Gibbons (1937) chose and established as satisfactory a comparatively speedy microdiffusion method of estimating the amine, suitable for routine analysis. Thus, the trimethylamine "value" of fish flesh apparently fulfilled the conditions required of a good objective index of quality.

Shewan (1937 and 1938a) studied the spoilage of chilled haddocks examining the correlation of the production of individual volatile amines and ammonia with bacterial counts and the sensory estimate of the general quality of the fish. The trawled haddocks, which were carefully gutted and washed immediately after catching and then kept stowed in boxes in plenty of ice without subsequent handling, passed through a characteristic succession of phases, fairly reproducible in its time relations. The organoleptic data are shown in Table VII.

As far as consumer acceptance is concerned, it has been found at the Torry Research Station that persons who are acquainted with fish in all its stages of spoilage, all definitely prefer fish that has not passed beyond Phase I. Fish still in Phase II is generally regarded as being quite good, although the more sensitive judges do not relish the flavor of incipient

TABLE VII

Organoleptic phases of the spoilage of carefully handled haddocks in ice (from Shewan, 1937; 1938)

Phase	Duration of Stowage in Ice (days)	Description of main spoilage changes
I	0	Perfectly fresh. No spoilage.
	3	Flesh slightly firmer than newly caught fish. Rigor mortis passed off.
	6	Flesh noticeably softer. Eyes opalescent, lost brilliancy, and slightly sunken. Very fresh "sea" odor absent: neutral odor.
II	9	Flesh softer. Surface "bloom" noticeably fading. Eyes grey and sunken. Slime becoming milky. Odor sweetish and "strengthening," but not stale.
	12	Flesh soft. General appearance stale. Increase in slime, becoming sticky, turbid and knotted. Odor definitely stale. Some "redness along the bone."
III	15	Flesh very soft. Surface very soft. Surface very slimy and unsightly; slime granular. Odor bad, somewhat ammoniacal. "Redness along the bone."
IV	20	Flesh very soft and very easily stripped from the bone. Slime colored yellowish or brownish. Odor putrid (ammonia, sulfide, indole, etc.) Much "redness along the bone."

spoilage that they discern, when the fish has passed well into this phase. To such, fish in Phase III is quite distasteful, if not unacceptable. All judges agree that fish at this stage is definitely stale; and even the least fastidious consider that fish is fast becoming unacceptable as it enters Phase IV.

It should be noted that the timing of the phases shown in Table VII relates specifically to the stated conditions of handling and storage. Thus, storage at a higher temperature, or less hygienic, less careful handling, has been found to result in a telescoping of the phases, so that each is of shorter duration.

From available European and North American data it seems that the description of events given in Table VII is generally valid under the same conditions for medium sized fish of the gadoid species, such as cod, haddock, whiting, pollock, etc. Larger fish appear to keep somewhat better, as do also the flat fishes, especially the halibut.

Fig. 1 shows the results obtained by Shewan (1937 and 1938a) for bacterial growth and the production of total volatile bases, ammonia, trimethylamine and dimethylamine, and the disappearance of trimethylamine oxide in the stored haddocks. The organoleptic phases of spoilage as described in Table VII, are indicated along the abscissa.

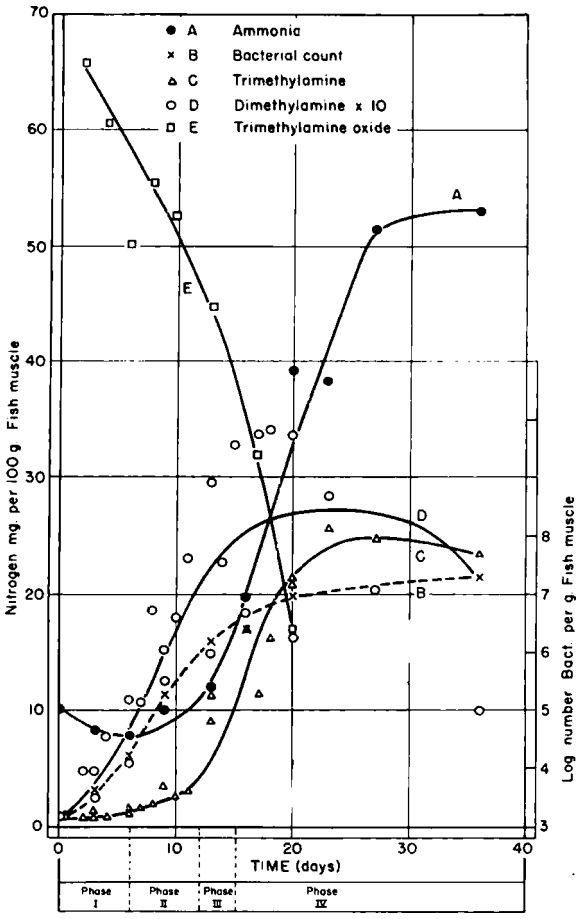

Fig. 1. Effect of storage period on bacterial count and decomposition of fish muscle.

It will be seen that the succession of bacteriological and chemical events is correlated fairly closely with the series of organoleptic phases. Bacterial growth has not gathered momentum until the end of Phase I, the period of indubitably fresh quality. During this period there is, therefore, no accumulation of noxious bacterial products, but dimethylamine—never present at any time in amounts of organoleptic significance—increases steadily from zero in the freshly caught fish, closely following the bacterial curve.

In Phase II the bacterial count and the amount of dimethylamine continue to increase steadily, whilst trimethylamine, arising from reduction of the oxide, has begun to increase rapidly in amount by the middle of the phase, i.e., at about 9 days, when the trimethylamine figure is about 3.5 mg.% (expressed on a nitrogen basis) and odor is beginning to strengthen. By the end of Phase II a condition of definite staleness has been brought about by the acceleration of bacterial growth up to a count

of $10^{6.5}$, and, as regards odor in particular, by the formation of trimethylamine to a level of about 7.0 mg.%. During Phase III in which the condition passes from definite staleness to incipient putridity, whilst the rates of increase of the bacterial count and dimethylamine diminish, the amount of trimethylamine continues to increase steadily, reaching 12-15 mg.% at the end of the phase, and ammonia for the first time begins to form rapidly, indicating the onset of proteolysis and putrefaction proper. In Phase IV in which the fish becomes putrid, the production of trimethylamine slows down, mainly due to the exhaustion of substrates, while the increase in ammonia is accelerated until about the twenty-third day, when production is retarded.

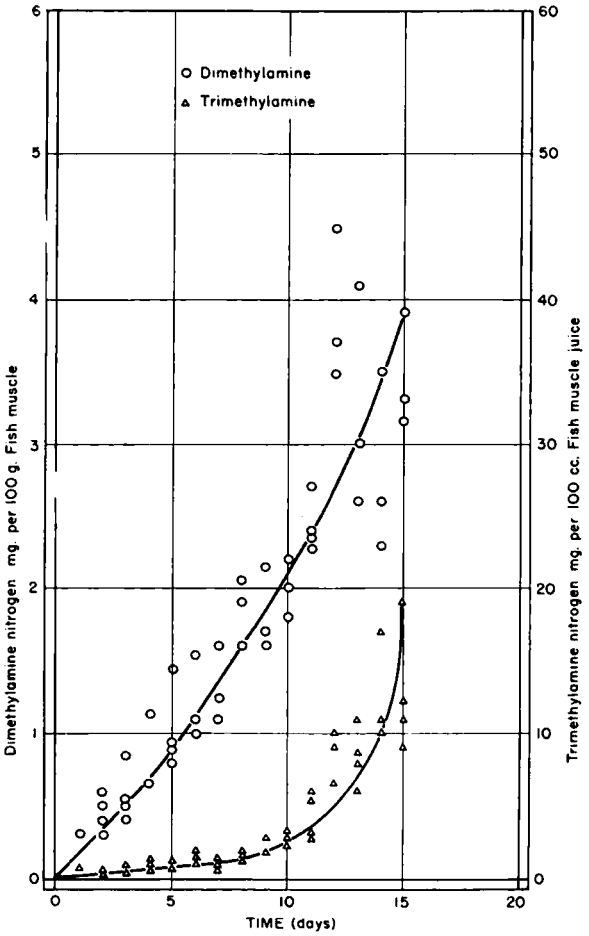

Fig. 2. Effects of storage period on formation of dimethylamine and trimethylamine in fish muscle.

Shewan (1938a) has collected the data relating to the dimethylamine and trimethylamine contents (Fig. 2), from four separate storage experiments with carefully handled iced haddocks. Each point represents the average values obtained from the mixed flesh of a sample comprising seven to ten fish.[1]

[1] It should be pointed out that the formalin method of Beatty and Gibbons (1937) used in these experiments for estimating trimethylamine actually determines a large proportion of the dimethylamine present. Dimethylamine was estimated directly and colorimetrically by Reay's method (1937). Thus from Fig. 2, it can

Shewan's data fully confirm Beatty and Gibbons' (1937) conclusion that the trimethylamine content of fish flesh is a useful index of general quality. It has been confirmed that spoilage odors are first detected by trained observers when the trimethylamine content has reached about 4 to 6 mg.%.

Referring to Table VII and to Fig. 2, an objective standard might be fixed for really fresh fish, i.e., fish that have not emerged from Phase I. Such fish should, on the average, contain no more than about 1.5 mg. % of trimethylamine (measured by the Beatty and Gibbons method). A second grade of fish that are of quite good quality and not to be regarded as definitely stale, should contain no more than about 6 mg. %. Fish containing more amine than this would be definitely stale or worse in quality, and a third grade of poor quality might be limited by a figure of perhaps 12 mg.% in order to exclude fish in which any appreciable proteolysis, as indicated by ammonia formation, has occurred.

The Beatty and Gibbons (1937) method of estimating trimethylamine is fairly rapid, but is still more time consuming than is desirable for the routine testing of many samples. Dyer (1945) has, however, devised a much speedier colorimetric method and removed this objection.

From Figs. 1 and 2, it is clear that the estimation of dimethylamine (which is as speedy as the colorimetric estimation of trimethylamine) furnishes an objective index of change well before any appreciable alteration in quality is noted by sensory observation. The diamine test, therefore, affords the best method so far discovered of measuring very early spoilage change in the gadoid fishes (cod, haddock, and allied species), and the present authors have found that in practice the combined application of the two amine tests provides a useful objective measure of quality in the case of these species over the whole range from perfect freshness to incipient putridity.

The trimethylamine estimation has been applied by several workers, including the authors, in following the spoilage of herrings, salmon, halibut, and other species, but more work is required clearly to establish the validity of the trimethylamine content as an index of deterioration in general quality in these cases. Thus, as Sigurdsson (1947) has shown, rancidity may result in marked loss of quality before trimethylamine production has commenced, as in the case of herrings chilled to −2°C. (28.4°F.). At higher temperatures, however, he suggests a figure of 5 to 7 mg.% as a limiting value for fresh herrings. The significance of chemi-

be seen that during the first week of storage in ice, the trimethylamine value obtained is largely to be accounted for by dimethylamine. We shall continue however to speak of figures obtained by the Beatty and Gibbons method as representing the tertiary amine.

cal tests of quality must clearly be studied for each species separately. Shewan (1938a), for example, has found that dimethylamine is not produced during spoilage, either in dogfish or in fresh-water perch. In the herring it is formed irregularly and only in very small amounts. In dogfish, even although trimethylamine oxide is present in much larger amounts, production of trimethylamine commences in the iced fish and reaches the same maximum some days later than in haddock. Again, ammonia production in the dogfish commences 4 or 5 days earlier than in haddock, owing presumably to the bacterial degradation of urea. Hjorth-Hansen and Bakken (1947) have found that sharks—also "urea" fishes—exhibit similar behavior.

Sigurdsson (1947) observed that the production of volatile acids in herrings stored at various temperatures closely followed that of trimethylamine and showed that the estimation of these acids would afford an equally good index of quality. Present methods for the estimation of the volatile acids appear, however, to be much less convenient and speedy than those for the estimation of trimethylamine.

As already noted, Dyer *et al.* (1946) have concluded that bacterial spoilage proceeds mainly at the external surfaces of the fish. In two earlier papers by Wood *et al.* (1942), and Dyer *et al.* (1944), the suggestion is made that objective tests, such as the estimation of trimethylamine, should be applied to the surface of the fish rather than to mixed samples of the whole fish. They show convincingly that during spoilage the concentration of trimethylamine increases earlier and much more rapidly at the surface. As far as trimethylamine is concerned, however, it does not appear that surface testing has been developed into a sound practical technique, showing advantage over composite sampling. Further investigation is desirable.

(2) *The surface pH test.* Wood *et al.* (1942) and Dyer *et al.* (1944) advocate measurement of the pH of the surface of fish by contact glass electrode as a suitable index of general quality. They have shown that for cod, haddock, and flounder, the pH of the surface is correlated with changes in quality as follows: Fresh—pH 6.2 to 6.8; Spoiling—pH 6.8 to 7.5; Spoiled—pH 7.5 and above. They claim that the method has been applied over a period of 2 years with satisfactory results. Elliot (1947) has examined the suitability of this method for quality grading, making some 10,000 pH estimations on fillets as they spoiled during stowage in ice. The best correlation between pH and condition as judged by the organoleptic method was obtained in the case of haddock, whiting and dabs. With the other species examined, pollock, cod, rose fish, and grey sole, wide variations in pH occurred among fillets in an equal state of freshness. Elliot rather doubts the general reliability of the surface pH

test in routine examinations of fish, stating that much more investigation is required into the factors affecting pH, such as catching method, area of capture, season, bacterial load, types of bacteria etc. The test has the one certain attraction of being extremely speedy and simple to carry out.

(3) *The titration test.* Stansby and Lemon (1933) proposed a method of following spoilage by measuring the fall in titration value (A) between pH 6 and pH 4.3 and a corresponding rise in titration value (B) between the original pH of the fish and pH 6. Cutting (1937 and 1938) and Collins *et al.* (1941) have criticized the theoretical basis advanced for the method, showing that the fall in the A value is largely explained by reduction of trimethylamine oxide, which "buffers" in this range, and not by autolysis, and that the rise in B value is accounted for by the production of volatile bases. Fitzgerald and Conway (1937) found no constant correlation between the two values, and Nickerson and Proctor (1935) found the results to be irregular. Cutting (1937 and 1938) confirmed this.

(4) *Miscellaneous tests.* The "tyrosine" estimation used, as noted earlier, by a number of workers to indicate proteolysis appears to be unsuitable as the basis for a system of quality grading. Values are too fluctuating both originally and in the course of spoilage, and the maximum observed percentage rise over the initial value is too small during the early stages of deterioration.

The determination of hydrogen sulfide cannot be recommended for the estimation of quality. Although usually there is a progressive increase as spoilage proceeds, initial values for this substance in fresh fish vary considerably, at any rate, in fatty fish; production is irregular—possibly from nonprotein sources—during early spoilage, the period for which a reliable test is really required.

Strohecker *et al.* (1937) and Lang *et al.* (1944) claim that the production of volatile reducing substances in fish is correlated fairly closely with recognizable stages of deterioration. The method no doubt requires further examination, but is at present ruled out as a routine technique on the count of complexity of apparatus used and low speed of operation.

Production of indole and skatole occurs too late in the spoilage process to be of any use in the grading of fresh fish. The estimation of these substances is rather to be used as an occasional confirmatory test of putrefaction.

Considerable investigation of the reduction of dyes by spoiling tissue is going on, and it is possible that a suitable dye or series of dyes may be discovered, whereby the various stages of deterioration may be indicated. No success, however, has yet been achieved in this direction.

For estimating the oxidative spoilage of fatty fish under chilling con-

ditions, the measurement of fat peroxide is at times helpful. Peroxide is, however, an intermediate product which is forming and breaking down simultaneously and it is not itself responsible for rancid flavor. Hence, correlation between the latter and the peroxide value, particularly in the early stages of spoilage, is by no means good. More sensitive testing is obtained when only the surface layer of fat is used rather than the total extracted fat. The method is not a speedy one and great care has to be observed to avoid destruction of peroxides by traces of alkali in the reagents used, and to employ only peroxide-free solvents.

Attempts to measure changes in the physical state of the fish flesh as, for example, alteration in electrical conductivity (Stansby and Lemon, 1933); in refractive index of muscle juice (Sidaway, 1941); and in firmness (Charnley and Bolton, 1938); Tauti *et al.* (1931) have not been successful in providing a sensitive method of assessing quality. The whole field relating to alteration in the physical and mechanical properties of fish muscle has as yet been inadequately investigated.

b. Bacteriological Tests. While bacterial counts obtained by cultural methods are valuable in research and, in special cases, for indicating the sanitary history and handling of fish, the methods employed are too slow for the routine testing of many samples. No satisfactory technique for direct counting of the microorganisms on fish has yet been devised, particularly in dealing with counts below a figure of 10^5 per g., which represents a very considerable degree of spoilage.

3. Conclusion

Of all the objective tests reviewed the most useful and reliable for the routine checking and grading of quality appear at present to be the trimethylamine and dimethylamine tests, although the latter is not applicable to all species. It is thought, however, that before the amine tests are likely to be generally accepted in the fish industry as the basis of a scheme of quality grading, more statistical data will require to be obtained in order to establish more clearly the extent of variations and the degree of sampling necessary. Thus, the data shown in Table VII and the curves drawn in Figs. 1 and 2, represent the average alteration during spoilage; but Figs. 1 and 2 show that there is a considerable scatter in the amine values obtained for groups of fish that have been subjected as nearly as possible to the same treatment. Individual fish in such groups have also been observed to vary slightly organoleptically in some particular, e.g., odor and appearance. Elliott (1947) has also shown that there is a scatter in the surface pH values of individual fish judged by the sensory method to be of identical quality.

Such factors as fishing area, method of catching, season, and the con-

dition of the fish probably affect the initial size and character of the bacterial load and the susceptibility of the fish to bacterial spoilage, so that even under identical conditions of storage, individual fish cannot be expected to spoil at precisely the same rate or in precisely the same manner.

Although closer and more extensive investigation is clearly necessary, such experience as has already been gained in applying the amine tests suggests that they may well afford at least as sensitive an index of quality as the normal sensory method of judgment.

VI. The Practical Control of the Quality of "Wet" Fish

In this section the authors have had chiefly in mind the conditions obtaining in the British fishing industry, the only one of which they have intimate knowledge, but the principles of good practice and the possibilities of improvement discussed clearly have a wider application.

1. The Handling and Stowage of Demersal Fish at Sea

a. Introduction. Since the beginning of the century there has been a marked increase not only in the total quantity of demersal fish landed in Britain, but also in the proportion of the catch coming from the distant waters, e.g., those off Greenland, Iceland, Bear Island, Spitzbergen, and the Murmansk Coast and the White Sea. Such voyages have extended the time at sea from 1-2 to 3-4 weeks, and it can be estimated from official statistics that in 1938 about half of the total catch—largely distant water fish—must have been stowed for more than 7 days in ice when it was landed; the situation today is substantially the same. The quality of the demersal fish landed, therefore, ranges from "perfectly fresh" to "unfit for human consumption," possibly about 2% on the average being actually condemned at the port. It is clear that chilling has very definite limits as a means of preserving fresh quality, which are frequently exceeded in actual practice. While this is so, there is considerable variation to be observed in the quality of fish caught at the same time on the same grounds when landed simultaneously by different vessels. The care with which handling, stowage, and chilling is carried out has obviously a very important influence upon the retention of quality and in normal times this is reflected in price. In order to demonstrate this and to show in what ways greater care should be observed and would repay attention, the Department of Scientific and Industrial Research (Great Britain) in 1928 carried out successful large scale experiments in handling and stowage at sea upon two commercial vessels. The results have been published by Lumley *et al.* (1929) in Special Report No. 37 of the Food Investigation Board. As a result of this work, and a

variety of surveys and experiments carried out in other countries, the broad principles of good practice in chilling, handling and stowage, have become clear.

b. Chilling. There is no doubt that temperature is the factor of overriding importance in the retention of quality. Ice has to perform the double function of cooling the fish from its temperature at the time of stowing, which lies somewhere between that of the sea water and that of the air, and of keeping it chilled until landing. In order to cool the fish rapidly and keep it chilled, the ice must be in actual and continuous contact with the fish, and must be sufficient in quantity to last the trip. Sufficient ice to meet these requirements is not always used. Cargoes of fish have been observed in which much of the fish at landing was no longer in contact with ice and the quality was poor in consequence. Clearly the amount of ice to be provided will vary with the season, length of trip, quantity of fish, type of fish room, etc. There is little published material regarding the quantities required under different conditions, but reference should be made to Lumley *et al.* (1929) and Knake (1946).

It is clear that fish holds ought to be insulated on roof, sides, and bulkheads. In this way overall wastage of ice is reduced, and, what is probably more important, is more uniformly distributed throughout the cargo, so that fish do not so readily become locally exposed. Dunn (1946) has estimated that in the hold of a noninsulated wooden fishing vessel the ice wastage arising from heat inleak during a week's voyage may well be of the same order as that caused by the cooling of the fish. In addition to insulation, the air in the fish hold may be kept chilled to just above the melting temperature of ice by means of a small refrigerating plant. Considerable commercial experimentation with chilled, insulated holds has been going on for some time, but no decision appears to have been reached concerning the necessity, at least in cold and temperate climes, of this further step, which laboratory experiment has shown to influence quality solely through preventing the fish from becoming inadequately covered with ice.

Earlier mention has been made of the work of Hess (1932) and Kiser (1944) on the temperature coefficient of growth of marine bacteria just above the freezing point of the fish, which is usually about −1°C. (30°F.). Referring to Hess's work, Huntsman (1931) makes the important point that every successive degree in cooling counts appreciably more than the last in preventing spoilage, and that in normal stowage in ice on board fishing vessels, the temperature of the fish may vary from 0 −5°C. (32 −41°F.). He suggests, therefore, that improved quality would result if fish were rapidly precooled to a tempertaure just above their freezing point as by immersion in circulating, chilled sea water. The

authors know of no sufficient investigation of this method, either as showing improvement in quality, which seems likely to be measurable, or as indicating practicable ways of applying it on board ship; although Huntsman, in the case of comparatively small vessels, suggests spraying the fish stowed on racks, or stowing them in tanks of chilled sea water. It would seem better perhaps, particularly in the case of larger ships, to explore the possibility of providing for a special prechilling stage prior to stowage.

c. Handling and Stowage. Lumley *et al.* (1929) found that haddocks which had been gutted and washed with great care and packed in sterile boxes buried in ice for periods up to ten days, tended to carry a smaller bacterial load, and less blood, slime, feces, and mud, and kept better than fish that had been taken from the general catch after handling by the crew in the normal way and stored under the same conditions. In the latter case the fish were less carefully gutted and washed, had come into contact with the deck, and had been in general more roughly handled. The precise relative importance for keeping quality of each of these various factors has never been ascertained. The results of the large scale trawler trials, however, which followed these preliminary experiments and the experience of other investigators (Huntsman, 1931; Lücke and Schwartz, 1937; Saath, 1937; and Heiss, 1937), has shown that the maximum retention of quality is achieved when fish are gutted and washed carefully, and handled, both on deck and in the processes of stowage and unloading, in such a way as to avoid bruising or puncture, and when all surfaces with which the fish come into contact are kept as free from dirt and microorganisms as possible.

The trawlers *Ben Meidie* and *Peter Carey* on which the trials of Lumley *et al.* (1929) were conducted were specially fitted with metal-lined deck pounds, which could easily be kept scrupulously clean by hosing with hot water. Fish, after being gutted in one deck pound, were transferred for washing and sorting to the opposite pound through which sea water passed in continuous stream and which was fitted with water jets so that, at sorting, any fish still requiring it could be given a very rapid, final wash. All baskets and the shelves in the fish room on which the fish were stowed were made of galvanized metal and, therefore, easily kept clean. The shelves were corrugated so that ice water containing slime, blood and microorganisms, was drained separately by way of special gutters into the bilges from each tier of shelves. This prevented the first-caught fish, stowed in the bottom of the hold, from being contaminated with water dripping from the later caught fish stowed above. The maximum number of shelves was employed in order to minimize crushing of the fish. Large fish were singly layered; smaller fish were

"bulked." In a number of tests the fish were stowed with ice in light wooden, closed boxes, which were stowed below on shelving. This procedure cut out at least three handlings of the fish, viz., stowage in the fish hold, unloading from the fish hold at the port, and packing in market boxes for auction.

The fish at landing were judged under code by a panel of representatives from the industry, who marked the fish for quality according to a scale based upon the best, then current, practice. According to this the judges considered that fish normally remained "fresh" as opposed to "stale" for 6 to 7 days in ice. The results of the experiments showed clearly that fish handled and stowed as described above, were generally rated as "6 to 7 days caught," when in fact they were "10 to 12 days caught." Boxing of the fish gave a definitely better result than ordinary shelf stowage. It was concluded that if measures similar to those adopted in the trials were taken, the period of "freshness" of iced, "white" fish could be extended on the average to about 10 to 12 days. The experiments showed, however, that this is the limit beyond which ice cannot preserve the fish in really good condition. This conclusion has been confirmed many times since in the laboratory, as Table VII illustrates. As already noted, the limit of preservation in good condition in ice is somewhat higher in the case of certain flat fishes, particularly the halibut, and for the larger sizes of gadoid fish. On the other hand, certain of the gadoids or related species, e.g., whiting and possibly hake, spoil somewhat more rapidly than cod and haddock.

It must be confessed that in commercial practice fish is, as yet, seldom handled with the care necessary to ensure the maximum preservation of quality within the limitations of chilling. No doubt there are economic and other reasons for this. The industry, nevertheless, is striving to improve its practice and continues, for example, to investigate the suitability of various types of metal, alloy, plastic, enamel, or other materials for the construction or surfacing of fish holds, pounds, shelves, containers, etc. The broad principles of good practice are now clearly envisaged, and, as earlier sections have shown, their scientific basis is more fully understood.

2. The Handling and Stowage of Pelagic Fish

In Britain by far the most commercially important species of pelagic or "fatty" fish is the herring. There, as perhaps is the case with most of the large pelagic fisheries throughout the world, the fish are caught fairly near the ports, so that the problem of their preservation at sea is distinctly different from that of demersal fish. In the case of the British herring, the fish, mainly caught overnight by drift nets, are landed about

8 hours on the average after catching; and it is not usual to ice the fish on board the fishing vessels, although a few boats carry their catches in boxes with ice. The fish, being relatively small and very numerous, are not gutted at sea. A very large proportion of the total annual catch is taken at a time when the fish are feeding well and are in consequence soft and oily. Deterioration in the gut cavity and the belly walls proceeds with great rapidity and fish frequently reach land already in burst or torn condition. As has been already stated, herrings deteriorate with considerably more rapidity, even in the chilled condition, than gutted demersal fish, and it has been observed that uniced herrings frequently have passed through the first organoleptic phase of real freshness, i.e., unaltered appearance, odor, and condition of the viscera, by the time of landing, i.e., within 8 hours of catching.

Experiments carried out on board fishing vessels just before World War II by Shewan and Reay (1939) showed that the most important step that could be taken to improve the quality of herrings as landed and during subsequent distribution was to chill the fish as soon as they are taken aboard. In addition, it was shown that the condition of the fish could be much improved by the substitution of shelving or boxing for deep, bulk stowage, which crushes and bruises the fish. Fish boxed and iced at sea and kept well iced on shore did not on the average pass out of the first phase (condition of real freshness) until some 32 hours after catching, not far short of the time required to cover distribution to any part of Britain.

Except perhaps where they are to be used for the manufacture of oil and animal feeding meal, which probably absorbs the major proportion of the world catch, it is clear that pelagic fishes, which as a class exhibit the highest rate of deterioration, should be thoroughly chilled and very carefully handled immediately after catching and during subsequent distribution.

3. Handling, Transport and Distribution on Land

The principles of good practice in preserving "wet" fish by chilling have been outlined and discussed in relation to the first stage in the progress from sea to consumer or processor. The same broad principles of adequate chilling, hygiene and care in handling apply without doubt to the further stages of handling, transport, and distribution on land. Even with the most careful treatment aboard ship, fish when it is landed has already completed part of its journey—sometimes a very considerable part—and is in consequence more perishable, demanding as great, if not greater, care than at catching. Once landed the fish has to face the hazards of temporary rises in temperature and further increase in bacterial

load as it passes from the port market, through the wholesaler's dressing, filleting, and packing depot, and thence by rail or road transport to the inland markets and the consumer. There appears, however, to be little published information concerning the magnitude of these hazards at the various stages of distribution. Here is a field for serious and exhaustive investigation.

4. Conclusion

It has been shown that even under the very best conditions of handling and stowage, chilling will not preserve the more important species of demersal fish in a "really fresh" condition for more than about a week, although a few days more must elapse before the fish is considered to be "definitely stale." A distant-water catch in part may frequently comprise fish which have been stowed in ice for periods ranging from six to eighteen days. Clearly chilling cannot solve the problems of bringing back the distant water catches in really fresh condition. One half of such fish may well be "definitely stale," or worse. In an attempt to solve this problem without radical change in practical procedure on board ship or during distribution, a considerable amount of research has been devoted to a study of the preserving power of ice containing bactericidal or bacteriostatic substances. It is not proposed to review the literature on this subject here. Reference should be made to the paper of Tarr and Bailey (1939) who adequately cover this ground. Although numerous compounds have been tested and the use of new ones is constantly being advocated, no really satisfactory preservative has yet been discovered. Many of the proposed chemicals, for example, produce undesirable changes in the appearance or flavor of the fish, whilst some give rise to corrosion of the walls and fittings of the fish hold, or containers, particularly if made of metal. The use of most of the substances proposed is prohibited by food and drug regulations. The more effective of these preservatives, in concentrations that would stand a chance of being specially permitted, delay the onset of putridity by at most about five to seven days; but the fish, during this period of extension, although not repulsive in odor or flavor, are frequently very soft in texture and of poor, unattractive quality. This criticism applies equally to the use of carbon dioxide, which has been found to be an effective bacterial inhibitor at concentrations exceeding about 40%, in conjunction with normal stowage in ice. In this connection the papers of Killeffer (1930), Coyne (1932; 1933a; 1933b), Stewart (1934a), Stansby and Griffiths (1935), and Vick and Howells (1937) should be consulted. Obvious practical problems would require solution before carbon dioxide gas stowage could be employed on a fishing vessel. There appears to be little hope at present,

therefore, of extending really satisfactory preservation by employing such adjuvant measures.

Within the past twenty years research has determined the conditions under which fish can be satisfactorily frozen and stored. If frozen rapidly and stored in protective packaging (or with an ice glaze) at temperatures between −20 and −30°C. (−4 and −22°F.), freshly caught fish retain their original quality, virtually without impairment, for periods ranging from 3 to 12 months depending on the temperature and the species (lean or fatty). There is little doubt that proper freezing and frozen storage affords practically ideal preservation of quality for periods covering all the likely requirements of the fish industry, and that the future lies with this method, which at this present time at any rate, has no rivals that come within measurable distance of it save perhaps canning in certain circumstances.

It seems clear that the problem of landing catches from distant waters in good condition, of exporting surplus fish, and of spreading seasonally abundant catches evenly through the year can only be solved in a manner that observes the growing preference for fish of the freshest quality by employing the latest improved methods of freezing and canning. Chilling will, however, continue to be necessary at various stages of the production and distribution chain, but it should be possible in the future when freezing and canning are more fully developed, to employ it without exceeding the demonstrated limits of its efficiency.

VII. General Conclusion

A general account has been given of the process of spoilage in marine fish, chiefly of those varieties of major commercial importance to the fisheries prosecuted in the Northern oceans. The chief features of existing knowledge concerning the physiological, biochemical and bacteriological factors affecting spoilage have been reviewed. The controlling influence of chilling and of careful and hygienic handling and stowing upon deterioration of quality has been evaluated, in relation to the needs of the industry and the acceptance of the consumer. In addition the possibility of checking the organoleptic estimate of changing quality by objective chemical, physical, or bacteriological tests has been discussed.

Apart from oxidative deterioration, such as the development of rancidity, specially characteristic of the fatty fishes and autolytic reactions that may be responsible in part for the softening of the flesh, the main agents causing deterioration in quality are bacteria of common soil and water types, which inhabit the sea and are present on freshly caught fish and on the surfaces with which the fish chiefly come into contact during handling

and stowage on board the fishing vessel and on shore during subsequent treatment and distribution as "wet" fish.

Knowledge, now beginning to accumulate, is, however, far from complete concerning variation in the character of the flora and in the bacterial load of freshly caught fish, of one or different species. This variation is no doubt attributable to such factors as type of aquatic environment, season, kind and intensity of feeding, and method of catching. Moreover, although it is clear in outline that the commencement and the speed of development of bacterial spoilage depends upon immediate *post mortem* changes in the muscle, particularly as they influence pH, much further investigation is required concerning the factors that may affect these changes, such as the extent of struggling, crushing, suffocation and change in pressure during the catching process, and the natural condition of the fish, which is influenced by season, feeding, reproduction, and possibly age and rate of growth.

Two main phases of the bacterial spoilage of marine fish have now been recognized, namely, the reduction of trimethylamine oxide to the amine coupled with the oxidation of lactic acid to acetic acid and carbon dioxide, and the degradation of protein, characterized by the formation of ammonia, hydrogen sulfide, indole, etc. This notable advance represents, however, only the first step in the elucidation of the complex of processes that must go on when a mixed flora lives and grows, with changing character, upon such a heterogenous medium as fish flesh.

A fuller knowledge is desirable on the one hand concerning the biochemical activities of the more important saprophytes, singly and in mixed populations, and on the other, concerning the chemical composition of fresh and spoiling fish. Attention should be specially focused upon the bacterial and tissue enzymic reactions occurring in the very early stages of deterioration, and upon the sites and routes of bacterial activity and attack. In this connection the suggestion recently made that bacterial activity normally proceeds almost wholly on the external surfaces and that diffusion maintains the supply of substrates and brings about the impregnation of the sterile flesh with bacterial end products challenges the older conception of the spoilage process as a steadily advancing bacterial invasion of the flesh.

A field of investigation that is virtually unexplored is that of the chemistry of the aroma and flavor of fresh and spoiling fish, raw and cooked. Whilst some of the nonvolatile extractives of the flesh no doubt contribute to flavor, volatile substances, as yet unknown, and present probably in very low concentrations are presumably responsible for the delicate aroma of very fresh fish. What are these substances? What happens to them during storage? And how much of the deterioration in flavor and

aroma is due to their escape or transformation and how much to the gradual formation of autolytic and bacterial products? The evolution of such substances as trimethylamine, lower fatty acids and hydrogen sulfide even in the extremely small amounts found in the earlier stages of chill storage no doubt all contribute to the slowly changing aroma.

It has been demonstrated that, in some species at least, trimethylamine and dimethylamine form regularly as storage proceeds, and that estimation of these substances furnishes a fairly sensitive objective index of quality that may well become the basis of a system of checking the grading of fish by the organoleptic method. Both these substances have begun to increase in concentration before odors of incipient staleness are observed—very much before in the case of the diamine—and, to different extents permit prediction of subsequent storage life. The investigation into the constituents responsible for aroma and flavor suggested above may well result in the discovery of further suitable, even better, objective chemical tests. The further study of spoilage by physical methods which should be pursued, may also furnish new ways of estimating quality.

The most important factor in controlling the spoilage of "wet" fish is temperature, but it has been shown in the laboratory and in large scale commercial tests on board fishing vessels that if care is taken to avoid increase in bacterial load by keeping everything with which the fish comes into contact as clean as possible and handling and crushing is reduced to a minimum, the storage life of fish chilled in ice can be considerably extended. In relation to the needs of the industry (for example, the transport of catches over long distances at sea or on land, and the fuller exploitation of fisheries that are markedly seasonal), and in relation to the growing preference of consumers for fish of indubitably fresh quality, chilling even under the most careful, hygenic conditions can only be regarded as a method of short term preservation, and should, as far as possible, be employed only as such.

The use of ice, the almost universal chilling agent, has resulted in greatly increased and more widely distributed supplies of "wet" fish, but a large proportion of the fish reaches the consumer and even the port of landing in inferior condition. The problem to be solved is that of providing fish of any species, in the freshest condition any where at any time of the year, and it cannot be solved in this manner. The solution, however, is at hand in the development of freezing and cold storage, and possibly in the wider application of canning, both at sea and on land.

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Spray Drying of Foods

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I. Introduction

This article presents a discussion of special subjects dealing primarily with the equipment and engineering involved in the spray drying of foods. The drying of particular foods, such as milk, coffee, and eggs, is not

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treated at length—as it deserves to be—since each such food would require comprehensive treatment of as great dimensions as the present article. The subjects presented are among those that lie within the interests and experience of the authors.

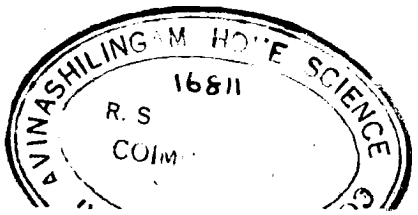
Only recently has spray drying begun to be treated from the chemical engineering standpoint as an important branch of the unit operation, drying. The technological status has been more that of a mechanical art than a science, but its maturity and importance are attested to by the fact that numerous universities, experiment stations, and industrial laboratories now have installations on which research worthy of publication is being conducted.

The food industry employs practically all the types of dryers found in the chemical industry—tunnel, drum, vacuum, flash, through-circulation, rotary and spray dryers. No single dryer is universal in its application. Tunnel and through-circulation dryers predominate for fruits, vegetables and preformed or caked wet solids (corn starch, gelatin slabs, coconut shreds, riced potato, etc.). For recovering solids from a liquid solution or slurry, drum, spray, and tray dryers (often of the vacuum type) find wide application.

The spray dryer is a means of converting the solids of the solution or slurry directly into powder or granular form by spraying the fluid into a stream of heated gas which evaporates water from the spray and from the resulting fine particles while still in suspension. It is unique not only in the brevity of drying time, generally reckoned in seconds, but also in the fact that, owing to the rapid evaporation of water, the solids are not heated to a high temperature. These characteristics are especially important for heat-sensitive foods where the time or degree of heating by other processes causes deterioration.

Because of the immediate utilization of absorbed heat for evaporation of water, the interiors of the fine suspended particles do not become heated to the temperature of the surrounding hot gases. In fact, in the case of most food sprays even the exterior does not become heated above 120°F. except in secondary drying or near the end of the drying period when the particle temperature rises above the wet bulb temperature of the surrounding air.

Spray dried milk powder finds preference over other dried milk powders where degree of solubility and naturalness of flavor have not been altered by overheating. Malt diastase retains its enzymatic activity after spray drying because it can be dried safely thereby, without encountering the time and temperature conditions that result in inactivation. With foods, retention of enzyme activity, vitamin content, and volatile flavors, and prevention of heat denaturation are among the reasons for preferring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spray drying to other methods. Denton *et al.* (1944) found that there was no loss during spray drying of the vitamins A, D, and riboflavin content of eggs although vitamin A was lost rapidly in subsequent storage of the powder. When spray drying foods containing volatile flavors, substantial percentages of the flavor are retained encapsulated in the powder. SO_2, for example (not a flavor but a stabilizer), was found by Charley (1940) to be retained practically quantitatively in spray dried fruit purees.

The following indicates the innocuous nature of spray drying in the case of oxygen-sensitive materials (Bullock and Lightbown, 1943): "The spray drying of inorganic salts gives some idea of the conditions to which a substance is actually submitted during the drying process. The most outstanding indication is that although large volumes of heated air are used, the atmosphere to which the droplet and moist powder are submitted is not an oxidizing atmosphere. This is clearly demonstrated in the drying of the ferrous salts. Apparently the particles are surrounded by an atmosphere of steam during the actual process of evaporation but, even so, it is remarkable that ferrous chloride, for example, can be spray-dried with the occurrence of only slight traces of oxidation."

Coulter (1947) found that although the amount of oxygen left in the container influenced the keeping quality of whole milk powder, the keeping quality of the powder was no more influenced by spray drying in air than in an inert gas, such as nitrogen. This worker determined that the spray drying of whole milk or other oxygen-labile materials in an atmosphere of nitrogen or carbon dioxide was "entirely practical." Commercial spray dryers have been designed for drying certain sensitive materials in the absence of oxygen or carbon dioxide.

From the standpoint of product quality, spray drying compares favorably with vacuum drum drying and high-vacuum, low-temperature drying. The economics of spray drying are usually more favorable than for these other methods of drying, and for large capacity installations where a high temperature differential is possible its efficiency is as good as, or better than that of most other types of direct dryers such as rotary dryers and tunnel dryers (Marshall and Seltzer, 1948). In addition, it produces particles of size and shape not obtainable by other drying methods. The material does not contact solid surfaces until it has become dry, a factor which often obviates flavor and color changes.

II. Commercial Spray Dryers

A spray dryer is a system of which the largest part is a chamber or tower within which the fluid is sprayed into moving hot air. The air is generally heated in an auxiliary unit by mixture with products of com-

bustion of an oil or gas burner (direct heating) or by passage through an interchanger where the source of heat is usually steam (indirect heating). In some dryers part or most of the powder is collected and discharged at the bottom of the chamber and in other dryers all the powder is carried by the exhaust air into powder collectors. Whether part or all the powder is recovered beyond the chamber, a powder collection device or combination of devices is necessary (such as cyclone separators or bag filters). Fans may be installed at the feed or exhaust end of the chamber or at both.

The design of spray towers depends fundamentally on the direction of air flow and its relation to the direction of spraying. There is no set formula that predicts the superiority of one combination over another for a desired drying job, although the recognition of some of the characteristics of each combination may serve as a guide in the selection of a dryer for a specific job. For example, the relative direction of spray and air may determine the particle size, granularity and bulk density of the powder. Domestic and foreign spray dryers used by the food industry have been classified in this section in accordance with the direction of spray dispersal and the relative direction of the drying air. All the designs encountered appear to fall into five principal categories:

1. Horizontal co-current
2. Simple vertical-downward co-current
   (a) where air has straight-line flow
   (b) where air has rotary motion
3. Complex vertical-downward co-current
4. Vertical-upward co-current
5. Vertical counter-current.

Some dryers have a complex sequence where the air may have cross-current flow, then co-current and finally counter-current. In order for a food manufacturer to appraise intelligently the application of a particular dryer for a proposed product he should review at least the theoretical mechanism of drying in systems proposed to him.

1. Horizontal Co-Current Dryers

The principal manufacturers of this type are Merrell-Soule (Borden Co.), C. E. Rogers Co., Barnhill and Buflovak Midwest Co. They are described as "boxes" because of the rectangular shape of the drying chambers in all but the one made by Buflovak. Numerous dryers of this type were built by the food manufacturers themselves during the past war, for egg drying. The design is simple, and less experience is required for construction and operation than for most other types of spray dryers. The head-space required is relatively low (about 17 ft.) thereby often permitting installation in an existing building. Low air velocities, in the order of 75 to

125 ft. per minute, are essential in order to favor settling of the major portion of the powder on the dryer floor. In operation, the time of rest on the floor is sometimes depended upon to effect a secondary drying. In the older dryers, the product is removed from the floor by interrupting the operation and shovelling the powder into manholes in the floor. The powder on the floor in such batch dryers does not reach a high temperature owing to the fact that it is continually being covered by fresh falling powder which serves to insulate the powder already on the floor. Milk powder of good quality has been produced in batch dryers, and sticky products which cannot be removed continuously have been handled with good results. The more modern dryers have continuous sweeps or conveyors on the flat or hopper-bottom floors.

Although some such dryers of custom design have cyclone dust collectors (Barnhill and Buflovak), most have fabric filters that remove the air-entrained powder which may amount to 20 to 25% of the total. Operation of this type of dryer is very simple, product recovery is generally good, and maintenance is moderate. Standardization of design is more advanced than with any other type of food spray dryer with the possible exceptions of the Swenson and Zizinia dryers.

Typical of this class of dryers is the C. E. Rogers Co. "700 pound" system, a dryer capable of producing up to 800 lb. milk powder per hour from 2400 lb. precondensed milk (evaporation of 1600 lb. water per hour) (Fig. 1). The Rogers Co. has standardized on a modular design which permits chamber construction in multiples of rectangles of a minimum width. In the "700 pound" dryer there extend through one vertical wall of the rectangular room four banks of spray nozzles at two levels, each bank with four nozzles, making a total of sixteen nozzles. Wall construction is galvanized sheet metal or stainless steel with 1 in. magnesia insulation on the outside of most walls. The rear section of the dryers as well as the hopper bottoms (when such are provided) are uninsulated. Feed liquid is pumped by a centrifugal pump through a milk heater, then through a strainer into a high pressure triplex pump. The triplex pump delivers the liquid to the nozzles at pressures over 1500 pounds per square inch (p.s.i.). Typical nozzles are of drill size No. 72 (0.025 in.). The product falling to the flat floor of the chamber is swept by a scraper conveyor to one side of the dryer into a long trough where it is received by a screw conveyor and discharged through a hole into the sifter. This flat floor is an improvement over the previous hopper-bottom floor which required more head space and necessitated the use of scaffolding when cleaning the chamber. Three 60°-angle hoppers, 20 ft. long, formed the bottom of the previous dryer. The product slid down the hoppers to narrow floors, about 2 ft. wide. The floor accumulations were scraped to center discharges by flat paddle conveyors which were operated by reciprocating shafts; the powder then fed to three sifters equipped with 24 mesh screens, one beneath each hopper.

Average milk drying conditions are: inlet air temperature 320°F., outlet temperature 170°F. Air is heated by steam-heated coils, and the steam load at maximum capacity is said to be 5600 lb. per hour at 100 pounds per sq. in. gauge (p.s.i.g.). The inlet fan delivers 15,000 cubic feet per minute (c.f.m.) at 60°F. and 1.25 in. static pressure (s.p.) and is driven by a 7.5 HP motor (7.1 operating horse power). An exhaust fan handles 18,000 c.f.m. at 170°F. and 3 in. s.p. with a 15 HP motor (12.4 operating HP). The hot air is conveyed to the dryer by four inlet air ducts, and at the end of these there is a distributor which diffuses the hot air into four 12-in. diameter ducts through the center of each of which extends a feed line with a spray nozzle. The exhaust air leaves from the rear end of the chamber after being deprived of entrained dust by filters within an attached box. Interposed be-

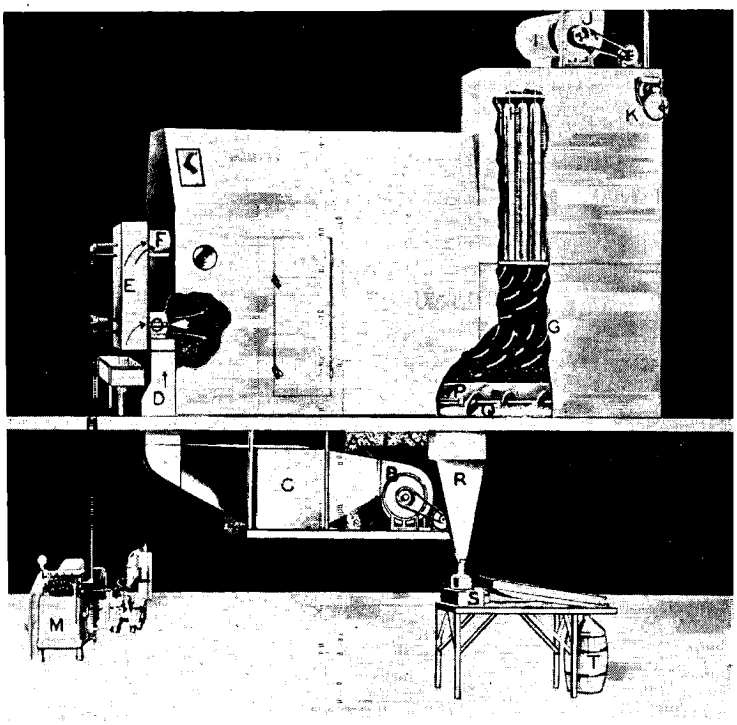


Fig. 1. Rogers spray dryer. Air enters the air filter A and is drawn into the outlet B, which forces it over the heating coils C through the air duct D into the air distributing head E, and then through the multiple air inlets F to dryer. Air picks up moisture from the liquid spray and passes through settling chamber G to air filter bags H. Air leaves the chamber through exhaust air duct I and exhaust fan J. Filter bags are automatically shaken by shaking device K.

Liquid to be dried is heated in preheater L and passes into high pressure pump M through liquid line N to spray nozzle O, where the liquid is atomized and comes in intimate contact with the incoming heated air entering through air inlets F. Moisture is picked up by the air. Solids from the spray fall to the floor where they are conveyed by a reciprocating conveyer P to one side of the drying chamber. The dried product is then conveyed by screw conveyer Q to chute R, then by gravity to sifter S, the finished product going directly from the sifter to package T. (Courtesy of the C. E. Rogers Co., Detroit.)

tween the chamber and the filter box is a baffle wall which extends about half-way down from the ceiling, thereby presenting a target for large particles which persist in flight to the end of the chamber. This wall also imposes a change in air direction which favors the dropping out of entrained particles. In the Barnhill dryer there are three parallel vertical partitions which force the outgoing air to follow a labyrinth path.

The use of cotton fabric bag filters as dust collectors proves to be very satisfactory in skim milk drying inasmuch as the outlet temperatures are not high enough to

deteriorate the fabric (drill cloth with an inside nap). Sustained use of temperatures above 180°F. will weaken the fabric and cause disintegration. Hygroscopic powders, unless brushed off or shaken down completely at shutdown time, are likely to fuse on the nap and can thereafter be removed only by laundering the bags. Warm air at about 140°F. may be circulated through the dryer during shutdown periods to prevent deliquescence of hygroscopic deposits on the filter bags.

There are 819 of these bags in the Rogers 700 lb. dryer, and so the magnitude of labor and expense for removal and laundering can be imagined. These are arranged in banks of six sets, each set attached to a separate shaker support. Most bags are 4 in. outside diameter and 7 ft. long. A control instrument times a shaking operation so that once every half hour each set of bags can be shaken a predetermined period, varying from 30 seconds to 4½ minutes. During this shaking period, the instrument pneumatically closes a butterfly valve in the outlet duct at the set being shaken. It correspondingly opens a butterfly valve in a duct leading from the discharge side of the exhaust fan. This creates a "blow-back" which removes accumulated material from the shaking bags. Each of the six banks of bags (each bank equipped with a shaker motor) accumulates powder for 25 minutes or longer and then is shaken. The maximum time for removal of powder from the floor by the rake and conveyor is 5 minutes. Therefore, total maximum time in the dryer is approximately 30 minutes. Vacuum in the dust-collecting section is normally 0.20 in. water gauge (w.g.) when the bags are clean. When shaking is required, approximately 0.20 in. static pressure is maintained while the bags are being blown back and shaken.

A potential disadvantage inherent in this type of dryer is the variable particle trajectory. Dried particles, in falling from the upper part of the spray cone, are likely to collide and agglomerate with incompletely dried particles in the center of the cone. The latter may thereby be removed prematurely from suspension in the drying gases. The manufacturer inclines to the belief that the velocity of the air carries the particles forward and keeps them from dropping until they are sufficiently dry so that they will not stick together.

The Merrell-Soule dryer is now manufactured by the Borden Co. solely for their own use. A typical installation is said to produce 1000 lb. powder per hour from a liquid containing 40% solids. As in the Rogers dryer, the chamber is a "box" with a flat bottom equipped with a mechanical sweep and a screw conveyor along an end wall. It differs in some significant details from the Rogers dryer. A single large centrifugal pressure nozzle (about No. 50 drill size) is inserted through an air orifice in the front vertical wall. The orifice contains tilted vanes so as to impart rotary motion to the air. Steam-heated air, not over 325°F., enters the orifice from a plenum and carries the spray forward horizontally with a vortical action. The spray cone persists only about 12 to 16 in. beyond the nozzle, and the mixing with air and dispersion is said to be very efficient. There is no rear baffle wall in the chamber as there is in the Rogers dryer, and as the central front of air reaches the rear section of chamber where the bag filters are suspended, there is an observable doubling-back or eddying which the manufacturer considers desirable for drying efficiency. This air turbulence and the secondary drying effected by the period of exposure on the floor of the chamber is said to be responsible for achievement of low moisture (1.5%) milk powder without deterioration of quality. Final moisture is always more costly and difficult to remove than initial moisture. Where low moisture powders can be produced without the need for secondary drying it is a factor that favors such a dryer.

The bag filters used in the Merrell-Soule dryer are of wide diameter (about 15 in.)

so that the number required for a given filtering area is less than in the Rogers dryer. The Merrell nozzle (Merrell and Merrell, 1916) is specially constructed and is equipped with inserts of tungsten-iridium which last for several months except when the fluid being atomized contains cocoa powder. (The latter material has been found to cause serious pump and nozzle erosion even with large nozzles.) The hard metal inserts have been found by the Borden Co. to be preferable to the perforated synthetic sapphires formerly used as inserts.

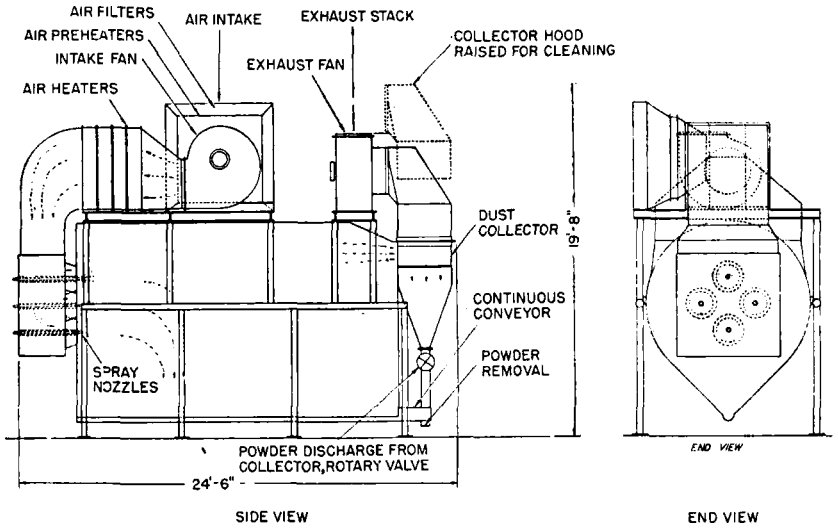


Fig. 2. Buflovak Midwest spray dryer No. 500. End view shows cross arrangement of spray nozzles and rounded shape of horizontal-type chamber. (Courtesy of the Buflovak Midwest Co., Mora, Minn.)

A horizontal dryer that departs from the box construction of the Rogers and Merrell-Soule is one recently developed by Buflovak Midwest Co. of Mora, Minnesota, a division of Blaw-Knox Corp. (Fig. 2). The chamber consists of a horizontal cylinder the lower half of which is shaped to a hopper bottom. The cubic volume of the chamber is much smaller for a given evaporative capacity than the rectangular "boxes" of horizontal dryers. A No. 500 dryer which is designed for producing 500 lb. skim milk powder per hour from 1250 lb. of 40% solids feed liquid (at 150°F.) has a chamber measuring 8.5 ft. in width and 18 ft. in length.

Because the elimination of corner areas results in diminution of chamber volume, the air velocity through the chamber is higher than in a "box" of comparable drying capacity. Air enters the front of the chamber at 300 to 320°F. and leaves the system at 180°F., the volume at the latter temperature being about 9000 c.f.m. The air velocity is near the upper limit (about 120 ft. per minute) of what is normally encountered in box dryers. The higher carrying velocity results in a lower percentage of powder being deposited in the chamber; approximately 50% is recovered in the dust collector.

An "Aerotec" powder collector is installed at the upper discharge end of the chamber. This consists of a cyclone-type separator with multiple tubular compo-

nents (84 in number) within a hopper-like housing. A high recovery efficiency (99.3 to 99.8% of skim milk powder sent to the collector) is claimed for this device. The powder from the collector passes through a rotary valve and is mixed continuously with chamber product being conveyed forward by a cadmium-plated screw conveyor that runs the full length of the chamber bottom. Vibrators are operated intermittently by a timer to remove powder that would otherwise cling to the walls of the chamber. No milk powder deposits are said to accumulate on the walls or in the collector, although the rear wall of the chamber presents a target where large particles of other sticky materials may tend to adhere. Milk powder having about 3.5% moisture is recovered.

The No. 500 dryer (see Fig. 2) has four nozzles at the front end arranged in cross fashion. These are generally of drill size No. 66 (0.033 in. diameter) with cores having four tangential grooves. The nozzles are inserted in short individual air-duct orifices which extend from a plenum box. Air is blown through an indirect steam-heater by a 7½ HP fan, and the humid air is discharged at the exhaust side of the dust collector housing by a 15 HP fan. Construction is of stainless steel, and the chamber is insulated with 2 in. magnesia block. The total cost including erection is quoted at $28,500 (as of July, 1948).

2. Simple Vertical Downward Co-Current Dryers

In this type of dryer the hot air is introduced at the top of a vertical chamber and engages the spray which is directed either horizontally (as from a centrifugal atomizer) or vertically downward (as from hollow cone or full-jet nozzles). The air may have streamline flow by virtue of introduction through a plain orifice or a perforated distributor plate, or it may have rotary motion as the result of introduction through a tilted-vane orifice or tangential inlets. Among the designers or manufacturers of the former type (straight line air-flow) are: P. T. Zizinia (Spray Drying Laboratories, Inc.), Thos. L. McKenna, R. H. Bishop Co., Spray Dehydration Inc. (Messrs. Suffern and Hyde). Those employing rotary motion are manufactured by Bowen Engineering Co., Instant Drying Corp. (formerly known as Industrial Associates), Kestner Evaporator and Engineering Co., Krause, and Drying and Concentrating Co. Some of these latter dryers have features that might possibly justify their being classified as "complex" rather than "simple." Bowen and Krause have varied their dryers in custom designing for specific jobs; the more recent Krause dryers are of the vertical upward co-current type.

The "straightline" type dryer requires a taller tower than does a "rotary" type for a given material in order to assure dryness by the time the powder reaches the bottom of the tower (generally a cone). The dryers devised by Dickerson (1926) and Holliday (1927) and described in early patent literature are of this type. The Zizinia dryer, which has evolved from the original Dickerson type into a standardized model of realtively low cost, has a cylindrical tower about 30 ft. tall and 8 to 9 ft. diameter surmounting a 45° or 60° cone. A top plenum chamber is partitioned from the tower by a perforated distributor plate. In old installations these plates had only about 4% open area, but in the more recent dryers the open area is greater. Inverted about the plenum space are two non-luminous gas burners (such as is made by Hone Co.) which serve as the heat source. Air for combustion of the gas is drawn by a single 5 HP fan which also serves to draw into the plenum the air to be heated. This single fan, located beyond a single cyclone collector, is said to handle approximately 2,000 c.f.m. with a pressure drop of 6 in. w.g. overall. A square port at the top of the tower, about 4 ft. below the distributor, serves

as the entrance point for the feed lines. Spraying is accomplished with nozzles, and from one to seven may be used. When several nozzles are used they are arranged in a 2 ft. circle with one nozzle at the center. The evaporative capacity is claimed to be 1,000 lb. water per hour when using entering air at 1000°F. (for chemical drying). Construction is of 20 gauge 18-8 stainless steel, and the curved plates are joined by vertical standing seams that are the sole vertical structural supports. The cylinder rests on an angle-iron frame below which is suspended the cone. For 45° cones, vibrators or knockers are generally required to prevent product accumulation.

Merits of this dryer are its low cost, about $10,000 for an 8 ft. diameter system (before installation), its light weight, about 2 tons for the tower, and its simplicity. All parts can be shipped in one 50-ft. box car and can be erected in one day. Construction is extremely simple. Although a high pressure feed pump may be used by the buyer, a simple gear pump capable of developing 100 to 150 p.s.i. is suitable for some jobs. Instrumentation is dispensed with for the sake of economy, although the buyer may install controls or recorders. For example, ordinary one-dollar thermometers are recommended for general use, and no safety controls are provided for the gas burners. The manufacturer claims to have had no explosions as a result of such design. Some users have replaced the burners with standard gas furnaces (e.g., Ross Eng. Co. or Gehnrich furnaces).

This dryer is not recommended for all spray drying purposes by the manufacturer, but apparently it is suitable for many food materials. Two installations have been observed by the authors which dry protein hydrolyzates, and one which dries a malt sirup. Inlet temperatures from 333 to 1000°F. may be used depending upon the operation. Several of the dryers are in operation in the manufacture of chrome tanning compounds. Owing to the fact that the spray is directed vertically downward there is danger of wetting the cone and causing subsequent product build-up. Care must be taken to insure uniform atomization and to avoid dripping from the nozzles. There is danger of overlapping of cones of the sprays which may cause agglomeration of the incompletely dried particles. These agglomerates, being heavy, descend at a fast rate, impinge on the cones and tend to cling there. In trying to use several nozzles in towers of small diameter there is also the danger of locating the nozzles too close to the walls in an attempt to avoid overlapping of cones. In such a case, undried particles are likely to hit the vertical walls and deposit there. When several nozzles are used (for example, on 9-in. centers) it is essential to use very high inlet temperatures in order to accomplish the drying very close to the nozzles. The dryer often works best with only one or two nozzles, provided that, in attempting to achieve a high capacity, too high a fluid pressure is not used since this may cause eddying of the spray and result in wall deposits. Such may be the case when using nozzles having very small orifices. When using wide-orifice nozzles without high inlet air temperatures the particles may be of too large diameter to accomplish drying in the relatively short distance of travel in air. It seems apparent that operating conditions must be delicately balanced with careful adjustment of nozzle spacing, fluid feed rate and gas temperatures.

This dryer may commend itself in cases where only a limited investment can be justified, where required capacity is moderate, where skilled construction personnel are not available, when fast delivery and erection of equipment are important, and where floor area or loading weight are limited. It lacks safety features (required by insurance companies) and instrumentation, and appears to be a rudimentary design. (Although the manufacturer is acquainted through long experience

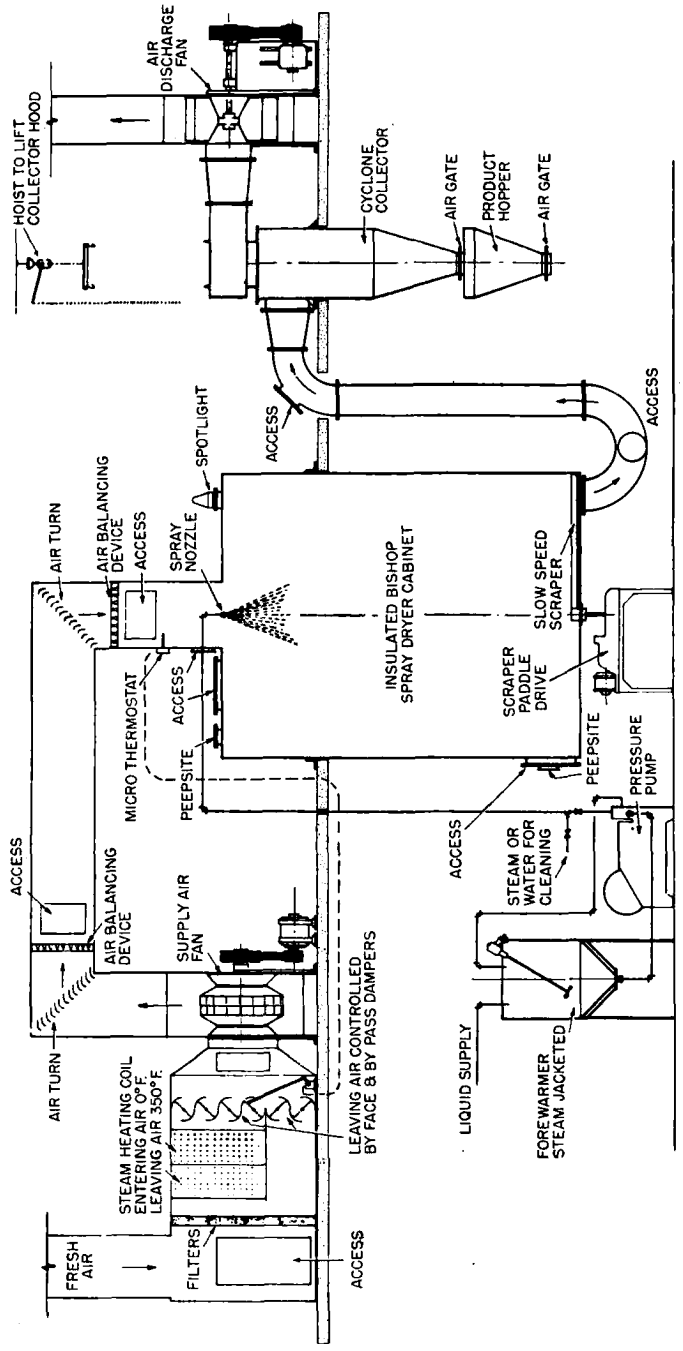


Fig. 3. Sectional diagram of Bishop spray dryer system. (Courtesy of the R. H. Bishop Co., Champaign, Ill.)

with the development of spray dryers, he has chosen to dispense with refinements in the interests of economy and simplicity.)

Dryers designed by McKenna and by Spray Dehydration, Inc. are of this same general design except that the nozzles may be located near the walls of the tower, and these spray horizontally or obliquely toward the center. A circular feed line surrounds the outside of the tower in some cases and serves as a header for the various nozzle lines. These dryers are intended primarily for filled soaps and detergents where straight-line air flow is essential, otherwise the bead-like particles tend to disintegrate as a result of friction with the tower walls or by impact with one another.

The Bishop spray dryer (Fig. 3) is a recently developed unit that has been applied in the manufacture of dried milk, ice cream powder, soybean albumin, yeast concentrate, protein hydrolyzate and soluble coffee. The chamber is a vertical cylinder, slightly taller than it is wide. The drying air, prefiltered and heated indirectly, is carefully straightened by vanes and a distributor before it enters the tower. The nozzle is positioned at the tower top directly at the center of the entering air duct. When more than one nozzle is required, a separate air duct is installed for each nozzle in the system, and the manufacturers claim that this practice is responsible for greater efficiency. The bottom of the chamber may be conical with the outlet air and dried material going out the bottom or the floor may be flat, in which case it is swept by a slow-speed scraper. The scraper moves the dried material to the discharge duct for the outlet air which is attached to the chamber floor near the junction with the tower wall.*

Both a supply fan and a discharge fan are employed in the Bishop dryer, the latter being located beyond the cyclone collector. The recovery efficiency using cyclone collectors, varies with the products being dried and ranges from 94 to 98½%. When drying valuable products some installations are equipped with bag collectors to remove the fine material from the cyclone exhaust. Some have scrubbers to wash the exhaust air where atmospheric pollution is a nuisance. The Bishop Co. claims to have had no trouble with deposition of product on the air outlet side of the dryer, probably because the two fans are regulated so that the pressure within the chamber is practically 0.0 in. w.g. If there were a vacuum it is believed that deposition would occur on the sides of the air outlet. The amount of air handled is said to be small for the size of the chamber, and the product is described as floating to the bottom like snow, instead of being drawn through by a rapidly moving air stream. To this slow movement of descending material they attribute the ability to have the temperature of discharged air lower than usual, thus improving the thermal efficiency.

In the Instant Dryer alluded to previously, the vanes in the plenum and orifice had been straightened by the food manufacturer so that streamline air instead of rotating air (as originally designed) is introduced to the tower. A centrifugal atom-

* Such a side location may tend to direct the air flow from the tower in the direction of the eccentric outlet. The present authors have experienced problems of wall deposition when drying hygroscopic materials in a system having similar side wall air outlets. The Industrial Associates dryer (now Instant Drying Corp.) has four such outlet ducts installed symmetrically at the bottom of the vertical walls. Each duct leads to a separate cyclone collector and fan (four in all). This arrangement routinely works very satisfactorily; however, should one fan fail to handle its share of outlet gas volume by a deficiency of as little as 15%, a tendency toward deposition on the opposite wall is noted.

izer is employed which produces a horizontal spray; such a spray may be more sensitive to air imbalance than that from a pressure nozzle where the spray produced is more nearly vertical. Generally it has been observed that the gas volume distributes itself fairly evenly among the four outlet ducts, the variation being about 10% or less.

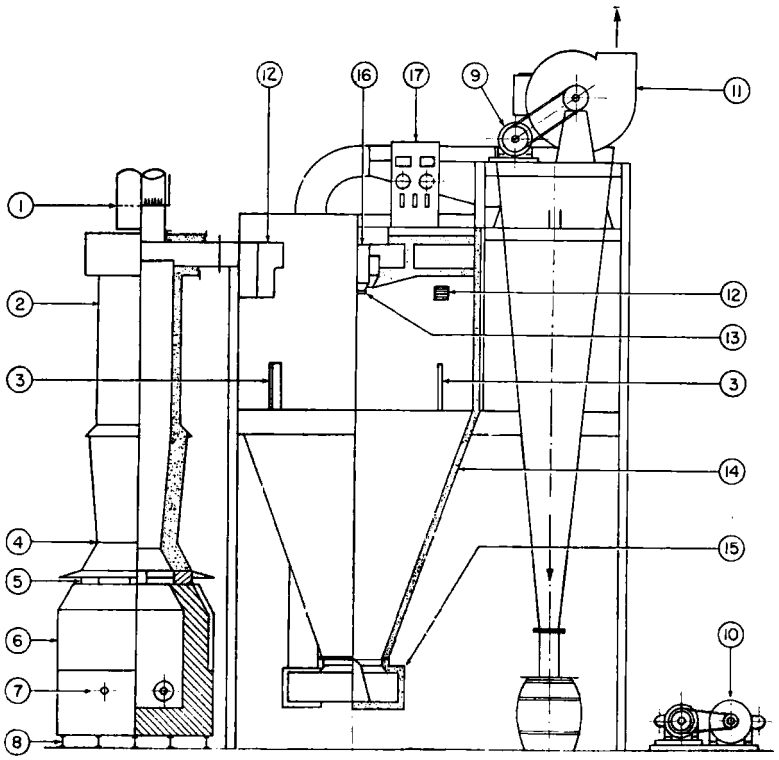


Fig. 4. Bowen spray dryer No. 4.

1. Stack damper
2. Furnace stack
3. Cold side inlet
4. Venturi mixer
5. Tempering air inlet
6. Furnace
7. Burner port
8. Furnace grill support
9. Main fan motor
10. Frequency generator
11. Main fan
12. Hot side inlets
13. Centrifugal atomizer
14. Conical drying chamber
15. Outlet scroll
16. Spray machine
17. Control panel for spray machine

The dryer tested by the authors was the minimum size designed by Instant Drying Corp., having a chamber diameter of 20 ft. and height of 18 ft. The four fans handled a total of 5200 c.f.m. as calculated to 70°F. The vacuum maintained within the chamber was 0.3 in. w.g. Operating under conditions where the evaporative rate was 800 lb. per hour with a temperature differential of 235°F. within the

chamber, the fuel consumption averaged 19½ to 20 gal. per hour. Direct products of combustion were mixed with the heated air. When producing 750 to 770 pounds of powder per hour the amount of product carried to the dust collectors ranged from 15 to 30% of the total depending upon the character of the powder. Only about 2% of the total powder was lost to the atmosphere as dust which represents very creditable performance on the part of the 20-in. diameter, Sirroco-type dust collectors that were used. In some more recent installations a single large fan draws the exhaust gases from all four collectors, and Buell cyclone collectors are used. The installed horsepower in the above dryer totalled 66 HP.

The Bowen dryer (Fig. 4) is typical of the type where vertical-downward co-current drying is accomplished with air having rotary motion imparted by tilted vanes within the entering air orifice. Bowen dryers are cylindrical and may have cone or flat bottoms, the latter being provided with a self-propelled air sweep. Chamber sizes range from the 30-in diameter to 30 ft. for the cone-bottom type and to 45 ft. for the cylindrical units. Most Bowen dryers heat air with direct products of combustion. A vertical oil- or gas-fired furnace of advanced design is employed, which is surmounted by a Venturi-type duct. Secondary air is drawn into the duct ahead of the constricted section. A stack outlet is built above this hot air duct for venting the gases at the start of firing. In addition to the vaned orifice, some recent Bowen dryers have an open vertical cylinder positioned centrally in the plenum into which the hot air must pour in order to discharge through the orifice into the drying chamber. The change in path imposes a pressure drop that is intended to distribute the air evenly to the orifice. The air leaving the orifice rotates in a direction opposite to that of the centrifugal atomizer, the intention being to induce more rapid drying.

Some recent Bowen dryers introduce part of the hot air from four inlets located symmetrically within the upper vertical walls. Such air is directed horizontally and is tangential with respect to the centrifugal atomizer from above which most of the hot air is introduced with rotary motion. Although these side inlets are said to increase dryer capacity, they have been found to be hazardous with food sprays. Their use should be limited to drying materials relatively insensitive to higher temperatures. The particles tend to burn or scorch when they impinge on the tower walls near the inlets. The devices for air cooling within the tower are useful when drying foods or thermo-plastic solids. The slanted vertical inlets for cool air located at several levels along the vertical walls (Bowen, 1937) serve effectively in cooling the walls and preventing sticking of some powders. These introduce a mass of rotating cool air adjacent to the wall for reducing the temperature of the dry particles quickly. The patented floor sweep (Bowen, 1934), being self-propelled by cool air drawn in by the slight tower vacuum, serves not only to blow the product into the discharge channel at the outer edge of the floor, but it also cools the floor and prevents sticking of the powder. Some large diameter installations have mechanically driven air sweeps.

In one type of the Bowen cone-bottom tower, advantage is taken of the cyclonic action of the rotating air for separating the product from the air. An outlet duct hooded with an inverted steep cone is installed within the middle of the tower and serves to discharge the spent air rising along the center-line of the tower cone. This type of dryer has been applied to the drying of milk products and is also used for drying detergents.

The atomizer assembly of the Bowen dryer consists of a high frequency motor rated up to 60 HP on the shaft of which is attached the atomizer disk. The motor

is installed within a heavy cylindrical cast steel housing through which is circulated cooling water to prevent overheating of the motor and feed fluid. Oil is circulated to the bearings of the motor with aspirators to remove excess oil and prevent leakage. The housing is lowered into a well in the tower top so that the atomizer disk extends below the vaned hot air orifice at approximately the *vena contracta* of the rotating entering air. The drying gases are generally handled by a single main fan placed on the outlet side of the system.

The Bowen dryer is provided with numerous recording and controlling instruments, the most important of which is the device for varying the fluid feed rate in accordance with the temperature of the outlet air. An evaluation and comparison with other control methods (such as where the fuel oil feed rate is regulated in accordance with outlet air temperature and the fluid feed rate is kept constant) is presented in the section dealing with instrumentation.

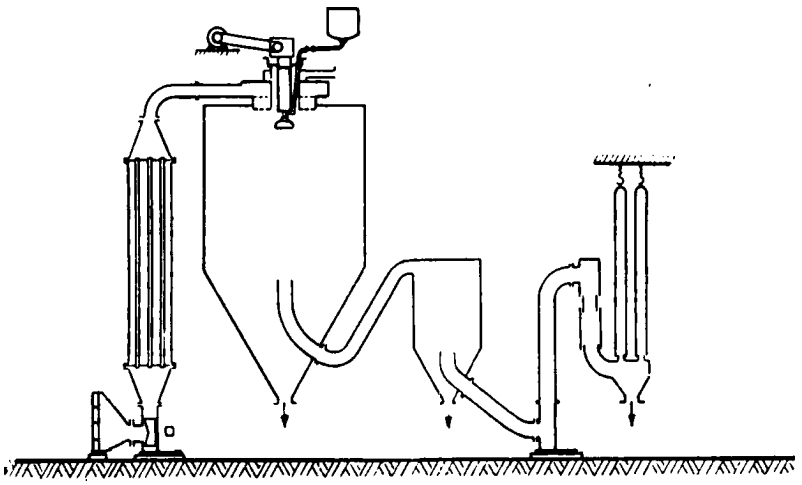


Fig. 5. Kestner spray dryer. Cross-sectional sketch shows principal design features. (Courtesy of the Kestner Evaporator and Engineering Co., Ltd., London.)

In addition to the 2½ ft. diameter table model dryer, Bowen has designed and installed pilot plant dryers of 5 ft. and 7 ft. diameter. Commercial dryers having diameters up to 45 ft. have been put into operation. A new dryer of 10 ft. diameter has recently been standardized for operations requiring intermediate capacity. This No. 4 unit with a capacity range of 5 to 20 tons product per 24 hours is a "package unit" which has all the design features of the larger cone-bottom types.

The Kestner dryer (Fig. 5) has a cylindrical chamber with a cone bottom. In one installation (see Kestner, Ltd., 1936) for milk drying the main source of heat is flue gas which is exhausted through a heat interchanger on its way to the chimney. A steam heater is also installed in the circuit to regulate the air temperature and can be used when the plant is starting up. The hot air is introduced with rotary flow from an orifice above a centrifugal atomizer disk. A smaller orifice concentric with the hot air orifice admits cold air to blanket the atomizer disk, the intention being to prevent milk from caking and drying on the disk itself. The vertical spindle, at the end of which the disk is suspended, is driven by a pulley through a single-stage

step-up gear. Using a standard motor rotating at 1440 r.p.m. the disk may be driven as high as 10,000 r.p.m., the speed of which is indicated by a tachometer coupled to the gearbox by a flexible drive. The powder falls to the bottom of the cone and is discharged through a rotary valve. An outlet air pipe with the opening facing upward is installed at the center of the widest section of the cone; this is fitted with inverted baffles to arrest entrained powder. The air then passes through a cyclone collector and bag collector in series (Williams, 1945). The Kestner dryer employs both an inlet fan and an exhaust fan so that the pressure within the chamber is close to atmospheric. Whereas in a dryer installed for potato drying the air is heated by passing through steam-heated tubes, one used for drying tanning extracts draws its hot air supply from a Universal oil burner (Ind. Chemist, 1939).

An interesting dryer which is included in this classification is that designed by Hall (1942, 1944) and sold by Drying and Concentrating Co. of Chicago (Fig. 6). In some important respects the dryer resembles the Swenson as in the use of a cyclone separator type of drying chamber and in the use of a pre-concentrating chamber as a dust scrubber. However, it differs sufficiently in some fundamental respects to justify its inclusion in the category of dryers having *simple* vertical co-current air flow. The hot air is introduced to the drying chamber through a top snail or scroll instead of through an annular bustle duct with louvre openings as in the Swenson dryer. After circulating along the walls of the turnip-shaped chamber the air, upon reaching the bottom of the cone, reverses itself and streams upward through the core. Owing to the fact that the fluid is atomized by a wheel-type centrifugal atomizer, the spent gases travel upward between the spokes which are arranged as fan-blades to assist in exhausting the air from the drying chamber. Such outgoing air does not encounter the main body of spray as is the case in the Swenson dryer. These differences are noted only to bring out the distinctions between the authors' conception of simple and complex downward co-current drying.

3. Complex Vertical Co-Current Dryers

In this classification may be listed the following dryers: Swenson (formerly known as Douthitt-Gray-Jensen), Niro, Mojonnier, Peebles, and Acme. The Swenson dryer is also discussed in the section dealing with thermal efficiency. The Acme dryer designed by Merlis follows the scheme of the Swenson fairly closely except that centrifugal atomizers are used in both the preconcentrator and the main drying chamber. In both these dryers the hot air enters through side louvres, into the drying chamber and the outgoing air travels through the sprayed fluid.

The Swenson spray dryer, manufactured by Swenson Evaporator Co., is shown in flow-sheet form (Fig. 7a) and in the accompanying photographs (Figs. 7b, 7c, and 7d). Most of the features of this dryer become apparent upon inspection of the pictorial flow-sheet. The system including the combined concentrator-dust collector has been regarded as exemplary in overall thermal efficiency and product yield. In the example illustrated, skim milk is preconcentrated in this device to about 28.5% solids and at the same time scrubs out of the exit gases the entrained milk solids. Such a system has the advantage of permitting the utilization of much heat otherwise wasted. Without the preconcentrator, the dryer would have a thermal efficiency of only about 50% because of the otherwise unutilized heat in the condensate and the wasted heat in the exhaust gases from the dryer. As should be expected, product yields are high for milk, only about 2% being lost, principally as accumulation in the system. When drying milk, it is customary to operate the dryer for 21 to 22 hours and then to shut down for washing. This is a regulation of food inspec-

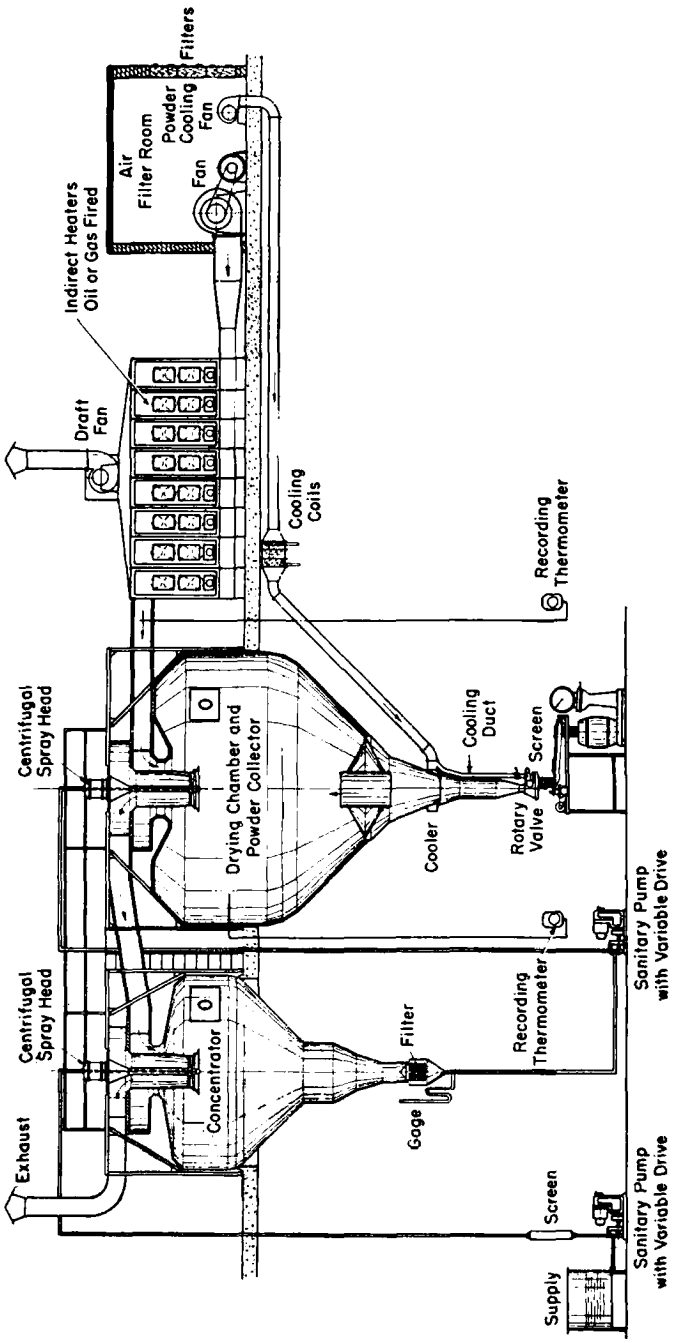


Fig. 6. Spray dryer of the Drying and Concentrating Co., Chicago, designed by J. M. Hall.

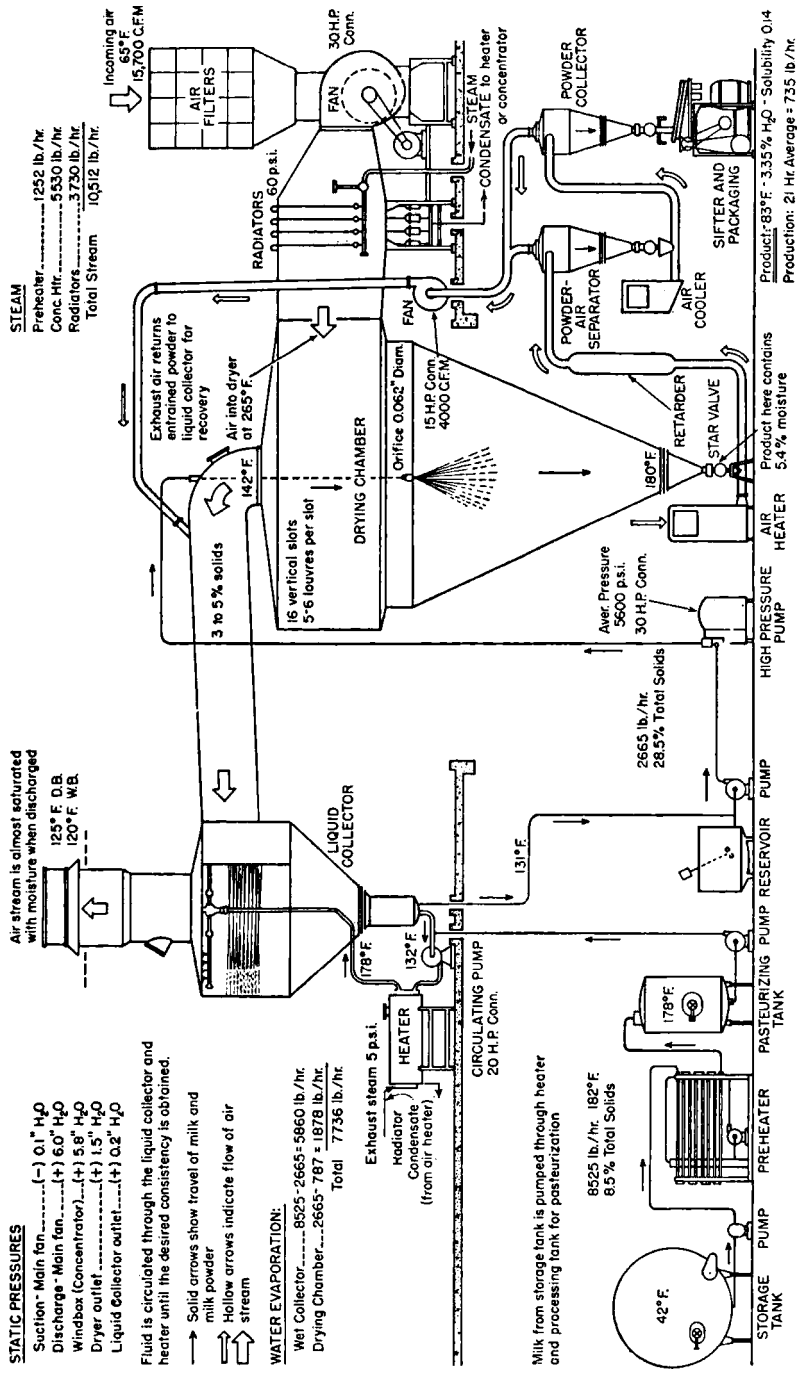


Fig. 7a. Swenson spray dryer, 18.0 feet in diameter, operating on skim milk. The flow sheet illustrates operating characteristics and data for heat and materials balance.

tion departments in most states; otherwise the dryer may run 48 to 60 hours between clean-ups. Washing the drying chamber is a relatively simple operation and consists of inserting a water hose through the top center; air fed to the chamber in the usual way circulates the wash water on the wall surfaces. A single operator can run the entire plant, although it is customary to have two shifts overlap at clean-up time so as to have two operators perform the cleaning job.

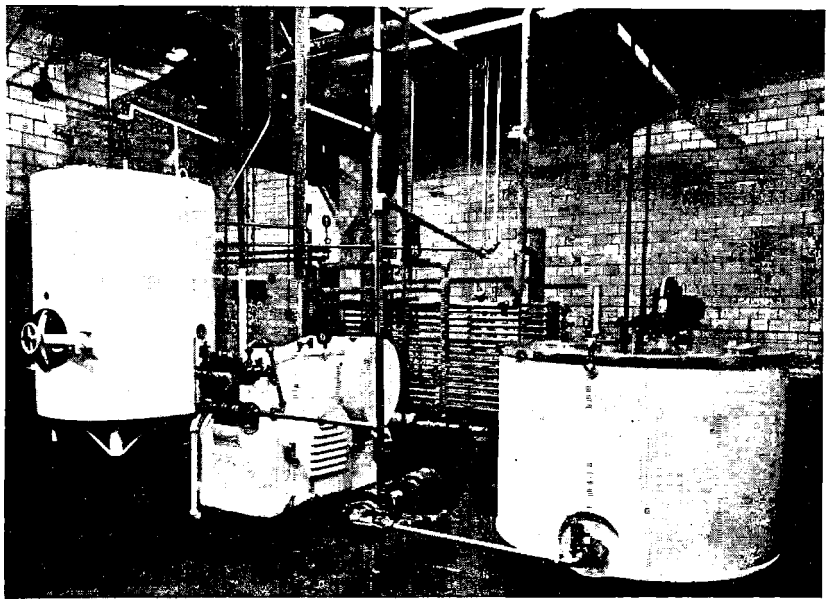


Fig. 7b. Feed tank and high pressure fluid pump for Swenson spray dryer.

The use of heat is unusually efficient in the Swenson dryer as demonstrated by the sample calculations shown in the section on thermal efficiency; about 1.4 lb. steam evaporate one lb. of water. Despite the attractiveness of this thermal efficiency, it should more fairly be compared not to other spray dryers alone but to complete dry milk systems, including vacuum evaporators and spray dryers.

For example, the thermal efficiency of the comparable-capacity Rogers "700 lb." dryer would be unimpressive by comparison if it were judged solely on the basis of a steam consumption of 5600 lb. per hour in the spray dryer to produce 800 lb. powder. The low pressure steam and hot condensate from this dryer may be utilized in a well-run plant for other heating operations, and the condensate should be returned to the boiler. The outlet air temperature of 170°F. (180°F. is maximum) is reasonably low. Starting with 9400 lb. skim milk at 8.5% solids, 7,000 lb. water may be removed in a double-effect evaporator where 1.5 to 1.7 lb. water may be evaporated per lb. steam in concentrating to 32-33% solids. Using the lower value, 1.5, the total steam consumption (vacuum evaporator and spray dryer) is estimated to be 10,270 lb. steam to evaporate 6,200 lb. water, or about 1.66 lb. steam per lb. water evaporated.

Considering the heat used in the Swenson secondary dryer section where milk

powder is dried from about 5.4% to 3.4% moisture, the total steam consumption in both systems is almost identical from the practical standpoint. Product recovery efficiency of the Rogers system also compares favorably with the Swenson system

Fig. 7c. Interior view of Swenson research laboratory showing the wet collector of the Swenson spray dryer.

because of the use of bag collectors for dust recovery. Additional considerations such as building construction and space requirements for the overall system, labor and installed costs are important factors in determining preference between two such well-standardized and accepted milk dryers. A chart (Fig. 8) provided by

Swenson Evaporator Co.* shows 1948 delivered costs for Swenson dryers of stainless steel construction. An installed Swenson system having the evaporative capacity discussed in the foregoing examples would be expected to cost about $100,000 exclusive of building changes or building construction.

Various features of the Swenson dryer are discussed elsewhere in this article. The fluid is sprayed at high pressure, about 5,000 p.s.i., through a single centrifugal-type nozzle positioned in the middle of the chamber at a level slightly above the top

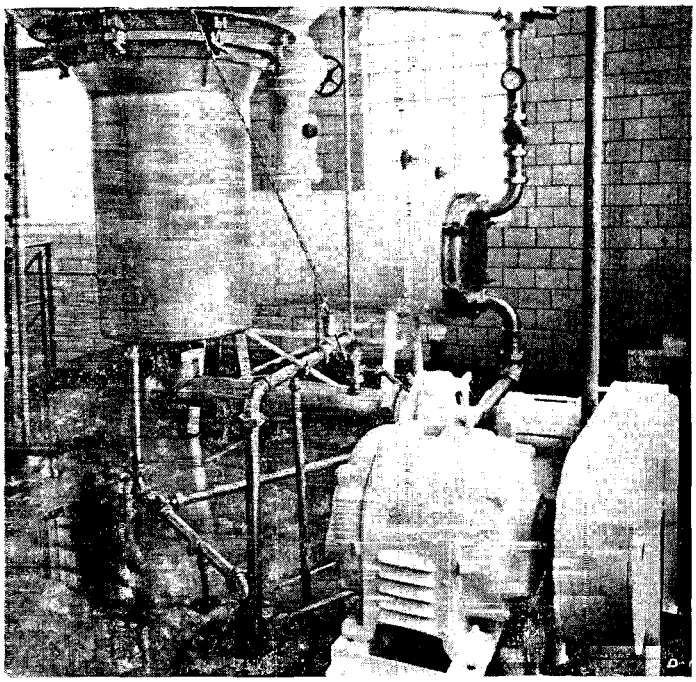


Fig. 7d. Recirculation system at the base of the wet collector of the Swenson dryer. The heater is shown at the rear.

of the cone. A hard-alloy orifice having a diameter of 0.062 in. is typical. The hot air entering the chamber through vertical louvres in the cylindrical section has rotary motion and descends the cone in spiralling fashion. It then spins upward, similar to the behavior of outgoing air in a cyclone separator, and passes through the spray emitted from the nozzle. Some of the sprayed particles, whose terminal velocities in this area do not exceed the air velocity, are carried by the exhaust gases into the wet collector where they are recovered by scrubbing. Most of the sprayed particles persist in their flight toward the chamber wall and circulate in the descending gases until they reach the bottom discharge where they leave through a rotary valve or an exhaust system. A secondary dryer system is standard for this

* Another cost chart showing also installed horsepower appears in *Chem. Eng.*, 54, 125, 1947.

dryer when moistures below 5% are required. A pneumatic conveyor system employing cooled air is installed beyond the primary air separator, and the final product is recovered in a secondary collector. The temperature of the powder emitted from the drying chamber is higher than that from most spray dryers, about 160°F., possibly due to contact with hot wall surfaces or recycling of some of the powder for long periods in the chamber. It is believed that its temperature before such heating is about 110°F.

A rotating, double-chain sweep is employed to keep the chamber cone wall clear of solids deposits. This sweep resembles in construction the supporting pieces of a kite. The lower end is pivoted to the cone bottom and the upper end is connected via a

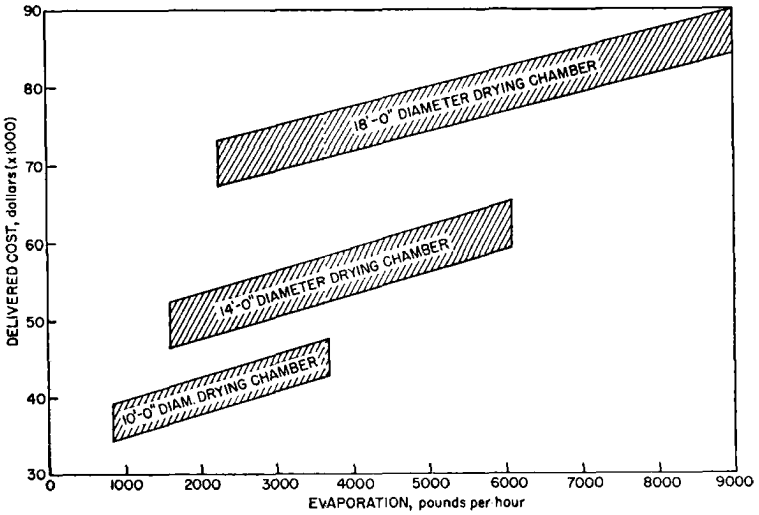


Fig. 8. Delivered costs of steam heated Swenson spray dryers, stainless steel construction (as of July 1948). Erection cost: 15-20%. (Courtesy of the Swenson Evaporator Co., Chicago.)

yardarm to a rod extending from the top of the dryer chamber. The chain is rotated rapidly by the action of the circulating hot air. As is characteristic of such sweep devices, the mechanism itself inevitably accumulates solids deposits.

The Swenson dryer has been widely used for egg powder production where, in most cases, a dry separator is installed instead of the wet collector. Some systems have a bifurcated outlet duct, so that either a dry separator or a wet collector may be used when such flexibility is required. The dryer has been employed for drying a wide variety of other foods such as cream, whole milk, ice cream mix, soluble coffee extract, pectin, protein hydrolyzates and starch. The wet collector is well-suited for starch recovery where the hazard of dust explosions is otherwise an important problem. For chemicals drying, inlet air temperatures up to 1000°F. may be effected by introducing direct products of combustion. Lower atomizing pressures, about 2,000 p.s.i., may be used for drying some chemicals.

In the Mojonnier dryer (Fig. 9) most of the hot air is introduced through a patented "air gun," orifice equipped with numerous vanes and baffles set above a

single nozzle in order to impart circulatory motion to the air. There are two sections to the "gun." A central section consists of three concentric rings with radiating vanes set at a 30° angle from the vertical. Outside these three rings of vanes is a wider ring containing turbine-shaped fins. There are six horizontal dampers in this outer section that are adjusted so that about 25% of the air passes through this ring and 75% through the vane section. It is claimed by the manufacturer that this combination produces an efficient mixture of air and spray within the chamber. The intention is to create a vortex within the tower such that there are no ceiling or wall-deposits despite the fact that no chains or brooms are used. At the top of the tower is a bustle that introduces hot or tempered air along the vertical walls

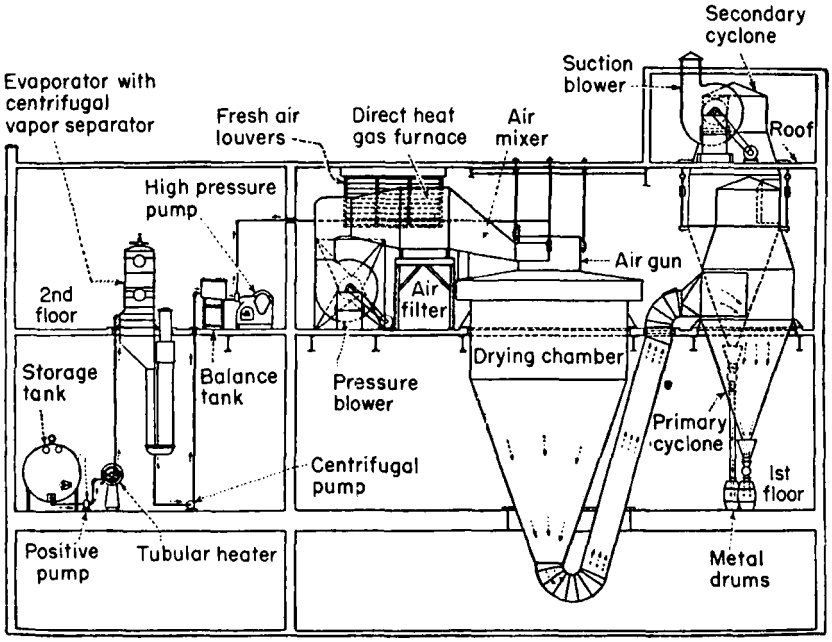


Fig. 9. Mojonnier spray dryer used in the manufacture of ice cream mix powder.

of the tower through four tangential jets. Air fed to the bustle may be about 185°F. as against 360°F. for air fed through the air gun. It is because of this practice that the Mojonnier dryer has been classified in this *complex* category.

Mojonnier prefers to use their own vacuum evaporator for precondensing as against a preconcentrator such as is standard with the Swenson dryer. Powder dried from pre-condensed milk has about 0.1% insolubles, whereas the heat treatment due to recirculation in the pre-concentrator is said to raise the insolubles content to a higher value. However, using a primary and secondary dust collector there is a loss of about 2% as fine powder, a factor which may for some users make the preconcentrator preferable.

The Mojonnier system has a considerably higher overall pressure drop than the Swenson system much of which can be attributed to the use of dry cyclone collectors. A dryer having an evaporative capacity of 1780 lbs. of water per hour and handling

20,000 c.f.m. of air has a 30 h.p. blower fan and a 75 h.p. exhaust fan. The overall pressure drop is said to be 15 in. w.g., the pressure drop within the dessicating chamber is 3 in. w.g., and the static pressure above the air gun is said to be ½ in. w.g. In the comparable Swenson system there is only a single blower fan of 30 HP. The total installed horsepower for the Mojonnier dryer is approximately 130 as against 93 HP for a comparable Swenson dryer.

The Mojonnier chamber consists of a 19 ft. diameter cylinder, 10 ft. tall, below which is a 70° cone, the overall tower height being approximately 18 ft. The air and product leave the cone together and travel upward through an inclined 30 in. diameter duct to two Day collectors in series. These are 9 ft. diameter chambers surmounted by peel-off domes about 7 ft. in diameter. Both collectors have 70°-angle cones. The exhaust fan is located beyond the secondary cyclone. Both cyclones discharge through six-vane rotary valves into powder sifters.

Day cyclone collectors have two distinctive features. One is that the outlet spool-pieces are double tapered with an hourglass effect intended to prevent deposit of powder on the inner walls of the duct. Another feature is that the ducts have louvre-like insertions known as Clark skimmers that are intended to peel out the dust carried to the wall by centrifugal action of the circulation air.

In one installation (Dean Creamery Co., Rockford, Ill.) the direct products of gas combustion join the heated air in drying a milk product, and no soot or dirt is said to be observable even in the first barrel of product (Corbett, 1948). A similar dryer has been installed using a Hauck oil burner for direct heating. In a typical installation, the principal control measure is the regulation of the fuel feed rate in accordance with the outlet gas temperature. A thermometer bulb inserted in the outlet duct of the primary cyclone activates a damper and regulates the gas valve via a valve positioner.

Whereas the Swenson and Acme dryers have cross-current flow of air with respect to the spray as the air rises to leave the chamber, the Niro dryer (designed by a Danish company with American offices at 52 Broadway, New York) which has a chamber of similar shape employs cross-current air initially which then turns downward with rotary motion (Fig. 10). The spray is introduced by a centrifugal atomizer near the top of the dryer. The hot air is led to the atomizer through an L-shaped insulated duct which enters horizontally through the cone and then extends upward centrally to a point below the centrifugal atomizer. The duct is surmounted by a conical distributor which consists of overlapping tilted vanes and which thereby imparts a rotary motion to the air leaving the duct. The direction of air rotation is the same as the disk. In the primary drying zone in the middle of the chamber the manufacturers have found that the temperature of moist droplets of heat-labile enzymes does not rise above 105-122°F. The powder is swept in cyclone-fashion to the bottom of the cone where about 80% is discharged through a rotary valve. The spent air turns upward at the core of the cone and is exhausted through a second L-shaped duct the opening of which faces the uprising air. This outlet duct is located on the vertical center-line of the chamber as is the inlet duct just above it. Both horizontal ducts are covered with peaked hoods of 60° angle which tend to minimize accumulation of powder. Cyclone collectors of the van Tongeren type are used for dust recovery, and an overall product collection efficiency of 99% is guaranteed by the manufacturer for some applications.

The Niro dryer has been operated for various milk powders in Europe for which the inlet air temperature is about 300°F. and the outlet air temperature about 180°F.

The special step-type Niro atomizer disk is described in the section dealing with

centrifugal atomization. [There is apparently less conservatism in Europe regarding the use of centrifugal atomizers for milk drying than there is in this country.] It is characteristic of this dryer to produce powders having high bulk density. There is said to be less tendency toward the formation of hollow spheres than in most food dryers. Although this is a disadvantage where large specific volume is desired for reasons of package display, for certain foods, such as gelatin and milk powder, the absence of air pockets facilitates dissolving. Bulk density of milk powders

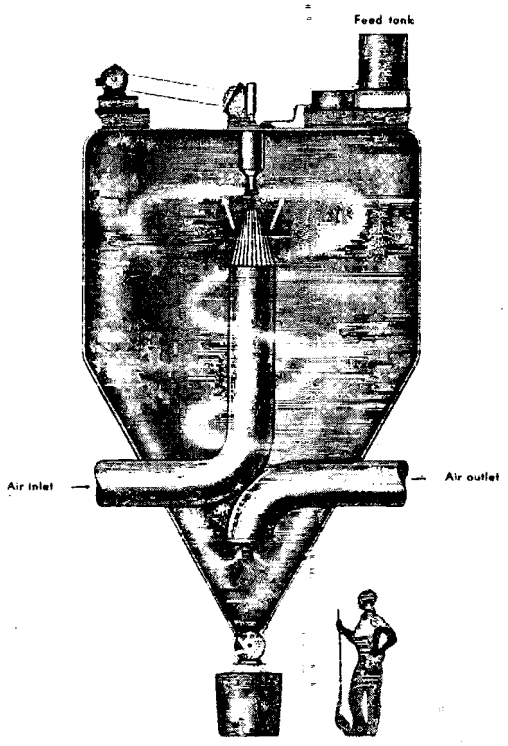


Fig. 10. Niro spray dryer. Phantom drawing shows method of introducing and exhausting drying air.

(whole milk, skim milk and sweetened milk) having 2 to 4% moisture is in the order of 34 lb. per cubic foot.

The Turbulaire dryer manufactured by the Western Precipitation Co. of Los Angeles is in its present design a vertical upward co-current dryer, but as originally developed by Peebles it was a vertical downward co-current dryer (Manning, 1931). Peebles and Manning (1943b) have evolved another dryer for Golden State Co., Ltd. of San Francisco which retains the vertical downward co-current principle but also incorporates several new features which have made the dryer especially successful in the drying of corn sirup (Fig. 11). The tower is essentially a standard cylindrical chamber with a cone bottom. A centrifugal atomizer is suspended from the

top of the tower. A shallow inverted truncated cone serves as the ceiling of the chamber and constitutes the lower wall of the hot air plenum. This ceiling is insulated, an important detail overlooked in the design of some other similar towers. The hot air at 320°F. flows out of the plenum through a conical-shaped collar just below the bottom edge of which the atomizer disk is centrally positioned. Part of the air doubles back and is drawn out of the chamber top through two or more vertical conduits located symmetrically near the outer edge of the conical plenum. This

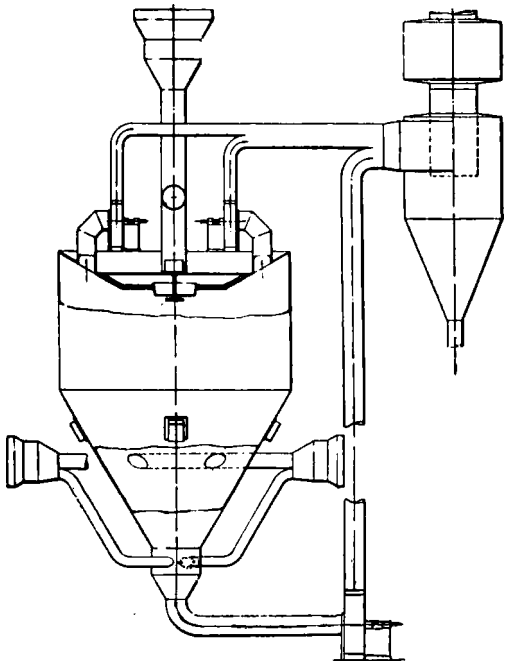


Fig. 11. Vertical co-current spray dryer such as is used for corn sirup. Peebles and Manning, U. S. Patent 2,333,333. Fresh hot air introduced to the cone partially replaces humid air.

outlet air which is at a temperature of about 235°F. when the corresponding entering air is about 320°F., carries out as much as one-third of the powder and delivers it to an air separator. The remaining tower air and powder descends to the cone bottom. Additional hot air, at about 200°F. corresponding to the above entering air temperature, is introduced tangentially from inlets at two levels into the cone and creates an intense vortical action. An optional design has bustle ducts surrounding the cone at one or more levels from which the newly introduced air sweeps downward along the walls of the cone. As an additional measure to prevent powder adhering to the cone, vibrators are sometimes attached to the cone walls. The air discharged from the bottom of the cone at about 190°F. carries the remaining two-thirds of the powder to the same main cyclone separator.

In a chamber measuring 18 ft. in diameter the volume of air discharged through the ceiling conduits is said to be 20,000 c.f.m. The volume discharged through the

cone bottom is said to be 8000 c.f.m., which is equal to that freshly introduced through the cone sides. For air entering with a humidity of 1.5 lb. water per 100 lb. dry air, the air leaving at both the top and bottom chamber outlets is said to contain approximately 3.5 lb. water per 100 lb. dry air. To this system of suspending the partly dried particles in a secondary zone of fresh hot air of lower humidity is attributed the ability to dry successfully hygroscopic fluids such as corn sirup and molasses.

The conditions of entering air and the method of spraying result in products consisting of hollow spheres and having low bulk densities. However, a high percentage of these spheres are shattered by the exhaust fans located between the drying chamber and the cyclone collector. Although it may be in the interest of raising the bulk density to locate the fans at such a point so as to obviate subsequent milling, this layout lacks the flexibility of systems having the exhaust fans beyond the collectors. In the latter, a hammer-mill may be used optionally for fragmentizing the spheres. Product recovery efficiency of 93 to 95% is said to be typical of this system. The gases may then be scrubbed for recovery of entrained solids.

The inventors claim special advantages for corn sirup dried in this system. Whereas the usual product prepared from the drying and pulverizing of slabs tends to cake in humid storage, the spray dried product does not cement as firmly. This relative stability is attributed to the "freezing" of the alpha form of maltose in a metastable state during instant drying.

4. Vertical Upward Co-Current Dryers

In this class of spray dryers, both the hot air and the spray originate in the lower middle section of the tower. The Turbulaire dryer with bottom-driven atomizer (manufactured by Western Precipitation Co.) is such a dryer (Fig. 12) although it is somewhat more complex than the ordinary co-current dryer. By atomizing very finely and employing very vigorous air circulation, high evaporative capacity is achieved in a relatively small chamber. In a tower of cylindrical shape, 9 ft. diam-

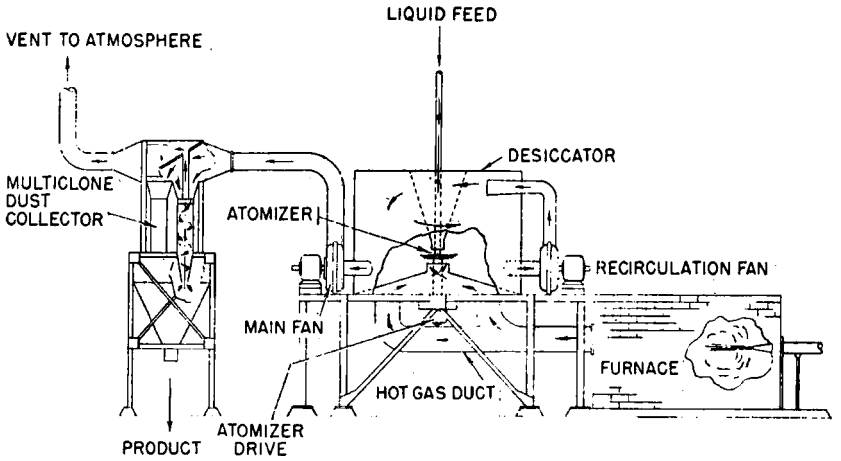


Fig. 12. Tubulaire dryer by Western Precipitation Corp. showing vertical upward co-current introduction of drying air, and outside recirculation of air.

eter and 7 ft. tall, it is possible to remove 1500 lb. water per hour with a 500°F. temperature drop of drying air. Such a chamber has a bottom of truncated conical shape through the center of which rises the shaft and housing for the centrifugal atomizer. The fluid is fed to the atomizer by a line extending down from the top of the dryer. The space beneath the cone serves as the plenum for the hot air which enters from bifurcated ducts that flank the atomizer drive mechanism. A pulley at the lower end of the atomizer shaft is connected via a woven fabric transmission belt (of the type used for Sharples centrifuges) to a larger pulley on a standard 3600 r.p.m. motor. By this device, disk speeds of 9,000 to 15,000 r.p.m. can be reached without the necessity of using special high-frequency, high-speed motors. The larger dryers having evaporative capacity of 3000 lb. water per hour have two hot air inlets. Most of the hot air (two-thirds of the total) enters the drying zone through an orifice that surrounds the atomizer housing just below the disk. About one-third of the hot air is introduced from the bottom to carry the spray upward and then turn downward along the walls; however, an outside fan connected via insulated ducts to the bottom and top of the chamber induces high velocity rotary motion of the air in the chamber. This mechanism is somewhat analagous to the operation of a propeller agitator immersed at the lefthand off-center position in a tankful of fluid. The by-pass duct system in the Turbulaire dryer takes partly cooled air out of the bottom of the chamber and returns it tangentially at the top side-wall, thus inducing rotary motion in the chamber. This turbulent action is responsible for high drying capacity. In most Turbulaire dryers the ratio of recirculating air to entering air is 2:1. In large chambers the ratio is 3:1, and in small chambers it is 1:1. A serious disadvantage for some food materials is that such by-pass agitation of air requires the passage of a large percentage of the powder through the by-pass fan which has a shattering effect. It is possible for some particles to remain in suspension and undergo several such passes through the outside system thus potentially causing additional time of exposure to warm gases at essentially outlet temperature.

This dryer, owing to the high speed atomizer (an 8-in. diameter cage bowl rotating at 12,000 to 13,000 r.p.m.) and the shattering effect of the by-pass fan, has the limitation of producing only very small size particles. It does an efficient job of drying where particle size need not be large. A solids separator is sometimes installed in the by-pass duct to remove powder that would otherwise be recirculated. Within the chamber is a mechanically driven sweep mechanism, supported by a pipe frame, which sweeps the vertical walls and floor with chains, and the ceiling and top cone with scrapers. Unfortunately, when drying hygroscopic foods that are sticky enough to adhere to the wall, they generally adhere as well to the parts of the sweep mechanism.

Western Precipitation Co. also manufacture a spray dryer having a top-driven atomizer which they regard as a "sirup-type dryer." This dryer would be classified as complex vertical downward co-current. The design is essentially that described previously in the patents by Peebles and Manning. Most of the air is introduced at the top center of the tower and is exhausted along with some entrained product through vents distributed symmetrically around the top cylindrical section. Some fresh hot air is introduced at two levels in the cone of the chamber to lower the humidity and to sweep the walls. At each vent point the air is drawn into a cyclone separator, of which there are eight in towers of wide diameter (16 ft.). A single large blower serves all separators. At the bottom of each separator is an individual powder blower that serves to convey the product material to a Multiclone separator

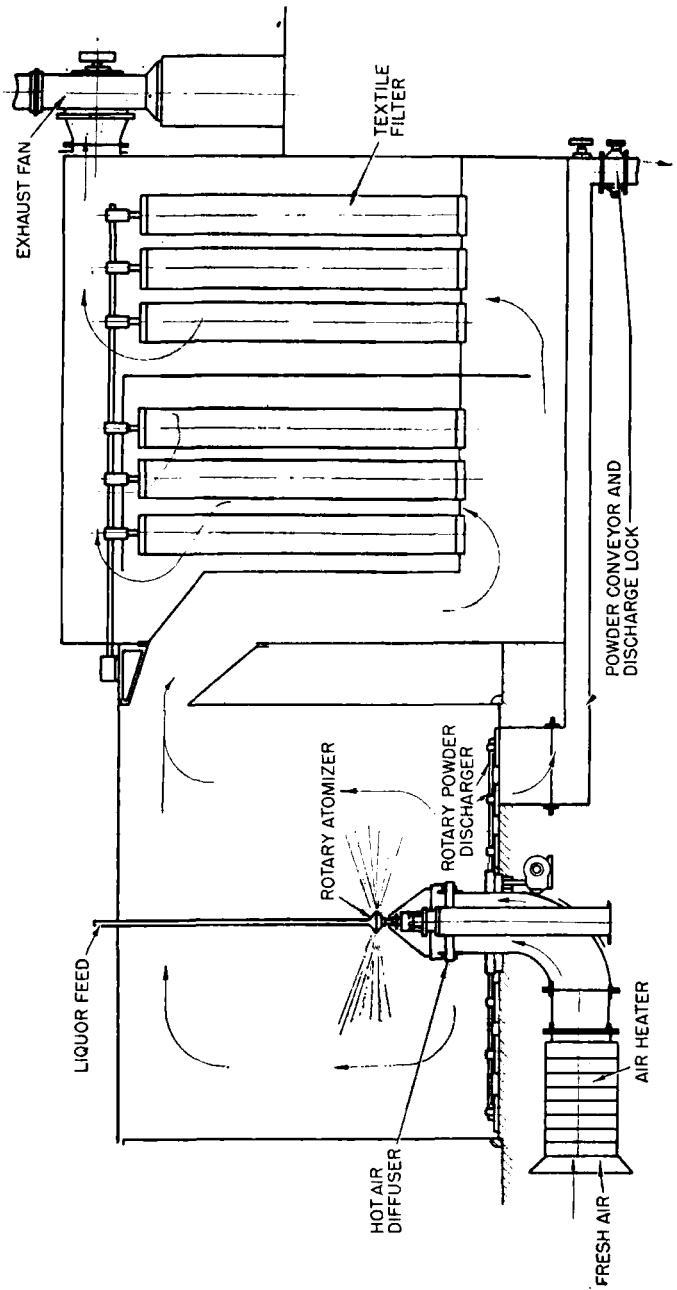


Fig. 13. Vertical upward co-current spray dryer by Geo. Scott and Son, Ltd., London.

where it joins the main stream of powder coming from the bottom of the drying chamber.

George Scott and Son (London) Ltd. manufacture a vertical upward co-current dryer that employs a centrifugal atomizer in the drying of pre-concentrated milk (Fig. 13). A recent installation for Torridge Vale Dairies Ltd., Torrington, Devon (Scott, Ltd., 1947) is believed by the makers to be the largest in Europe and is capable of producing about 1,200 lb. powder per hour from a concentrate containing 42 to 43% solids. The atomizer is bottom driven by a direct connected high-speed, high-frequency motor enclosed in a conical hood within the chamber. The disk is fed by a line descending from the roof of the chamber. Air is indirectly heated by steam and enters the chamber through the center of the flat-bottom floor; it is drawn into the cylindrical chamber by a single exhaust fan (located beyond the chamber) and is emitted with horizontal rotary motion beneath the atomizer through a large-diameter circular distributor equipped with vertical adjustable vanes. The hot air inlet duct, the atomizer drive and a floor-rake drive are enclosed in a streamlined conical shroud which is several feet in diameter at the base and rises to almost half the height of the chamber. The diameter of this milk dryer chamber is 22 feet. Most of the powder falls to the glazed ceramic tile floor where it is swept into a floor opening by the paddles of a slowly rotating floor scrapper.

Air is discharged tangentially from the chamber through a side opening below the roof of the chamber and is drawn through a battery of cotton sleeve filters. These sleeves are blown back and are automatically shaken periodically in order to drop the recovered powder in a V-shaped hopper located beneath the filter section. The product, along with powder from the main chamber, is continuously conveyed from the hopper through a rotary valve into a powder sifter. This powder recovery system is essentially the same as that used with some Rogers dryers in this country.

The Krause spray dryer (Oetken, 1931), or one of its several modifications, appears to have anticipated, in most of the fundamental details, the Scott dryer just described. Instead of a flat roof the top of the chamber is cone-shaped in adapting it to a central round outlet duct. This duct leads the air to a dust collection chamber containing cotton sleeve filters. In another modification wherein the vertical upward co-current principle is also employed, instead of admitting the hot air from a central distributor as in the Scott dryer, the hot air enters from a side annular plenum constructed by building a tilted circular wall enclosing the bottom corner of the flat-bottom chamber to a height of about 3 ft. along the vertical wall and to a width of about 2 ft. along the floor.

The Krause dryer (Fig. 14) first appeared in Germany about 1910, and by 1942 it was believed by Bühler (1942) to be the most widely used system in Europe. In addition to towers having underdriven atomizers, Krause has constructed a variety of towers wherein the centrifugal atomizer is inserted through the top of the drying chamber. Bühler estimated the distance traveled by a particle in descending circles in a Krause dryer by observing the drying of high concentration gelatin. Feathery strands are produced, which in a tower employing pressure nozzles and straight-line air flow requires about 46 ft. of height for satisfactory drying, whereas in a Krause chamber having rotary air motion, a height of about 16.5 ft. (in a tower of the same diameter) is sufficient. He calculates the total distance traveled, which includes 2½ turns in the tower, to be almost 130 ft., thereby illustrating that tower height is not as important as the actual distance traveled by the particle.

Low bulk densities are claimed for soap powders dried with the Krause system,

as would be expected with a co-current dryer. A density of 0.4 or lower is reported by Bühler. (Soap and detergent beads made in this country with other dryers are sometimes below 0.2 lb. per cu. ft.)

Zahn and Co., Berlin, manufacturers of the Ravo-Rapid dryer (Fig. 15), presented about 1939 (Kesper, 1939a, b; Zahn, 1930) a modified version which resembles in many essential respects the features of the Scott and Krause dryers. The drying chamber is a vertical cylinder with a flat bottom that is swept by a rotating rake. A bottom driven centrifugal atomizer is fed by an overhead line. Whereas most

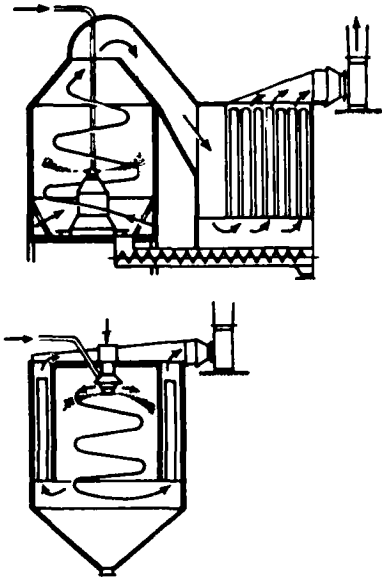


Fig. 14. Krause dryers of the upward and downward co-current types also showing alternate arrangements of bag filters.

of the drying air is blown tangentially through the wall of the chamber at a level below the atomizer disk, part of the hot air is introduced through two distributor ducts located along the vertical center line of the chamber, one duct rising through the floor and surrounding the atomizer housing, the other duct being suspended from the top of the chamber. These ducts discharge hot air directly above and below the atomizer disk almost with jet effect (Fig. 15). The volume of air introduced through each duct (each has a separate blower) can be controlled so as to deflect the spray upward or downward (Zahn, 1943a, b). Zahn illustrates the use of the flared distributor outlets of these ducts and shows that the outlets can be telescoped up or down in order to adjust the apertures below and above the rotating disk. The mixture of air and spray flows horizontally where it joins the spiralling air introduced through the side wall. Whatever particles fail to fall to the floor are carried with the outlet air into either a bag collector chamber or into a preconcentrator scrubber.

Kesper (1939a, b) states that this technique of introducing drying air produces exceptionally uniform particle sizes. The speed of disk rotation is said to have an

optimum value for each case, the general peripheral speed being 490 ft. per second (which is equivalent to an 8-in. diameter disk rotating at about 12,000 r.p.m.). Kesper does not report the average particle sizes, but it is apparent that the particles must be very fine (probably below 40μ). Fine atomization as a rule will result in more uniform particles than will coarse atomization.

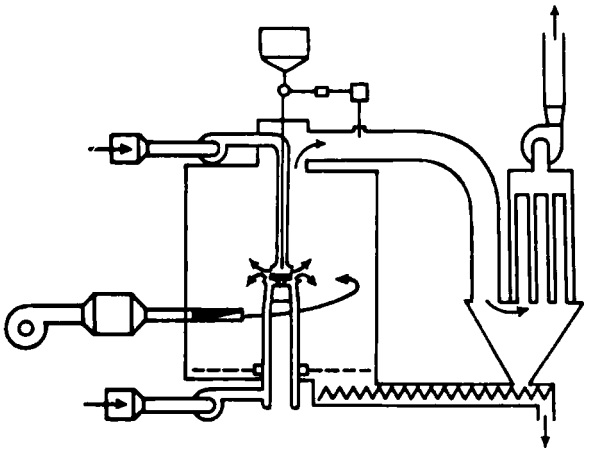


Fig. 15. Zahn spray dryer. Drying air is introduced from above and below the centrifugal atomizer, and the mixture of air and spray flows horizontally to join the rotating air introduced through the side wall.

5. Vertical Counter-Current Dryers

In this type of dryer the fluid is sprayed vertically downward from the top of a tower or is sprayed diagonally or horizontally from several stations along the vertical walls. Hot air is brought in at the bottom of the tower and travels upward in straight-line flow to a discharge duct at the top of the tower. The heavier particles descend counter-current to the flow of air and are recovered from the floor of the tower. Light particles are carried upward by the air stream and are recovered in cyclone collectors and bag collectors. In view of the fact that the spray encounters partly cooled air, there is less tendency toward the formation of hollow spheres; the product generally consists primarily of solid spheres or granules. Agglomeration of particles is common in the counter-current tower because, as the falling particles dry during their descent, some attain a lower relative density with respect to the air and are either carried upward and collide with and adhere to wet particles, or their downward falling rate may be so reduced that heavy, moist particles of higher velocities collide with them. The product accordingly has less uniform appearance and is less bead-like than what is obtained from a co-current dryer. Because of the inherent nonuniformity of the product, most manufacturers using counter-current dryers have not believed that special mechanical provisions for uniform atomization, such as is obtained with centrifugal atomizers and pressure nozzles, are justified; two-fluid atomizers, using air or steam, are used because of the high capacity per nozzle, the lower maintenance requirement, and the lower cost of equipment. In counter-current dryers, the driest particles fall through

the zone of hottest gases, and consequently this dryer is not suitable for most heat-sensitive foods. This type of dryer is used almost exclusively by the soap industry. Where high final moistures are desired, as is the case in soap drying (10 to 13% moisture), counter-current drying proves to be satisfactory.

Soap dryers of the counter-current type are built very tall, 40 to 100 ft. high. For the sake of construction simplicity and economy, the tower shape is often rectangular instead of cylindrical although most soap towers are cylindrical in design with a cone bottom. It is generally considered that the cylindrical tower provides more uniform distribution of the drying air and hence offers a higher thermal efficiency. The floor may also be a flat bottom which is swept by a rake or may be a hopper bottom with an endless belt conveyor which continuously removes the product to the outside and discharges it into a screw conveyor. Air is direct-heated to 400°F. or higher and is introduced through the walls of the tower a few feet above the floor. In a typical case, soap containing about 62-66% solids at a high temperature, about 210 to 225°F., is pumped from a receiving tank or tempering tank through strainers to two-fluid nozzles at about 40 p.s.i., where it is atomized by steam at about 100 p.s.i. Both external and internal mixing types are used, the former being favored more often. Spraying may be carried out at several levels with two or more nozzles at each level. A rectangular tower about 100 ft. high and 20 ft. wide using about 100,000 c.f.m. drying air at 400°F. can dry as much as 30,000 lb. of fluid soap per hour to a product containing 11 to 13% moisture. The quantity of water removed is only about half that evaporated in the drying of a comparable weight of food solids. Powdered soaps may have moisture contents ranging from 8 to 25% and consist of 50 to 92% anhydrous soap with the balance of solids composed of various builders such as sodium silicate, sodium carbonate and trisodium phosphate.

Bag filters and wet scrubbers (such as the Ducon) are commonly used to remove soap fines suspended in the exhaust air from cyclone collectors. Ultrasonic vibration, which has been applied for the agglomeration of carbon dust (Business Week, 1947), is being tested in the soap industry.

The alternate method for manufacturing powdered soap which involves the use of chilling rolls, tunnel dryers, and grinders is less economical than spray drying for large scale production (in excess of 1500 lb. per hour) and is said to produce more dust. The powder entrained in the exhaust air of the spray dryer is recovered in collectors and may either be blended with the fluid soap in the crutcher or it may be air-classified to remove the exceptionally fine particles (such as cause sneezing) and the bulk of it blended with the main product.

Another interesting use of the counter-current tower is in the spray cooling or spray crystallization of laundry products. Wurster and Sanger, Inc. of Chicago, Ill. (Wurster, 1931) design such towers for the manufacture of soap powders (Fig. 16). These towers, a diagram of which is shown, are not less than 60 ft., and are preferably at least 90 ft. high in order to provide adequate crystallizing time during the fall of the sprayed particles. The cross sectional area varies with the capacity but has a minimum of 20 square feet. A typical fluid contains 20% soap, 40% soda ash, and 40% moisture. The fluid is atomized with air in view of the fact that moisture due to steam would be less desirable in the cooling tower. Circulation of air within the tower is effected by the use of fans in recessed positions at several levels along the vertical walls. As the atomized particles descend through the tower the sodium carbonate takes up the water for crystallization, and the particles cool and harden.

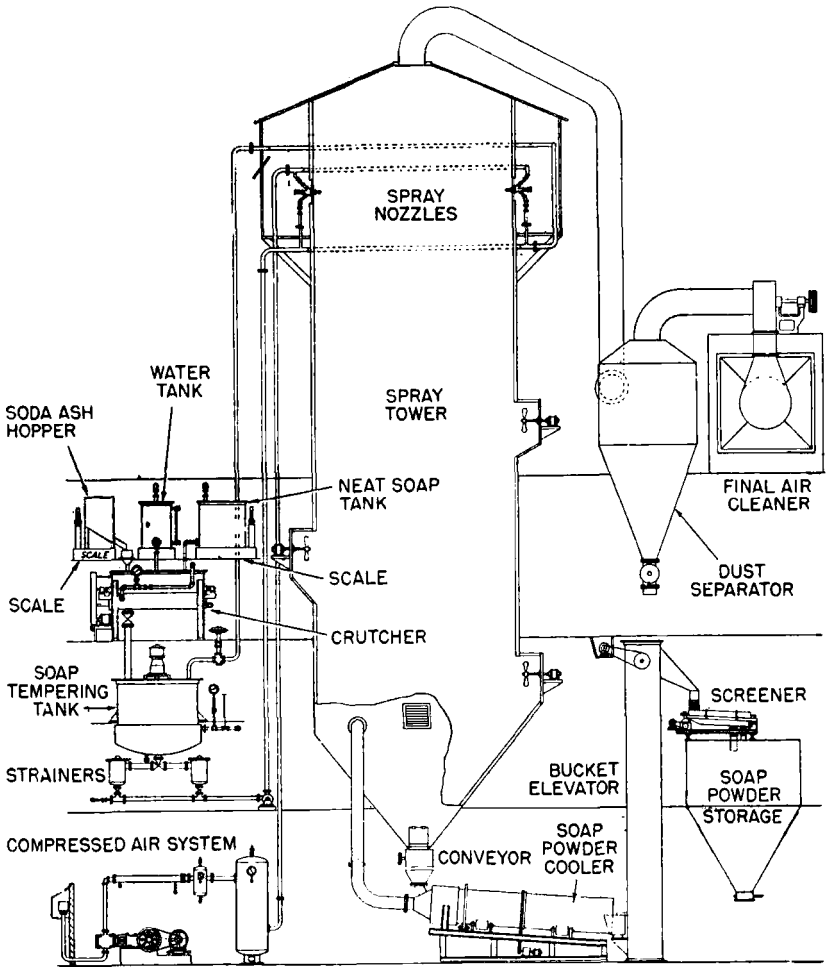


Fig. 16. Vertical counter-current spray tower for soap powder production by spray cooling (Wurster and Sanger, Inc., Chicago).

Air at atmospheric temperature is used. Some evaporation occurs which assists in cooling the powder.

Kestner spray dryers have been used for production of powdered toilet soap, in which case hot air is used, and for filled soap powders, in which case unheated air is used for spray cooling (Reavell, 1944). Since Kestner dryers are of the co-current type, many of the soap powders consist of hollow spheres, although the manufacturers claim that solid spheres can be produced by altering the operating conditions of the spray dryer. The bulk density of spray cooled powder is shown to be varied within wide limits, 21 to 41 lb. per cu. ft., by varying the composition of the fluid (fatty acid content, soda ash content, and moisture). By employing air having rotary motion, it is possible to use much shorter soap drying towers. The Kestner dryer

illustrated by Reavell can be installed in an area 36 ft. by 25 ft. and requires an overall head space of 45 ft. The cylindrical section is 15 ft. diameter and 15 ft. high, and the 60° cone bottom including the rotary valve is not over 15 ft. long.

6. Laboratory Spray Dryers

Laboratory spray dryers may be purchased as standardized assemblies for general work or may be custom designed and constructed to order for drying a specific type of material. These range in size from table models capable of producing 100 g. dried product to pilot plant dryers that can produce 100 lb. samples. Although most installations are used for experimental or development purposes, some laboratory-size dryers are used for commercial drying of special products such as nucleic acids, amino acids and drugs where only small quantities are required. Many laboratory dryers are installed in plants having full-scale spray dryers, and the former are used to test the effect of modifications in formula composition on the flavor, solubility, and appearance of the dried product with minimum expenditure of materials and time. Laboratory dryers are used in numerous research laboratories for drying sample solutions prepared in the course of product development and for storing the test products in powder form. If the dryer is suited for handling the fluid to be dried, such factors as relative dryability, aroma retention and color retention can be studied. Tests can be carried out to test the effect of solids concentration and of various additives such as drying aids and flavor fixatives.

In view of the fact that laboratory dryers have small drying chambers, the drying time must be considerably less than in commercial dryers. This imposes the necessity of atomizing the fluid very finely; whereas most spray dried food powders are in the particle size range of 50 to 125 microns, the powders from small test dryers are generally found to be in the range of 5 to 50 microns. Sludges, slurries, or suspensions that cannot be atomized so finely are likely to deposit on the chamber walls in partly dried condition. In a large research organization where a wide variety of products are being developed, any standard small spray dryer may be found to be a valuable instrument for successfully testing, for example, six out of ten materials. However, where the laboratory dryer is required for work being done on a single product, it is wise to test the dryer before purchase in the test laboratory of the manufacturer or, if it can be arranged by the manufacturer, on a dryer in another customer's laboratory.

Because laboratory spray dryers in some respects permit less variability and have less flexibility than commercial-size dryers, the experimenter may encounter cases where it is not possible to dry certain materials. Tests should then be carried out, if possible, with commercial-size dryers

before concluding that the material is not dryable by spray drying. Most manufacturers can arrange such tests either with their own facilities or at another's plant. On the other hand, if a material dries satisfactorily using a laboratory dryer, the research man may be confident that it can be spray dried commercially. Drying temperature conditions and product properties will probably be found to change, generally in the direction

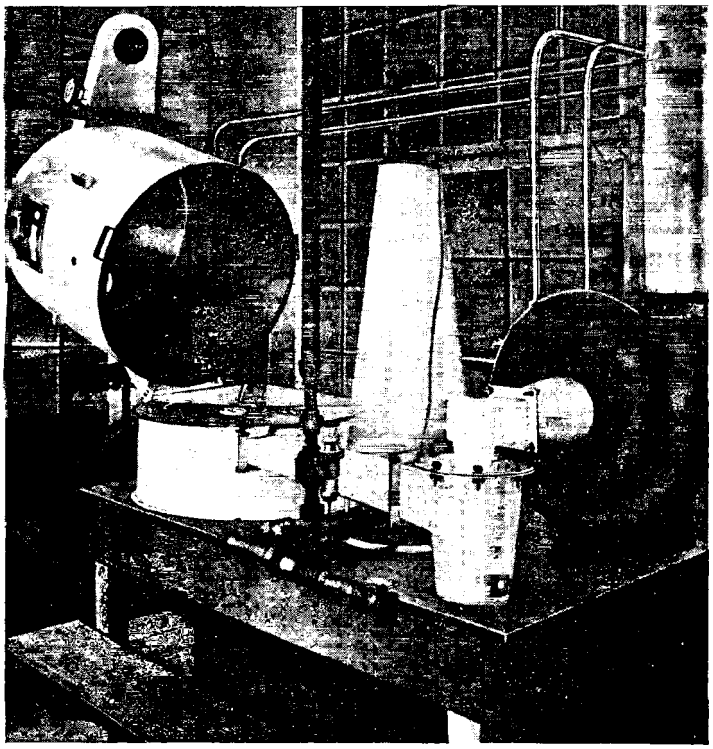


Fig 17. Bowen laboratory table model spray dryer in open position for cleaning.

of improvement, when the operation is transferred to full-scale equipment. As a rule, only one who is well experienced in the spray drying art is in the position to predict the results of commercial drying (particle size, bulk density, free flowing characteristics, solubility, etc.) from observing tests, successful or otherwise, carried out on a laboratory dryer.

Since compactness, convenience, and simplicity are more important than thermal efficiency and cost of heating in a laboratory unit, the most widely sold models have the air heated by either gas or electricity. Steam heating is used in some cases. The atomizing device may be either a

centrifugal atomizer or a single nozzle of the two-fluid type. The chamber may have a cone bottom or a flat bottom, and one or more air separators may be installed. An air compressor is required for providing air under pressure either for driving the centrifugal atomizer or as the atomizing agent in a two-fluid nozzle.

The Bowen table model spray dryer (Fig. 17) is an adaptation of one of their principal commercial designs. It incorporates typical features such as the centrifugal atomizer with a disk that resembles the commercial type, a hot air orifice with a ring of tilted vanes for imparting circulatory motion, cooling-air inlets (at an angle to the radius) at two levels in the chamber wall, a self-propelled air sweep on the flat floor and an efficient air separator. The entire assembly, with the exception of a 3 HP air compressor (100 p.s.i. pressure is needed), is erected on a table 6½ ft. by 3 ft. and requires not over 8½ ft. clear head space. The inside of the chamber can be viewed during operation, and the entire chamber can be tilted to an inclined position for recovery of wall and floor deposits (which, having been air-cooled, are frequently of as good quality as the product recovered from the air separator). The authors have spray dried samples containing as little as 200 g. solids with 90% overall product recovery. The entire system can be disassembled, completely washed, and reassembled in 1 hour.

Air is heated by passing over gas jets on the way to the chamber plenum. Temperatures as low as 200°F. have been used successfully for drying foods having delicate flavors, and temperatures as high as 750°F. have been used for less sensitive foods. The air enters the chamber with rotary motion opposite to the direction of rotation of the disk. Since the chamber is only 30 in. in diameter and 30 in. tall, the atomization must be very fine in order to insure drying. The atomizer disk, 2 in. in diameter, is rotated up to 50,000 r.p.m. by suspending from the shaft of an air-driven Onsrud turbine.

Where only small samples are needed this dryer performs satisfactorily, the capacity being about 1 gal. of fluid per hour. The fluid may be added by gravity or from a feed vessel under pressure. Previous to some recent improvements, viscous solutions or suspensions presented feeding problems because of the narrow feed tube and the narrow aperture in the atomizing disk. Both the feed tube diameter and the disk aperture have been enlarged in the latest units. Bowen has installed laboratory models 5 ft. in diameter and pilot plant models 7 ft. in diameter.

A recently-developed laboratory spray dryer incorporating several desirable refinements is one manufactured by Glengarry Machine Works, Bay Shore, New York (Fig. 18). The atomizer is a two-fluid nozzle which sprays upward from its position in the cone bottom. Hot air is

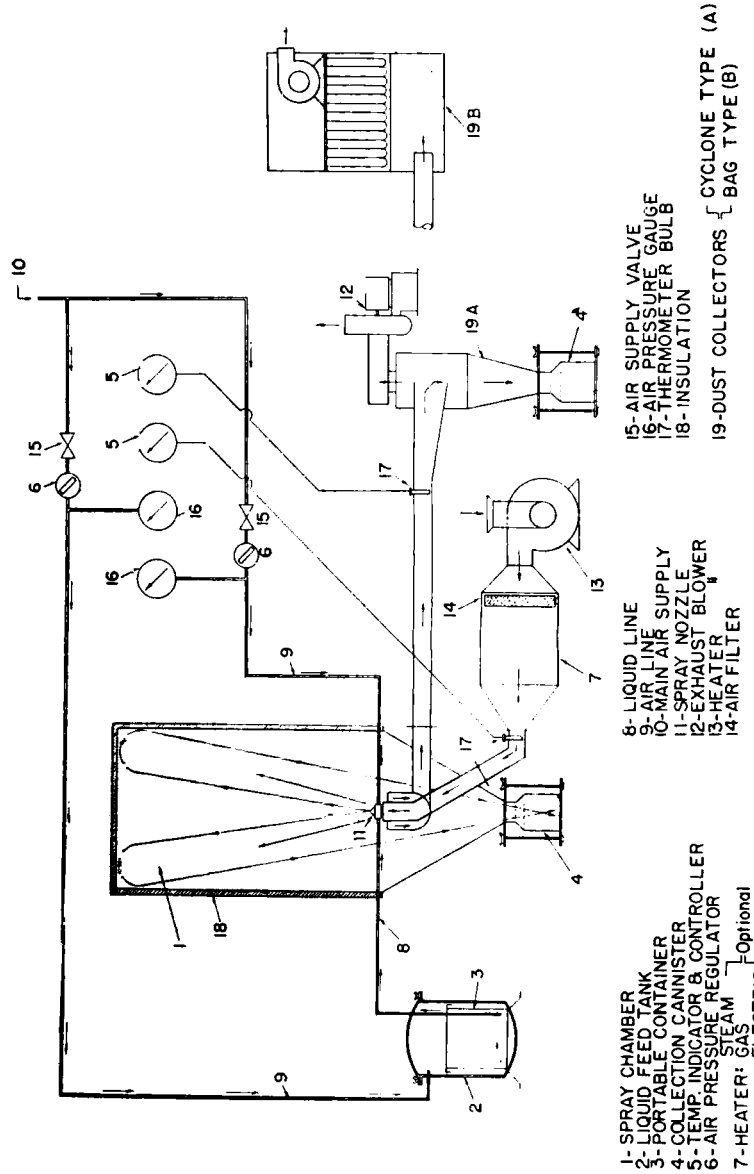


Fig. 18. Flowsheet of Glengarry research spray dryer. (Courtesy of Glengarry Processes, Inc., Bay Shore, New York.)

introduced from a duct from beneath the nozzle so that the gases and particles blow upward fountain-like to the top of the chamber, then reverse along the outside and are exhausted through the cone bottom to a series of Buell air separators in series, the last of which contains a bag filter. Product recovery efficiency is said to be 90-96% for some materials. There are two fans, a blower (¼ HP) that delivers 250 c.f.m. air at ¾ in. pressure drop and an exhaust fan (½ HP) that discharges the same volume at 4½ to 5 in. pressure drop. Four banks of electrical elements heat the air, three of which are manually controlled and one automatically controlled. An air safety switch cuts out the heaters if a fan motor fails. A 2 HP compressor capable of delivering 3 c.f.m. at 100 p.s.i. is required for the two-fluid atomization. This Glengarry system is neatly designed and fabricated and is very well instrumented for a laboratory model. Its ease of operation and cleaning is attested to by the fact that as many as ten test runs can be carried out with it in a day.

A test dryer of greater capacity that is more suitable for installation in a pilot plant or a plant area rather than in a laboratory is that manufactured by Western Precipitation Corp. This is a ruggedly constructed model and can be supplied with several optional arrangements of collectors. The chamber is a vertical cylinder, 4 ft. inside diameter, surmounting a cone bottom. Air is electrically heated to as high as 600°F. by strip-heaters in a cabinet and is introduced through both a tangential inlet at the top of the vertical wall and through a small plenum cap above the chamber. Most air is fed through the top and descends in a straight-line flow. Fluid is fed by gravity or from a "blow-chest" to a two-fluid nozzle inserted through the top of the chamber where it is atomized by compressed air delivered at 35 to 65 p.s.i. The air containing the powder is drawn out of the chamber by a fan fitted to the bottom of the cone and is blown into a single-tube Multiclone separator. A bag filter can be provided as a secondary collector, or a "cold powder system" may be purchased. This consists of a fan that draws the powder from beneath the primary and secondary collectors along with room air as the carrier and coolant and delivers it to another Multiclone collector and a set of stocking-type bag filters. The main fan is 1½ HP and is rated for 225 c.f.m. at 250°F. A manually operated chain sweep is installed along the inside of the cone of the chamber; this can be jiggled by a handle located at the bottom of the cone. This dryer has much higher capacity than the aforementioned laboratory dryers, 25 lb. water per hour for a 500°F. temperature differential in the chamber.

Several laboratories and universities have dryers designed and constructed by research men or students. A cyclone-type dryer of simple design and inexpensive materials, described by Woodcock and Tessier

(1943), was built at the National Research Laboratories, Ottawa, Canada (Fig. 19), using 16 gage galvanized sheet metal with soldered joints and seams. The main drying chamber consists of a cylinder 2 ft. in diameter and 14 in. high surmounting a 4 ft. cone. Air enters the top sides from a bustle duct through adjustable louvres. The exhaust air is removed through a central duct at the top of the drying chamber and the product is collected at the bottom of the cone. Air is supplied by a turbine-type fan delivering up to 90 c.f.m. and is electrically heated. Exhaust air

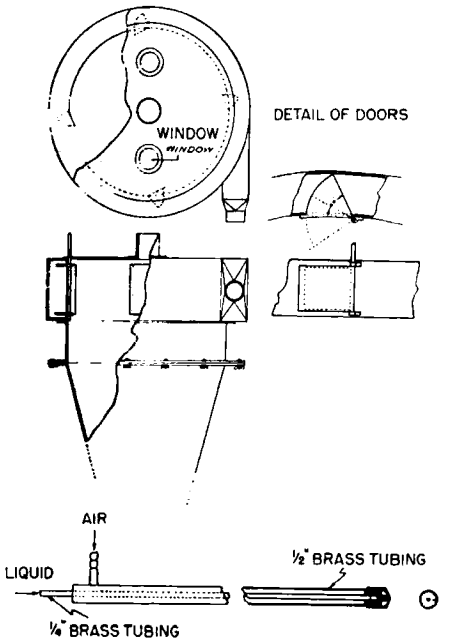


Fig 19. Laboratory spray dryer of the National Research Council, Canada, showing some design details of chamber and spray nozzle. (Woodcock and Tessier, *Can. J. Research* 21, 1943.)

passes through a small cyclone collector. A two-fluid nozzle projects downward from the top of the chamber; compressed air at 5 to 25 p.s.i. is used.

Many government laboratories have small commercial dryers. A United States Department of Agriculture laboratory in Washington, D. C. has a Swenson model. The Eastern Regional Laboratory (U. S. Dept. Agr.) in Philadelphia has an Acme dryer (Acme Coppersmithing & Machine Co., Oreland, Pa.). The Western Regional Laboratory (U. S. Dept. Agr.) in Albany, Calif., has a Mojonnier dryer (see also Coulter, 1947). Most types of dryers have at one time or another been custom produced

in small scale and, by arrangement with the manufacturer, can be inspected at the plant of installation.

Laboratory or small-scale dryers of foreign manufacture are available also. One is produced by Kestner Evaporator and Engineering Co. Unlike the larger plants which are fitted with a rotary type of atomizer, this small dryer is fitted with a nozzle atomizer and is therefore limited for use only with solutions, whereas the rotary atomizer can be used for suspensions and slurries.

III. Atomizing Devices

A fundamental requirement for efficient drying is the conversion of the liquid into a spray of uniform droplet size and the wide dispersion of the spray over the cross-section of the tower. The ideal condition for most food products would be to have all droplets of the same size so as to insure homogeneous drying and heating of the particles and so as to facilitate product recovery. Atomization and dispersion can be accomplished in a variety of ways.

Pressure nozzles and centrifugal atomizers are both used in the food industry. The two-fluid nozzle, which employs either steam or air, is rarely encountered outside the soap or chemical industry although it has some merits that justify its consideration for food drying. Another type of liquid atomizer is the impinging-type nozzle where a high-velocity fluid jet is shattered by impact against a target. H. G. Houghton (1941) shows the following classifications under pressure nozzles: hollow-cone nozzles, solid-cone nozzles, fan nozzles, and impact nozzles. Only the first of these, i.e., pressure nozzles having a hollow-cone spray pattern, is commonly encountered in food spray drying. Solid-cone sprays are used occasionally in soap drying. They are necessarily small in size because of the high capacity and have tiny internal vanes or spinners which are much smaller than the cores of hollow-cone nozzles.

1. Nozzles

In the pressure nozzle the disruption of the fluid into a spray is, by some investigators, primarily attributed to the turbulence set up within the nozzle. The conversion of energy due to pressure is essentially completed when the forces overcome the internal viscosity and surface tension so that the fluid is emitted into the air as a spray. In atomization, work is expended to overcome surface tension and to increase the total surface of the fluid. "Atomization energy" equals the product of surface tension and total new surface created. Dispersion consists of the work to bring the fluid into the tower space under pressure and to carry the particles against gas resistance. The energy required for atomization is

only a small part of the total expended in pressure injection, the major part being expended in overcoming friction (Marshall and Seltzer, 1948). In the centrifugal atomizer the fluid is distributed uniformly to the edge of the rapidly rotating bowl or disk and is emitted at high velocity either directly as a spray or as a thin flat sheet which breaks into filaments or ligaments, which then form droplets. In the two fluid atomizer the energy for disruption of the liquid is supplied primarily by air or steam. This device may be either an ejector-type nozzle or one in which the gas jet is directed at right angles to the fluid orifice.

There is as yet no established law for atomization. Castleman (1931) attempted to show that there exists a physical background for explaining the mechanism of atomization by nozzles. He is an exponent of the theory that air friction is of pre-eminent importance in atomization (as opposed to those who support the theory that atomization is determined primarily by the turbulence created within the nozzle). Castleman assumed that pressure atomization and two-fluid atomization are physically similar and involve "first, ligament formation as a necessary step between the large mass of liquid and the discrete droplets; second, that Rayleigh's analysis of the rate of collapse of liquid columns applies to these ligaments." He cites the photometric evidence of Sauter (1928) of the atomization of water by an air stream. As the air speed was increased the mean radius of the drops decreased rapidly, finally asymptotically becoming 6 μ at an air speed (at the orifice) of 330 to 395 ft. per second. Scheubel's (1927) spark pictures show that the ligaments are clearly visible up to air speeds of 345 ft. per second. This asymptotic effect and the fact (shown by others) that particle homogeneity increases with increase in air speed may be a very useful principle for spray drying. It would indicate that there are limitations, for example, in increasing the speed of centrifugal atomizers for reducing particle size. The curves of Reavell (1944) and Philip (1935) shown for variation of particle size and bulk density as a function of bowl speed, which superficially appear to be at variance with Sauter's and Scheubel's observations, may be shown to have some common ground of agreement when the colloidal properties of the fluid are considered.

Castleman concluded from a correlation of Rayleigh's (1878) work on the rate of collapse of liquid columns with the observations of Sauter and Scheubel that the following describes the simple process of liquid atomization in an air stream: "A portion of the large mass is caught up (say, at a point where its surface is ruffled) by the air stream and, being anchored at the other end, is drawn out into a fine ligament. This ligament is quickly cut off by the rapid growth of a dent in its surface and the detached mass, being quite small, is swiftly drawn up into a spherical

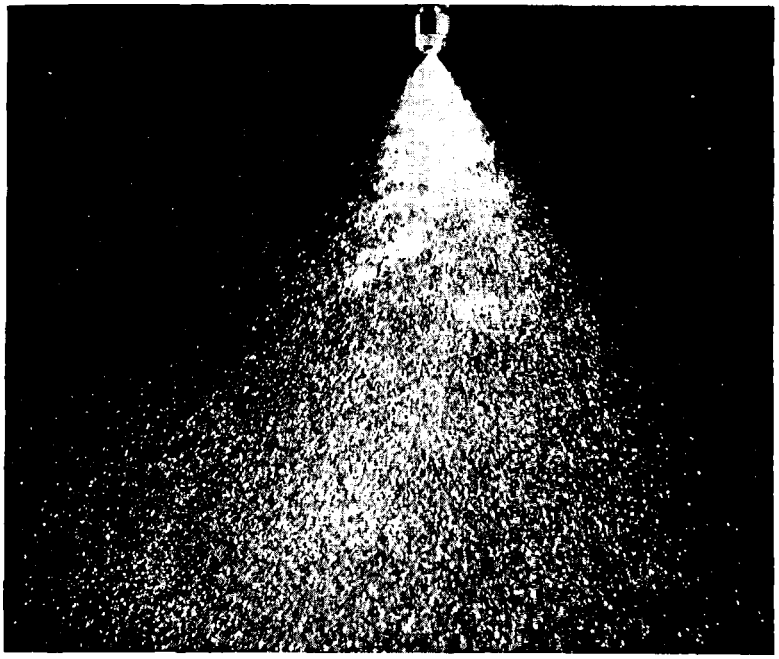


Fig. 20. Solid cone spray of water from "Fulljet" nozzle (Spraying Systems Co.) operating at 100 p.s.i. water pressure. Stroboscopic photograph shows undulations due to air turbulence.

drop. . . . The higher the air speed, the finer the ligaments, the shorter their lives and the smaller the drops found. . . ." Castleman develops the theory mathematically from considerations of mean sizes of ligaments, life periods of ligaments, and the influence of air speed on drop sizes.

Kleinschmidt (1941) extended Castleman's conclusions: "The other common method of forming filaments is to spread the liquid out into a thin sheet. This sheet then draws up into a filament on its free edge, and this filament, in turn, breaks down into droplets. Such sheets or films of liquid, when projected into air at a high velocity often exhibit another and extremely interesting phenomenon. The rapid relative motion of the film of liquid and the surrounding air sets up turbulence in the air which, reacting on the liquid film, causes it to wave or flap, just like a flag flying in the breeze. At its free edge, this flapping often becomes so violent that the film actually rolls up and joins itself into a tube which breaks away from the sheet and, being unstable, as a solid filament is, necks down and breaks into droplets.* In this case, however, the droplets are hollow,

* Fig. 20 is a stroboscopic photograph showing undulations in sprayed water characteristic of the "flag-waving" described by Kleinschmidt.

having enclosed a considerable amount of air. Often these particles also enclose other smaller particles previously formed. These hollow particles are usually very thin-walled and present a large surface, which is frequently desirable." It should be emphasized that this is not the sole mechanism for the formation of hollow spheres. Various observers have noted that during the drying of viscous solutions to viscid spheres, the case-hardening of the exterior may reduce vapor transfer, cause expansion of gas within the particle and thereby give rise to a tiny balloon. The latter mechanism is believed to account for the difference in density obtained in co-current towers compared with counter-current towers.

Fogler and Kleinschmidt (1938), in their excellent comprehensive treatise on spray drying, present stroboscopic photographs to support the mechanism just described. Taken along with Scheubel's, Sauter's, and Castleman's developments of the ligament-to-drop theory, the evidence seems convincing, although some workers regard the "flag mechanism" for hollow droplet formation as being the exceptional occurrence. Several important characteristics of two-fluid atomization are presented. In addition to formation of small droplets by the break-down of filaments, particles of the fluid may be blown off the liquid surface by the high-velocity gas jet, and these dry to much larger sizes than the particles of filament origin. Formation of such "primary" particles and the uneven distribution of energy in two-fluid atomization accounts for the wide range of particle sizes. Other effects such as high-frequency sound vibrations * and pressure pulsations affect or disturb the uniformity of atomization. Air or steam expansion in the two-fluid nozzle causes temperature drops near or below freezing. Primary particles may be chilled by these low temperatures and, if thermoplastic, may set to irregular shapes. The forte of the two-fluid nozzle is that very viscous fluids can be atomized. For food manufacturers it presents an alternative to the expensive—though more elegant—centrifugal atomizer for handling heavy slurries or pasty fluids.

The energy available in a pound of air or steam is considerably more than can be bestowed by pressure to a pound of liquid. For example, Kleinschmidt and Fogler (*loc. cit.*) show how air expanding from 210 p.s.i. gage to atmospheric pressure will release 21,600 ft.-lb. per lb. of energy and steam will release 15,600 ft.-lb. per lb. Water passing from 2000 p.s.i. gage to atmospheric pressure will release only 4600 ft.-lb. per lb. Marshall (see Marshall and Seltzer, 1948) shows, however, that only a small percentage of this total energy is actually utilized in atomization.

* Ultrasonic vibration is currently being tested to determine its effectiveness in promoting more rapid drying in spray chambers and also in promoting agglomeration of fine particles for dust collection. (Ultrasonics, Inc., Cambridge, Mass.)

Kleinschmidt and Fogler tend to emphasize that atomization occurs externally in pressure nozzles, the first effect being the formation of the film. They duly demonstrate the effect of pressure in the spray-liquor line and illustrate the influence of the spinner construction of the nozzle on the rotative motion in the spray.

In contrast to the importance of air friction stressed thus far, other investigations of atomization have tended to demonstrate that the degree of turbulence set up within the nozzle is of controlling or of greater influence than air friction. DeJuhasz (1931) and DeJuhasz *et al.* (1932) carried out experiments to determine which physical forces participate in atomization and dispersion. They found these phenomena could not be accounted for solely by air friction forces although the latter have an important influence on spray characteristics. At partial vacuums, even one-fiftieth of an atmosphere, it was found that sprays were atomized and dispersed essentially to the same extent as in air at atmospheric pressure. Increasing air density was found to widen the spray and reduce penetration. Static charge was found to have no influence. In the earlier paper, DeJuhasz inclined to the belief that under the conditions of oil-engine injection (pressure nozzles) the liquid emerges from the nozzle in atomized or disintegrated state. (Castleman has disputed this view, pointing out that the life period of ligaments fine enough to break up into small drops of spray is very brief, in the order of 10^{-5} seconds.) The authors concluded that dispersion increases with increase in pressure, decrease in fluid viscosity and increase in air density. Cone angle increases with increasing fluid pressure, increasing air density and decreasing fluid viscosity. At a certain pressure the spray cone angle attained its greatest value (10°); at the same time the breakup distance (at which the cone no longer persists) decreased to less than 0.5 in. It was found that "the dispersion of a spray issuing from a circular orifice can be represented by a bell-shaped flux curve, resembling a curve of probability, having the densest portion in the axis of the spray, which is enshrouded in coaxial cones of diminishing flux values. Under certain circumstances, the flux curve shows a rather flat top or even a depression in its central portion." Of interest in spray drying was the observation that rotary motion imparted to the jet by a centrifugal nozzle reduced the break-up distance as did also any imperfections of the orifice. The character of the flow, whether laminar or turbulent, was found to be related to the Reynold's number (*Re*) in the orifice. When *Re* exceeded 6000 the sprays had fully developed cone angle and short break-up distance. DeJuhasz has recently (1948) compiled a bibliography on literature dealing with sprays.

Holroyd (1933) calculates mean drop diameters for various orifice diameters using experimental data of Kuehn (1928) in one case, and in an-

other for similar orifices but varying injection pressures using data of Lee (1932). From these values for drop diameters he demonstrates the constancy of the factor $D/d^{\frac{1}{3}}$ in the first case, and $DP^{\frac{1}{4}}$ in the other case (where D = mean drop diameter, d = orifice diameter, P = injection pressure). Such agreement he attributes to the probable validity of the equation based on the turbulence theory. Holroyd suggested an experimental method for determining drop-size distribution wherein a high melting wax or alloys of low wetting point would be sprayed and the solid spray particles collected for study by sedimentation methods. Fogler and Kleinschmidt used a similar technique of spraying molten tallow into cold air and obtained photographs to demonstrate the opposing theory (namely, that the drops are formed principally as a result of air friction, the mechanism being the disruption of sheets to form ligaments and their breakdown into droplets).

Marshall and Seltzer (1948) conclude that only turbulent jets are of importance in spray drying and that it would be unlikely to encounter viscous flow in spray drying.

Assuming that atomization is caused by turbulent motion of the fluid as it leaves the orifice, Holroyd developed equations based on dimensional reasoning. He related the mean angular velocity of the fluid (ω) to the diameter of the orifice (d), the velocity of flow (v), density (ρ) and viscosity (μ) of the fluid as follows:

$$\omega = \frac{v}{d} \Psi \frac{(dv\rho)}{\mu}.$$

Centrifugal forces in the jet counteract surface tension to cause drop formation, effects of viscosity and orifice diameter being considered unimportant:

$$D^3 = k \frac{\lambda}{\rho\omega^2}$$

where D = mean diameter of the drops
λ = surface tension
k = a constant.

An equation for the mean drop diameter is developed:

$$D = \left(\frac{\lambda d^2}{\rho v^2}\right)^{\frac{1}{3}} \phi\left(\frac{dv\rho}{\mu}\right).$$

Iwanami (1939) studied the flow of viscous fluids, four kinds of water glass, and three kinds of oil, through sharp-edged diaphragm orifices and showed that the limit of laminar flow lies in Re 5 and that, in this range, the relation between the coefficient of discharge, C, and Re is: C =

$0.22\mathrm{Re}^{0.5}$. Re is expressed by $dv\rho/2\mu$ and C, by $v/\sqrt{2gh}$, where d is the diameter of the orifice in centimeters, v the mean discharge velocity at the orifice in centimeters per second, μ the coefficient of viscosity in poises, ρ the density of the fluid in grams per cubic centimeter, and h the head at the orifice in centimeters. Nukiyama and Tanasawa (1939) developed mathematical expressions for the mean diameters of drops of alcohol-glycerin-water solutions atomized from air nozzles and pressure nozzles.

Nukiyama and Tanasawa have published a series of six papers during 1938-1940 in *Trans. Soc. Mech. Engrs. Japan,* which contain data on gas-atomizing nozzles (carburetors), and have developed a useful empirical equation for estimating average drop diameter of the spray as a function of the liquid properties (density ρ, viscosity μ and surface tension σ) and the atomizing variables as follows:

$$D = \frac{585\sqrt{\sigma}}{v\sqrt{\rho}} + 597\left(\frac{\mu}{\sqrt{\sigma\rho}}\right)^{0.45}\left(\frac{1000Q_L}{Q_a}\right)^{1.5}$$

where D = mean diameter of the drops, microns (a drop with same ratio of surface to volume as total sum of drops),

v = relative velocity between the air stream and the liquid stream, meters per second,

$\frac{Q_L}{Q_a}$ = ratio of volume flow rate of liquid to volume flow rate of air at vena contracta,

σ = liquid surface tension, dynes per centimeter.

Another empirical equation expresses the data on distribution of drop size in sprays. In the 5th paper of their series (*Trans. Soc. Mech. Engr. Japan* 6, 7-15, Feb. 1940) Nukiyama and Tanasawa show excellent photographs of the pattern of liquid atomized by means of an air stream. These photographs resemble the ones shown by Fogler and Kleinschmidt and lend support to the ligament-to-drop theory of the latter authors. Lewis *et al.* (1948) have corroborated the above equations for gas atomizing nozzles and have found qualitative agreement in the case of liquid spray nozzles. The latter authors have extended the work of Nukiyama and Tanasawa, have reduced the data to relatively simple straight-line plots for drop diameters and drop size distribution and have found that data obtained by the Japanese on laboratory models are applicable to large Venturi atomizers. Their results indicate that the use of hot gases or steam instead of compressed air will improve the atomization of viscous liquids when the ratio of liquid to gas is high, but not when it is low.

Larcombe (1947) presents empirical rules for water and other fluids atomized by pressure spray nozzles as follows:

1. Drop size (diameter) is approximately inversely proportional to the square root of the pressure.
2. Drop size increases approximately as the square root of the viscosity.
3. Drop size increases approximately directly with surface tension.*
4. Drop size increases approximately directly with density.

Larcombe's work in the pressure range of 5 to 1000 p.s.i. is below that normally used in food spray drying in this country (*i.e.*, 500 to 6500 p.s.i.).

The nozzles used in spray drying that depend solely upon pressure for atomization are generally of the centrifugal type, having one or more

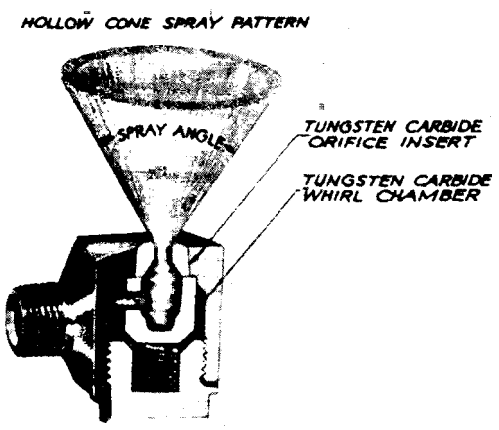


Fig. 21. Whirljet spray nozzle (Spraying Systems Co.) showing tangential inlet and rotary motion in vortex chamber.

tangential inlets into a central vortex chamber. The fluid emerges from the orifice as a film around the periphery of the orifice which film breaks into a cone of sprayed particles. The simplest type of centrifugal nozzle, one having a single inlet drilled on a tangent to the circumference of the vortex chamber, is exemplified by the Whirljet spray nozzle manufactured by Spraying Systems Co. (Fig. 21). This produces a hollow-cone spray pattern. A steel spring holds the vortex chamber against the insert. Another means of imparting rotary motion in the vortex chamber is to insert a core having slanted grooves, numbering two or more, just ahead of the

* Larcombe states: "The same rules can be applied also to approximate the size distribution. If liquids of different properties are sprayed the same size distribution will be obtained at different pressures."

vortex chamber which may be the space within the orifice insert itself (Fig. 22).

In the range of pressures used for spray drying, 500 to 6500 p.s.i., the capacity of pressure nozzles operating with common fluids conforms with the standard relationship of being approximately proportional to the square root of the pressure. Inspection of capacity tables for spray drying nozzles such as are issued by Spraying Systems Co. of Chicago shows the constancy of the relationship. For example, a typical spray drying nozzle equipped with a No. 80 orifice (0.0135 in. diameter orifice) shows

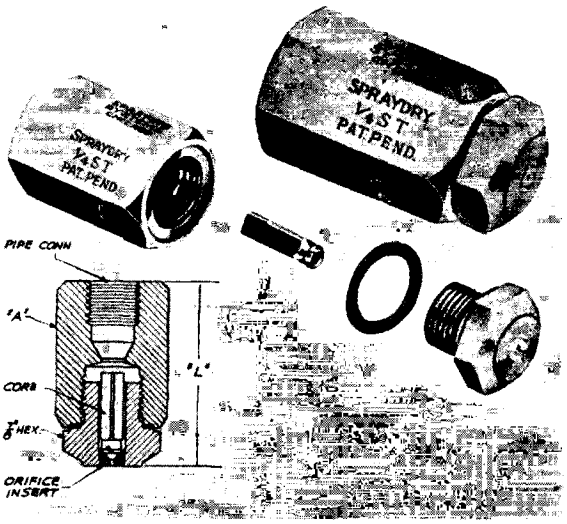


Fig. 22. Pressure-nozzle with core having slanted grooves ahead of tiny vortex chamber. (Courtesy of the Spraying Systems Co., Chicago.)

a constant factor of 1.6 for volume of water divided by the square root of pressures ranging from 1000 to 6000 p.s.i. A No. 62 nozzle (0.038 in. diameter orifice) shows a constant factor of 4.38 for the same pressure range. The capacity at constant pressure is approximately proportional to the area of the orifice, Houghton (*loc. cit.*) states, although the orifice does not run full. This relationship can be demonstrated to hold for nozzles dimensioned proportionately in a range of orifice sizes characteristically used for spray drying, say from drill size No. 44 (0.086 in. diameter) to drill size No. 80 (0.0135 in. diameter). Where nozzle capacities are tabulated for water, the manufacturer recommends multiplying by a conversion factor for eggs, 40% and 24% solids, of 0.7 and 0.8, re-

spectively. The conversion factor for milk is said to be approximately the same.

For a pressure nozzle of given construction the spray angle, or included angle, increases with increase in orifice diameter and with pressure.

A short pitch spiral or groove within the nozzle core creates a wide-angle spray, whereas a large pitch spiral or groove creates a small angle. Data (Table I) for a typical spray drying pressure nozzle abstracted from Catalog No. 22 of Spraying Systems Co., shows the effect of orifice diameter and pressure on capacity. In all cases a flat-top core is used. The number of grooves as well as the depth and width of the grooves increases in proportion to the increase in orifice area, the intention being to have much of the pressure drop occur in the orifice. Since considerable turbulence occurs in the space between the core top and the orifice, any imperfection, even microscopic, on the surface of the core top can cause irregular spraying or spurting by interfering with the circulatory motion imparted to the fluid by the slanted grooves. The small extra cost for having the core tops polished to a mirror finish can generally be justified by the benefits of a practically faultless spray cone which produces very uniform atomization and reduces the tendency toward wall deposition. Cores having dished or cupped tops are represented by the manufacturer as providing relatively better atomization at lower pressures. Such cup top cores have shorter lives than flat-top cores and are rarely superior to polished flat-top cores.

As a guide to those desiring to design nozzles, the methods used by S. M. Doble (1947) for relating the critical dimensions of the spray noz-

TABLE I

Capacity and Spray Angle of Pressure Nozzles (Type 5, Spraying Systems Co.)

Orifice		Capacity, pounds water per hour at the following pressures (p.s.i.):						No. of grooves in core	Spray angle
Drill Size No.	Diam., inches	1000	2000	3000	4000	5000	6000		
80	0.0135	33.5	47.5	58.3	66.6	75	83.3	2	56°
72	0.025	74.2	105	128.3	148.3	165.8	181	2	70°
65	0.035	226	320	392	452	505	555	4	68°
60	0.040	267	376	462	533	596	653	4	73°
55	0.052	348	496	608	702	785	858	4	83°
52	0.0635	702	993	1218	1400	1568	1717	4	75°
49	0.073	827	1167	1433	1651	1850	2013	4	80°
42	0.0935	1609	2275	2782	3208	3590	3930	6	83°

zle with capacity, pressure and spray cone angle should be very useful. He presents nomographs and tables showing how it is possible to anticipate the exact dimenisons necessary for a nozzle to deliver any quantity of water from 4 to 1800 gallons per hour (g.p.h.) in the pressure range of 20 to 100 p.s.i. By the correct selection of the number, shape, and size of the channels in the cores or distributing washers and the orifice diameter any spray cone angle up to 150° can be obtained. This range of pressures is lower than what is usually used in spray drying, and the orifice diameters used by Doble are of higher order, $\frac{1}{16}$ to $\frac{1}{2}$ inch. However, his experimental techniques and his treatment of data should serve as a pattern to workers doing quantitative research on spraying.

Doble ascertained for the pressure range of 20 to 90 p.s.i. that volume delivered (g.p.h.) by a nozzle is proportional to the square root of pressure at the nozzle in accordance with the expression,

$$V = K\sqrt{P}.$$

K varies with the size and number of slots or channels and the orifice diameter but is constant for a given combination of dimensions. For example, K is 1.9 for a nozzle having two channels $\frac{1}{16}$ in. in diameter and an orifice $\frac{1}{16}$ in. in diameter; for a nozzle having four channels of $\frac{1}{4}$ in. in diameter and an orifice of $\frac{1}{2}$ in. in diameter, K is 124.3. Volume distribution and the apex angle of the spray cone (the angle between the inner and outer spray cones) were also measured by Doble and related to the nozzle dimensions. Two channels invariably gave an angle greater than did four channels.

Larcombe (*loc. cit.*) found that the relationship, $V = K\sqrt{P}$, although closely followed by some nozzles, does not hold for all nozzles. In the vortex-type nozzle the value of K was not found to be constant. Doble and Halton (1947) applied cyclone formulae to provide a theoretical explanation for the empirical relationships found between nozzle capacity, spray cone angle, and pressure drop.

For the sake of high capacity in a given installation and for high thermal efficiency it is common to use multiple atomizers, although design and operation can be simplified by employing a single atomizer wherever possible. A single atomizer reduces the amount of attention and inspection required of the operator and minimizes the difficulties encountered within the tower such as variable particle trajectory with resultant variation in extent of drying, agglomeration of particles following collisions, and possible accumulation of material on the wall.

In addition to the disadvantages of having multiple nozzles in a spray tower there are the limitations such as high pressure drop in small nozzles and the shorter life of the orifice inserts. For example, in spray drying a

corn sirup solution through, say, nozzles equipped with No. 72 orifice inserts at a pressure in the feed-line manifold of 3000 p.s.i. the solution may have to be diluted to about 40% solids in order to obtain a satisfactorily low viscosity. A single No. 29 nozzle may atomize satisfactorily a 60 to 65% solution of corn sirup solids at about the same volumetric rate but with a significantly higher rate of powder production.

Although the orifice inserts are ordinarily manufactured from hardened stainless steel, those having small drill sizes show extensive widening after 12 to 16 hours of continuous use. Their openings increase by one or two drill sizes, and very frequently the openings will become oval-shaped thus precluding uniform atomization. The orifice inserts are inexpensive and are replaced readily in the cap using a hand press having a special ram and base fixture. If the fluid being atomized contains suspended solids, abrasion may reduce sharply the life of small orifices. Cocoa slurries have been known to erode No. 72 orifices beyond further use after less than four hours of spraying. Large orifices have longer lives before erosion causes distortion of the spray. Some dryers are designed for operation with multiple nozzles which spray horizontally from a side wall; these generally inherit the necessity of requiring small nozzles. Although satisfactory for milk drying, they sometimes do not operate well with suspensions ordinarily not regarded as being especially abrasive.

Core tips as well as orifice inserts show wear after about a day of continuous use. The principal effects are widening and deepening of the grooves and flattening of the top. Unless polished cores have been used from the start it will be found that the nozzles atomize more smoothly after a few hours' use owing to the fact that natural wear accomplishes the same effect as machine polishing.

Some commercial dryers have been standardized in design to operate with a single nozzle of large capacity. A single large orifice reduces the problem of plugging due to suspended solids. Frequently these are manufactured especially by or for the dryer manufacturer and are equipped with hard abrasion-resistant orifices. Synthetic sapphires, industrial diamonds or tungsten carbide have been cut or ground successfully. The core tips may also be tungsten carbide, or stellite. For spraying milk at 2000 p.s.i. tungsten carbide nozzles such as are made by Spraying Systems Co. have a life 25 to 30 times that of hardened steel nozzles. Whereas, in some cases, hardened steel nozzles wear to an appreciable extent in 1 to 2 hours when spraying cocoa, tungsten carbide nozzles run 100 to 200 hours at 2000 p.s.i. One large dairy company manufactured their wear-resistant nozzles in their own shop. Although nozzle manufacturers now provide a choice of hard materials for nozzle inserts and cores, the food manufacturer sometimes finds the softer conventional nozzles preferable

because the parts can be polished more readily to perform smoothly. Cost of nozzles is rarely an important factor in spray drying economics.

In the interest of achieving a certain bulk density not obtainable with a single large orifice, a manufacturer may feel compelled to use multiple nozzles. Instead of arranging these symmetrically within the tower using a separate pipe line to each nozzle, it is possible to obtain a large head or nozzle body equipped with from two to seven nozzles or nozzle caps. This makes possible the use of a single fluid feed line. For food work, where uniform drying is essential, multiple heads should not have more than three orifices due to the fact that more than three cause cone overlapping.

Most large nozzle bodies, whether for single or multiple nozzles, are equipped with cylindrical strainer screens to withhold particles that might obstruct nozzle passages.

Tanasawa (1940) reported on the effect of various arrangements of nozzles on the characteristics of the spray. With pressure nozzles he advises that there should be little resistance to air currents, and eddies should be avoided. Streamlining of the tower cross-section is advisable. Parallel-arranged nozzles were found to be better than criss-cross arrangement.

Eddying is more often encountered when using pressure nozzles than when using centrifugal atomizers (rotating disk or bowl), especially where only a single nozzle is employed, the spray of which does not cover a large percentage of the tower cross section or where the tower is not streamlined. The type of eddying or reverse gas currents encountered with the Merrell-Soule dryer is said to be of advantage in the drying of milk. The vortical action of the air and its reverse eddying along the side walls is believed to be important in reducing the moisture of the powder to 1.5% without deterioration of quality. This manufacturer possesses a Krause dryer equipped with a centrifugal atomizer but prefers the product obtained by nozzle atomization.

Several food laboratories have done experimental work with two-fluid atomizers using model dryers such as are manufactured by Western Precipitation Co. and Glengarry Machine Co. The principal limitation that discourages their use by the food industry for full-scale production is the wide range of particle sizes produced.

Two internal-mixing types of two-fluid nozzles are illustrated, one having internal vanes for imparting rotary motion comparable to the action within some pressure nozzles (Fig. 23); the other (Fig. 24) is of simpler construction and is intended for low capacities. An external-mixing two-fluid nozzle is shown in Fig. 25.

An outside-mixing type of two-fluid atomizer claimed to have especial

merit for atomizing fluids of high viscosity with relatively low pressure gas (air or steam at not over 4.5 p.s.i.g.) is shown schematically by Hunkel (1937) in Fig. 26. The fluid emitted slowly from the lower channel is engaged by the vertical gas stream which, according to Hunkel, entrains the small fluid particles at the end of the fluid film. Fine atomization is achieved only when the fluid channel orifice is tapered gradually to a sharp point. In practice, Hunkel recommends that the fluid channel be movable at point A so as to vary the angle, a, for achieving the desired particle size. Remarkable flexibility is claimed by varying the gas pressure between only 3 and 4.5 p.s.i.g., which is estimated to produce the relative speed of air to fluid encountered in centrifugal atomization.

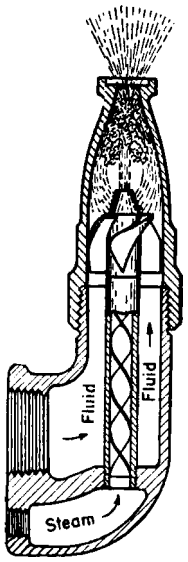


Fig. 23. An internal mixing two-fluid nozzle showing internal vanes for imparting rotary motion. (Courtesy of the Spray Engineering Co., Somerville, Mass.)

The present authors suggest the possibility of using the principle of this Hunkel nozzle for cases of food drying where turbulence within the centrifugal atomizer or pressure nozzle is destructive to the colloidal properties of the dried product, e.g., egg albumen. The standard spray nozzles used in spray dryers are recognized to have homogenizing action which, although useful in the drying of some foods, alters the properties of some products. In cream products the reconstitute is likely to have a curdled appearance; when used for whipping it tends to look "runny." Pyenson and Tracy (1948) found no noticeable differences in whipping properties of whipped reconstituted cream-mix made from a product sprayed with various size nozzles. Spray pressure had no significant effect on the overrun obtained when the reconstituted cream-mix was whipped. However, drainage (from the whip) was nearly doubled as the spray pressure was increased from 200 p.s.i. to 2800 p.s.i. The product dried with 200 p.s.i. spray-pressure produced a whip of good firmness, satisfactory dryness, and small, uniform gas cells.

Edeling (1944) constructed a model nozzle (20 mm. wide) of the Hunkel type using Plexiglas for visibility and studied the particle sizes of atomized fluid droplets accumulated on the surface of castor oil. The entire nozzle behaves like a jet-aspirating pump where the liquid can be fed almost with its own head pressure alone. It was found that the spray left the nozzle as spherical particles in a spray jet of irregular composition. Coarser particles existed in the spray on the side beneath the fluid orifice than on the other side, whereas a cloud of fine mist surrounded the entire spray jet. Instead of flowing to the sharpened orifice in thin

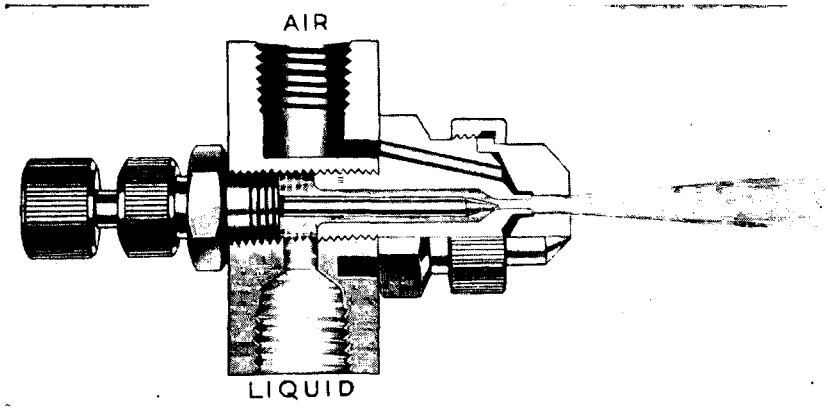


Fig. 24. An internal mixing two-fluid nozzle of simple internal construction. (Courtesy of the Spraying Systems Co., Chicago.)

films, the fluid actually was observed to dam up before it encountered the air stream. Single lamellae peeled off from the strongly vibrating surface in contact with the air jet, which lamellae were immediately disrupted into droplets beneath the edge of the fluid orifice. Significant foaming was observed. For fine atomization, high pressure or low fluid feed rate was necessary. Raising or lowering the temperature of the air was of no consequence. Addition of 10% of a surface tension-reducing compound ("Nekal") to water, which halved its surface tension, was not found to cause any measurable difference in atomization.* Nor could any difference be found as a result of higher viscosity. Glycerin, with a viscosity about 500 times that of water, produced about the same size droplets as water.

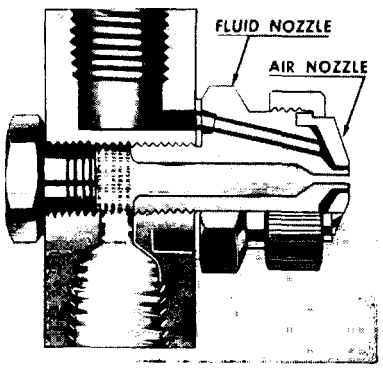


Fig. 25. External mixing two-fluid nozzle. (Courtesy of the Spraying Systems Co., Chicago.)

Using a wide fluid channel and low fluid pressure, atomization was intermittent. The nozzle worked best when, for a fluid channel width of about 2 to 4 mm., the orifice at B was tilted 0.2 to 0.4 mm. to position B'. The gas orifice, about 1.5 mm. wide had to extend as close as possible to the fluid orifice. For each centimeter of fluid nozzle width 15 liters per hour of fluid could be atomized. To achieve a spray made up of droplets of 0.1-0.2 mm. with very viscous liquids, air pressure of 3 to 4.5 p.s.i. is necessary. The amount of air thereby introduced is about equal in weight to the fluid atomized and is only 3 to 5% of the total drying air introduced into the spray dryer.

* This is not a good test of the effect of surface tension. Surface active agents show effect *only* at surface boundaries.

Edeling describes a commercial adaptation of this nozzle having fluid introduced from two sides into the air stream. This proved to be unsatisfactory, primarily because of incrustation of the nozzle. The hot air must be so regulated as not to return material to the nozzle, otherwise the nozzle becomes "bearded" and will cause dripping of fluid, as was the case with tannin extract. An expedient for reducing this condition was to bring fresh air, hot or at room temperature, into the "dead space" beneath the nozzle by using a narrow diffuser.

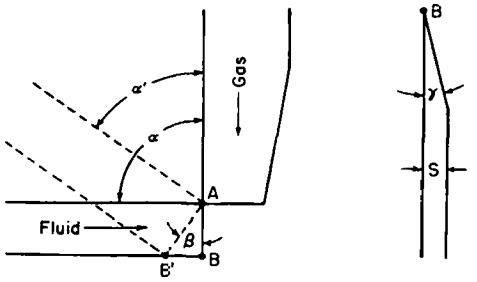


Fig. 26. Special outside mixing two-fluid nozzle according to Hunkel (1937). The fluid orifice may be inclined as shown by dotted lines, thereby reducing the angle between the orifice from α to α'. The fluid orifice is sharpened to taper from thickness S to a point at B, γ being the angle of the taper.

2. Centrifugal Atomizers

Pressure nozzle atomization has several limitations, the most important being that the nozzles become obstructed by large particles when spraying a suspension. It is possible to spray dry numerous coarse slurries of food materials using the centrifugal atomizer where pressure nozzles would be impractical. Potato slurries have been dried commercially by Bowen (1931) and by Kestner dryers (see Williams, 1945) employing centrifugal atomizers with less damage to starch cells than would occur using nozzle atomization. Suspensions containing sugar crystals have been dried without disintegrating the crystals. Thick, pasty, malted cereal mixtures containing 60% or more solids have been sprayed with centrifugal atomizers where nozzles would have been out of the question. Mixtures containing abrasive solids, such as cocoa matter, have been atomized without the excessive wear such as occurs in the triplex pump of the alternate system. A suspension of salt crystals (NaCl) can be spray dried using the centrifugal atomizer to yield a unique form of free-flowing spheres consisting of cemented crystals.

The centrifugal atomizer is generally a bowl or disk rotated at the end of a shaft either directly driven by a high speed, high cycle motor or by a belt-driven standard-speed motor. The fluid is delivered to the atomizer by gravity feed or by low pressure pump. There are wide variations in

atomizer diameters and speeds. The small 2-in. disk of the Bowen table-model dryer is rotated at speeds up to 50,000 r.p.m. by an air turbine whereas the wheel-spoke type atomizer of the Drying and Concentrating Co. dryer is 30 to 32 in. in diameter and is direct-driven by a standard 3450 r.p.m. motor. The centrifugal force at the edge of the latter is approximately equal to that of a 4-in. disk rotating at about 9800 r.p.m. [since centrifugal force is proportional to the product of the radius and $(\text{r.p.m.})^2$]. There is fairly good flexibility in the size of droplets and size distribution (and accordingly, bulk density) depending upon such factors as disk design and peripheral speed—which depends on diameter and rotational speed.

The main function of most centrifugal atomizers is to accelerate the fluid up to the linear velocity of the largest diameter of the disk. High friction losses or elimination of slippage within the atomizer are necessary in order to assure continuous acceleration. Several standard disks are provided with obstructing devices such as perforated or slotted walls, spokes, reversed impeller blades, and steps.

Since there would be too much slippage of the fluid over a flat disk resulting in inefficient acceleration, the simplest centrifugal atomizers have concave interiors, somewhat similar to soup plates (called "bowls"). The Kestner atomizer is simply an inverted bowl or bell of almost hemispherical shape, and the Krause disk is a top-fed saucer the edges of which flare out horizontally.

Another simple centrifugal device that promotes the acceleration of the fluid resembles a rimless wheel. The fluid is fed to the hub and is emitted through hollow spokes or tubes. Some Krause and Ravo-Rapid dryers employ atomizers of this type, and, in the U.S., the Drying and Concentrating Co. standardizes on a wide diameter atomizer having nozzles at the discharge ends of the radial tubes or spokes. The spokes in this case are fan blades having tubular passages. The atomizer accordingly serves also as an exhaust fan which assists in removing the air from the dryer chamber (Hall, *loc. cit.*). Western Precipitation Corp. also builds a radial-type centrifugal atomizer having hard linings; the tubes do not have nozzles at the orifices.

A number of manufacturers (Bowen, Western Precipitation, Peebles, and Instant Drying) provide disks which are modifications of a basket centrifuge. Some have vertical slots in the outer wall, an example being a Peebles disk of 14 in. diameter with slots spaced apart laterally ¼ in. on centers, each slot being ⅛-in. wide and ½-in. long (see Schopmeyer, 1947). Typical of Peebles-designed dryers, this "basket" atomizer develops unusually high centrifugal force owing to its wide diameter and high speed, about 14,500 r.p.m. An "exploded" photograph of such an

atomizer manufactured by Western Precipitation Corp. is shown in Fig. 27. Some atomizers have wide, round perforations (as much as 1 in. in diameter) in the outer walls; or, in cases where the outer wall has a reverse horizontal lip, there may be circular or elliptical cutouts in the top and bottom edges. These atomizers can be used with very heavy sludges. The contour of the disk is such that the center is slightly elevated and serves as a splash plate for the fluid fed from a distributor. The fluid is

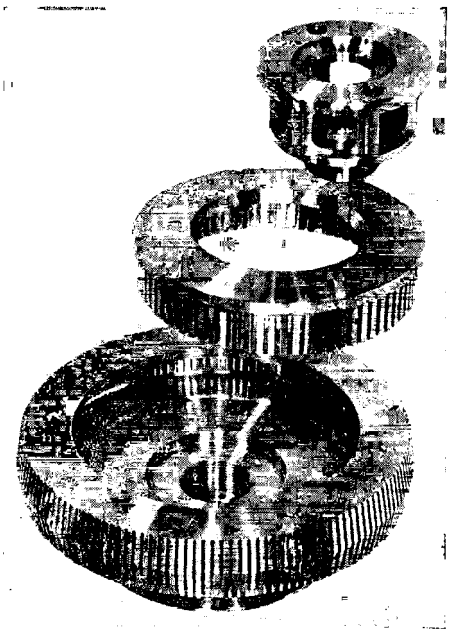


Fig. 27. Basket-type centrifugal atomizer disassembled to show interior vertical slots. Atomizer is bottom-driven but top-fed. Courtesy of the Western Precipitation Corp.)

flung horizontally against the solid section of the outer wall and is then emitted from the perforated edges. Series of sprayings and impacts are believed to insure more even distribution of uniformly thin films (Wilson *et al.*, 1935). Such films disperse into smaller ligaments and droplets.

Some atomizer rotors resemble centrifugal pumps with standard turbine-type impeller blades or sigmoidal-shaped blades (Fig. 28). Sometimes these are arrayed in reverse direction with respect to the disk rotation so as to resist fluid slippage. One effectively designed disk consists of an inverted bowl having a close-fitting cover plate of slightly smaller diameter than the bowl interior. The fluid is forced through nar-

row apertures within the bowl as well as at the edge where it is emitted. In this type as well as in others, such as the Kestner atomizer, the provision of round distributor openings or weirs near the center of the disk serve not only to spread the fluid evenly along the inner wall but undoubtedly provide valuable friction.

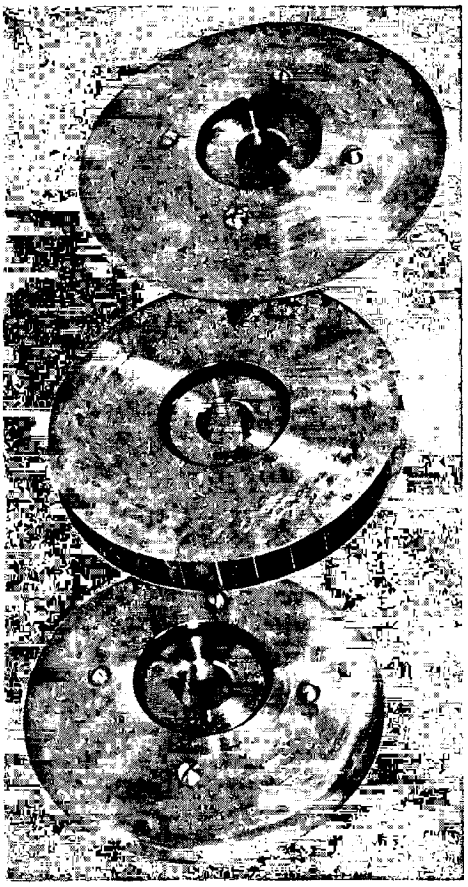


Fig. 28. Three types of Bowen atomizer rotors—disk, turbine (vane), and bowl. (Courtesy of Bowen Engineering, Inc., Garwood, N. J.)

Convolutions of the fluid path can be created by step-wise construction of the disk interior. An interesting utilization of steps is in the Niro rotor (Fig. 29). In the middle of the wheel is a cone consisting of small steps, onto which the fluid is fed. The fluid is said to be atomized as it is flung horizontally, but the spray again reforms a fluid on vanes or

on the vertical edge of the bowl. The reformed fluid then again atomizes as it leaves the bowl through oval openings. This mechanism is regarded as "two-stage atomization" by the manufacturers. A modification of this Niro rotor, which is said to effect "two-phase atomization," has two rows of holes on vertical axes and is said to have a mixing action when atomizing two immiscible liquids simultaneously. Nyrop (1940) developed this atomizer disk which is really two disks, one above the other, separated by a plate and each centrally provided with step-like cones. By individually feeding each disk section it is said to be possible to dry

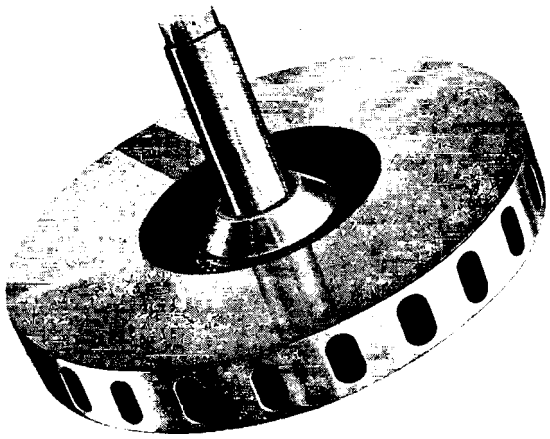


Fig. 29. Niro atomizer bowl showing "steps." (Courtesy of the American Niro Corp.)

separately two liquids with different hydrogen ion concentrations without causing neutralization.

Homogenizing atomizers are designed also by Peebles (see Manning, 1931) and Bowen, which contain, in one case, two concentric vertical walls with vertical milled slots and, in another, flap-like outer baffles which can reform the once-sprayed fluid. The interior parts act as baffles and have the general overall purpose of bringing the material up to rim speed.

Atomizer disks may be cast or machined, generally from stainless steel or aluminum. They must be balanced statically and dynamically before use. The material of construction and the thickness of the atomizer walls must be sufficiently strong to withstand the stresses developed at high speeds. Hydrostatic pressure may be ignored for most atomizers

since stresses due to the action of the fluid are minor compared to those of rotation, and stresses due to centrifugal force may be determined for the rim and the bore. Axial stresses are small and may be ignored. A formula for the stress on a thin steel cylinder (rim) is as follows:

$$S = 1.03 \times d^2 \times (\text{r.p.s.})^2$$

where S = stress, p.s.i.
d = diameter of atomizer, in feet
r.p.s. = revolutions per second.

The stress at the rim of an 8-in. atomizer bowl rotating (without fluid feed) at 10,000 r.p.m. exceeds 12,000 p.s.i. The stress at the bore of the usual atomizers exceeds that at the rim and may be three to four times as high. (Designers of disks may refer to Church, 1944.) Design of centrifugal pumps and blowers involves stress considerations very similar to those encountered in designing centrifugal atomizers. Keyways or holes in the bore tend to prevent stress concentrations that might start fatigue failures by breaking up tangential stress lines. Impeller vanes and shrouds contribute to dead load since they do not contribute to strength.

Centrifugal atomizers may be fed by several methods. In cases of underdriven atomizers it is customary to feed from the top of the chamber through a vertical pipe. Examples of dryers having such underdriven atomizers are the Krause (see Oetken, 1931), Zahn (see Kesper, 1939a, b), the Turbulaire manufactured by Western Precipitation Corp., and that of George Scott and Son (London) Ltd. Some overdriven atomizers are fed by hollow rotating shafts such as in dryers of Drying and Concentrating Co., Ravo-Rapid, and Krause. In underdriven top-fed atomizers the need for a distributor in the feed line is eliminated although the atomizers themselves sometimes have internal construction that favors uniform fluid distribution. Some overdriven atomizers are fed by separate lines that are slightly oblique to the solid rotating shaft. This is the method employed by the Bowen, Kestner, and Niro atomizers and also that of Instant Drying Corp. Such feed lines either discharge into stationary distributor chambers supported by the housing or into a rotating slotted or perforated cup built above the atomizer. Manufacturers of some "homogenizing" atomizers, such as Niro, attach considerable importance to the uniformity of distribution in order to insure a uniform product with a narrow range of particle size distribution. Of course, grossly non-uniform feeding will result in sidewall deposits. A type of atomizer encountered in the chemical industry but not in the food industry consists of a

wheel with impellers or a drum which skims the surface of the fluid and flings drops into the hot air stream (Faber, 1939; Dakin, 1942).

Early centrifugal atomizers used to be turbine-driven using steam, but electric motors are used universally in modern dryers. Special high-

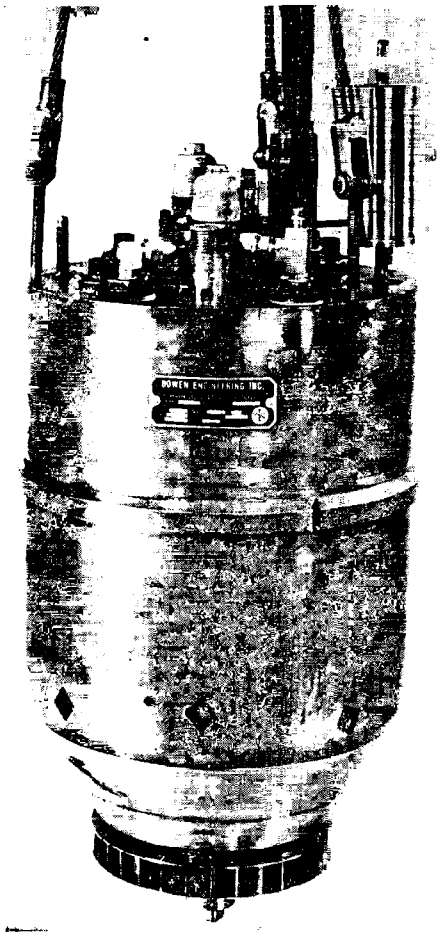


Fig. 30. Bowen atomizer assembly with turbine (or vane) type rotor, top driven by a special high-speed, water-cooled motor within the above housing.

speed motors may be direct-connected to the shaft as in the Instant Dryer and Bowen models, (Fig. 30), or standard speed motors may be used with step-up gears or pulleys or a combination of both (e.g., Niro, Turbulaire, and Krause). When an overdriven atomizer is directly connected to the extended motor shaft, such as in the Bowen atomizer, the

assembly must be lowered into a well set into the top of the chamber. Provision must be made in such a case to cool the motor by circulating cool water through a jacket. Instant Drying Corp. has an air-cooled motor seated on the roof of the chamber. The atomizer is suspended a proper depth within the chamber by attaching it to a long spindle-like shaft (about 3 ft.) connected to the motor shaft by means of a flexible coupling. The lower end of the long shaft turns within a thrust bearing consisting of several horizontal carbon brushes held by light spring tension against the shaft. Because feed fluid frequently gets into the lower bearing, whether of the brush type or ball-bearing type, Instant Drying Corp. has recently redesigned their drive so that the lower bearing consists of the heavy ball type at the bottom of the motor. The shaft has been shortened to extend only about 8 in. below the bottom bearing, thereby reducing the problem of balancing of shaft and bowl. This redesign makes necessary the deepening of the well into which the motor is seated at the top center of the tower. In both these atomizers the motors are small and lightweight despite the fact that they may be 10 to 15 H.P., and these factors facilitate removal and inspection by the use of a chain hoist to raise the entire assembly. High frequency motors (120 to 160 cycles), which also are capable of rotating at three or more times the speed of standard motors, make small size possible. A motor generator set is required for such motors in order to convert standard 40 or 60 cycle current.

The spraying effect achieved by centrifugal atomization is not only dependent upon centrifugal force but also must depend upon the frictional influence of the outside air. Some atomizers have the incidental property of pumping air. Certain manufacturers have attempted to minimize this by using shields to prevent induced pumping by the outside surfaces as well as by effecting liquid seals in the disk interiors. With some atomizers that have an exceptionally high centrifugal force, ordinary observation cannot detect the presence of a film leaving the rim; with others, particularly of the disk type, it is possible to perceive a thin sheet or film which tears into a fine spray under the action of surface tension and friction with the outside air. Observing the action with water being fed to the disk, the width of the sheet from the edge of the disk varies from less than $\frac{1}{16}$ up to $\frac{1}{2}$ in., depending upon the feed rate, disk diameter, and speed. The vertical component in still air appears to be essentially horizontal when the disk has only a narrow vertical lip, say $\frac{1}{2}$ in. or less. With wider lips or rims there is also a vertical component, according to the designers of centrifuges. A. K. Doolittle (1934) has patented an atomizer bowl which he claims imparts a downward velocity component to the fluid emitted.

Air is believed to help the spraying of some fluids when an unfilled atomizer is used although it may conceivably be undesirable for others. Since most centrifugal atomizers act as centrifugal pumps, the fluid feed rate should be adjusted to the range of their pumping capacities. Inadequate feed could result in useless power consumption due to excessive pumping of air, whereas excessive feed could result in poor atomization, spillage, and spattering from the outside surfaces of the disk.

Whereas most centrifugal atomizers run partially empty, some, such as the radial or spoke type with nozzles, run full of liquid (Fig. 31). Since the former have only an ordinary fluid head (due to the fact that only thin films exist) only the centrifugal force needs to be considered for practical purposes. In the latter full atomizer, the hydrostatic pressure behind the

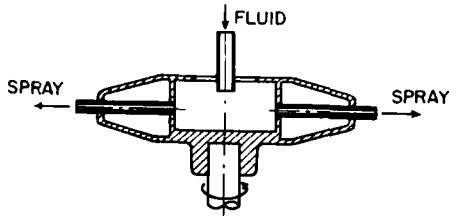


Fig. 31. Krause radial type centrifugal atomizer, bottom-driven. The fluid is fed to the hub and is emitted through hollow spokes. (Oetken, 1931 and Edeling, 1942.)

nozzles is an appreciable factor, and it produces a combination of pressure nozzle atomization as well as centrifugal atomization. For example, consider a 12-in. diameter bowl running at 7,250 r.p.m. full of liquid that is discharging through nozzles installed at the periphery. The centrifugal force (which is proportional to $\omega^2 r$) would be 9,000 times gravity, whereas the hydrostatic pressure generated by the fluid in the bowl (which varies as $\omega^2 r^2$) provides additional 970 p.s.i.* Such hydrostatic pressures enable discharge through nozzles. In such full bowls it is not necessary to keep the fluid under feed-pump pressure in order to obtain heads of 1000 p.s.i.

When using the spoke-type or bowl-with-nozzle-type of centrifugal atomizer the fluid must become accelerated to the peripheral speed before it can be emitted, which is a desirable feature. However, the fluid is thereby concentrated at only a few points. A more open disk, on the other hand, may not bring the fluid up to full rotational speed but may distribute the fluid sheet along the entire peripheral edge, a factor which would favor good atomization. For this same reason (spreading the sheet out thinly), impeller-type atomizers are very efficient for some uses. The liquid is considered to flow as films on the vanes perpendicular to the

* From data provided by George J. Strezynski, De Laval Separator Co.

plane of the disk. For a mathematical treatment involving volumetric flow rate, see Marshall and Seltzer (1948).

A full-running centrifugal bowl having a peripheral slot for discharging liquids subjected to full hydraulic pressure has been developed recently by De Laval Separator Co. for homogenizing fluids such as cream. The fluid must be well screened to prevent interference with the automatically adjusted slot; slurries and coarse suspensions cannot be handled satisfactorily. A 7-in. diameter bowl operating at 12,000 to 15,000 r.p.m. develops about 1200 p.s.i. hydrostatic pressure. A centrifugal disk imparts two velocity components to the liquid, one peripheral (V_p) and the other radial (V_r).* The power consumption depends upon the material being homogenized and varies between 5 and 10 HP.

The De Laval Co. has observed that the power consumption for centrifugal homogenization is approximately equal to that for mechanical homogenization. This is interestingly analagous to a power comparison of nozzle atomization versus centrifugal atomization.

For a given fluid, centrifugal atomization usually requires somewhat more power than does pressure nozzle atomization. In both cases, mechanical factors other than pure atomization are responsible for much power consumption. In nozzle atomization there are pressure drops in lines and nozzles, the theoretical energy required for atomization being only a small part of the total expended. In centrifugal atomization,

* Mr. Strezynski of De Laval Separator Co. has calculated the effects of depth of liquid in a centrifugal bowl on the discharge velocity and kinetic energy of water. An 18.5 in. o.d. bowl running at 6000 r.p.m. is assumed. The tabulated data given below show how the radial velocity, the resultant velocity, and the kinetic energy per pound of water discharged through the orifices will vary with the depth of liquid in the bowl:

Depth of liquid in bowl (in inches)	Radial velocity (V_r) (in feet/sec.)	Resultant velocity (V) (in ft./sec.)	K.E./lb. water (in ft. lbs.)
7.25 (full)	473	682	7225
6.00	453	670	6975
5.00	430	654	6650
4.00	398	633	6240
3.00	354	606	5700
2.00	300	575	5140
1.00	216	537	4480
0.50	156	516	4140
0.25	109	504	3940
0.10	72	496	3820
0.00	0	491	3740

power is consumed in converting cycles for high-frequency motors or there are transmission losses when gears and belts are used. Some work is expended in overcoming friction when rotating the disk in air and some is used in pumping air. Some work must be accounted for by the fluid friction losses within the atomizer due to contact of the fluid film with the metal surfaces and also with the air.

Where a fluid is accelerated to the linear velocity of the outer edge of a disk (without slippage) the maximum theoretical net power horse-power consumption is given (Marshall and Seltzer, 1948) by the following expression:

$$HP = 1.02 \times 10^{-8} W (Nr)^2$$

where W = rate of fluid feed, lb. per minute
N = r.p.m.
r = outer radius of the disk in feet.

Where slippage occurs, the net power would be less than is calculated by the above expression. Flower (1941) shows an equation for power lost in accelerating fluid through a centrifuge wherein the power value is half that shown in Marshall's expression. Marshall's expression has been confirmed in practice, as far as centrifugal atomizers are concerned. Others (Western Precipitation Co.) have found that practically the calculated horsepower is required above the small amount necessary for air pumping, windage, mechanical losses in the bearings, etc. Where total power consumption is in the order of 10 HP this total loss is in the magnitude of 2 to 3 HP. Figure 32 is a general theoretical net power curve prepared by Marshall for the case of liquid acceleration without slippage.

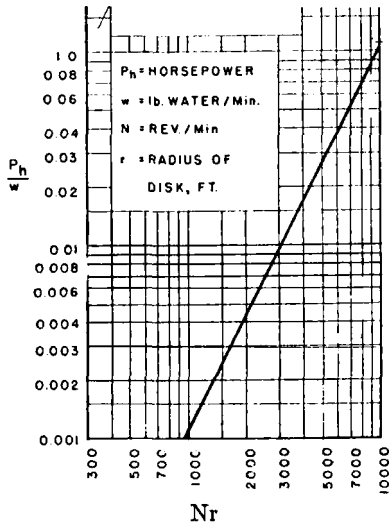


Fig. 32. Theoretical net power consumption for vaned disk atomizers.

Philip (1935) of the Kestner Co. regards the power required for centrifugal atomization as a relatively inexpensive item. He shows diagrams of typical power consumptions when employing Kestner disks at various rotational speeds and feed rates with a fluid of average viscosity and density. Philip cautions that such curves do not apply exactly in practice, because in order to maintain a constant size and form of particle

the power relationship must be established between feed rate and atomizer speed. Such would be anticipated from inspection of the horsepower equation. By raising either the feed rate or rotational speed there would be required an increase in power necessary for accelerating the fluid to the peripheral velocity. However, the force of a given centrifuged liquid upon which the degree of atomization depends is related to the product of disk radius and rotational speed. In order to impart the same amount of energy to each pound of liquid at the higher feed rate it would be necessary to increase the disk speed or change to a disk of greater radius. Philip states that particle size variation is not very marked over a wide range for variation in feed rate, so that there is no need to vary disk speed for variations in feed rate within 20 or 30% of normal.

3. Dispersion

Some aspects of dispersion have already been discussed in the foregoing description of the mechanism of nozzle atomization. Having effected the atomization of the fluid, the manner in which it is dispersed in the tower space determines the practicability of the drying operation. Control of dispersion (or mixing with the tower gases) is important for insuring at least primary drying before wall surfaces are reached. Also, the trajectory or path of the particles must be controlled beyond the period of primary drying so as to insure sufficient travel distance for complete drying. Such travel distance may be assured by designing the tower tall enough or large enough to permit sufficient travel distance, or by imparting to the drying gases a rotary motion so as to extend the travel distance of the particles to be dispersed therein. Unless sufficient travel distance is imparted to the particles, the drying gases must be kept at abnormally high temperatures, especially the outlet gas, which results in reduced thermal efficiency.

The persistence of the sprayed particles in the path or travel pattern imparted by the atomizing device depends upon factors such as the manner of travel and the air density. DeJuhasz *et al.* (1931-1932) showed that dispersion increases with increase in fluid viscosity and air density. Spray-cone angle increases with increasing fluid pressure and air density and with decreasing fluid viscosity. For liquid fuels the break-up distance was found to decrease to less than 0.5 in. when pressure was increased to attain the greatest cone angle. Their description of the fuel dispersion, having the densest portion in the axis of the spray, is not typical of many sprays encountered in food drying. In spray drying it is more desirable to have hollow-cone sprays so as to favor hot air penetration. Dense sprays tend to promote reagglomeration during drying.

Flat, horizontal sprays from centrifugal atomizers are penetrated more quickly than the oblique sprays from nozzles (Wilson, 1935).

Persistence of particle travel in the direction imparted by the spray device is highly dependent upon the speed and direction of the hot air stream. In some of the simpler tall towers, such as are designed by P. T. Zizinia and T. L. McKenna, the sprayed particles can be seen to persist as uniform cones for a distance of 2 ft. from the nozzles, depending upon the pressure within the nozzles. Such towers employ straight-line flow of air by introducing the air from a plenum into a perforated distributor sheet at the top of the tower. This provides an even, but low, air velocity through the horizontal cross-section of the tower. In one such tower the gases above the drying zone were found to travel at the low velocity of about 75 ft. per minute. Owing to the gas volume contraction caused by cooling, the velocity below the nozzles was reduced to 60 ft. per minute.

A similar effect was noted in a tower containing a centrifugal atomizer. Air flowing from the top plenum into a wide orifice flowed essentially in straight-line fashion into the tower at so low a velocity that the vertically-sprayed particles persisted for about a foot or more before being turned downward by the air stream. In Bowen dryers where the hot air is introduced similarly but is imparted a rotary motion by vanes within the orifice, the air engages the spray from the centrifugal atomizer at approximately the *vena contracta*. In such dryers the spray pattern appears to persist for less than a foot from the disk.

In towers having low velocity air with straight-line motion, eddying is likely to occur and is frequently manifested by deposition of powder on the walls. One cause is the turbulence created by the large shrinkage of gas volume in the primary drying zone. Another cause is that hot insulated walls promote upward travel of warm air.

Scott (1932) shows the following terminal velocities for particles of density 1.0 and sizes such as are encountered in spray drying:

Diameter, microns:	10	30	60	90	120
Terminal velocity, ft./sec.:	0.008	0.086	0.31	0.70	1.24.

The terminal velocity in still air is calculated from Stokes' law, expressed as follows in c.g.s. units, since viscosity is usually given in these units:

$$U = \frac{2}{9} \cdot \frac{\rho_1 - \rho_2}{\mu} \cdot gr^2$$

where U = terminal velocity, cm./sec.
ρ_1 = density of the particle, g./ml.
ρ_2 = density of the air, g./ml.

g = acceleration due to gravity, 981 cm./sec.^{2}
r = radius of the particle, cm.
μ = viscosity of air, poises.

Scott estimates that a drop of liquid milk of specific gravity 1.03 in air (50°C. and sp.g. 0.0019) will have a terminal velocity of 0.15 ft. per second.

Blizard's (1924) observations on terminal velocities of powdered coal and coke are cited. Coal particles of 80 micron diameter projected downward into still air with a velocity of 100 ft. per second were reduced to a terminal velocity of less than 1 ft. per second after travelling a few inches. Air resistance, the magnitude of which is implied by such values, is stated to be proportional to the amount of diffusion between the particle and the air, and accounts for the extraordinary rates of evaporation. Total drying time cannot be estimated from the initial high velocity of the drops leaving the atomizer since this initial high velocity will be lost very quickly.

Modifications of Stokes' law for one-dimensional and also two-dimensional motion in a centrifugal field are developed by Lapple and Shepherd (1940). These equations and some work on the trajectories of sprayed particles indicate the complex factors involved even for motion of particles in a gas at rest. Allowances must be made in such equations for the case of spray drying where, except for the few instances of straight-line co-current air flow, the path of the particle is three dimensional and where complicated phenomena are encountered such as agglomeration, "particle clouds," or "hindered settling" due to high particle concentration. Entrainment of air is also an important complicating factor, making the relative viscosity quite uncertain. A simplified form of Stokes' law for constant settling rate of spherical particles in streamline two-dimensional motion in a centrifugal field (such as would be the case in cyclone separators or in spray dryer chambers having cyclonic motion of air) is given as follows:

$$U_r = \frac{V^2_t\,(\rho_s - \rho)\,D^2}{18\mu r} D^2$$

where U_r = particle velocity in a radial direction, ft./sec.
V_t = gas velocity in a tangential direction, ft./sec.
ρ_s = actual particle density (not bulk density), lb./cu. ft.
ρ = gas density, lb./cu. ft.
D = particle diameter, ft.
μ = gas viscosity, lb./(ft.) (sec.)
r = radius of curvature, ft.

For such spherical particles in streamline motion an alternate method of expressing Stokes' law is in terms of drag coefficient, C, and Reynolds number, Re:

$$C = \frac{24}{Re}.$$

C is defined by the expression

$$\frac{2F}{\rho U^2 A}$$

where F = frictional force on the particle, poundals
A = particle area projected normal to direction of motion, sq. ft.

C may be determined by reference [see Fig. 1 or Table 1 of Lapple and Shepherd (1940)] to the Reynolds number where

$$Re = \frac{DU\rho}{\mu}.$$

Because of the high rates of heat transfer to clouds of solid particles, the technique used in spray drying suggests their adaptation to the heating of finely divided materials, such as chemicals. Johnstone *et al.* (1941) investigated the factors essential for the design of such heating equipment. In the case of spray drying, heat is transferred to sprayed and falling particles primarily by convection. Radiation from the hot walls of the chamber is minor by comparison, at the average temperatures encountered in spray drying.

Meyer (1937) estimated that a radiator temperature of about 2000°F. was necessary in order to obtain the same rate of heat transfer as can be obtained by forced convection in a spray dryer, thus demonstrating the non-feasibility of spray-drying by radiant heat. Another deterrent to the use of radiant heat is that the water vapor would be expected to rise to a temperature approaching that of the radiating walls by absorbing radiant heat.

A particle of about 41 microns diameter surrounded by air in a spray dryer was estimated to have a heat transfer coefficient of 240 BTU/(hr.) (sq. ft.) (°F.), and the heat transfer rate was calculated to be in the order of 50,000 BTU/(hr.) (sq. ft. of particle surface) where the temperature gradient is 200°F. This indicates the extraordinary rate of heat transfer attained in spray drying.

Johnstone *et al.* (1941) determined the terminal velocities and drag coefficients of particles of sand, carborundum, and aloxite which had densities in the order of 2.65 to 3.92 g. per ml. and particle sizes from 300 to 600 microns. Since these values are of much higher order than what

are encountered in spray drying, the terminal velocities are of much higher order, 5.5 to 16 ft. per second. Heat transfer coefficients of such particles, heated as clouds in a vertical cylindrical furnace having a wall temperature of 858°F., were found to be in the range of 45 to 55 BTU/(hr.) (sq. ft. of particle surface) (°F.). These authors conceived the heat transmission to take place not by radiation, but principally by convection from the wall to the gas and from the gas to the particles.

Marshall and Seltzer (1948) show curves relating time for evaporation of pure liquid drops to the initial diameter of the drops and demonstrate the effect of various average temperature differences between the drop and its surroundings. These drying time curves should be useful for approximation in cases of materials forming very porous particles and for drops with low solids content and no tendency to case-harden.

Rapid mixing of the spray with the drying gases is important for some food materials in order to insure evaporative cooling of all the particles at the same time by all the air. In some wide towers having straightline flow of air the particles leaving the primary drying zone encounter an annulus of much hotter gases and may, without the benefits of evaporative cooling, tend to undergo some flavor deterioration. A milk product having a subtle developed flavor was found to be impaired by drying in such a dryer, although for numerous other less sensitive food materials this dryer was perfectly satisfactory. Rapid mixing alone in such a case is not sufficient to insure non-impairment of a sensitive material.* If such mixing occurs in an inverted cone and the spent air is withdrawn through the top, as in a cyclone collector, the large particles are thrown to the wall and are drawn off at a bottom outlet equipped with an air-seal device (e.g., in the Swenson dryer). A large number of particles however, are carried upward through the center of the cone by the emitting gases and are carried into a wet collector for recovery (up to 5% by weight). Such material is exposed to a reheating and redrying such as may impair solubility and flavor. Also, in being carried upward through the tower some of the small particles are engaged by the fresh spray and are again carried into the descending spiraling gases. Such action, although resulting in good thermal efficiency, may produce high product temperatures (up to 160°F.) and a non-homogeneous flavor. These remarks allude to special materials of unusual heat sensitivity and

* In some dryers the hot air is made to circulate in direction counter to the rotation of the sprayed particles, the intention being to effect more favorable mixing. In view of the fact that the particles reach the terminal velocity within only a few inches of the atomizer, it is questionable whether such counter rotation of air is of any importance.

are not intended as adverse criticism of certain such dryers that have operated very favorably on a wide variety of food products.

Some dryers, such as the Turbulaire, dry effectively in relatively small chambers by imparting exceptionally high turbulence to the hot air and also by atomizing to very small particles from high-speed, wide-diameter disks. In addition to the formation of very small particles, which may be a limitation for certain types of food products, this particular dryer recirculates part of the hot air through an outside duct and fan, which practice exposes some of the smaller particles to repeated heating as well as resulting in shattering by the fan. The main exhaust fan is sometimes installed between the chamber and the product collector which again has a hammer mill action in shattering the particles.

In the selection or design of a spray dryer for handling heat sensitive materials, considerations such as the recirculation of fine particles or their excessive travel in hot air should be regarded as importantly as air temperature and product collection efficiency. In some soap dryers employing counter-current air flow the fine particles may be exposed to the hot air several times as long as heavy particles in being carried upward and through two or three collectors. Use of such dryers would preclude satisfactory results with, say, orange juice powder where the product collected in a secondary separator is frequently below standard in color and flavor.

In horizontal co-current dryers, satisfactory mixing may be achieved with straight line air-flow by using several air orifices with fine nozzles, as in the Rogers dryer, or with circulatory air flow induced by tilted vanes in a single large orifice, as in the Merrell-Soule dryer. In both dryers the rectangular chamber serves as a collection box for most of the powder, the major propelling effect of the air dissipating itself before the air leaves through ample outlets in the rear of the chamber (through long cloth sleeves that serve as filters ahead of the outlet duct). A characteristic of both dryers is the variable trajectory of the particles, some being deposited almost below the nozzles and others being carried by the air stream to the far end. It would seem that some classification of particles occurs in accordance with size, weight (density), and position in the spray cone and that such classification might not be related to optimum drying time. Manufacturers of the Rogers dryer claim, however, that the particles remain air-borne until they are completely dried. Both dryers have operated satisfactorily with milk, citrus juices, and malt diastase, even though the latter two are heat sensitive and exceedingly hygroscopic.

4. Bulk Density

Attempts to control or vary bulk density of product (weight per unit volume) by a seemingly intelligent approach have often proved futile because very few spray dryers are designed, intentionally or otherwise, to provide much flexibility. One important reason is that although particle sizes change as dryer conditions or feed liquid conditions are altered, the distribution of particle sizes may be such as to result in only small or minor change of bulk density. Philip (1935) relates, as an example, the drying of a saponin solution having abnormally low surface tension. At low atomizer speeds the dried particles are spherical and solid. Increasing the disk speeds up to a critical point produces smaller but solid particles and the bulk density remains constant. At a critical speed larger, hollow particles are formed. From this point on, increase of atomizer speed produces lower bulk densities, the effect being then inversely proportional to the atomizer speed. The particle size may increase until it becomes a fragile hollow sphere which shatters into fragments. Reavell (1928) showed that at such a condition the bulk density then tends to increase because the particle fragments can pack more densely (Fig. 33).

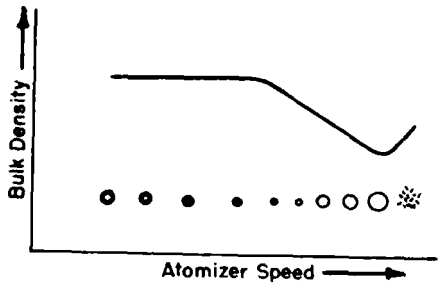


Fig. 33. Bulk density of saponin powder dried from solution; atomized at various bowl speeds in the Kestner spray dryer.

This curve had been published originally by Philip (1935) showing the slope to the left but with an arrow pointing downward along the ordinate. It has been established through correspondence with the Kestner Evaporator and Engineering Co., Ltd. (Mr. D. A. Johnson) that the ordinate scale, bulk density, increases conventionally in the upward direction and that the arrow direction was originally incorrect.

Philip states that ordinary solutions may not exhibit such a wide range of behavior within the attainable range of atomizer speeds. Some solutions may produce only solid particles, and others may not be dryable when attempting to obtain other than hollow particles by reducing atomizer speeds. With some liquids, spherical particles persist even at the

upper limit of atomizer speed. Suspensions do not necessarily follow the same trend as solutions. The Kestner Co.* reports that in their experience comparatively few materials tend to form hollow spheres; for instance, inorganic salts do not normally give this type of product. Hollow sphere formation, they have found, is usually encountered with materials of a rather elastic nature such as soap, although tannin extract and coffee extract also give rise to hollow sphere formation.

The Kestner Co. (Leaflet No. 264) has apparently devoted careful study to achieving a specified bulk density, and they claim that a very light powder can be produced without difficulty and that in most cases a dense powder can also be obtained with the Kestner Patent Spray Dryer. Most solutions have been found to follow these rules:

1. Increase of atomizer speed decreases bulk density.

2. Increase of solids in solution increases bulk density. For a soap solution having equal parts soap, soda ash and water, Reavell (1944) of the same company found, however, that the bulk density of the spray dried powder increased linearly with increase in atomizer speed (Fig. 34),

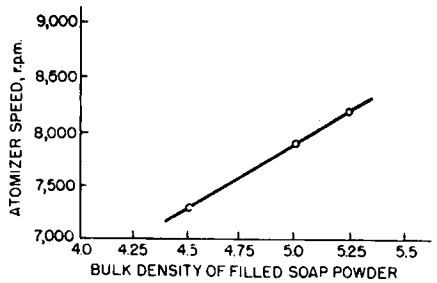


Fig. 34. Relation of bulk density of filled soap powder to speed of rotor when atomized (Reavell, 1944).

a case which appears to be an exception to the above rule. We are informed by the Kestner Co. that the atomizer speeds used by Reavell were below those at which hollow spheres are formed.

The present authors have found that food solutions or suspensions can be dried under conditions where the bulk density is inversely proportional to the atomizer speed (Fig. 35). Less flexibility was found with pressure nozzles. In one case, a food solution was treated with "Naccanol," a surface tension reducing agent, so that a very marked decrease was found in tensimeter readings. The treated solution produced powders of the same bulk density as the control solution when dried under identical

* Communication from Mr. D. A. Johnson.

conditions and with a variety of nozzle sizes. Using a centrifugal atomizer for the untreated solution, a given disk was varied widely in speed to produce only minor changes in bulk density, although the bulk density did appear to decrease slightly with atomizer speed. Changing the disk to one of identical design but with wider diameter appeared to displace the bulk density curve slightly upward and to the left.

The curve shown in Fig. 35 is for an aqueous food extract having 40% solids that was dried by the authors using two different centrifugal

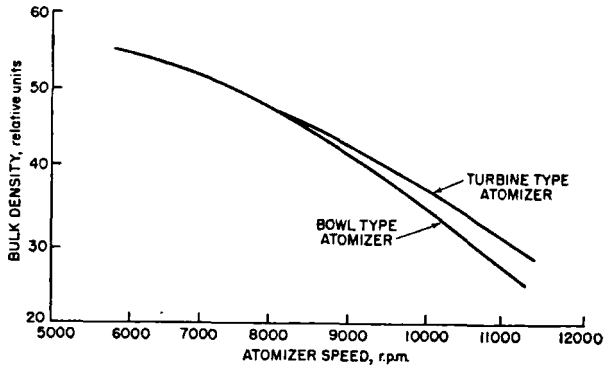


Fig. 35. Variation of bulk density of food solution "Y" with change of atomizer speed. Type of rotor is shown to influence bulk density of the same material at the same rotor speed.

atomizers, all drying conditions being constant. This curve resembles part of the curves shown by Philip (*loc. cit.*) and Reavell (*loc. cit.*), but increasing the speed of the disk to the highest limit did not produce shattered spheres with a resulting increase in bulk density. However, hammer-milling of the product increased the bulk density.

In their Leaflet No. 264, the Kestner Co. exhibit a curve for a solution of an unidentified material "K" showing the variation of bulk density with solids concentration of the liquid fed to the atomizer. This conforms with their second rule showing that bulk density increases almost linearly with increase of dissolved solids (see Fig. 36).

Whereas the milk and coffee solutions may be expected to follow this rule, the trend of bulk density is just the opposite for solutions of hydrophilic colloids, such as gelatin, Irish moss, and pectin. Owing to the marked increase of viscosity as concentration increases, the higher solids solutions form large hollow spheres which result in low bulk density. In view of this tendency, an impracticable voluminous product is obtained when spray drying solutions of gelatin (made with gelatins having gel

strengths of over 150 Bloom) at solids concentrations exceeding 15%. This is true for Irish moss solutions exceeding about 3% solids. The rate of solution of such fluffy air-filled particles is exceedingly slow because of poor wettability. Siehrs (1945) overcomes these undesirable properties by mixing about 90% of the spray-dried product with 10% of the original gum solution thus forming a dough. The dough is extruded to form pellets which are then dried on a belt in a tunnel. The dried pellets are milled to produce a dense powder.

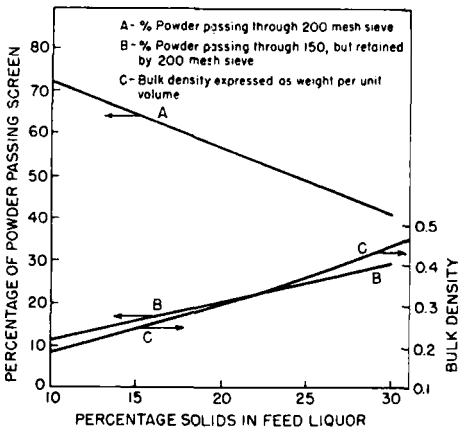


Fig. 36. Variation of bulk density with concentration of dissolved solids. (Adapted from leaflet No. 264, Kestner Evaporator and Engineering Co., Ltd.)

A principle well-known to the chemical and soap industries but apparently little recognized in the food industry is that bulk density is affected by the temperature of the air that the spray first encounters, high temperature tending to produce low bulk densities. This is attributed to the fact that very hot air promotes balloon formation due to rapid drying and case-hardening of the particle exterior and to subsequent expansion of trapped moisture. If low bulk density (or high specific volume) is desired, then care should be taken to direct the hottest air at the freshly formed spray (see Fig. 37). The purest sort of co-current drying such as is achieved by introducing the hot air from directly above or below or behind the atomizer should produce the most extreme results. Dryers of Bowen Engineering Corp. and Instant Drying Corp. are designed to achieve very high inlet gas temperatures, 500°F. or higher, by introducing direct-heated air (including combustion gases) directly above the atomizer. Mojonnier dryers having the so-called "air gun" orifice for introducing hot air above the nozzle can probably simulate the bulk

density results obtainable with the aforementioned dryers provided that the air is very hot (as is the case in some Mojonnier dryers which use products of combustion of natural gas) and provided that the hot blast effect is not diluted too much by the practice of introducing part of the drying air at a lower temperature through a bustle surrounding the top section of the dryer.

The practice of introducing hot air through a bustle duct and through tangential jets or louvres at about atomizer height (as in the Swenson

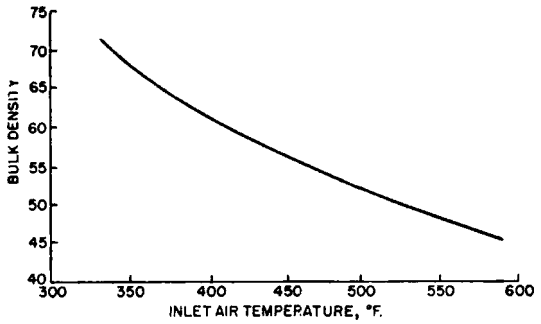


Fig. 37. Variation of bulk density of food solution "X" spray dried with various inlet air temperatures. (Bulk density is shown in relative units.)

dryer) is not purely co-current since the spray must traverse areas of relatively cool and partially spent gases before it meets the hottest air. The Rogers dryer and the Merrell-Soule dryer have nozzles inserted directly in hot air orifices, although the gas temperatures employed are relatively low. These three dryers are popular in the milk powder industry where a granular product is frequently preferred to feathery, fluffy particles. Precondensing of milk to 20% solids or higher prior to spray drying is not only more economical from the standpoint of fuel and power consumption than spray-drying fluid milk directly, but it also makes possible the spray drying of the milk to a more dense and more rapidly-soluble powder. Hunziker (1926) reports that the dense powder made from condensed milk is less subject to entrainment and is recovered more readily. He also reports other measures for increasing milk powder density such as by increasing nozzle orifice diameter, reducing fluid pressure, and "superheating" the milk (heating at 212°F.). Such heating reduces the solubility of the product. Precondensing is said to affect the solubility to only a negligible extent provided that the forewarming and condensing temperatures do not exceed 160°F. The present authors have

also found that increasing the temperature of most food solutions of the non-hydrophilic type fed to the dryer increases bulk density.

For a fixed inlet gas temperature there appears to be a tendency toward lower bulk density when the outlet gas temperature is high also. When the outlet temperature is lowered for a fixed inlet gas temperature the moisture content of the product rises and bulk density tends to increase.

Lamont (1929) shows data for the increase of average particle size and the decrease of bulk density when gas temperatures are raised in the drying of gelatin, malt sirup, soap and sodium silicate. The spray drying was done in a tower where hot air (335 to 490°F.) was introduced directly above the atomizing nozzles, conditions that are conducive to the formation of hollow spheres and low bulk density. He also shows that increase in temperature of soap fluid markedly increases the bulk density of the dried powder. It will be noted that the fluids dried by Lamont are of the hydrophilic colloid type that increase rapidly in viscosity with increase in solids concentration. As should be expected with such solutions, increase of solids in solution decreased the bulk density of the powder. For example, a 20% gelatin solution produced a powder having a density of 0.055 g. per ml., whereas a 10% solution produced a powder having a density of 0.065 g. per ml.

Whereas fine atomization as a general rule will create small particles and accordingly produce high bulk density, there are cases where the conditions favoring fine atomization (such as increased disk diameter and increased disk speed) tend to produce lower bulk density. This is true when high inlet gas temperatures are employed, and it is probably caused by particle inflation resulting in the formation of large thin-walled spheres.

Relatively low air temperatures in the atomizing zone will favor high bulk densities and the formation of solid spheres. A means employed in the soap industry to accomplish this without the sacrifice of thermal efficiency is drying by counter-current flow of hot air (Burkhart and Marceau, 1939). In the case of certain foods that must be dried to low moisture contents this method would present the hazard of over-heating the product. As the particles settle within the soap tower, generally a very tall one, some overtake slower particles or encounter other particles being carried upward by the air. Collisions result in agglomeration which may be objectionable for many food products although not disadvantageous for some laundry products.

Any means of filling voids or interstices between spray dried particles or fragments will tend to increase bulk density. Hammer-milling of part or all of the product as it leaves the tower is sometimes resorted to in

order to increase bulk density. Helical or cut-flight conveyors are known to have been used for shattering large hollow spheres. A principal explanation for the relative non-flexibility of pressure nozzles in permitting adjustments of bulk density is that regardless what orifice sizes are used, the range of particle size is so wide that there is generally abundant dust or fines to sift into the interstices.

Vapors entrapped in the fluid being atomized decrease bulk density. The practice of vacuum concentrating food solutions before drying results in the reduction of bulk density of the powder produced therefrom. Centrifugal atomizer disks that pump air (especially when running below capacity) may possibly have an influence on product volume, although the authors have not found the effect to be of as noticeable magnitude as when air is introduced by agitating the fluid prior to drying.

Larcombe (1947) attributes to pressure nozzles that impart rotary motion to the liquid as it passes through the jet, the tendency to produce low density powders by virtue of the hollow structure of the particles. In accordance with the photographic evidence presented by Fogler and Kleinschmidt (1938), he describes the predroplet stage as a thin, cone-shaped filament or sheet which, because of turbulence, builds up waves that curl back and form hollow tubes and subsequently break down into hollow spheres. Pressure nozzles that do not impart rotary motion expel the liquid in threads, which break down into the more solid particles characteristic of high density powders.

In the previous section on nozzle atomization with two-fluid nozzles it has been shown that drop diameter decreases as the relative velocity between the gas stream and liquid stream increases (or D is inversely proportional to v). Variation of the velocity of air passing the atomizer should, therefore, be expected to be of some influence on bulk density. High air velocity would be expected to operate to create small particles and result in high bulk density, unless the fluid is of the type that produces inflated particles when engaged by air streams having high temperature.

IV. Product Recovery and Handling

Three types of powder collectors are in general use to separate spray-dried products from drying air. These are the dry centrifugal (or so-called cyclone) collector, bag filters, and wet scrubbers. The selection of a dust collector or combination of collectors will depend on such factors as specific gravity and particle size of product, whether or not a high proportion of product is collected at the base of the drying chamber, and the effect of various types of collectors on product quality.

The usual dry cyclone collector originally adopted from such units as used by the wood-working industry (sawdust and shavings collectors) offers the advantages of lower first cost, design which is fairly well standardized, and the utmost simplicity in operation and maintenance. Such collectors are roughly ten feet in diameter in the 10,000 to 15,000 c.f.m.

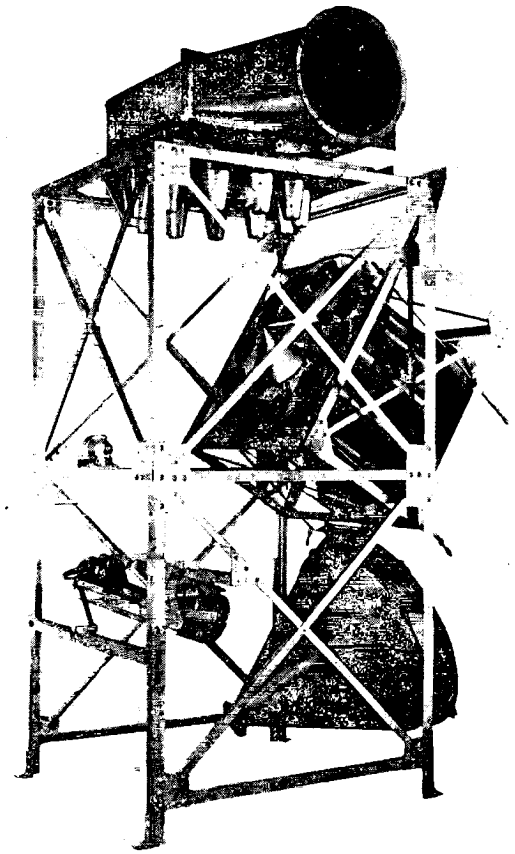


Fig. 38. Multiclone collector of demountable type shown in opened condition for cleaning. This type is recommended by the manufacturers for use in food plants. (Courtesy of the Western Precipitation Corp.)

capacity range. The efficiency of such collectors is apt to be poor on light density food products, with losses on particle sizes less than 40 microns being particularly high. These losses have been reduced on certain installations by the use of relatively small diameter collectors operated in parallel.

The Western Precipitation Company of Los Angeles, California, manufactures the "Multicone" unit (see Figs. 38 and 39) which features numerous parallel operated collectors of unusually small diameter. The maker claims very high recovery on particles as small as 5 microns (1 micron = 1/25,400 inch). Manufacturers' catalog files should be consulted for information on the numerous collectors available. However, another example of "Multiple-parallel" collectors is the unit manufactured by the Buell Engineering Company of New York. Although this collector does not in general incorporate the extremely small diameter of the Western Precipitation unit, it employs the patented Van Tongeren "shave-off" principle which, according to the manufacturer, increases the efficiency of a collector of given size considerably. In part, the principle consists of admitting the dust-laden air through a tapered scroll curved around the cylindrical section. Another feature is the by-pass channel

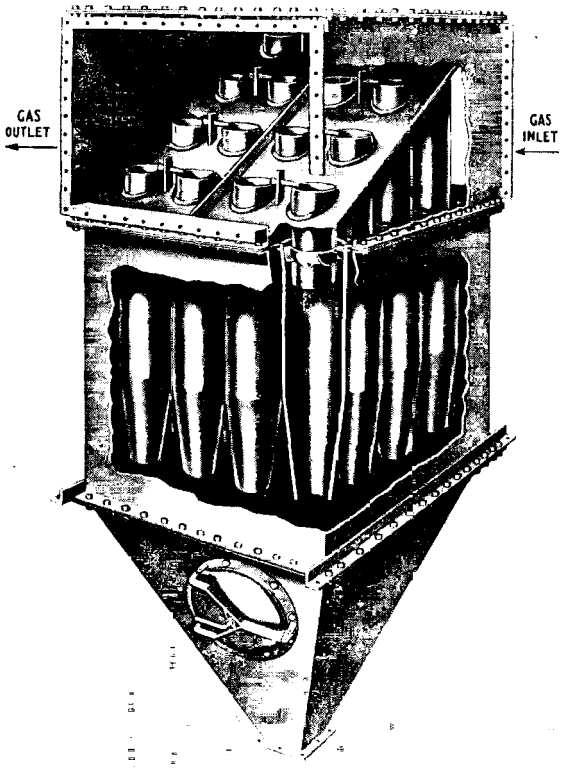


Fig. 39. Western Precipitation Corp. Multiclone collector showing multiple parallel-operated units.

forming a box-like appendix along the vertical side of the cylinder which is intended to "shave-off" dust reaching the walls and short-circuit it to the cone.

All parts of a food spray drying system should be readily accessible for inspection and cleaning; in this connection, collectors should not be overlooked. When small-diameter collectors are selected, the "take apart" and accessibility features of these units become especially important. This is due to the tendency of these units to clog more easily than equipment of large diameter. Moreover the designer should not overlook the increased pressure drop across small diameter collectors when he selects fans and determines power requirements. Many variables relating to the product are encountered by the collector designer. Whenever possible, a proposed design should be performance tested on the actual product to be recovered before a recommendation becomes final. The collection efficiency of units of various diameters indicated by Anderson (1941) is believed to be representative of published information.

The following observations by the authors are indicative of striking differences in efficiency on collectors of different diameters. A spray-dried food product having an apparent bulk density in the order of 0.20 g. per ml. and containing up to 25% by weight of particles under 20 microns in size was manufactured in two separate plants. Slight differences in processing up to the point of collection is not believed to account for the considerable difference in recovery. In one plant four parallel collectors, each handling approximately 2500 c.f.m. were used. These collectors were of somewhat special design (Sirocco-type manufactured by American Blower Corporation), relatively tall, and about 20 in. in diameter. Recovery efficiencies in the order of 97% were achieved. However, it was necessary to employ chains, suspended full length and allowed to rotate around the inside of the collectors, to prevent "bridging" of product at the base of the units.

In another plant two large collectors about eight feet in diameter, handling about 14,000 c.f.m. were employed. These units were connected in series—one followed by the other in the air stream. Overall recovery was in the order of 90% (only 3% recovered in second collector). The recovery of even the 3% lost in the first case cited, would, in terms of annual volume, easily pay for an elaborate scrubber or bag filter installation in less than a year. Mutilples of smaller diameter collectors were not suggested because much of the material lost varied in size from 4 to 7 microns—deemed small for any dry cyclone collector—and severe operating difficulties due to plugged collectors were anticipated. Satisfactory second stage recovery of the particular product by either wet scrubbers or bag filters has been achieved.

In a large proportion of food spray-drying plants, consideration has been given to either wet scrubbers or bag filters in place of or to augment the recovery obtained with cyclone collectors. Electrical precipitation has been suggested as an alternative but so far as is known to the authors no installation is in use in connection with a food spray-dryer. Overexposure of heat-sensitive materials to the air temperature through the electrical precipitator while product accumulates on the charged plates has been given as one objection to this recovery method. High initial cost for the large volumes of air involved is another objection to electrical precipitation.

The choice between wet scrubbers and bag filters is usually made on the basis of a number of factors. Both types of equipment have enjoyed long and successful use with food spray dryers. A recovery of 95-98% of all solids fed to either type of unit is expected for adequately sized, well-designed apparatus. Scrubbers or bag filters are often employed as secondary collectors after dry cyclones. The choice of a scrubber on the standard Swenson installation illustrates the advantages of dual purpose where the scrubber not only recovers some of the product but also improves the system thermal efficiency by employing the spent dryer gases to build up concentration of feed liquor for the dryer by evaporation in the scrubber. Whether or not wet scrubbing has an adverse effect on product quality should, whenever possible, be determined by obtaining reliable experimental data before purchase of equipment. It has been found difficult to prevent growth of microörganisms in certain installations (e.g., with egg powder) inasmuch as conditions of temperature and moisture in a scrubber may be almost ideal for such growth. Heating in the scrubber recirculation system has been thought to insolubilize a small portion of certain spray-dried products. Utmost care should be employed to provide scrubbers of completely sanitary design with the absence of cracks, corners, or ledges where product can possibly deposit. The purchaser should be certain that all parts of a proposed scrubber are readily accessible for easy and thorough cleaning. Steam connections to strategically located orifices within the scrubber are sometimes employed to provide assistance in cleaning and sterilizing these units. Pasteurization of solution from the scrubber is sometimes required.

The present authors have experimented with various types of scrubbers including the packed column and bubble cap variety. In these the essential idea is to bubble the air, carrying recoverable dust, through one or more films of water. On a particular product, pilot units of these types failed in their purpose due to losses caused by intense foaming, the foam with product in solution being carried out of the unit. The open type of simple centrifugal scrubber, e.g., the Swenson, or centrifugal multi-stage

type, e.g., the Ducon (see Fig. 40), are relatively free of losses due to foaming since their operation is foam-breaking rather than foam-making in character as a result of the centrifugal cyclone effect.

If scrubber treatment has no deleterious effects on a product, the evaporating capacity of such a unit in terms of overall plant evaporating requirements should not be overlooked. It is common practice to re-

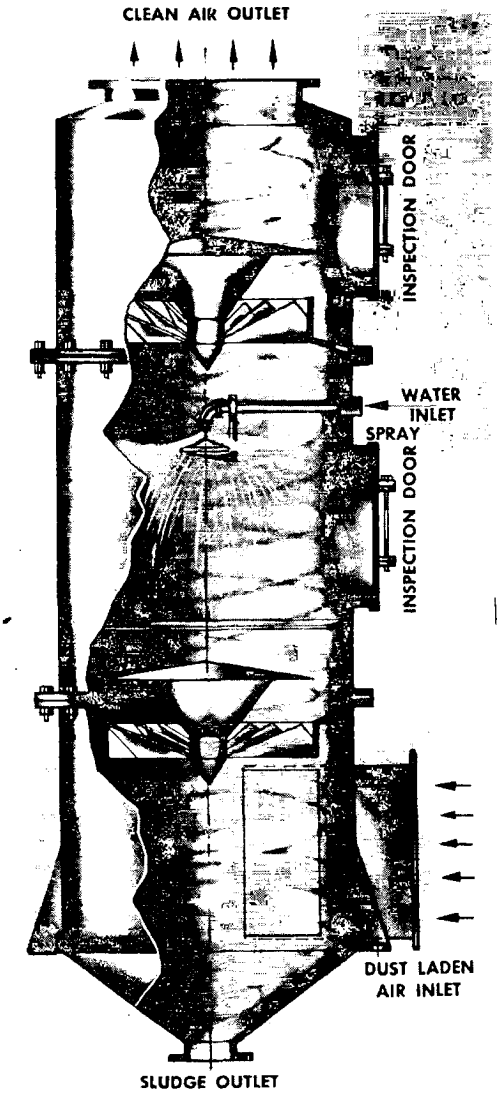


Fig. 40. Centrifugal scrubber collector, multistage type. (Courtesy of the Ducon Co.)

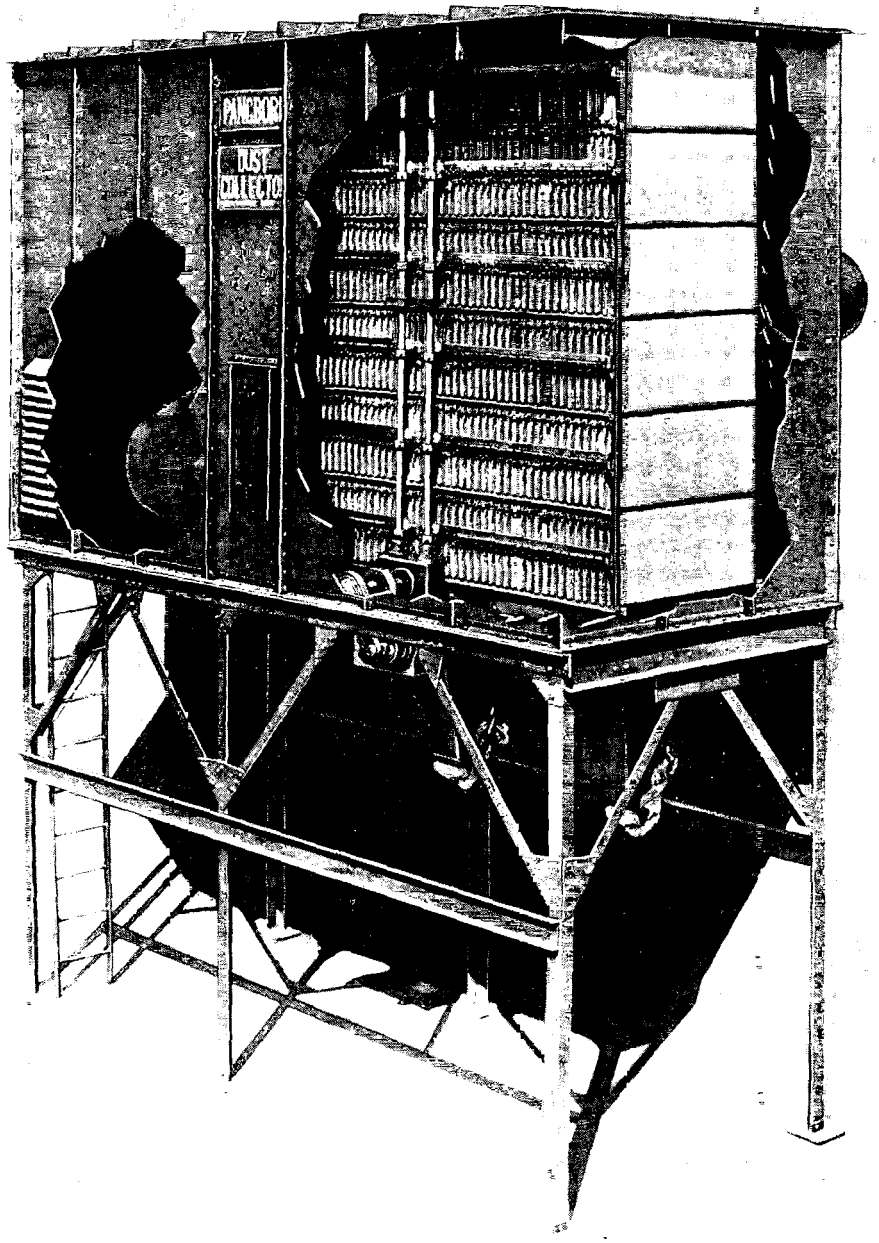


Fig. 41. Cloth screen dust collector of steel frame construction. Phantom view is of clear air side. (Courtesy of the Pangborn Co., Hagerstown, Md.)

circulate scrubber solution. The pumping rate for such solution generally varies in a range of 1 to 16 g.p.m. per 1000 c.f.m. of air handled.

It is desirable to locate the scrubber at the air exit of the spray dryer system. A short, straight discharge connection from the scrubber through the roof is widely favored since cleaning difficulties, condensation on discharge ducts, and expenditure for corrosion resistant duct-work are thus minimized.

Scrubbers employed for recovery of insoluble industrial wastes or by-products are commonly provided with open top "sludge" tanks. Material settles to the bottom of one compartment and solution overflows to another for recirculation to the washer. Such tanks allow foam to release and provide head on the suction side of pumps. Float valves then readily control makeup water requirements. However, such open tank arrangements are not recommended for most applications in the food industry. Contamination is easily introduced with such equipment. Even a small head of foam can remain undisturbed for days at a time in such a tank thus allowing microörganisms to multiply. Instead of an open tank, a small reservoir or catch basin constructed as an integral part of the base of the scrubber is favored.

The use of a bag filter installation is illustrated and discussed in another section dealing with the Rogers dryer. This bag filter arrangement is an integral part of the Rogers dryer which is rather widely employed in connection with drying of milk products. The W. W. Sly Manufacturing Company produces a bag filter wherein the filter units are of the flat screen type. The operation of flat or tubular units is similar.

The cotton bags in general use thus far have maximum temperature tolerance in the 160-180°F. range, above which their operating life is shortened. Bag material other than cotton cloth, furnished in units by the American Wheelbrator Company of Mishauaka, Indiana, withstand over 275°F. temperatures according to the manufacturer.

The Pangborn collector of the type illustrated in Fig. 41 has been in successful service as a final collector on soluble coffee for several years, according to the Pangborn Company of Hagerstown, Maryland.

There are apt to be up to 1000 filter bags on a food spray dryer installation in the 1500-pound per hour evaporation range. The bags are mechanically shaken on a predetermined time cycle. The product usually falls into hoppers and is blended continuously with materials from other parts of the system. It may be necessary to heat or dehumidify the bag house during shutdowns to prevent plugged bags in the event conditions fall below the dew point temperature. If pre-shrunk cotton or other material is employed for bag construction, laundering should be feasible, should it become necessary.

The effect of either bag filters or a wet scrubber on product quality and yield should be checked on a test unit before purchase, whenever such tests are possible and practical.

A dust collector of unique design (the "Aerodyne") is shown in Fig. 42. This unit is manufactured by A. B. Rosenblods (Pantenter) of Stockholm, Sweden. The manufacturer claims high collection efficiencies and cites a long list of European concerns claimed to employ the device for collection of food dust after spray drying. Milk, blood, and glucose are among the products named. Operation of the unit appears to depend on the greater mass of material to be collected—due to its momentum—not making the abrupt turn followed by the air in passing through the fins of circular Member A. The authors have had no experience with such a collector. However, the manufacturer's description of its operation follows:

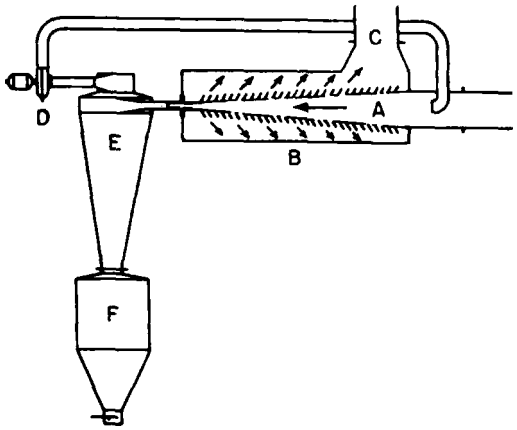


Fig. 42. Aerodyne dust collector, showing principle of operation. (A. B. Rosenblods, Stockholm.)

The filter element A, which is conically shaped in this case, is built into a housing B. The dust-laden air, which enters the filter through the wide end of the cone is cleaned just before passing through the apertures in the cone and finally escapes through the outlet C in a cleaned condition. The dust separated out inside the cone is swept away by the air current to the apex of the latter, from whence it is withdrawn, together with a small amount of air, by the fan D. A secondary separator E with a receptacle F is connected between the cone apex and the fan D. Dust not collected in the secondary separator is carried back to the filter inlet and thus has to pass through the system again, in some cases repeatedly, until its separation from the circulating air has been completed.

The filter cone A is readily accessible and can be removed by hand quickly and easily.

The area required for a filter of this design is only about 2% of that necessary for a cloth filter of equal capacity.

V. Product Cooling Devices

After a heat-sensitive, thermoplastic product such as molasses is dried, cooling may be required to enhance keeping quality and prevent the material from lumping in the shipping container. Once the product is separated from the humid air of the dryer, water jacketed screw conveyors have been found to be fairly satisfactory as coolers. Power and cooling water requirements are nominal. It is believed that the better designs of such coolers are of the open or "U" shaped type with well-gasketed, tight-fitting covers. Slip-on clips are preferred to screwed fasteners for facilitating removal and cleaning, in order to keep these covers securely in place.

One alternative method of product cooling is to employ conditioned air in a product conveying system. Under favorable climatic conditions and product characteristics, atmospheric air may suffice for cooling in such a system. However, if conditioned air is considered, contact cooling in a jacketed screw conveyor is suggested as a less expensive alternative. If, for reasons of plant layout, the product is air-conveyed for a distance of several floors, such an air conveyor may in itself provide sufficient product cooling.

VI. Heat Supply

Three general types of heaters can be utilized to supply the heat for spray dryers. Steam coil heaters placed in the supply air duct, as illustrated in the section describing the Swenson dryer, have been employed. Secondly, there is the direct-fired indirect heater, as illustrated by the Ross Engineering Company unit in Fig. 43. The selection of fuel for such an indirect unit is not governed by spray dryer problems but is generally motivated by the same considerations governing the selection of industrial fuel in any specific geographical location. Thirdly, there is the direct fired heater discharging the products of combustion directly into the supply air stream to the spray dryer. For example, the Ross unit becomes such a heater if the heat exchanger at the top of the unit (Fig. 43) is omitted.

Surprising as it may seem to those not familiar with spray drying practice, many delicately flavored materials such as tomato juice and lemon juice have been successfully spray-dried with heat from a direct fired furnace. The milk producers generally seem to favor indirect heating. However, at least one plant operating on a dairy product has, as far as it is known to the authors, experienced no quality difficulty by the use of direct firing (Corbett, 1948). The fuel saving of direct over indirect heating is ordinarily considerable. If a direct-fired furnace is em-

ployed, combustion should, of course, be complete and without a trace of smoke. A "warm-up" or auxiliary stack should be provided so that the furnace can first be brought up to temperature. Then the products of combustion (flue gases) can be diverted to the spray dryer after junction with the main volume of drying air. Fuels for food drying—by direct heat—are generally limited to natural or manufactured gas or no heavier

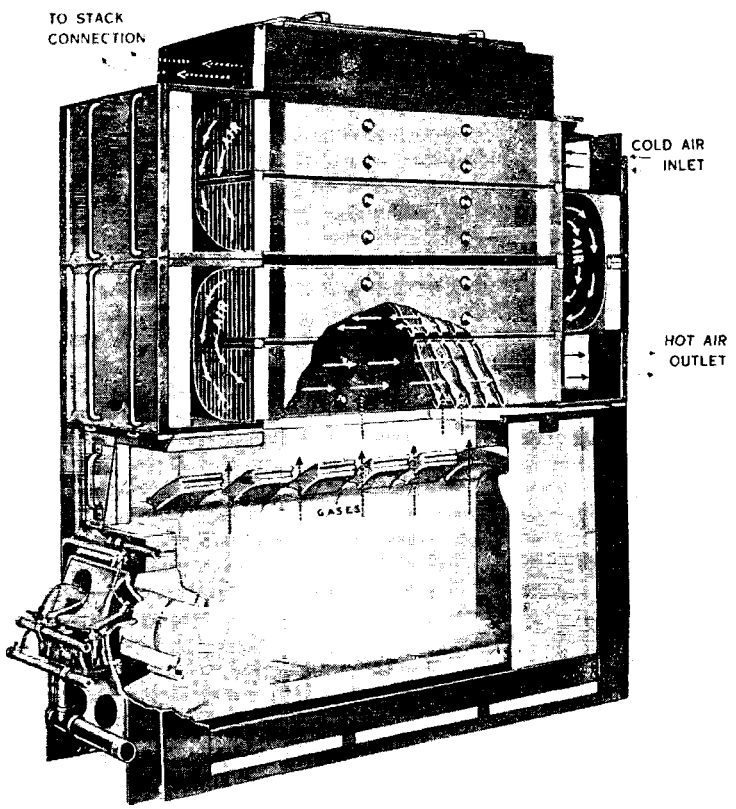


Fig. 43. Ross Engineering Corp. indirect heater fired by oil or gas.

than No. 2 furnace oil. Heavier oils have, however, been employed for drying tannins and industrial chemicals.

In furnaces for food drying, it is of the utmost importance that complete combustion take place in the combustion chamber before the products of combustion are discharged to the supply air. For example, Greene *et al.* (1948), discovered that burning natural gas directly in the supply air stream (no separate combustion chamber) of an egg spray dryer may

have produced formaldehyde due to incomplete combustion which reacted with the protein of the eggs, producing off flavors and insolubility. When a combustion chamber was provided and the products of complete combustion were delivered to the air stream, this difficulty was eliminated.

The matter of furnace design is one for well-qualified experts since numerous technical and experience factors are involved. For example, if the combustion chamber is too large, the furnace will have a tendency to create smoke. If it is too small, capacity limitations and rapid destruction of refractories are to be expected. The quantities of primary and secondary air must be carefully proportioned. Adequate insulation between refractory and furnace shells must be provided. Skilled workmanship, particularly on installation of refractories, is of no small importance.

It is sometimes economically advantageous to utilize existing plant steam capacity to heat dryer supply air in the range below 350°F. and then to provide either direct or indirect-fired heaters for the balance of the heating requirement. Inlet temperatures of 500°F.-600°F. are sometimes desirable to provide proper product density and higher thermal efficiency in the drying operation.

The furnace or heating unit should be located as near as possible to the dryer inlet. Long supply ducts, even though well-insulated, are likely to introduce high thermal losses, according to data on existing dryers.

1. Industrial Burner Types

The direct fired oil furnace generally employs either horizontal rotary burners or some form of industrial oil burner which utilizes steam or compressed air for atomization. The horizontal rotary burners are similar to those used on industrial boilers with the exception that lighter grades of furnace oil are employed, thereby obviating the necessity of such devices as preheaters and viscosity compensating valves. The steam or compressed air atomizing burners do not require high speed motors or other moving parts and are manufactured by Hauck Manufacturing Co., National Airoil Corp. and others. The standard natural gas burners successfully used in power plant work are adaptable for use in direct fired furnaces but proper combustion must be provided as previously indicated. Among the manufacturers of gas burners are National Airoil Corp. of Philadelphia, Gehnrich Co. of New Haven, and the Hauck Manufacturing Co. of Brooklyn. The latter company issues a very useful manual entitled "Industrial Combustion Data," wherein are presented classifications of oil and gas burners, showing the air and power requirements involved.

2. Safety Devices

Fires and explosions have been reported in spray dryer operations. It is, therefore, important that the designer consider safety as an integral function of any installation—not as an afterthought. A few of the obvious precautions will be outlined here. However, every plant has safety requirements peculiar to its own operation. The purchaser of equipment is, therefore, urged to require the sanction of his insurance service before approval of plans is permitted.

a. Explosion Vents. A combination of certain circumstances can render some food dusts explosive if ignition is provided by static discharge or otherwise. If such an explosion is improperly confined, the results can, of course, be disastrous. Hence, explosion vents are a desirable feature of many installations. One large insurance underwriter suggested 1 sq. ft. of venting area per 20 cu. ft. of dryer volume for safe operation. However, this factor may vary on different installations. Explosion vents generally consist of light weight panels held in place only by their own weight (on top of dryer) or panels secured by spring latches or other approved fasteners at the sides of the dryer.

b. Sprinklers. Fire extinguishers, in the form of water nozzles capable of handling large volumes of water, are sometimes installed in the upper portion of the dryer. Such nozzles have long been a feature of certain chemical dryers and are generally controlled manually.

c. Purging of Explosive Mixtures. Controls for automatic mandatory pre-ventilation of a dryer before start up are usually required by insurance companies particularly where a direct-fired system is employed. Such pre-ventilation is designed to provide several complete air changes in the drying system, thus guarding against explosion of accumulated materials involving the product or, for example, a mixture of combustible gas leaking from a main gas burner or gas pilot.

d. Flame Reversal at Furnace. If, for any reason, the main dryer fan (or fans) fails to continue in proper operation on a direct-fired system, flame and smoke can readily be discharged to the furnace room through secondary air openings. The authors have seen this happen and can testify to the seriousness of such a condition. Preventive measures approved by insurance companies generally involve air flow switches which cause fuel and burner shut down in the event of a fan failure.

e. Excessive Dryer Temperature. A high temperature limit switch at the dryer inlet or heater discharge is a common provision to prevent temperature from exceeding a predetermined setting. This control functions to shut off fuel and burner and to lock them out of operation, following

which restarting the dryer requires manual resetting, purging, etc. This control is again particularly desirable on direct fired systems.

f. Protection of Equipment. The requirements for protection of equipment will obviously vary greatly from one installation to the next. A typical spray drying system may have a "draw-through" system with the principal fan after the dryer and collectors. The inlet duct may provide substantially constructed shut-off dampers, involving the warm up stack or cleaning routine. If the fan is operated with such a damper closed, dryer and collection equipment may be in danger of collapse by the vacuum created. Hence, it is not only wise to instruct operators but also to provide positive lock-out switches to render fan operation impossible unless all operating conditions are in order. There are many other protective considerations such as those involving automatic shut-off of feed solution to the dryer, cooling and lubrication of critical equipment, etc., details of which are beyond the scope of this article. Casualty insurance experts can frequently render very valuable assistance at no extra cost to concerns covered by them.

VII. Materials of Construction

Stainless steel has been a favorite material of construction for food spray dryers in spite of its cost, which is considerably higher than plain steel (with protective coating) or aluminum. Stainless steel may not be an absolute requirement in all food dryers. The dairy products industry has found it advantageous in the liquid phases of its operations and can probably justify it for its spray dryers, but eggs have been dried successfully in properly coated plain steel dryers. How the coatings (furnished by various manufacturers specializing in finishes for food equipment) stood up in service is not known to the authors. Their availability should not be overlooked when materials of construction are considered, however. It is understood that aluminum has been rather widely used for spray dryer construction in Europe, and it is expected that aluminum should stand up well in connection with the drying of products not destructive to it. Each application should be analyzed on its merits but the use of aluminum may well find greater acceptance than it has to date. Plain steel with a surface layer of stainless, known as stainless clad, has been used for dryer construction and is an excellent material if the stainless side can be obtained with a satisfactorily smooth finish. It is suggested that purchasers of spray dryers should insist on smooth interior finishes at least to the degree known among metal finishers as "2B." Smooth finishes will sometimes assist in minimizing wall deposits.

Inasmuch as a spray dryer and auxiliary equipment may be designed

by those not well versed in the sanitary requirements of a food plant, the design should be carefully checked in this respect.

VIII. Economics of Spray Drying

Although it is true that depreciation of dryer investment, cost of its operation, including labor, offer a large element in production expense along with indirect costs, the cost of raw materials is frequently far in excess of the total of all other costs. This should not be overlooked as a matter of perspective in an economic analysis of a new product. How-

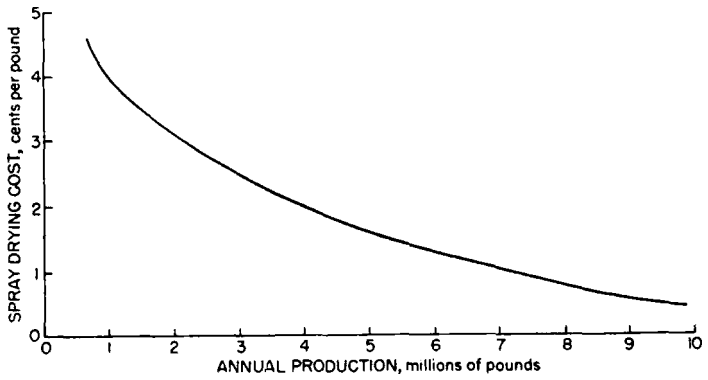


Fig. 44. General cost curve for spray dried food products, showing relation of cost to production volume.

ever, a spray dryer installation producing 1000 lb. per hour of finished product can easily cost upward of $100,000 at the present time. Therefore, the annual production requirements over which such expensive equipment can be spread must be considered. The accompanying cost chart (Fig. 44) is at best an approximation and is presented only for the student or others totally unfamiliar with spray drying costs, to give them a very general picture. The curve is based on preliminary estimates for about a dozen proposed installations. These installations would have involved production rates of 750 to 1500 lb. of product per hour. Direct firing with No. 2 fuel oil or natural gas was assumed. Concentration of feed solution to the dryer was in the order of 50 to 60% solids. Thermal efficiencies in the 40 to 50% ranges were assumed. The costs include equipment amortization, maintenance, fuel and power, labor (including shift differentials) and operating overhead but do not include a building to house the dryer. The unit costs at low production rates could probably be lowered somewhat by the use of smaller simplified dryers. The

curve is based on stainless steel construction; other materials might suffice for a specific product. However, the high unit costs of spray drying a small annual volume of material should not be overlooked.

IX. Control of Product Accumulation on Inside Surfaces of Dryer

One of the fundamental weaknesses of spray drying equipment is sticking of the product to the inside surfaces of the dryer. Poor yields, contamination by overheated product, and production time lost for extra cleaning of the dryer are the results of such wall deposition. Engineering research should be directed to the end that the product never reaches the dryer walls or does not adhere if the walls are reached. The first approach means prevention rather than cure and suggests dividends in terms of smaller, less expensive dryers for a given load, along with higher efficiencies.

Relatively poor, nonuniform atomization is probably at the core of the wall deposition problem for the reason which follows. The typical spray dried product may range in size from less than 10 microns up to 200 microns. These particles probably started out as a mixture of small and large droplets of feed liquor leaving the atomizer. The larger droplets, having larger mass and an appreciable initial velocity, can be assumed to travel farther before drying is complete. If the surfaces of the dryer are hit sticking occurs. Drying temperatures and air distribution are therefore intended—consciously or otherwise—to handle the largest drop size. If drop size and uniformity were under better control the wall deposition problem would be much less troublesome and dryer design itself might be vastly simplified. Such control would also be highly desirable in relation to product quality and density regulation.

Present expedients for keeping dryer surfaces clean, employed singly or in combination, are summarized by the following:

(a) Mechanically driven brushes or rakes

(b) Suspended chains or sweeper blades which brush the walls while being rotated around the dryer

(c) Admission of air through slots at the sides of the dryer, thereby moving product away from the walls and providing a blanket of relatively cool air near the walls.

Mechanically driven brushes are generally unsatisfactory due to loose bristles contaminating the product and due to the fact that such brushes soon become so "gummed up" with product as to render them inoperable. Rotating rakes on the floors of flat-bottom dryers have been employed with success on certain materials by dryer manufacturers such as Instant Drying Corp., Bishop, and Scott. However, experience with these rakes

would not lead to any blanket recommendations for their use. Considerable difficulty was encountered owing to certain spray dried foods sticking between the rakes and the floor with attendant product losses and shutdowns.

The use of chains or sweeper blades as described in the section on commercial spray dryers is helpful on easily dried products such as skim milk. However, such chain-type devices appear to be of little value on products such as citrus juices or sweetened whole milk. In addition to wall deposition, the chains themselves are apt to become heavily coated with product which tends to form lumps that are easily overheated.

The method of admitting room temperature air or tempered air through slots in the dryer walls sometimes tends to reduce wall deposition. The volume of such air can very easily be excessive—causing such a reduction in drying efficiency that wet particles promptly coat the dryer walls and build up heavy product accumulations. The effectiveness of the method in its judicious application to certain products is granted, bearing in mind the loss in thermal efficiency and fan capacity requirements.

A variation of admitting air through slots in the dryer walls is found in the air sweeper used in the Bowen flat-bottom dryer for control of floor accumulations. This sweeper revolves on a center bearing support and is driven by the reaction of air admitted through a slotted trailing edge disposed at a suitable angle to the floor of the dryer.

The present authors have devoted no little effort to controlling side wall deposition. Much of this effort has been toward extension—through entirely independent conception of ideas—of the Bowen air sweep (Settelmeyer, 1949). We term the device an "Air Broom." This device, still in the experimental stage, shows considerable promise. Essentially it consists of a motor driven tube which moves slowly within a few inches of the walls and bottom of the dryer. An external blower unit provides the high pressure air for multiple orifices directed at the dryer surfaces. The orifices must be as near as possible to the dryer walls and so spaced on the tube as to keep all dryer surfaces clean. It was found that cable or angle iron braces should not be employed as the supporting structure on account of heavy product accumulation on such members. Supporting framework, consisting of tubes cooled and cleaned by air (supplied by the main tube), functions well. The product must be kept from all supporting rollers and structural work by means of air jets. The air quantity requirements for a broom in an average sized commercial dryer have been found to be only 10% by weight of the total drying air.

The experimental "Air Broom" was developed in a tall cone-bottomed cylindrical tower 18 ft. in diameter. Some 16 linear feet of cone surface plus 40 feet of side walls are cleaned by the device.

Insulation of the Drying Chamber

Upon first consideration, good engineering practice would dictate the insulation of spray dryer walls in the interests of thermal efficiency. However, it is rather easily demonstrated that metal temperatures at dryer walls considerably exceed the product temperature due to the evaporative cooling effect applied to the product while it is still in the dryer. In the case of thermoplastic heat-sensitive materials such as tomato juice powder the metal temperature of the dryer walls may easily be sufficient to cause serious flavor and color deterioration. In certain cases the insulation has been removed from the dryer walls with resultant decrease in wall deposits when handling products which are either heat sensitive or have a greater tendency to adhere to insulated hot metal. In the soap industry, supply air to the heater is sometimes drawn through a sleeve surrounding the dryer walls. Thus the radiation losses are to a larger degree recovered for use in the dryer than when insulation is employed. The authors have used this method successfully with a food dryer. The combination of insulation removal and air sweeping has been particularly beneficial.

X. Spray Dryer Instrumentation

Automatic control of spray dryer operations, excepting safety devices, are generally confined to regulation of inlet and outlet temperatures and the rate of liquid fed through the atomizing device.

Atomizing pressures, in the case of nozzles, or speed of rotation of centrifugal atomizers—although usually requiring precise setting—are conveniently and satisfactorily controlled by manually set regulators. The automatic control of air volumes through the dryer, although of theoretical or research interest, is rarely justified commercially.

A basic instrument system, essentially non-automatic, is illustrated by the Swenson dryer application. The inlet temperature is held at a predetermined point by regulating the flow of steam to the heater by means of a thermostatically controlled valve. The thermostatic element or bulb is located in the air stream leaving the heater. Once the desired inlet temperature is obtained changes in control settings are rare. In the basic Swenson system the outlet temperature is held to a pre-determined value by manual adjustment of the pressure on the feed to the atomizing nozzle —thus regulating the feed rate to the dryer. A dual pen recorder is generally employed to provide a record of inlet and outlet temperatures. Actual control of outlet temperature is, however, achieved manually.

If product density requirements and concentration of feed solution are

reasonably constant such a system functions quite adequately, offering low first cost with operating simplicity. The habit of frequent observation of temperatures is soon acquired by operators.

In most commercial operations dryer outlet temperature is the governing control factor. Outlet temperature controls final product moisture content, for example. Other factors being equal, dryer capacity is directly proportional to the difference between inlet and outlet temperature. Inlet temperature, as a rule, is easily regulated. Hence fairly close regulation of outlet temperature, just above the point of wet product sticking to the dryer walls, is almost always desirable. In practice, it has been found that non-uniform regulation of outlet temperature can be one of the causes of operating difficulties such as excessive sidewall deposition.

Two methods of automatically regulating outlet temperature are widely employed. Type one: With inlet air temperature held constant, the predetermined outlet temperature governs flow of dryer feed liquid through a suitable flow control valve. Type two: The heat input to the dryer is controlled (by a fuel or steam valve) in accordance with outlet temperature. The inlet temperature, of course, fluctuates with evaporative loads in this second type of system. However, such fluctuations are not troublesome as to product density or overheating under typical operating conditions.

Both types of systems are in very satisfactory commercial operation. In either system it is important, as a safety precaution, to provide a maximum firing rate or upper limit inlet temperature control entirely separate from the dryer control system.

It will, of course, be apparent that the type 1 system (outlet temperature controlling liquid feed rate) can more readily be applied to a centrifugal atomizer system in view of present difficulties in automatic regulation of pressures in the range of several thousand p.s.i. encountered in the use of single-fluid pressure atomizing nozzles. The type two system of controlling heat input by outlet temperature is readily applied to either atomizing system.

The first system is sometimes favored when drying heavy slurries due to the difficulties in regulating the feed rate. The feed rate of heavy slurries can be rather easily regulated to either centrifugal atomizers or two-fluid nozzles.

There are variations and combinations of the basic systems described above. It is intended that a concept of the three systems, in wide commercial use, will provide a point of departure for the reader to evaluate problems peculiar to his own situation.

The fundamental principles of instrumentation, although outside the scope of this work, should be understood by those involved in the selec-

tion and operation of controls. The matter of process and apparatus lags are of particular importance in spray drying. The fundamentals of automatic control, including a discussion of lags and basic controls is set forth in the excellent paper by Peters and Olive (1943).

XI. Humidity Problems

The spray drying process commends itself especially well when it successfully produces free-flowing powders from fundamentally hygroscopic solids. Examples of such materials are fruit juices such as orange and lemon, malt diastase, protein hydrolyzates, tomato juice, ice cream mixes containing sucrose, sweet chocolate, and molasses. Drying aids such as corn sirup solids of low-dextrose equivalent (D.E.), pectin, starch, sodium carboxymethylcellulose, casein (and its derivatives), and various gums are added in many cases to fluids that are otherwise considered undryable in the available equipment or that are prone to cake after drying.

Exponents of spray drying are slow to admit that any food material is undryable; the solution of the drying problem is generally thought to be a matter of using proper drying aids, or developing a special spray dryer. As a rule, dryers have been installed and satisfactory operating conditions established for fair and good weather conditions, and a gamble is taken as to how much interference with production humid weather will cause. In some plants used for drying malt diastase, the managements have found it not feasible to attempt operation at all during the summer months. These are in New York City and San Francisco and have entirely dissimilar spray dryers. In other cases, where the product is less hygroscopic, *e.g.*, skim milk, only extreme humidity conditions have adverse effect on dryability. Coulter and Kitzes (1948) have secured data showing the effect of variation in the humidity of the air to the dryer on the moisture content of the dry milk. They report that "this effect is of little practical importance at humidities below that of saturated air at 80°F., but using saturated air at 100°F. either the capacity of the dryer must be materially reduced or the moisture content of the powder will be increased." These authors assume that during the constant rate drying period the rate is proportional to the humidity difference $(H_s\text{-}H_a)$, where H_s is the saturated humidity at the temperature of the surface of the solid and H_a the absolute humidity of the air. The falling rate period of drying was observed to begin at a critical moisture content which they regard as evidence that the particles attain the wet bulb temperature of the air. Inasmuch as the wet bulb temperature is higher at high humidities, the temperature of the milk particles during the constant rate period would increase with increase in the humidity of the air.

The present authors have developed a conception of the "primary drying zone" in the dryer as that area beyond which the falling rate period or unsaturated surface drying begins. Boundaries of the zone have been found experimentally within dryers by obtaining thermocouple readings and also by actual sampling within the dryer (although the latter method was indicative in only a qualitative way). The distance from the point of atomization at which the air temperature ceased to drop sharply was regarded as the boundary, since the rate of temperature drop is proportional to the rate of drying. In order to produce a non-sticky powder it is necessary that sufficient drying occur outside the primary drying zone to reduce the moisture content beyond a low, critical range. Although, quantitatively, the amount of water vaporized at this falling rate stage is usually proportionately small, the effective drying is actually determined in this secondary zone. Within the latter zone, which is by far the greater volume of the dryer system, the humidity of the air is fairly close to that determined at the exhaust stack. Since the drying rate or so-called evaporation potential at this stage is proportional to the difference between vapor pressure of water within the incompletely dried particles and the partial pressure of the water vapor in the air, the authors have regarded the absolute humidity of the stack gases (or outlet air) as being the controlling criterion of successful second-stage drying. In most dryers the time for passing of the powder through the dryer system is fixed and so the ultimate moisture content of the product must depend primarily on the combination of dry and wet bulb temperatures or, accordingly, on the humidity of the air. An analysis of the humidity conditions for determining or predicting operability involves the use of very simple psychrometry. Such an analysis demonstrated, in a commercial process with a standard set of conditions (inlet air temperature, outlet air temperature, and fluid feed rate), why it was necessary to modify these conditions in order to produce a powder of low moisture content. The evaporative rate, since the constant drying rate period had been passed and the falling rate period was in effect, was found to be reduced in proportion to the temperature and the total moisture in the outlet air.

The dryer selected for demonstrating the interpretation of psychrometry was one that was direct-heated by fuel oil and in which the products of combustion mixed with the fresh inlet air. The amount of fuel oil consumed in heating the air varied from about 16 g.p.h. to 22 g.p.h. The fan capacity was constant, 14,000 c.f.m. air at 200°F., the standard outlet air temperature. The weight of water being evaporated per hour was normally 1000 lb. The factors contributing to the total moisture in the outlet air were the moisture in the incoming air, the water formed by

combustion of the fuel oil, and the water evaporated from the fluid fed to the dryer. The calculation of total moisture in the outlet air shows plainly that the conditions of drying require modification during humid summer weather in order to maintain suitable drying. An obvious adjustment is in the reduction of the fluid feed rate or the elevation of inlet air temperature (Table II).

Inspection of this table shows that normally in the summer in the New York City area the humidity of the outlet air will be 35% higher than in the winter. This is a severe enough handicap even for materials that dry moderately well (*e.g.*, soluble coffee). An occasional humid spell in the summer is likely to result in outlet air humidities about 50% higher than during normal winter weather. During such days, unless special precautions are taken, problems are likely to develop, such as deposition of product on the floor or cone bottom of the dryer, caking in conveyors and rotary valves, and excessively high moisture content in the collected product. Although it is evident that a reduction of the fluid feed rate to 40% or 50% of the usual production rate (see item 5 in the table) will result in outlet air humidities comparable to winter conditions, considerations such as higher production costs per unit of output and the hazards of product collection (even in dehumidified rooms) in extreme cases dictated the policy of operating only during seasons of favorable humidity * in the instance of the malt diastase manufacturers mentioned previously.

Dehumidification of part or all the entering air is an expedient that has been considered for dryers producing hygroscopic powders. For example, a manufacturer desiring to spray dry corn sirup in a plant located in a damp, misty region along the Mississippi River could not be guaranteed operability by one spray dryer manufacturer during the warm season unless he invested in a large dehumidifier for the inlet air. The decision for purchase depended in part upon whether there was sufficient profit margin despite the increased capital investment and operating cost. The unit eventually chosen was a Peebles dryer wherein most of the humid hot air which has accomplished much of the drying is displaced by fresh hot air at a lower level in the tower.

In addition to measures such as reduction of fluid feed rate and dehumidification of entering air, raising the drying air temperatures, both inlet and outlet, is effective provided that the higher temperatures do not impair the product. For example, in spray drying a citrus juice it was found that operation during humid weather resulted in excessive moisture levels in the powder, a condition which promoted caking during storage. The product moisture was reduced below the critical by simply raising

* Chemical Engineers' Handbook, 2nd Edition, McGraw-Hill, N. Y., for weather conditions in principal U. S. cities (pp. 1109-1111).

TABLE II

Influence of Weather Conditions on Moisture in Outlet Air [a]

Weather conditions [b]			Source of moisture			
			A. Entering air	B. Combustion of fuel oil	C. Evaporation of water from feed solution	D. Total moisture in outlet air (sum of A, B, and C)
	Dry bulb temp. °F.	Relative humidity %	lb. water per minute			
1. Normal winter weather for month of February	32.7	58	1.89	2.78	16.65	21.32
2. Dry summer weather 7/3-5/46	78.4	42	7.03	2.28	16.65	25.96
3. Normal summer weather for month of July	78.3	58	9.94	2.15	16.65	28.74
4. Humid summer weather 8/5/46	83.2	67	13.35	2.02	16.65	32.02
5. Humid summer weather 75% of normal production	83.2	67	13.35	1.77	12.50	27.62
50% of normal production			13.35	1.52	8.33	23.20
40% of normal production			13.35	1.39	5.83	20.57

[a] Unpublished data of authors.

[b] Weather records for 12:00 noon in New York, N. Y. (Meteorological Summary, U. S. Dept. of Commerce, Weather Bureau, 1946).

the temperatures of both inlet and outlet air from 5 to 10°F., taking care at frequent intervals to ascertain that no discoloration was occurring in the powder because of overheating.

Where drying to a low moisture content is not as important as the ability to remove the powder in free-flowing condition, many powders can be rendered relatively non-sticky by cooling the particles while they are still suspended within the air in the dryer system. Such cooling is generally accomplished by admitting substantial volumes of unheated air either for admixture with the main body of hot air leaving the main drying zone or for cooling near the interior dryer surfaces where viscid particles are otherwise likely to impinge and stick (such as in the Bowen dryer). Provision for employing such techniques generally involves special dryer design features that may be called into operation only during humid weather, or, in the case of very hygroscopic materials, may be used throughout the year. One type of spray dryer (Peebles or Golden State Co., Ltd.) used for drying corn sirup removes practically the entire volume of hot air from the tower after it has become humidified by passage through the primary drying zone. Additional hot, non-humid air is then introduced to the lower section of the dryer tower so that the still-sticky particles are transferred to a zone of low humidity drying air. These design features are dealt with in the section on commercial spray dryers.

Some foods naturally contain colloidal ingredients such as pectin, which serve admirably as drying aids. In spray drying tomato puree, if proper care has been taken in heat-inactivating the pectin-destroying enzymes during the "hot-break" operation, the natural pectin that survives assists measurably in either drum drying or spray drying. Corn sirup solids (low in dextrose equivalent), flour or gums may be added also provided they are compatible with the use for which the product is intended. Charley (1940) found that, whereas various plum purees could be dried easily using a Kestner spray dryer, one batch in which the pectin had been intentionally hydrolyzed with an enzyme was impossible to dry. The pectin content of the untreated product was high, about 7% on the dry basis.

Secondary Drying

Another means of compensating for poor or insufficient drying under humid conditions is the employment of a secondary system wherein the incompletely dried powder from the spray dryer is further processed to a lower moisture content. Such a system may consist of a combination of pneumatic conveyor and cyclone separator using fresh hot air if the powder primarily requires cooling. Both types have been in-

stalled in conjunction with dryers such as the Swenson where the product can be removed conveniently from the cyclone-type drying chamber no longer suspended in the original air. Milk powder from the main drying chamber may have as much as 5½% moisture and may be dried to 3½% or less moisture by the secondary hot air stream. In some dryers, such as the Mojonnier, where the cone bottom of the main drying chamber does not serve as a cyclone separator, the duct leading from the dryer to the primary separator may be intentionally extended so as to increase the time that the powder remains in contact with the drying gases. In some installations the duct is a long, inclined one equipped with mixing paddles so that the powder is agitated in order to dry more effectively. Despite the fact that vapor pressure of the powder is high enough so that it is not yet in equilibrium with the humid air, drying is more favorable when fresh, less humid air can be used for secondary drying.

During the recent war when egg drying received such extraordinary impetus it became recognized that spray dried whole egg powder is stable only if it has a much lower moisture content than could ordinarily be attained by single stage spray drying. It was essential to dry to moistures below 2%, the shelf life of egg powder at that level being about three times as long as at 5% moisture. Greene *et al.* (1947) found that for drying egg powder to less than 2% moisture in co-current type dryers where the humid outlet air had vapor pressures ranging from 52 to 75 mm. Hg (0.045 to 0.065 lb. H_2O per lb. dry air) temperatures far in excess of 170°F. would be necessary. Such high temperatures would be detrimental to the product quality. In order to produce low moisture powders with the dryers being used, the humidity of the outlet air could be reduced only by such extreme measures as significantly reducing the feed rate or by increasing greatly the air flow. Since fresh air rarely exceeds a humidity of 0.013 lb. H_2O per lb. dry air, much lower temperatures can be used for further drying with fresh air. In a typical case, the powder emitted from the main dryer through a rotary valve at the rate of 700-750 lb. per hour was resuspended in 2400 c.f.m. air entering at 220°F. and leaving through a cyclone separator at 184°F., the secondary dryer system consisting of 60 ft. of 10 in. diameter tube discharging into a 5 ft. collector. Powder at 3.8% moisture was dried thereby to 1.3% moisture. Several redryers of this type were installed in plants drying eggs for the Army, and their performance is claimed to have been satisfactory in all cases. A volume of redrying air of 1000 c.f.m. at 200-220°F. per 500 lb. egg powder per hour (containing 3.4% moisture initially and 1.5-2.0% moisture as it leaves the system) may be regarded as a suitable ratio of air to powder. Egg powder of such low moisture content regains

as much as 1% moisture unless it is cooled with dehumidified air or in a closed system.

XII. Evaporative Capacity and Thermal Efficiency

When objectively studying the engineering performance of an installed spray dryer as a drying apparatus there are two criteria that are subject to experimental and mathematical evaluation. These are the evaporative capacity, expressed as pounds water evaporated per unit time under standard practical operating conditions, and thermal efficiency, expressed as the percentage of the total heat that is utilized productively for heating and evaporating water. Important as evaporative capacity and thermal efficiency may be to the engineer, they are secondary to the achievement of good quality and high yields.*

* W. S. Bowen (private communication) emphasizes that the capacity of a drying chamber is controlled in many operations by the wall condition. If the particle size and feed rate are uniform the capacity of the dryer can be employed to the evaporative limit. But if there are fluctuations the wall condition is determined by the maxima in particle size or feed rate. Variations or pulsations in inlet temperature occasioned by poor instrument control of fuel feed also require that the liquor feed be adjusted for the minimum outlet temperature; if it is adjusted for the maximum temperature, the walls will become wetted when the temperature cycles downward.

The conditions necessary for ideal wall conditions (and economical fuel use, as well) are:

1. Steady inlet air temperature.
2. Steady flow rate of liquid feed.
3. Uniformity of particle size.

The last condition is more important when the initial particle trajectory is at right angles to the chamber walls (as from a centrifugal atomizer) than when the spray is parallel to the walls (as from nozzles).

Non-steady liquid feed may be caused by several factors, such as:

1. Pulsations or surges due to uneven pumping.
2. Imperfect design of the throttling or control valve on the liquid line to the atomizer or on a circulating by-pass.
3. Large uncorrected lags which give rise to cycling of the valve-setting control. The outlet air temperature is most commonly employed for controlling either the fuel feed rate or the liquor feed rate. In either case it is imperative that the response be smooth and even and not result in a "hunting" type of curve.

Particle size uniformity is determined primarily by the characteristics of the spraying device. Regardless of the device used, the finer the atomization the more uniform will be the particle sizes. Most manufacturers, however, do not desire products having the preponderance of particles below 50μ.

Thermal Efficiency

In estimating roughly the overall thermal efficiency of a spray dryer, workers have customarily divided the quantity of useful work (B.T.U. for heating and evaporating water) by the heating value of the fuel consumed. This conception of thermal efficiency may be refined by making allowance for the heat gained or lost by the solids in the spray fluid. This simplified version of thermal efficiency requires no consideration of atmospheric datum temperature such as appears in the expression by Fogler and Kleinschmidt (1938).

Fogler and Kleinschmidt state "with the exception of the relatively small amount of heat that can be put directly into the spray liquor as it is being pumped to the atomizer, the primary heat cycle of the spray-drying operation consists in heating the drying gas from the atmospheric datum, T_0, to the temperature, T_1, at which it enters the drying zone of the spray chamber. Here heat is given up in heating the spray liquid, in evaporating solvent from it, and in radiation losses from the chamber walls, following which the gas leaves the drying zone at a temperature, T_2. If the radiation loss is stated as a percentage, R, of the total temperature drop in the drying zone, then the useful work done in the drying zone is proportional to $(100 - R)$ per cent of $(T_1 - T_2)$, and the thermal efficiency of the spray chamber operation may be stated as:

$$\frac{\left(1 - \frac{R}{100}\right)(T_1 - T_2)}{T_1 - T_0}."$$

The usefulness of this expression is limited by the fact that R, the percentage radiation loss, must be determined experimentally or by equating the expression with another involving either the total heat supplied by the fuel or the weight of air passing through the dryer in unit time.

Energy supplied to the dryer may be expressed as the heat supplied by the air, $W_a C_p^a \ (T_1 - T_0)$, or may be expressed as the total thermal output (in BTU) of the fuel consumed. Likewise, following Fogler and Kleinschmidt's terminology, useful energy may be expressed as:

$$(W_a C_p^a T_1 - W_b C_p^b T_2)$$

where W_a = weight of gas (air) entering dryer
W_b = weight of gas (air) leaving dryer
C_p^a = average specific heat of air entering dryer (humid heat)
C_p^b = average specific heat of humidified air within dryer (humid heat).

T_1 should more properly be defined as the temperature of the drying

gas leaving the heater in view of the fact that considerable radiation loss may occur before the gas enters the drying zone. (Losses as high as 10% of the total heat have been found at high inlet temperatures.)

It has been observed that numerous spray dryers used for food draw their inlet air supply from within the building enclosing the drying chamber. Accordingly, intentionally or otherwise, some of the heat lost from the chamber walls by radiation is utilized in preheating the inlet air. By designing the dryer chamber with an outer shell through the annulus of which the air inlet supply for the heater is drawn a fuel economy of 15% or more can result due to utilization of the radiation losses. Such a device not only renders expensive insulation unnecessary but also serves to cool the walls, thereby reducing wall deposits in certain cases. In the case of this special "air-cooled" dryer where radiation may actually be promoted, the application of Fogler and Kleinschmidt's index for thermal efficiency may erroneously indicate inferior performance. Several commercial dryer designs use measures for cooling chamber walls which generally consist of introducing cool or tempered air through slots in the chamber walls. In such cases, the use of terms for weights of air and final air temperatures result in an involved expression.

A simple definition of overall thermal efficiency (O.E.), expressed as percentage, is as follows:

$$\text{O.E.} = \frac{\text{Heat Supplied for Evaporation of Water} + \text{Heat Change in Solids Dried}}{\text{Heat Input}} \times 100$$

The divisor may be determined by metering the quantity of fuel consumed or may be determined by temperature and air volume measurements on the heated air. In the case where indirect steam is used for heating the air, unless the condensate is employed in a preheater for heating the fuid fed to the dryer or returned to the boiler, or otherwise used advantageously, the heat lost with the discarded hot water must be regarded as part of the heat input and will accordingly lower the value for thermal efficiency. In the Swenson milk dryer the condensate and exhaust steam from the air-heating radiators are sent through a preheater wherein the milk is heated to about 175°F. prior to being sprayed within the concentrator.

The second term in the dividend (Heat Change in Solids Dried) will generally be a compartively small negative value in view of the fact that in most cases of food drying the fluid fed to the dryer is at a higher temperature than the product powder. In most spray drying operations in the food industry the powder is removed from the dryer at a temperature only slightly above the wet bulb temperature and considerably

below the dry bulb temperature. It is rare to find product temperatures exceeding 140°F. when the powder is removed continuously from the dryer system, and more generally the temperature is under 120°F.

It is often illuminating to draw up a heat balance for the drying chamber alone, independent of the air heating system ahead of the chamber or the powder and dust collection systems beyond the chamber. In such a case the thermal efficiency may be expressed for this limited (but important) system using the same form as above, but regarding Heat Input as that quantity of heat actually delivered to the drying zone. This thermal efficiency value may be regarded as the percentage of heat used for actual drying; the remaining percentage is that heat lost by radiation.

Although some food spray dryers have inlet air temperatures as high as 1000°F., the majority operate with inlet temperatures below 600°F. A high percentage of food dryers are indirect-heated by steam; in such cases the inlet temperature is generally not higher than 350°F. Outlet temperatures generally range from 170 to 270°F. It is apparent that the higher inlet temperatures favor higher thermal efficiency even though in a given dryer the radiation losses are likely to be higher. Assuming for purposes of illustration that there are no radiation losses, in a dryer system wherein 70°F. air is heated to 600°F., then is fed to the dryer chamber and leaves the stack at 200°F., the maximum thermal efficiency will be in the order of 78%. If the same air is heated to only 300°F. and leaves the stack at 200°F., the maximum thermal efficiency will be in the order of only 35%. Such a low efficiency would be very disturbing to engineers in the inorganic chemicals industry; it is not uncommon, however, in the food industry where dryer efficiency is far less important than product quality.

Where precautions must be taken for heat sensitive foods, very high inlet temperatures are rarely encountered in practice. Despite the fact that the temperature of the main bulk of the powder is heated to a temperature that exceeds by not more than a few degrees the wet bulb temperature of the gases within the dryer chamber, it has been found by practical experience that in many food dryers a portion of the inlet hot air tends to by-pass the main drying zone and encounters partially dried particles that can no longer be protected against rise in temperature by the high evaporative rate of water. High inlet temperatures (500°F. or over) would be destructive to food quality in such dryers. There are also instances where the wet bulb temperature corresponding to a very high dry bulb temperature is excessive for particularly sensitive materials; such was found to be the case in drying a special cultured milk having a delicate developed flavor. Moreover, accumulation of some material on the walls frequently results in discoloration or charring and, when the

deposit falls into the product, it affects the color and flavor adversely.

A common technique for recovering some heat from outlet air is to exhaust it through a preconcentrator where it is in intimate contact with a spray of dilute feed liquid. This practice not only conserves heat by preconcentrating the feed liquid, but the scrubbing action of the spray also results in almost complete recovery of the fine powder in the air stream. The most notable spray dryer incorporating this feature is the Swenson milk dryer. Overall solids recovery of 98% is claimed for this dryer when operating on skim milk. Typical temperatures for the air discharged to atmosphere are said to be 125°F. dry bulb and 120 to 124°F. wet bulb. In one test run the air was observed to be practically saturated, with a dry bulb temperature of 125°F. and wet bulb of 124°F. For the overall milk drying operation, including preconcentrating, preheating, and spray drying, the steam consumption is said to be only 1.4 lb. steam per lb. of water evaporated. A combined flowsheet and heat balance for a Swenson dryer is shown at the end of this section, and a discussion appears in the section on commercial spray dryers.

Considering the importance of the knowledge of thermal efficiency and evaporative capacity in relation to the optimum economical operation of a spray dryer, it is surprising that few food manufacturers have gone to the trouble of evaluating a dryer as they would a newly installed steam boiler. An engineer or chemist with the assistance of a single operator can accomplish a complete set of simple tests in only two or three shifts of operation, spraying only water into the chamber. The data and charts obtained not only serve as a guide to the production capacity and fuel consumption to be expected under any given set of inlet and outlet air temperature conditions but also uncover imperfections in design or installation that may possibly be corrected.

The method employed by the present authors has consisted of spraying and evaporating water in the dryer chamber under nine different combinations of inlet air temperature and outlet air temperature. This can be accomplished in three sets of runs with three temperature combinations in each, as follows:

1. Low inlet air temperature range; a. Low outlet air temperature, b. Medium outlet air temperature, c. High outlet air temperature.

2. Medium inlet air temperature range; a. Low outlet air temperature, b. Medium outlet air temperature, c. High outlet air temperature.

3. High inlet air temperature range; a. Low outlet air temperature, b. Medium outlet air temperature, c. High outlet air temperature.

The rate of water fed to the system must be kept constant and uniform during the run, thus maintaining a constant outlet temperature. A run of 15 to 20 minutes duration under steady state conditions is

sufficient to relate the evaporative rate (or capacity) to the particular combination of inlet and outlet temperatures. Each set of three runs provides three points each for the construction of three curves, plotting outlet air temperature vs. pounds water evaporated per hour, the inlet air temperature being constant for each curve. From these three master curves a family of curves can be derived from which evaporative capacity may be read directly or interpolated for any set of temperature conditions encountered in actual production. Such a family of curves is

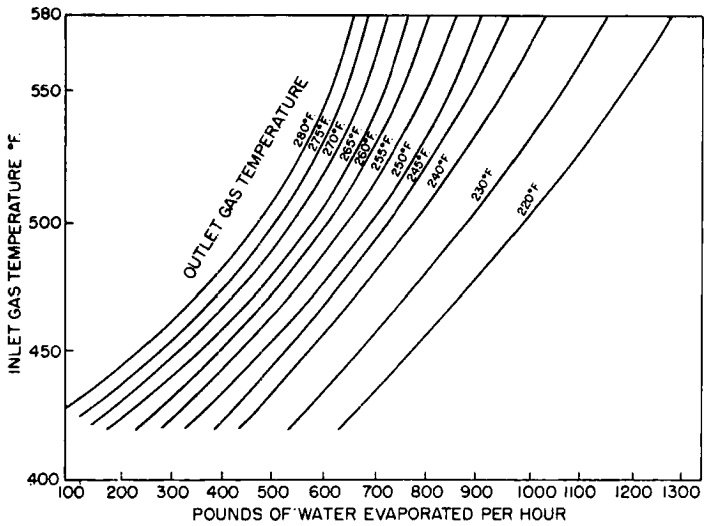


Fig. 45. Relationship of evaporative capacity to gas temperature conditions obtained during efficiency test on a vertical co-current dryer. The air volume was constant.

illustrated in Fig. 45. These curves represent the drying of water fed to the spray dryer at 75°F. Since more typical feed conditions are 150°F. and 50% solids (which contribute a small amount of heat), in actual practice the evaporative capacity should be about 10% higher than what is predicted by these curves. It is advisable to exceed the range of temperatures, both low and high, anticipated in the drying of a particular food so as to cover ranges that may be required in the future for drying other foods.

It is interesting to observe that the curves become straight lines as the outlet gas temperature diminishes. This is interpreted to correspond to the decrease in radiation loss as the mean temperature of the air within the tower is diminished. Actually, the radiation loss is closely related to the outlet air temperature, inasmuch as most of the surface area within

the dryer chamber is swept by air only slightly hotter than is indicated by the outlet air temperature.

If, in addition to the measurement of inlet and outlet temperatures, observations are made at the same time of air temperatures entering and leaving the heater and of air volume entering or leaving the system, quite a complete set of heat balances can be developed. To illustrate, the data used in preparing Fig. 45 have been augmented with additional observations, and a set of performance and efficiency values have been derived as shown in Table III.

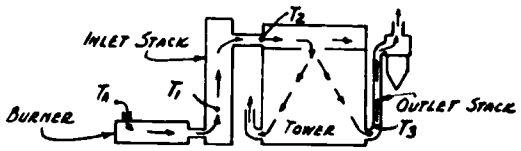
The spray dryer tested in this instance was on old one that had been reconstructed for a war-time job. Its efficiency as judged by these tests is obviously unimpressive. The dryer was improved in several major respects following the tests so that under operating temperature conditions that would have caused evaporation of only 530 lb. water per hour, the evaporative rate was raised to 800 lb. water per hour. The design of this dryer had been modified so that there was straight-line air flow instead of the original rotary air flow in the dryer chamber. Owing to the fact that the distance of travel, allowing for a parabolic trajectory, was only about 25 ft., it was necessary to operate with high outlet air temperatures in order to dry the product sufficiently before it reached the floor. The average temperature of the powder leaving the dryer was 114°F.

From the standpoints of evaporative capacity and thermal efficiency, the best operating conditions were: high inlet air temperature and low outlet air temperature. Under these conditions a high percentage of total heat (amounting to almost 10%) was lost in the stack between the heater and the spray tower. This loss could be reduced by elevating the position of the burner so as to shorten the distance of hot air travel. Both methods of installation are common and, assuming that there are no special building restrictions, the choice of burner location can best be arrived at by estimating the cost of alternative construction and difference in fuel consumption. The value of the fuel lost in the example given may be in the order of a thousand dollars annually, depending on the hours of dryer operation.

Another design detail of this dryer resulting in decreased thermal efficiency was brought to light by a series of profile readings of temperature at several cross-sections on the horizontal plane in the dryer chamber (Fig. 46). The readings were taken by inserting a thermocouple on an aluminum "fishing pole" through various sight holes in the chamber wall. It was found that, although the hot air from the heater entered the tower at, say, 475°F. (regarded as inlet air temperature), by the time it poured out the plenum chamber through a ceiling orifice and into the main dry-

TABLE III

Performance and Efficiency Tests on Spray Dryer



Test	Gas temperatures, °F.				Overall heat balance (Per cent of total heat)				Thermal efficiency in tower	
	Outside air, T_0	Bottom of inlet stack, T_1	Top of inlet stack entering tower, T_2	Bottom of outlet stack leaving tower, T_3	Loss in inlet stack by radiation, %	Overall efficiency. Useful work; heating, and evaporating water, %	Loss in tower by radiation, %	Loss with outlet gases, %	Heat used for actual drying, %	Heat * lost by radiation, %
"E." Low Inlet Temp.										
Run #1										
Low outlet temp.	72	440	430	226	4.02	32.5	22.3	41.3	59.2	40.8
Run #2										
Med. outlet temp.	72	440	431	252	4.01	21.5	26.4	48.2	44.9	55.1
Run #3										
High outlet temp.	72	437	430	272	4.02	12.4	30.0	53.7	29.3	70.7
"F." Med. Inlet Temp.										
Run #1										
Low outlet temp.	75	545	511	231	9.13	37.2	21.2	32.3	63.8	36.2
Run #2										
Med. outlet temp.	75	547	510	252	9.17	31.9	21.9	36.9	59.3	40.7
Run #3										
High outlet temp.	75	545	510	272	9.18	28.4	21.2	41.0	57.2	42.8
"G." High Inlet Temp.										
Run #1										
Low outlet temp.	73	605	565	224	8.41	43.0	20.7	28.2	67.5	32.5
Run #2										
Med. outlet temp.	73	600	557	250	9.79	34.9	20.5	33.0	61.0	39.0
Run #3										
High outlet temp.	73	600	561	271	9.13	30.7	23.3	37.2	56.8	43.2

* Before improvement of insulation.

ing zone within the tower it had cooled to 360°F. It is unlikely that convection currents could travel up into the orifice because the downward velocity there is high. It is more probable that convection currents cooled the sheet metal ceiling that separates the plenum chamber from the drying chamber. Accordingly, the hot gases were partly cooled as they were being drawn to the main drying zone.

The main drying zone was found to be within a 2-ft. radius of the atomizer. Within this zone the temperature dropped very sharply; outside this radius the temperature changed relatively slightly. Much of

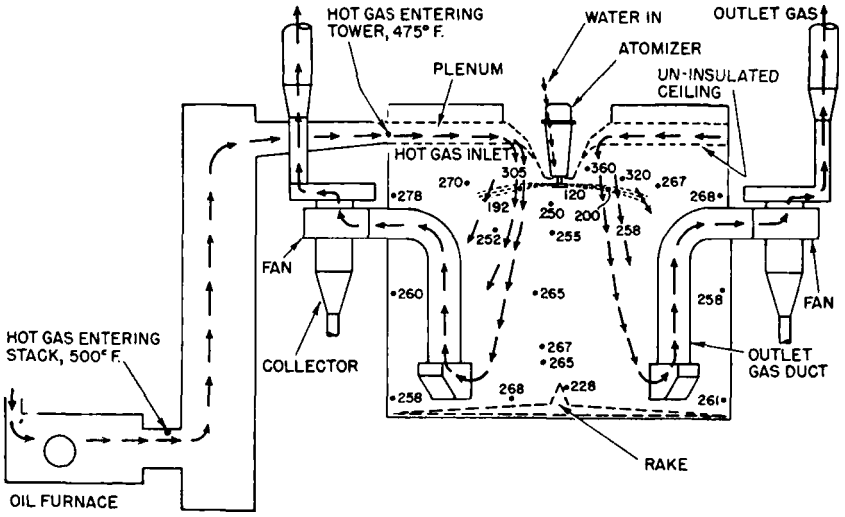


Fig. 46. Gas temperature readings in °F. at various zones in a vertical co-current dryer. A significant thermal change is shown before the gas leaves the plenum chamber and enters the spray chamber.

the driving force or drying potential must have been lost by the dissipation of heat before the gases reached the main drying zone. Even though the heat was transferred to drying gases, much heat had by-passed the main drying zone where it would have been more effective. It is quite probable that the outlet air temperature could have been reduced with the same ultimate drying effect on the powder had the heat introduced to the drying chamber been used more efficiently in the main drying zone.

Unintentional by-passing of heat or hot air around the primary drying zone is found occasionally in commercial spray dryers and should be avoided or corrected if the manufacturer is concerned about the heat sensitivity of the powder or the thermal economy of the process. Such by-passing can be detected more readily in dryers having straight-line

air flow (whether co-current or counter-current) than in dryers having rotary air motion.

By simply insulating the sheet metal partition between the plenum and the drying chamber the unintentional heat transfer can be effectively

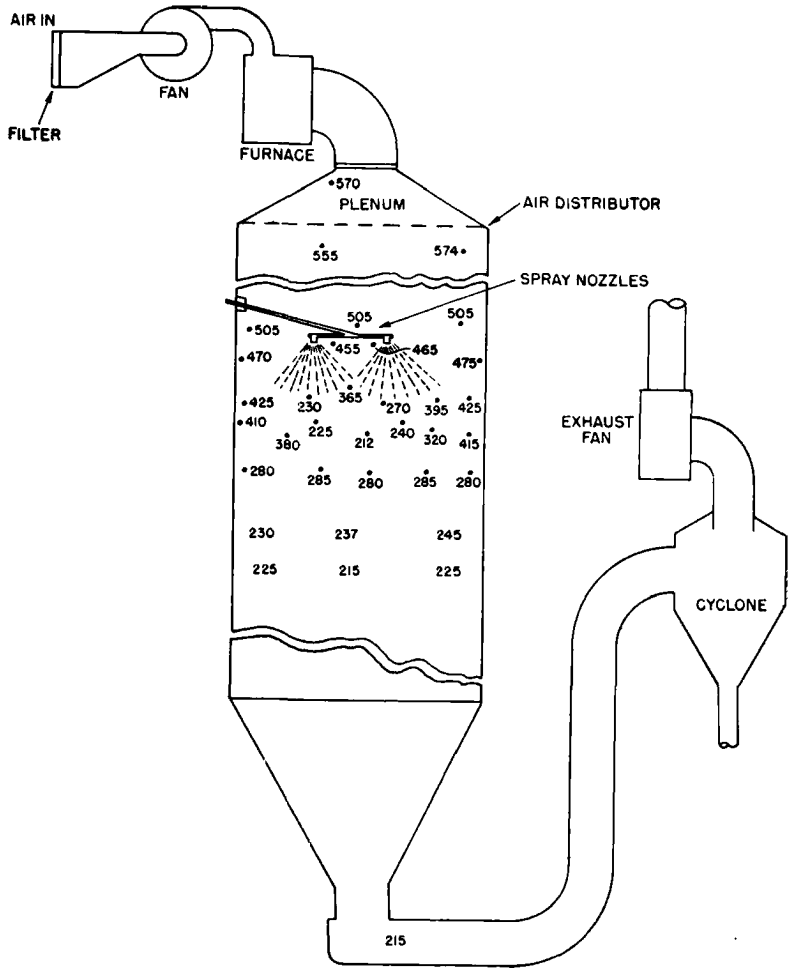


Fig. 47. Gas temperature readings in °F. in a tall spray tower. By-passing of hot air is shown to occur around the primary drying zone.

reduced in the first dryer. In the second dryer (see Fig. 47), since the perforated distributor sheet admits streamline hot air at a uniform velocity across the entire horizontal cross-section of the chamber, one means of utilizing the hot air efficiently is to use a sufficient number of

spray nozzles or to use atomizers having wide spray-cone angles so as to cover as wide an area as possible without coating the walls. Towers of narrow diameter are less subject to such by-passing than wide diameter towers.

Heat and Materials Balance for 18 ft. Diameter
Swenson Spray Dryer Operating on Skim Milk *

A. Milk Balance

Product: 735 lb. at 3.35% H_2O = 710 lb./hr. bone dry
Assume about 5% powder carry-over from dryer to wet collector and about 2% solids loss in entire system.
Dryer feed: 710 × 1.07/0.285 = 2665 lb./hr. at 28.5% total solids (T.S.)
Wet collector feed: (710 × 1.02)/0.085 = 8525 lb./hr. at 8.5% T.S.

Wet collector balance	*Dryer balance*
Feed at 8.5% T.S. = 8525 lb./hr.	Feed at 28.5% T.S. = 2665 lb./hr.
Product at 28.5% T.S. = 2665 lb./hr.	Product at 3.35% H_2O = 787 lb./hr. (before carry-over and losses)
Evaporation = 5860 lb./hr.	Evaporation = 1878 lb./hr.

Total evaporation = 7,736 lb. H_2O/hr.

Product distribution:

Total product	= 787 lb./hr.
Packaged product	= 735 lb./hr.
Entrained and lost product	= 52 lb./hr.
Powder carried to wet collector	= 37 lb./hr.

B. Heat Requirements

(1) *Preheating feed* (with steam at 5 p.s.i. gage)
8525 × (182 − 42) × 0.95 = 114,300 B.T.U./hr.
Allowing 5% radiation loss,
114,300/961 × 0.95 = 1,252 lb. steam/hr.

(2) *Heating dryer air* (assume solids drying at 110°F.)
Heat input: basis—100 lb. feed at 28.5% T.S. producing 29.5 lb.

Heat supplied by feed: 100 × (131 − 110) × 0.84 =	(−) 1,765	B.T.U.
Latent heat of vaporization: (100 − 29.5) × 1029 =	73,000	B.T.U.
Heating vapors: (100 − 29.5) × (142 − 110) × 0.45 =	1,016	B.T.U.
Heating product: $29.5 \times \left(\frac{180+142}{2} - 110\right) \times 0.4$ =	602	
	72,853	B.T.U./100 lb. feed

72,853/70.5 = 1035 B.T.U./lb. H_2O evaporated
Heat to dryer: assuming 10% radiation and trap losses,
1878 × 1035/0.90 = 2,160,000 B.T.U./hr.
2,160,000/0.25 × (265 − 142) = 70,250 lb. air/hr. to dryer
70,250 × 13.26/60 = 15,530 c.f.m. at 65°F.
Steam at 60 p.s.i. gage = 70,250 × 0.24 (265 − 65)/904 = 3,730 lb./hr.

* Data supplied by courtesy of Swenson Evaporator Co., Harvey, Illinois.

(3) *Wet collector* (evaporation at 125°F.)

a. Evaporation from cooling dryer exhaust:
$(67{,}600 + 1{,}820) \times 0.25 \times (142 - 125)/1014 = 291$ lb. H_2O/hr.

b. Evaporation from cooling skim milk feed:
$8525 \times (178 - 132) \times 0.95/1014 = 367$ lb. H_2O/hr.

c. Evaporation from heat input to shell and tube heater:
$8525 - 291 - 367 - 2665 = 5202$ lb. H_2O/hr.

Steam to heater: (at 5 p.s.i. gage)

Heater input: $5202 \times 1026/0.95 = 5{,}625{,}000$ B.T.U./hr.
Radiator condensate flash: $3780 \times (308 - 227) = 306{,}500$ B.T.U./hr.
$5{,}318{,}500$ B.T.U./hr.

$5{,}318{,}500/960 = 5{,}530$ lb. steam/hr. at 5 p.s.i. gage

$$\frac{5{,}318{,}500}{8.33 \times 0.94 \times 60 \times (178 - 132)} = 246 \text{ g.p.m. circulating through wet collector}$$

Summary of steam use:

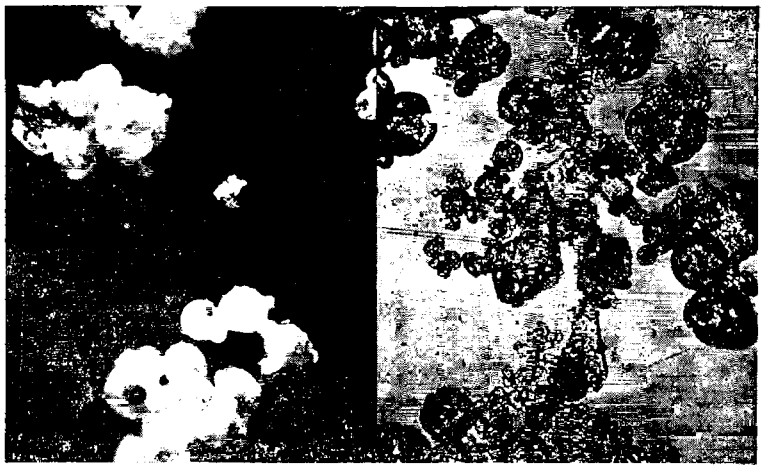
Steam for preheater at 5 p.s.i. gage	—	1,252 lb./hr.
Steam for radiators at 60 p.s.i. gage	—	3,730 lb./hr.
Steam for wet collector system	—	5,530 lb./hr.
Total steam	—	10,512 lb./hr.

$$\frac{10{,}512}{7{,}530} = 1.396 \text{ lb. steam/lb. } H_2O \text{ evaporated}$$

In addition to the 14,900 c.f.m. air at 65°F. introduced for drying, 4000 c.f.m. air is shown in the flow sheet (Fig. 7a) to be introduced for secondary drying and cooling of the powder. The evaporation obtained from this conveying air is small and has not been taken into account in the heat balance. The 5% powder carry-over figure and the 10% radiation and trap losses represent maximum values determined by Swenson in actual operations. The average carry-over from the drying chamber to the wet collector is said to be 3% but some plants show up to 5%. The 2% powder loss is principally due to accumulation in the system since there is practically no dust loss.

XIII. Photomicrographs of Spray Dried Foods

Sodium chloride:

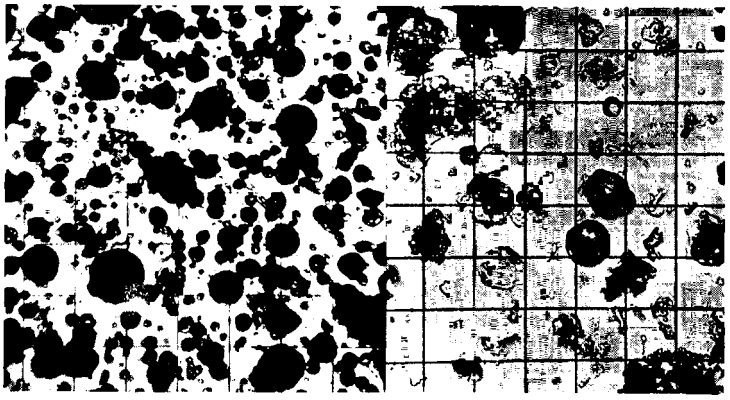


(a) Magnified 60×

(b) Magnified 100×

This product was obtained by spray drying a suspension consisting of fine crystals added to a saturated brine, the final level being 40% total solids. A dark field photograph (a) shows the perfectly spherical nature of the particles. A standard photograph with an illuminated field (b) is also shown wherein some of the spheres were crushed to demonstrate that the spheres consist of cemented crystals. This product was sprayed with a centrifugal atomizer.

Coffee:

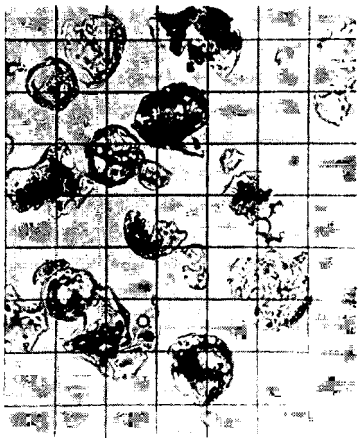


(a) Magnified 60×

(b) Magnified 200×

(a) This photograph shows a wide range of particle sizes and is fairly typical of most soluble coffees.

(b) This photograph shows a fraction of the above coffee powder screened through a 38 micron opening (400 mesh). Note the transparent shells and the fragments of shattered spheres. The thin walls and shell-like particles are typical of low-density powders.

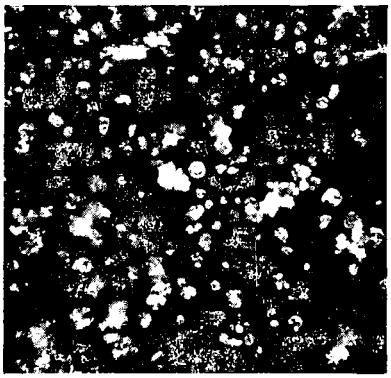


(c)

An enlargement of b (400 ×) shows blow holes and spheres within spheres. These powders were obtained by centrifugal atomization.

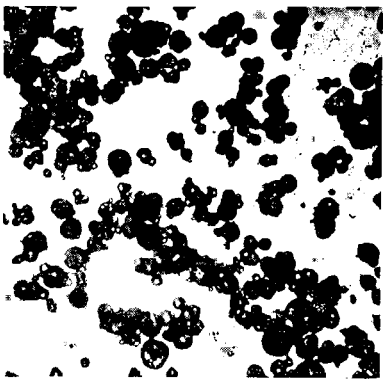
Pectin:

Pectin.* This dark field photograph shows spherical particles obtained with the Niro centrifugal atomizer.



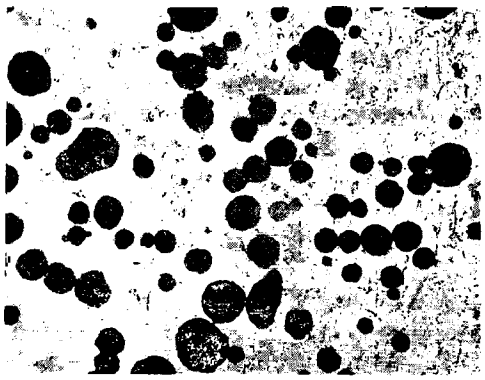
Milk:

Milk.* Another Niro dryer product shows a preponderance of particles in a uniform size range.



Yeast:

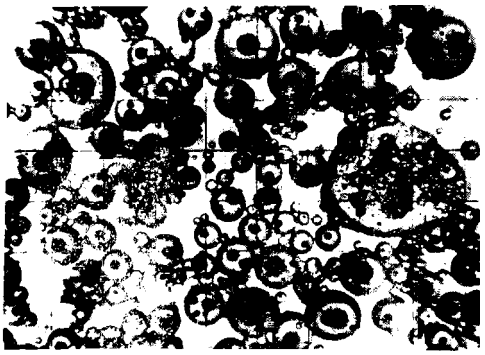
Yeast.* The particles are shown to be spherical with little tendency toward agglomeration.



* Photographs by courtesy of Niro Corp.

Lemon Juice Powder:

This was spray dried from a 50 to 60% solution using pressure nozzles.



Magnified 60×

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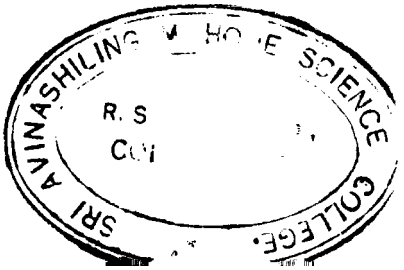

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Example: Austin, J. J., 229 (see Stewart), *257* means that Stewart *et al.* will be mentioned on page 229, the *et al.* accounting for Austin. This article can be located under Stewart in the list of references. Numbers in italics refer to the pages on which references are listed in bibliographies at the end of each article.

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