THE EFFECT OF FRESH AND HEATED GROUNDNUT OIL ON BLOOD CHOLESTEROL AND LIVER VITAMIN A CONTENT IN ALBINO RATS.

By

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I. INTRODUCTION

Food which is one of the prime necessities of life influences the health, well being and logevity of human beings. Nutrition has been defined by Stare (1952)[1] as the science of food and its relation to health. It deals with the constituents of food, the "nutrients", namely, proteins, fats, carbohydrates, vitamins, minerals and water, and the processes through which they are utilised by the body. Proteins, fats and carbohydrates are energy yielding factors. They release energy for work, growth and maintenance of life.

Basu (1946)[2] points out that oils and fats are important sources of energy in the diet. They furnish more than two times the energy that can be supplied by proteins or carbohydrates. In addition to their high caloric value, their components, the essential fatty acids are the carriers of the fat soluble vitamins A, D, E and K. Fats and oils help to make food palatable and impart satiety to meals.

The average consumption of fats and oils in India is low when compared to other countries. According to the F A O (1963)[3] the intake of fats and oils in India during 1955-1958, was only half an ounce per Consumption Unit, while the allowance recommended by Aykroyd et al (1963)[4] was two ounces. Subramanyan (1957)[5] refers to the low fat intake as one of the causative factors of vitamin A defeciency in South East Asia. While the consumption of fat is low, the treatments given to fat during cooking, particularly in deep fat frying, reduce its usefulness because of decomposition.

Sweetman and Mackeller (1954)[6] point out that fats and oils function in cookery as spreads, shortenings, salad oils and frying media. According to Fitch and Francis (1949)[7] frying is one of the most common uses of fat in cookery, since many kinds of food materials can be fried to give tasty preparations.

Raju and Rajagopal (1958)[8] observed that much of the oils used in Indian homes was subjected to varying conditions of heat, depending upon the nature of the foodstuffs in which the oils or fats were incorporated. They further stated that in the process of frying, oils were heated in open pans for long durations. The residual oils from such operations were used again, supplemented sometimes with fresh oils. Perkins (1960)[9] states that heat treatments of fats may result in loss of their nutritional values. Reheating may also accelerate the oxidative and other undesirable changes in fats. Rao (1960)[10] points out that iron pans which are commonly employed for frying purposes in India, may lead to the contamination of oil and its consequent rancidil what extent such reheated and contaminate quality of diets needs to be investigated.

Lowe *et al* (1958)[11] point out that changes taking place in oils and fats due to deep fat frying have been studied only on a commercial scale. Information regarding changes occurring in fats due to heat treatment in the Indian homes is not available. Therefore, this study was designed to investigate the saturated and unsaturated fatty acid contents of fresh and fried oil, and the effects of fried[12] oil, in comparison with raw oil, on the growth, liver vitamin A content, and blood cholesterol levels of young weanling albino rats fed at ten per cent level.

II REVIEW OF LITERATURE.

The literature regarding fats and oils have been reviewed under the following heads:

A. History of fats and oils
B. Chemistry of fats and oils
C. Role of fat in nutrition
D. Changes occurring in fats and oils due to heating.
E. Fat in cookery.

A. History of Fats and Oils.

McCollum *et al* (1944)[12] reported that Magendie (1783-1855) was the first to study the nutritive effects of three types of food substances namely carbohydrates, proteins, and fats. McCollum recorded that around 1912 Stepps found rats failed to grow on fat free diets and concluded that fats and oils contained substances essential for growth. McCollum and Davis (1913)[13] discovered that the essential substance present in fats was vitamin A and concluded fats served as carriers of vitamin A. Burr *et al* (1932)[14] recommended that fat was essential in nutrition like amino acids, minerals or vitamins because they contain certain indispensable, unsaturated higher fatty acids. Knudson *et al* (1940)[15] pointed out that moderate levels of fats and oils were favoured the utilisation of calcium in the rats. Basu (1946)[2] stated that fats and oils were important sources of energy, being two times as effective as proteins or carbohydrates.

B. Chemistry of Fats and Oils.

Bloor (1943)[16] defined lipids as a group of naturally occurring compounds and substances found in chemical combination with them. Fats represent the most abundant and widespread class of lipid in nature. They are found abundantly in nuts and seeds.

West and Todd (1961)[17] state that animal and vegetable fats are complex mixtures of the glycerol esters of fatty acids. Fats from different sources differ because of the differences in the glycerides composing them. Hawk et al (1947)[18] classify lipids as, simple lipids, compound lipids and derived lipids.

Simple lipids are esters of fatty acids with certain alcohols. They are further classified according to the nature of alcohol as, fats and oils and waxes. The difference between fats and oils is that oils are ~~fats~~ liquids at room temperature. Waxes are esters of fatty acids with a long chain aliphatic and aromatic alcohols such as sterols.

Compound lipids are esters of fatty acids which on hydrolysis yield other substances in addition to fatty acids and alcohol. The important members of this group are, phospholipids, glycolipids, aminolipids and sulpholipids. Derived lipids are substances liberated during the hydrolysis of simple and compound lipids. The derived lipids are fatty acids, aliphatic alcohols and sterols. According to West and Todd (1961)[17] there are two types of glycerides of which fats are composed. Simple glyceride with the general formula,

$H_2C—O—CO—R$

$HC—O—CO—R$

$H_2C—O—CO—R$

and mixed glyceride with the general formula

$H_2C—O—CO—R_1$

$HC—O—CO—R_2$

$H_2C—O—CO—R_3$

on hydrolysis fats yield glycerol and fatty acids. Kleiner and Orten (1958)[19] define fatty acids as the acid occurring in natural triglycerides. It belongs to a group of mono carboxylic acids ranging in length from four to twenty four carbon atoms including only the even numbered members of the series. There are two types of fatty acids, saturated and unsaturated. Hawk et al (1947)[18] state that fatty acids which contain ten or less carbon atoms in their molecules are liquids at room temperature, the others are solids with melting points rising with molecular weight. The common saturated fatty acids are, buteric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic and lignoceric.

According to Mitchell (1946)[20] the unsaturated acids are characterised by the presence of one or more double bonds in the molecule. They have been further classified according to the number of double bonds. Hawk et al (1947)[18] pointed out that because of the presence of the double bonds, the unsaturated fatty acids are more reactive than the saturated acids, the reactivity increases with the increase in the number of double bonds. The representative of the unsaturated acids are, oleic, linoleic, linolenic and arachidonic acids.

Holman (1954)[21] pointed out that linoleic, linolenic and arachidonic acids are essential for human beings and they are called essential fatty acids. According to him essential fatty acids are a group of naturally occurring poly unsaturated fatty acids.

C. Role of Fats in Nutrition.

The nutritive values of fats and oils are due to their essential fatty acid content and vitamin carrying capacity. Fat perform physiological, biological and biochemical functions in the nutriture of the body. These functions are reviewed under the following headings:

(1) Effects on growth, reproduction and lactation,

(2) Protein sparing action,

(3) Interrelationship with vitamins,

(4) Cholesterol level,

(5) Effects on the skin,

(6) As source of energy,

(7) Deficiency of fats and,

(8) Requirements.

1. Effect on growth, reproduction and lactation: Turpeinen (1938)[22] and Smedly et al (1940)[23] concluded that the principal unsaturated fatty acid required for growth was arachidonic acid. Turpeinen (1938)[22] reported 100 mg. per day of linoleate was the optimum level for growth. Martin (1939)[24] observed 20 mg. of linoleate promoted optimum growth. Mackenzie et al (1939)[25] recorded satisfactory growth and reproduction, when linoleate fed at the above level.

Burr et al (1940)[26] and Hume et al (1940)[27] showed that when 5 mg. of linoleate was given, a marked growth response may be obtained, but when 10 to 15 mg. of its was given there was a marked effect on growth. Burr (1942)[28] stated that no absolute value can be given for the amount of linoleic acid which should be considered optimum. Decker et al (1950)[29] pointed out that essential fatty acids have a key position in the formation and development of new cells. They observed that a chronic insufficiency will lead to deficiency in mature mice. In accute fatty acids deficiency death may be the result.

Greenberg et al (1950)[30] demonstrated that when linoleate and linolenate were added together, linoleate apparently "sparked" linolenate so that the activities of the combination were greater than would be predicted from individual activities.

Dietary fat plays an important part on growth. Deuel et al (1951)[31], Greenberg et al (1950)[30] (1951)[32] reported that rats receiving adequate amounts of linoleate grew rapidly, even better when cottonseed oil was included in the diet. Fenton et al (1951)[33] observed that when hydrogenated diet containing sufficient amount of corn oil to supply the EFA, growth of rats was increased. Deuel et al (1955)[34] stated that when completely saturated coconut oil was added to the diets, containing, marginal amounts of essential fatty acid a supressing effect on growth, was noted.

Evans et al (1934)[35] Mackenzie et al (1939)[36] had shown that when female rats grown to maturity, on fat free diet, they always showed reproductive failure. Scheer et al (1947)[37] subjected weanling rats and young adult rats to caloric restriction with a resultant marked decrease in body weight. They noted that the weight loss was less rapid, mortality minimum, and recovery on re-institution of ad libitum feeding, quicker and reproductive capacity superior in rats receiving diets containing fats. After a comparison of fat free diet with 5 per cent of hydrogenated fat or corn oil, Fumerrow et al (1952)[38] concluded that a dietary source of unsaturated fat such as corn oil was necessary for normal reproduction and lactation of rats.

2. Protein sparing action: Another feature of the metabolism of fats is their protein sparing action. Forbes et al (1946)[39] (1946)[40] proved that digestability of protein and the retention of nitrogen were improved by increasing the fat content in diets of growing rats and mature animals. Willman and co-workers (1947)[41] explained that fat is able to spare protein, where carbohydrate is completely ineffective.

Pearson and Panzon (1949)[42] have arrived at the same conclusion. They report that loss of essential amino acid in the urine and stool was considerably reduced when corn oil was administered.

3. Inter-relationship with vitamin: Evans and Lepovsky (1929)[43] were the first to suggest that an inverse relationship existed between levels of dietary fat and the thiamine requirement of the rat. The apparent reduction in the requirement of thiamine resulting from the addition of fat to a low fat diet was regarded as a 'sparing action'. But according to Kemmer and Steenbock (1933)[44] the requirement did not result in an increase in the concentration of thiamine in the body tissues. McHenry (1937)[45] (1937)[46] had considered thiamine as a limiting factor in fat synthesis. However Munkenbush et al (1942)[47] indicated that thiamine may have no more specificity for fat synthesis than pantothenic acid or pyridoxine may have.

Sarma et al (1947)[48] pointed out that oleic acid caused growth inhibition in diets containing sub-optimum levels of pyridoxal and pyridoxamine. But Williams and Feiger (1946)[49] (1946)[50] showed in a bioassay that oleic acid replaced biotine with lactobacillus. Deuel (1950)[51] stated that fats were important in the absorption of the fat soluble vitamins from the gastrointestinal tract. He also observed that vegetable oils were the most satisfactory foodstuff from which tocopherol could be obtained. Fish liver oil is one of the potent source of vitamin A and D. Vegetable oils especially palm oil contains large amounts of provitamin A, carotene.

Rathmann (1957)[52] reportes that Engel in 1942 found that oils were necessary for the optimum action of choline as lipotropic agent in rats. The lipotropic actions of inositol could be demonstrated only in the absence of unsaturated fat. He further points out that in 1938 Birch and Salmon reported that an acrodyia like dermatitis was caused by the deficiency of vitamin B_6 and unsaturated fatty acids. He reports that the subsequent studies proved that the curative power of linoleic acid was enhanced by vitamin B_6.

4. Effect on the Cholesterol level: Deuel et al (1955)[53] showed that when hydrogenated coconut oil was the sole fat in the diet, appearance of deficiency symptoms was rapid than a fat free diet. Rathmann (1957)[52] reports that a high proportion of cholestrol existed in the blood serum as esters of unsaturated fatty acids. These esters were easily mobilised and soluble in blood than the esters of unsaturated fatty acids.

5. Effect on the skin: Hanson (1937)[54] reported that the levels of unsatura-

ted fatty acids in the blood of the eleven children suffering from eczema were below normal. The eczema was substantially reduced by the administeration of cotton and linseed oil. The plugging of follicular openings with a dense, compact layer of keratin has been observed in rats on essential fatty acid deficiency. This was histologically similar to the follicular hyper keratosis in man. Burr (1942)[28] and Harris et al (1954)[55] stated that only linolenic and arachidonic esters cured the skin abnormalities.

6. As source of energy: The important nutritive value of fats and oils is their concentrated energy. While dry protein and carbohydrate yield only 4.1 calories per gram, fat yields more than 9.3 calories per gram. Anderson and Lusk (1947)[56] indicated an increase in the working capacity of rats on high fat diets. Samuels and co-workers (1948)[57] confirmed the earlier results of Anderson and Lusk that rats fed on diets containing 86 per cent of calories from fats, survived longer and accomplished greater amount of work, during a subsequent period of fasting, than animals which had received diets containing a similar proportion of carbohydrate or protein.

7. Deficiency of fat: Evans et al (1934)[35] observed that male rats on fat free diets lost fertility. The animals deficient in the essential fatty acids showed impairment of spermatogenic development.

Hecker et al (1950)[29] noted that in dog, a low fat diet resulted in a significant decrease in the proportions of the essential fatty acids in the skin. Kramen et al (1953)[58] reported that fat deficient diets or lack of essential fatty acids in diets may under certain conditions give rise to visible pathological changes in the skin such as hypermia, dryness and scaliness.

Deuel et al (1955)[53] reported that rats placed on a high carbohydrate diet with no added fat at weanling become deficient in essential fatty acid in about twelve weeks. He also pointed out that the presence of certain completely saturated fats in the diet, for example, hydrogenated coconut oil hastened the appearance of some of the essential fatty acid deficiency symptoms. Alfin-Slater et al (1958)[59] report that essential fatty acid deficiency could be produced in rats by feeding an essentially high carbohydrate diet containing no added fat.

Macfarlane et al (1940)[60] reported that the intrinsic system as well as extrinsic system required lipid material for coagulation to take place. Houser (1958)[61] reports that it was demonstrated very early that tissue thrombo plastin was completely inactive if lipids were removed by treatment with organic recombination of the lipid protein. An active principle in blood is lipid in nature.

8. Requirements: Burr and Burr (1930)[62] state that the daily requirement of fat for maximum growth of rats has been variously set from 20 to 100 milligrams. Goldsmith (1953)[63] states that fat is needed in human nutrition for growth and replacement of tissues, lipid secretions, and energy. The requirement of the optimal level of fat in the diet is not definite yet, since fat may be formed from carbohydrate or protein.

Aykroyd (1960)[4] recommends two ounces of fat per consumption unit as the optimum intake. The Food and Nutrition Board of the Nutrition Research Council of the U.S.A recommends that an adequate diet should contain essential fatty acids to the extent of one per cent of the total calories.

D. Changes occurring in Fats and Oils due to Heat.

Perkins (1960)[9] stated that heat treatment resulted in a loss of nutritional value of the fat. Heated fat obtained from deep fat frying of foods may be organoleptically acceptable in the human diet, but exerts a growth depressing effect when fed to animals.

Perkins (1960)[9] points out that three types of degenerative changes occur in fat or oil: autoxidation (oxidation of a fat or oil at temperatures upto 100 degrees), thermal polymerisation (between 200 - 300° C. in the absence of oxygen) and thermal oxidation (oxidation at about 200° C. in the presence of oxygen.)

The effects of heated oil are reviewed under the following heads:

Effects on:

1) Growth,
2) Vitamins,
3) Internal organs,
4) Reproduction and lactation and
5) Chemical changes.

1. Effects on Growth: Whipple (1930)[64] reported that when dogs were fed autoxidised fat, they decrease rapidly in weight exhibited diarrhoea with some haemorrhage, developed loss of hair and skin lesions and ulcers and eventually become weak and died. Morris et al (1943)[65] found that rats fed diets containing lard heated to 300° C. for 120 minutes failed to grow. Crampton et al (1951)[66] pointed out that ingestion of certain oils, heat polymerized at 275° C. has been shown to depress rat growth and efficiency of feed utilisation. This reflects a lowered nutritive value of those

oils which varies in severity with the degree of unsaturation of the oil, the length of time of heating each oil and the level at which the heated oil is incorporated in the diet. The factor or factors responsible for these adverse nutritional effects appear to be present in the oil itself, and act in a manner directly toxic to the animal.

Goodman _et al_ (1952)[67] showed that thermally oxidized corn oil at 200° C. exhibited definite growth depressing action under both ad libitum and paired feeding under laboratory conditions. Kaunitz _et al_ (1955)[68] showed that the inclusion of 15 to 20 per cent of refined cottonseed oil aerated and heated to 95° C for 200 to 300 hours, in the diet led to rapid loss of weight accompanied by diarrohea, occurrence of large livers and death within three weeks.

Raju and Rajagopal (1955)[8] also observed that rats fed unsaturated oils which had been submitted to prolonged heating at 300° C did not grow. Kaunitz (1956)[69] proved that polymeric material formed during the autoxidation of fat depressed growth in rats even in the presence of abundance of dietary factors. Johnson _et al_ (1956)[70] (1957)[71] found that corn oil oxidised at 200° C for 24 hours exerted a definite depressing effect on growth and butter oil did not show such depression.

2. _Effect on vitamins:_ Irwin _et al_ (1938)[72] demonstrated that oxidised fats were not completely absorbed. Raju and Rajagopal (1955)[8] points out that heated oils were poorly absorbed, produced cancereous lesions and caused paralysis resembling that due to vitamin E deficiency. Pavcek _et al_ (1946)[73], Kummerow (1948)[74] and Holman (1950)[75] showed that the essential fatty acids,

carotene vitamin A and biotine were destroyed during the autoxidation of fats. De et al (1946)[76] observed loss of vitamin A and carotene upto 63 to 69.5 per cent during the frying of puris in ghee. Alfin-Slater (1958)[77] showed that unsaturated oils heated at about the same temperature for one or three hours caused vitamin E deficiency when fed at 15 percent level. This deficiency was corrected by vitamin E supplementation. Kaunitz (1950)[69] has reported that riboflavin exerted some protective effect on animals fed on rancid fat.

Roy (1960)[10] reports that the absorption, utilisation and storage of vitamin A and carotene were considerabl reduced in the case of animals receiving heated oils. Vitamin A and many of B complex vitamins except riboflavin and nicotinic acid were destroyed to varying extents when diets were mixed with heated oils and kept. Perkinds (1960)[9] points out that animals fed on corn or cottonseed oils oxidised at 100° C for long periods of time developed vitamin A deficiencies and eventually died.

3. <u>Effects on Internal Organs:</u> Morris et al (1943)[65] reported that rats on heated lard developed occasional leisons in the liver, heart, kidney or stomach, of the animal that survived for one year. Bernheim et al (1952)[78] showed that products of fatty acid oxidation inhibited the activity of the enzyme. They reported the inactivation of succinoxidase, cytochrome oxidase and choline oxidase by rancid fats. Herisset (1954)[79] found that the rate of enzymic hydrolysis of pancreatic lipase was retained by oxidised peanut oil. Raju and Rajagopalan (1955)[8] reported that the liver weights of rats receiving heated oil were significantly higher than those of the controls. Heated oil also produced fatty infilte-

ration of the liver.

4. Effects on Reproduction and Lactation: Farmer et al (1951)[80] showed that the female rats receiving diets containing heated oil produced only smaller number of litters. They further noticed that heated oil had an adverse effect on the reproduction and lactation in the rats.

5. Chemical Changes: Goodman et al (1952)[67] pointed out that some of the changes occurring in fats and oils due to deep fat frying were decreased in iodine value, increased in refractive index, free fatty acid content, acetyl value and drop in smoke point.

Johnson et al (1957)[71] studied the chemical changes that take place during thermal oxidation of edible oils. They found conjugation decreased and free fatty acids increased during frying.

Perkins (1960)[9] reported that the autoxidation of ethyl linoleate with oxygen led to the formation of conjugation ethyl linoleate hydroperoxide which inturn oxidised and formed higher peroxidic polymers, or combined in other ways to form non-cyclic branched chain products. If unsaturated peroxides are present, both saturated and unsaturated aldehyde and ketonic products may arise. The changes taking place in thermal polymerisation are primarily those of the normal "Diels Alder Reaction." The poly unsaturated acids undergo thermal polymerisation after conjugation to form polymeric products.

E. Fat in Cookery:

The principal uses of fats and oils in cooking are to give richness and flavour to cooked foods and as shortenings and salad dressings.

Fitch and Francis (1949)[7] defined shortening as the property of fat, which influences favourably the texture of a baked product.

Frying is one of the common methods of cooking of foodstuffs in hot fat. If the layer of fat in the frying pan is shallow, the process is called sauteing or pan frying says Fitch and Francis, (1949)[7].

Blunt et al (1915)[81] reported that the factors which affected the smoking temperature of a fat or an oil were the amount of free fatty acid present, the surface of the oil exposed, and the presence of foreign particles. The fats with highest per centage of free fatty acid would have the lowest smoking point. The smoking temperature for a particular fat is lowered when exposed surface area of the fat is increased. The wider the diameter of the cooking vessel, greater is the lowering of the smoking temperature. The presence of finely divided foreign particle lower the smoking point.

The smoking temperature affect the absorption of fat by the food stuffs. Absorption is greater in fats with low smoking points. Lowe (1955)[82] has listed the factors governing the amount of fat absorbed by foods during frying as, time of cooking the total surface of the food, the smoking temperature and the composition of the oil and the nature of foods. The longer the food is cooked, the greater the fat absorption and in higher temperatures less fat is absorbed.

Many factors affect the surface area covered by the fat, such as the nature of the fat, its concentration, the temperature the other ingredients and their concentrations, the manipulation and extent of mixing.

III. EXPERIMENTAL PROCEDURE

Experimental procedure consisted of:

A. Selection of a common fried preparation and the frying oil for this study.

B. Standardization of the recipe and the frying process.

C. Chemical analysis of the heated and fresh groundnut oil.

D. Determination of the nutritive value of the fried oil by studying its effects on weanling albino rats with regard to growth, liver vitamin A content and the blood cholesterol level.

A. Selection of a Common Fried Food Preparation and the frying oil.

For the selection of a commonly used fried food preparation and the frying oil for this study, a survey was conducted in selected families in Coimbatore city, to find out the fried food preparation found in their menus, the oils used for frying foods and the conditions of frying with special reference to the temperature and duration of frying. The survey involved:

1. Selection of the method of survey
2. Selection of the sample
3. Developing the interview schedule
4. Conducting the interview, collecting and interpreting data
5. Selection of the commonly used fried food preparation and the oil.

1. Selection of the Method of Survey: The interview cum questionnaire method was chosen for this survey, because it enabled the investigator to collect, correct, first hand information from all classes people regardless of their status of literacy.

2. Selection of the sample: Among the students in II Year B.Sc Home Science class of Sri Avinashilingam Home Science College, thirty families were selected by random sampling by including every alternative family. The selection was made from families of college students, because of their interest and co-operation.

3. Developing the interview schedule for the study: A schedule for the interview as given in Appendix I was developed to elicit information on the following: Why fats and oils are used by the home maker in daily diet, the types of food preparation made with fats and oils, the types of fats and oils used, methods employed for cooking with fats, temperature of the frying medium; whether fats are heated till the smoke comes, duration of heating of fats in frying; preferences for certain oils and fats and reasons for not using others and whether the fried oil is reused, mixed or not mixed with fresh groundnut oil.

4. Conducting the interviews, collecting and interpretation of the data: The investigator was introduced to the families by the students concerned. The students explained to the members of their families the purpose of this study, and helped in establishing rapport. Thereafter information required was collected by putting the questions in a simple, understandable manner and recorded the answers. The temperature and the duration of heating oil during the preparation of puris were actually observed and noted using a thermometer graduated upto 250°C. The information was then tabulated and interpreted.

5. Selection of the commonly used fried food preparation and the frying oil. The fried food preparations commonly used by the thirty families surveyed are

given in Table I.

TABLE I.

THE FRIED PREPARATIONS COMMONLY USED IN THE FAMILIES.

Preparation.	Frequency.	Number of families.
Puris	Weekly	15
	Fortnightly	8
	Occasionally	7
Vadai	Weekly	2
	Fortnightly	5
	Occasionally	23
Bujji	Weekly	1
	Fortnightly	4
	Occasionally	25
Bonda	Weekly	nil
	Fortnightly	4
	Occasionally	26
Applam	Weekly	4
	Fortnightly	20
	Occasionally	6

As seen from Table I, puris were the preparation most commonly used. Hence it was selected for the study.

And from the survey it was found that the groundnut oil was used by 75 per cent of the homemakers. So this oil was chosen for this investigation.

B. Standardisation of the Recipe and the Frying Process.

The standardization of the recipe and the frying process for this study included:

1. Standardization of the recipe.
2. Procurement and storage of materials.
3. Standardisation of the cooking process.
4. Selection of the equipment.
5. Procedure for making puris.

1. Standardization of recipe: The recipe for puris in this study was standardised on the basis of the information secured on the ingredients and the proportion in which they were mixed for preparing the puris by the thirty homemakers.

The ingredients of the standardized recipe ~~is~~ are given in Table II.

TABLE II

THE STANDARDIZED RECIPE FOR PURIS

Ingredients.	Quantity (gms.)
Wheatflour	590 ($3\frac{1}{2}$ cups)
Water	210 (1 cup)
Salt	1 teaspoon
Groundnut oil for frying	275 ml.
Temperature of frying	192° C to 197° C.
Number of puris	60
Time taken for frying puris	30 minutes.

2. Procurement and storage of Materials: The oil was bought from a retail shop once a week. The oil was stored in a dry tin with a tight cork.

The flour was bought once in a month and kept in tightly covered tin, salt was kept in a bottle. The preparation of the puris was standardised based on the information secured from the survey regarding the size and weight of the dough, temperature of frying oil and the time taken for frying.

3. Standardisation of cooking temperature: For frying puris the source of heat was a kerosene stove (Janatha). In order to maintain the temperature the wicks were adjusted and the same level of kerosene was poured daily into the stove. From the survey the temperature of the frying medium was found to be 192° C to 197°C.

The time to fry the puris was kept to be 30 minutes.

4. Selection of the equipment: For preparing the puris the following equipment were used:

1. An iron kadai
2. Spatula
3. Measuring cup
4. Pastry board and Roller
5. Janatha stove
6. Thermometer stand
7. Thermometer graduated upto 250°C.

An iron kadai was selected because it was used in most of the houses for frying purposes.

5. Procedure used for making puris:

a. Seived the flour and measured,

b. Added salt to the seived flour

c. Made the flour into a dough using the measured quantity of water

d. Covered the dough for 5 minutes

e. Made the dough into small balls of 10 grams.

f. Rolled it to 3" diameter

g. Poured the measured quantity of the oil in the heated pan

h. Heated the oil up to 175° C

i. Fried the puris one at a time when one side was cooked, that was after 0.3 minutes turned to the other side and fried for 0.2 minutes. After every 5th minute checked the temperature and maintained it between 192°C 197°C.

After all the puris were fried, the oil was cooled and the weanling albino rats were fed at 10 per cent level in the synthetic and poor rice diet. The oil fried once was never reused again.

Using this standardised recipe, puris were prepared daily around 7 A.M. using fresh oil for a period of 8 weeks which was the duration of the rat feeding experiment. Everyday the left over oil was used for feeding the rats. This left over oil was never reused for frying.

C. Chemical Analysis.

The thiocyanogen value of the fresh and heated oil was determined by the method given by Jacobs (1959)[83] and the iodine value was determined by Wijs method given by Jacobs (1959)[83]. From the above values the linoleic and oleic and saturated glycerides were calculated. The procedure for iodine number and thiocyanogen value are given in Appendix II and III respectively.

D. Determination of the Nutritive value of the Fried Oil:

The nutritive value of the heated oil was determined by comparing the effects of feeding young albino rats with fresh and heated oil with reference to growth, blood cholesterol, liver weight and liver vitamin A.

The experimental procedure in this regard involved:

1. Selection and maintenance of rats,
2. Sandardisation of the diets.
3. Recording food consumption.
4. Recording the weight of the rats.
5. Other observations.
6. Determination of the liver vitamin A content and blood cholesterol level.

1. Selection and Maintenance of the Rats: ~~Thirty two~~ Twenty eight weanling albino rats, of thirty days old, weighing 61.85 gms. on an average were selected for the study.

The rats were divided into four groups ~~eight~~ Seven in each with equal sex distribution. The rats were maintained in individual cages with ad libitum supply of ration and boiled cooled tap water for eight weeks.

The rat cages were made of aluminium with raised screen bottom, rectangular in shape with the following diamensions, length 12", width 8" and height 9" (figure 1). Movable trays were provided for the collection of the food droppings and feces. Each cage was provided with a card as shown in Appendix IV to record the data, weight, food intake and remarks.

A glass bottle carrying drinking water was fixed in inverted position to the side of each cage, with a tight cork through which, a small metal tube supplied water to the rats as they sucked.

Food was served on small enamel plates. An "Ohaus Balance" with animal box graduated to first decimal in grams was used to record the weight of food and rats. (Figure II). The bottom trays were cleaned daily after weighing the left over food. The cages were cleaned once in two days with soap, water and phenyl.

2. Standardisation of the diets: The diets selected for the experiments were,

a. Basal synthetic diet + 10 per cent heated groundnut oil.

b. Basal synthetic diet + 10 per cent fresh groundnut oil.

c. Basal poor rice diet + 10 per cent heated groundnut oil.

d. Basal poor rice diet + 10 per cent fresh groundnut oil.

The synthetic diet was chosen because the effects heated oil could be detected quickly. The poor rice diet was chosen because it is typical of the South Indian Diet.

The previous works on this subjects were done on 10% level. In order to compare the results of the present investigation with those of the earlier workers, 10% level was adopted. Also at this

FIGURE I.

THE RAT CAGE.

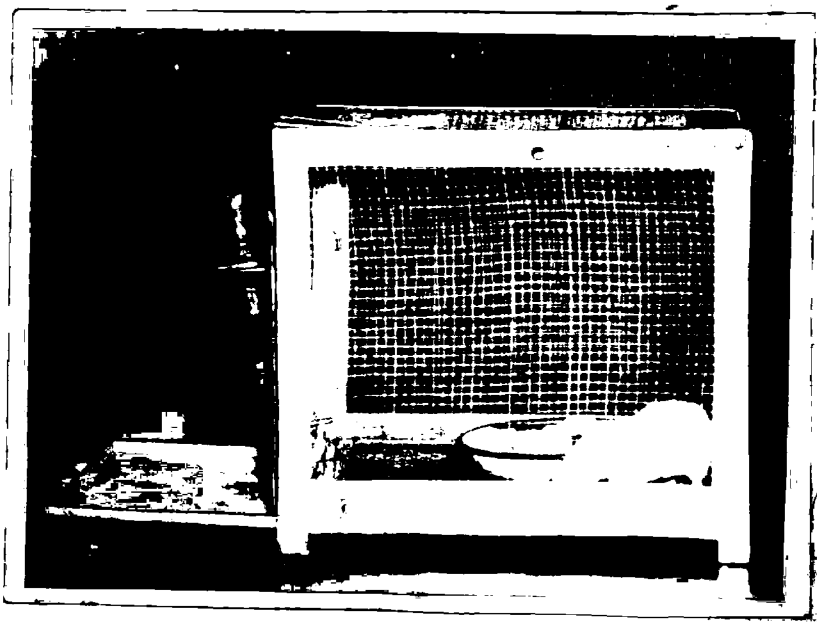
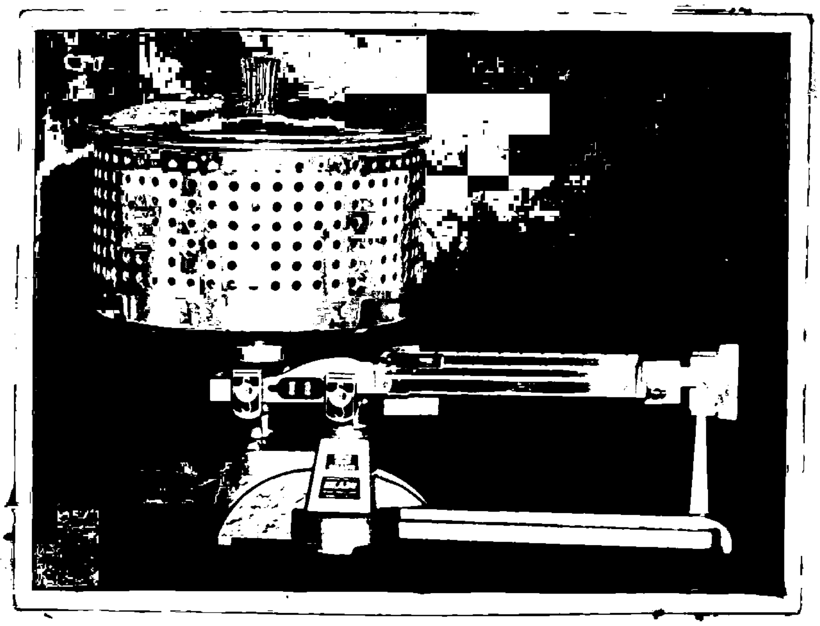


FIGURE II.

THE WEIGHING BALANCE.



level any effect which may be due to the effect of "heated oil" may be easily detectable. The ingredients needed for the preparation of the synthetic diet is given below:

TABLE III.

EXPERIMENTAL SYNTHETIC DIET.

Ingredients.	Per cent.
Corn starch.	60
Sucrose.	10
Casein.	12
* Salt mixture (1957)[18]	4
** Vitamin mixture (1959)[84]	4
Groundnut oil.	10

* given in Appendix V ** given in Appendix VI.

The ingredients needed for the preparation of the synthetic diet were bought as a lot except oil, and stored in tightly covered containers. The oil was weighed and incorporated in the daily diet, at the time of feeding.

<u>Basal poor rice diet:</u> The poor rice diet of South India used in this study was of the same as that used by Theophilus and Mason (1949)[85]. The composition of the poor rice diet is given in Table IV.

TABLE IV

THE BASAL POOR RICE DIET.

Ingredients.	Percentage.
Parboiled rice milled flour	89.70
Red gram dhal	3.00
Brinjal	4.30
Plantain	2.10
Groundnut oil	0.43
Mutton	0.26
Coconut	0.21
Distilled water	ad libitum

Devadas (1950)[86] analysis of the nutrient content of the poor rice diet is as follows:

Protein	7.05 per cent
Fat	12.00 per cent
Carbohydrate	74.00 per cent
Calcium	0.02 per cent
Phosphorus	0.15 per cent

In order to incorporate 10 per cent of the groundnut oil 11.9 per cent of the rice was reduced. Because it forms the largest percentage of the diet.

The rice and red gram dhal were made into a flour in a mill. The vegetable, mutton and coconut were ground and mixed with rice and red gram dhal and the mixture steamed for 25 minutes in an idly vessel.

Then made into balls of 60 grams each equivalent to 33.3 grams of raw food.

3. Recording the Food Consumption: Food consumption records were maintained to study the relationship between the rates of growth and nutrient intake. Devadas (1950)[86] regards an increase in food intake as indicative of an increase in growth, which in turn is the action of the nutrients present.

In order to give the rats as much as they wanted to eat, feeding was ad libitum. The amount of food given daily was such that about 10 to 15 grams of food would be left over. The daily intake of food was noted by weighing the left over food and deducting it from the original weight and was recorded in the card.

In order to determine the evaporation of moisture from the poor rice diet, aliquotes of the diet was left in an empty cage at the same time every day and weighed on the subsequent mornings. From this procedure it was found that the moisture loss was 3.5 per cent on an average. From this the necessary correction was made while calculating the food intake of the rats.

4. Recording the weights of the rats: The rats in each group were weighed on the first day of the experiment. The weights gained by them to the nearest of one tenth of a gram were recorded for each week.

5. Noting other observation on rats: The observation other than weight made daily were: General appearance, activities, conditions of the eyes, skin, tail, coat and wiskers, and symptoms of deficiencies were

recorded.

6. Determination of blood cholesterol and liver vitamin A of the Albino rats: At the end of the experimental period the animals were sacrificed to determine the blood cholesterol and liver vitamin A of the albino rats.

The rats were killed by giving a blow to the head and the blood was collected. 0.5 milli littre of the blood was taken for the estimation of cholesterol by Bloor's method.(1958)[87]. The details of the procedure is given in Appendix VII.

The liver was quickly removed cooled and dried in between the folds of a filter paper and weighed to the fourth decimal and vitamin A was determined by the Antimony trichloride method as given by Jacobs (1959)[83] with slight modification. The procedure is given in Appendix VIII.

IV RESULTS AND DISCUSSION

The results of the study on the effect of fresh and heated groundnut oil on blood cholesterol and liver vitamin A content in albino rats are discussed under the following heads:

A. Chemical Analysis of Fresh and Heated Groundnut Oil.

B. Food Intake and Weight gain of albino rats.

C. Alterations in Blood Cholesterol level and

D. Changes in Liver Weight and Vitamin A content of rats.

A. Chemical Analysis of Fresh and Heated Groundnut Oil.

The iodine and thiocyanogen value of the fresh and heated groundnut oil were determined. From these two values the linoleic glyceride and saturated glyceride content of the heated and fresh groundnut oils were calculated. The results are given in Table V.

TABLE V

CHEMICAL COMPOSITION OF FRESH AND HEATED GROUNDNUT OIL

Oils.	Iodine Value.	Thiocyanogen Value.	Linoleic Glyceride Per cent.	Oleic Glyceride per cent.	Saturated Glyceride per cent.
Fresh groundnut Oil.	101.6	81.5	24.4	63.5	12.1
Heated groundnut Oil.	91.8	74.5	20.93	32.1	48.07

From the data given in Table V it is seen that the iodine value of the heated groundnut oil was lower than that of the fresh oil. This finding is in accordance with Fulmer and Manchester (1908)[88] Goodman and Block (1952)[67], Johnson *et al* (1956)[70] and Johnson *et al* (1957)[71]. These workers found that the iodine value decreased and saturation increased on heating oils.

From the analysis, it was also found that the percentage of oleic and linoleic glycerides of the heated oil decreased. According to Rao (1960)[10] linoleic glyceride is prone to heat damage. On the other hand the saturated glyceride increased on heating the oil.

B. Food Intake and Weight Gain of Rats.

Food Intake: The food intake of rats during the experimental period was analysed and the average food intake is given in Table VI. The statistical analysis revealed that the intake of food between the groups fed on heated and fresh oil incorporated with synthetic and poor rice diets was not significant.

TABLE VI

AVERAGE FOOD INTAKE OF EXPERIMENTAL RATS DURING THE EXPERIMENTAL PERIOD (in Grams)

Groups.	I. Week	II Week	III. Week	IV Week	V. Week	VI Week	VII. Week	VIII Week
Group I	7.42	7.60	8.10	9.10	9.20	9.54	10.20	10.33
Group II	6.30	7.80	7.90	9.10	9.51	10.11	11.11	11.20
Group III	15.74	16.57	17.84	19.10	19.72	21.10	21.80	22.80
Group IV	15.37	17.20	18.12	19.20	20.51	21.11	22.11	22.9

Group I Synthetic diet with 10 per cent heated groundnut oil.

Group II Synthetic diet with 10 per cent fresh groundnut oil.

Group III Poor rice diet with 10 per cent heated groundnut oil.

Group IV Poor rice diet with 10 per cent fresh groundnut oil.

The food intake of individual rats in all the four groups are given in appendices IX and X.

Weight Gain of Rats: The effect on growth albino rats as indicated by change in body weights, has been used as an index for the assessment of the nutritive value of foods. According to Devadas (1950)[86], growth is essentially a nutritive function, and is therefore a measure of the nutritive performance. The data regarding the average weight gain of the rats are given in Table VII. The weekly gains are also illustrated in fitures III and IV. The statistical analysis of the data is given in Table VIII. The individual rats gain in weight is given in Appendix XI.

TABLE VII.

MEAN WEIGHT GAIN OF RATS FED SYNTHETIC AND POOR RICE DIETS WITH 10 PER CENT HEATED AND FRESH GROUNDNUT OIL.

Groups.	Initial average weight (gms)	Final Average weight (gms.)	Weight gained (gms.)
Group I.	62.1	91.5	29.4
Group II	61.8	104.8	43.0
Group III	61.8	78.4	16.6
Group IV.	62.1	83.1	21.0

Group I synthetic diet with 10 per cent heated groundnut oil.

Group II Synthetic diet with 10 per cent fresh groundnut oil.

Group III Poor rice diet with 10 per cent heated groundnut oil.

Group IV Poor rice diet with 10 per cent fresh groundnut oil.

TABLE VIII.

THE ANALYSIS OF VARIANCE FOR INCREASE IN WEIGHT OF RATS FED SYNTHETIC DIETS AND POOR RICE DIETS WITH TEN PER CENT OF FRESH AND HEATED GROUND NUT OIL.

Sources of variation	Degree of Freedom	Sum of Squares.	Mean Square.	*F ratio obtained	F value 5%	1%
<u>Groups I & II</u>						
Total.	17	37289.55				
Between groups	1	36106.26	36106.26	774.1**	4.50	8.66
Error (within groups	16	1182.29	73.89			
<u>Groups III & V</u>						
Total	17	56782.54				
Between groups	1	55599.92	55599.92	757.3**	4.50	8.66
Error (within groups)	16	1182.62	73.91			
<u>Groups II & IV</u>						
Total	17	97851.61				
Between groups	1	85160.79	85160.79	107.4**	4.50	8.56
Error (within groups)	16	12690.82	793.17			
<u>Groups I & III</u>						
Total	17	1502.13				
Between groups	1	142.60	142.60	1.6	4.50	8.56
Error (within groups)	16	1359.59	84.97			

* F Ratio according to Snedecor (1946)[88] is a ratio of the variences (or mean square) between th e experimental varience and within replicates. If P, the probability of its occurrence, by chance is less than .01, the difference obtained in the experiment as a whole are very significant.

** Significant beyond 1 per cent level.

GROWTH CURVES OF ALBINO RATS FED ON SYNTHETIC DIET WITH 10% FRESH AND HEATED GROUNDNUT OIL.

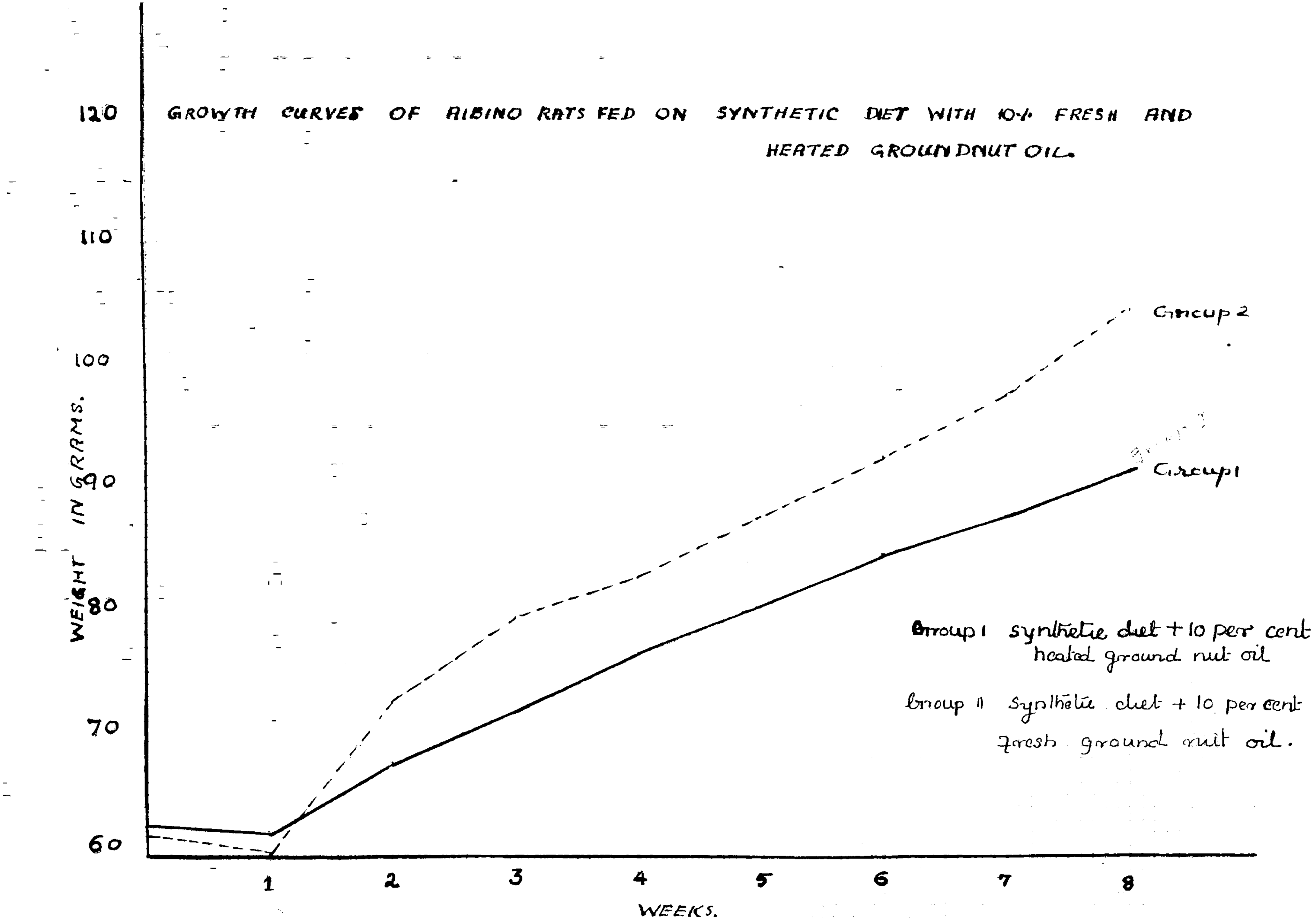


FIGURE. 3.

GROWTH CURVE OF ALBINO RATS FED ON POOR RICE DIET WITH 10% FRESH AND HEATED GROUND NUT OIL.

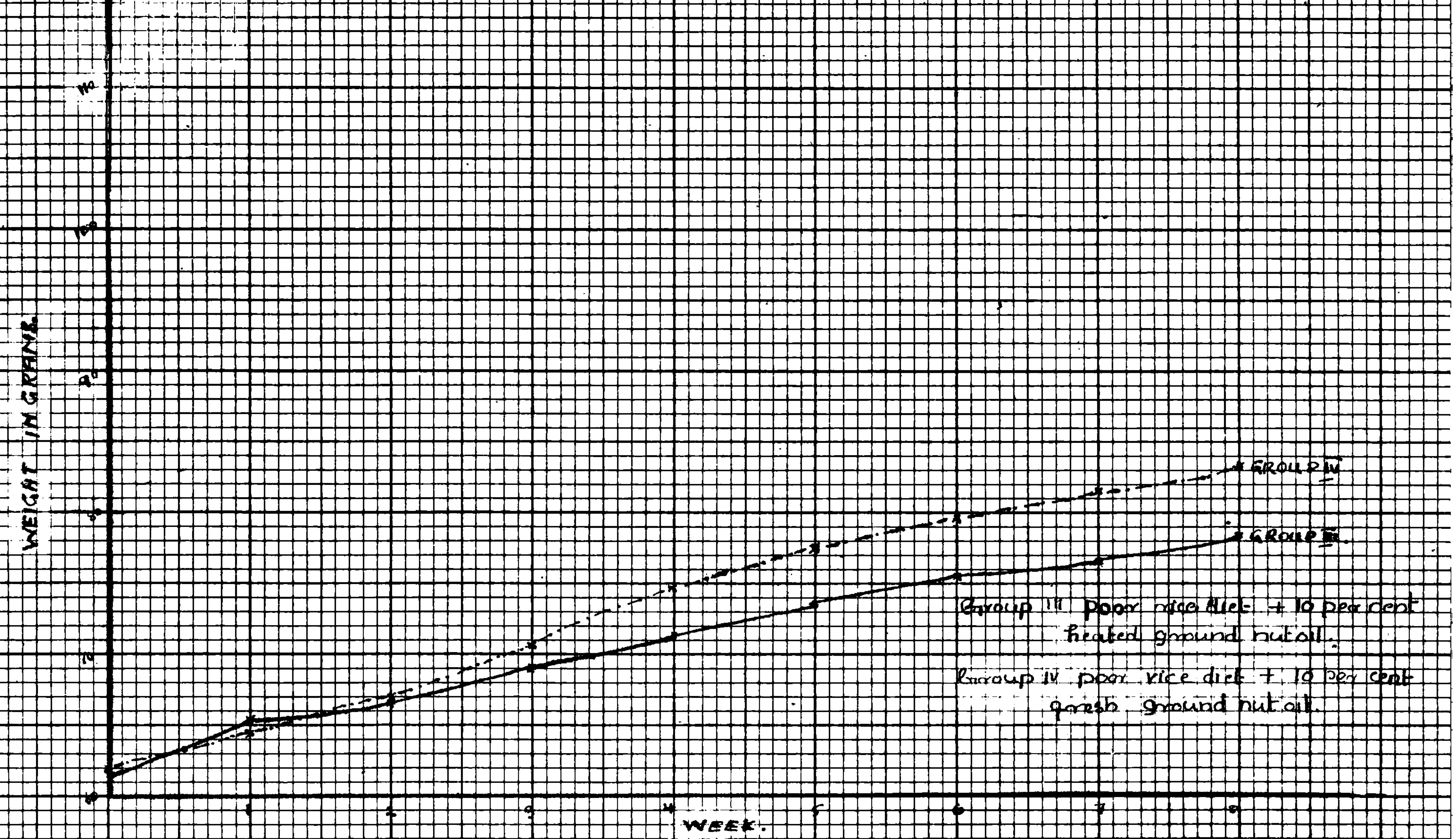


FIGURE 4.

Table VII shows that the growth of the rats on the heated groundnut oil was poor. This finding is in accordance with that of Whipple (1932)[64], Crampton (1951)[66], Goodman (1952)[67] and Kaunitz et al (1955)[68] The difference was significantly greater between the rates fed synthetic diet with fresh and heated groundnut oils, than between the poor rice diets with 10 per cent heated and fresh groundnut oils. This reason may be that fat exerts negative influence when incorporated in the imbalanced diet like the poor rice diet or the constituents of the poor rice diet, were not adequate to utilise the fat (Devadas 1950)[86].

From the Figure III it is evident that there was decrease in weights, during the I week of the experiment, only in the case of the rats on the synthetic diets and not on the poor rice diet rats. This may be due to the change from the natural stock diet to the synthetic diet. With 10 per cent fresh ground nut oil. The weight gained by the rats on the synthetic diet with 10 per cent groundnut oil was 43.83 grams while rats on synthetic diet with heated groundnut oil gained only 29.4 grams. In the case of the rats on poor rice diet with heated groundnut oil the increase in growth was 15.6 grams while that of the rats on fresh oil group is 21.1 grams.

Table VIII reveals that the difference in the gain of weight of the rats on synthetic and poor rice diets with 10 per cent heated and fresh oil was significant beyond 1 per cent level. The rats maintained on the poor rice diet gained in weight without decrease. However, the gradient was low in the case of heated oil.

<u>Other observations:</u> The coat, tail, whiskers and eyes were observed

daily in all the rats. At the end of the third week of the experiment two rats on poor rice diet with heated groundnut oil lost their hair on the sides and abdomen (Figure v.). In the case of poor rice diet with 10 per cent groundnut oil the hair started falling fro 35 days onwards (Figure VI). The coat of the rats on poor rice diet with heated oil was more rough r than those receiving the fresh oil, with rice diet group. Except for these changes on coat and hair, the whisk rs, eyes and tails were normal. Figures VII, VIII, IX and X shows experimental rats of the four groups.

FIGURE V

DEVELOPEMENT OF ALOPECIA IN RAT, FED ON POOR RICE DIET WITH TEN PER CENT HEATED GROUNDNUT OIL.

FIGURE VI

DEVELOPEMENT OF ALOPECIA IN RAT, FED ON POOR RICE DIET WITH TEN PER CENT FRESH GROUNDNUT OIL.

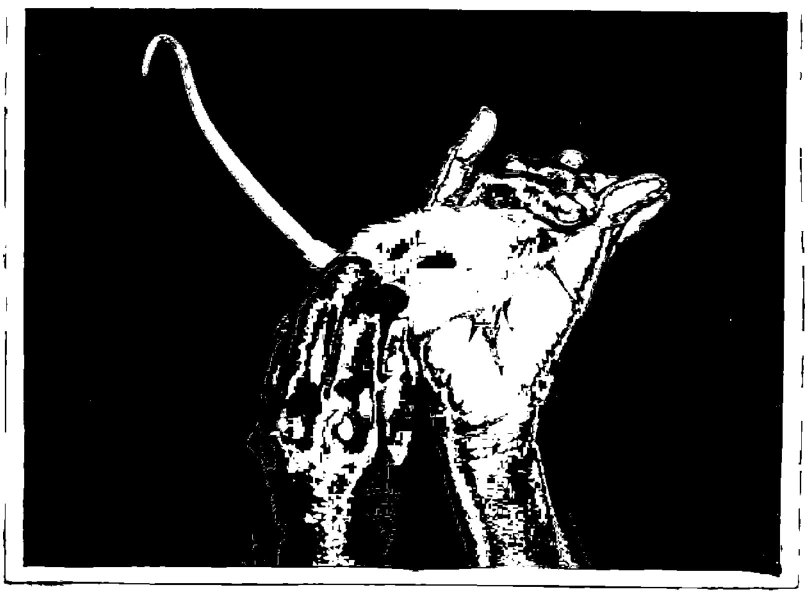


FIGURE VII

RAT FED ON POOR RICE DIET WITH TEN PER CENT HEATED GROUNDNUT OIL.

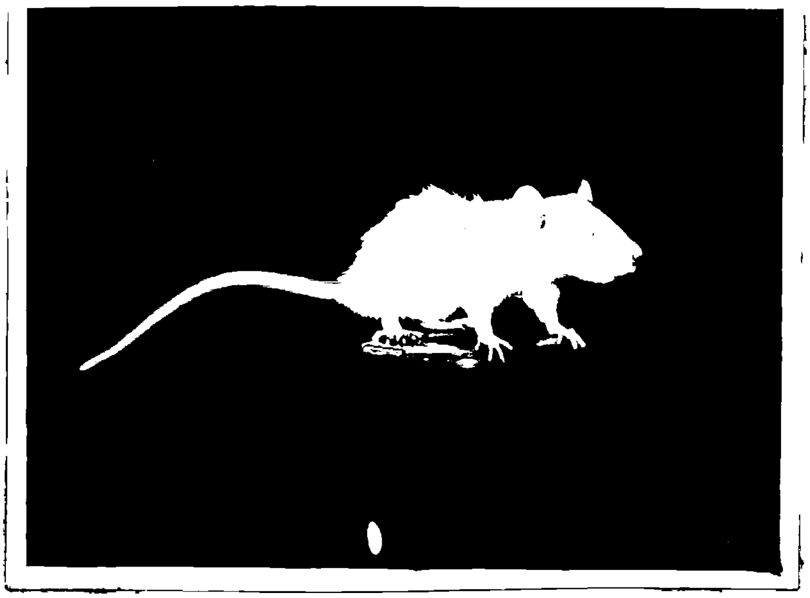


FIGURE VIII

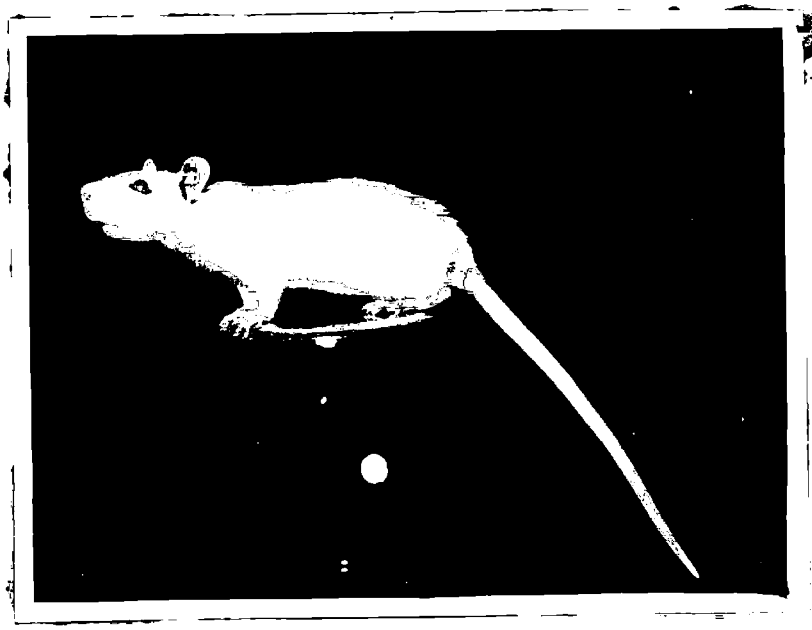
RAT FED ON POOR RICE DIET WITH TEN PER CENT FRESH GROUNDNUT OIL.

FIGURE IX

RAT FED ON SYNTHETIC DIET WITH TEN PER CENT HEATED GROUNDNUT OIL.

FIGURE X

RAT FED ON SYNTHETIC DIET WITH TEN PER CENT FRESH GROUNDNUT OIL?



C. Alteration in Blood Cholesterol.

The average blood cholesterol levels of the experimental albino rats were analysed and are presented in Table IX and Figure 11 The analysis of the data is given in Table X and the blood cholesterol level of individual rats are given Appendix XII.

TABLE IX.

AVERAGE BLOOD CHOLESTEROL LEVEL (Mg/100) of EXPERIMENTAL RATS.

Groups.	Cholesterol level (Mg/100).
Group I	64
Group II	52.9
Group III	62.7
Group IV	54.7

BLOOD CHOLESTEROL LEVEL OF THE EXPERIMENTAL RATS.

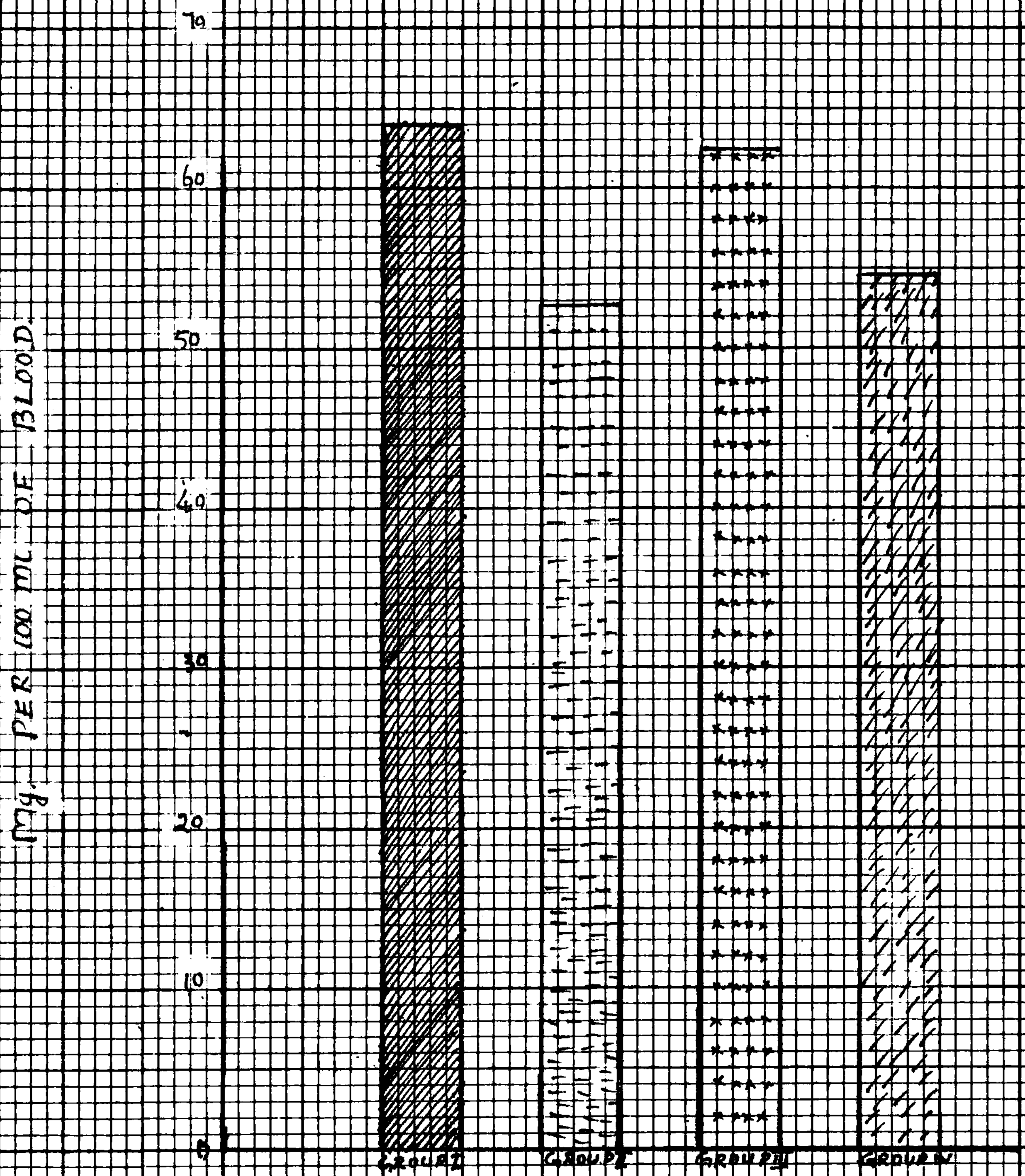


FIGURE. XI

Group I Synthetic diet + 10 percent heated ground nut oil

Group II Synthetic diet + 10 percent fresh ground nut oil.

Group III Poor rice diet + 10 percent heated ground nut oil.

Group IV Poor rice diet + 10 per cent fresh ground nut oil.

TABLE X

ANALYSIS OF MEAN VARIANCE OF THE BLOOD CHOLESTEROL LEVEL IN THE SYNTHETIC AND POOR RICE DIETS WITH 10 PER CENT HEATED AND FRESH GROUNDNUT OIL.

Sources of Variation.	Degree of freedom	Sum of Squares	Mean Squares	F Ratio observed.	Value of F at the probability level of 5%	1%
<u>Group I & II</u>						
Total.	13	408	31.4			
Between groups	1	380.7	380.7	165.5**	4.75	9.33
Error (within groups)	12	27.3	12.3			
<u>Group III & IV</u>						
Total.	13	309	23			
Between groups.	1	262.6	262.6	67.3	4.75	9.33
Error (within groups)	12	46.4	3.9			
<u>Groups I & III.</u>						
Total.	13	52.9				
Between groups	1	1.7	1.7	.39	4.75	9.33
Error (within groups)	12	51.2	4.26			
<u>Groups IV & III</u>						
Total.	13	25.67				
Between groups	1	3.1	3.1	1.66	4.75	9.33
Error (within groups)	12	22.51	1.88			

The data in Tables IX and X and figure X clearly indicate that the mean cholesterol level of blood in the gruops of rats receiving the synthetic, and poor rice diets with heated oil was higher than in the group received synthetic and poor rice diets with fresh oil. The analysis of variance (Table X) shows that the signicicance in beyond one per cent level.

D Changes in the Liver Weight and Vitamin A Content of the Liver

(a) Liver Weight

Table XI presents data on the Liver weight as the percentage of the body weight.

TABLE XI

THE LIVER WEIGHT OF RATS AS PERCENTAGE OF BODY WEIGHT

Groups	Liver weight as percentage of body weight	Standard deviation
Group I	3.521	± .2029
Group II	2.760	± .2160
Group III	4.460	± .2118
Group IV	3.920	± .3710

It is evident from the Table XI that the weight of the liver in Rats fed with heated oil was greater than that of the group fed with fresh oil in both cases. Kaunitz et al (1955) pointed out that when frats were fed with heated oil (95° C for 200 to 300 hours), developed enlarged livers. Raju and Rajagopal (1957) reported that the liver weight of heated oild group are significantly higher than that of their controls. Rice *et al* (1957) noted a slight increase in the liver weight of the group fed with heated oil.

The liver weight of the individual rats are given in appendix XIII

<u>Liver Vitamin A Content:</u> The average vitamin A content of the liver of the experimental rats are given in figure XII. From the figure it is evident that the fresh oil had higher vitamin A content than the heated oil group. Table XII gives the analysis of mean variance for vitamin A content in liver.

AVERAGE LIVER VITAMIN A CONTENT OF FOUR GROUPS OF RATS.

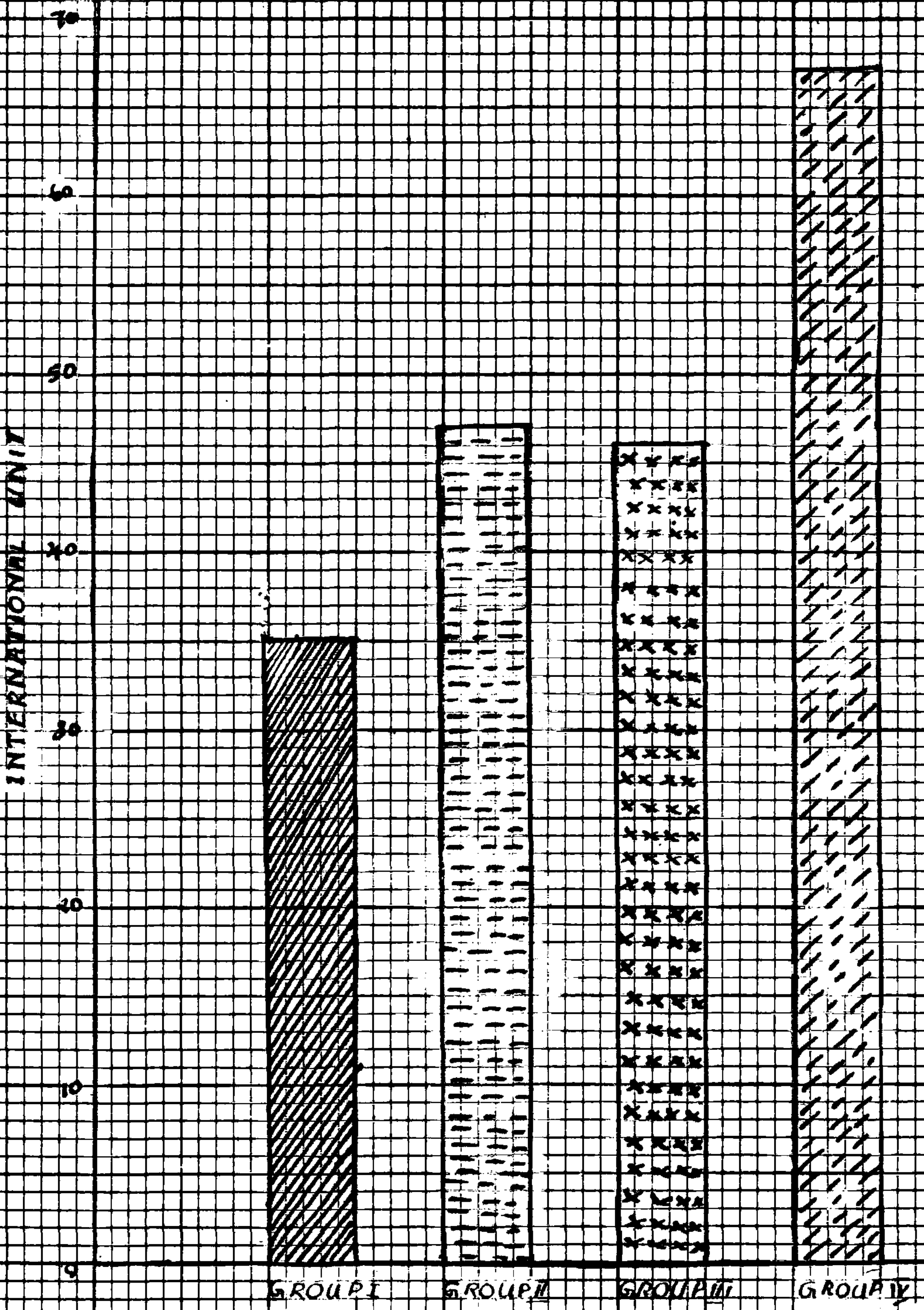


FIGURE XII

Group I Synthetic diet + 10 percent heated ground nut oil.
Group II Synthetic diet + 10 per cent fresh ground nut oil
Group III poor rice diet + 10 percent Heated ground nut oil
Group IV poor rice diet + 10 percent fresh ground nut oil.

TABLE XII

ANALYSIS OF MEAN VARIANCE OF VITAMIN A CONTENT IN THE LIVER OF THE EXPERIMENTAL RATS

Sources of Variation	Degrees of freedom	Total sum of squares	Mean squares	F.Ratio	Value of VF. at the Probability level of 5%	1%
Group I & II						
Total	13	669				
Between groups	1	539	539	49.66	9.33	4.75
Error (within groups)	12	130				
Groups III & IV						
Total	13	34081				
Between groups	1	1757.8	1757.8	32.43	9.33	4.75
Error (within groups)	12	650	54.1			
Groups I & III						
Total	13	403.1				
Between groups	1	320.6	320.6	53.07	9.33	4.75
Error (within groups)	12	72.5	6.04			
Groups II & IV						
Total	13	1937.3				
Between groups	1	1483.5	1483.5	36.49	9.33	4.75
Error (within groups)	12	453.8	37.56			

From the analysis it is evident that the vitamin A content of level of the fresh oil group was significant beyond one per cent level. This confirms the results of the studies by Pavcek *et al* (1942)[73], Kummerow *et al* (1948)[75] Witting *et al* (1958)[89] and Perkins (1960)[9]. Vitamin A content of the liver of individual rats are given in Appendix XIV

SUMMARY AND CONCLUSION.

The present study was designed to investigate the effects of fresh and heated groundnut oil on growth, blood cholesterol and liver vitamin A in albino rats. Four groups of rats, seven in each group were selected for the study. One group on the synthetic diet and one on poor rice diet, were fed on heated groundnut oil, and the other groups were fed on fresh groundnut oil. A summary of the results of the study is given below:

1. The growth of synthetic diet group receiving heated oil was 15.55 per cent less than the rats receiving the fresh groundnut oil. The same result was observed in the case of poor rice diet group also. The weight gained by the poor rice diet group was less than synthetic diet group.

2. Cholesterol level was 9.1 per cent greater in synthetic group, receiving heated oil than the fresh oil group. In the case of poor rice diet group, fed on heated oil the cholesterol level was 8.2 per cent greater than the fresh oil.

3. The liver weight of the rats fed on heated oil was greater than the fresh oil in both the poor rice diet and synthetic diet groups.

4. The liver vitamin A content was greater in rats fed on fresh groundnut oil than the rats fed on heated oil in both the poor rice diet and synthetic diet groups.

5. From the chemical analysis it was observed that the iodine value and the percentage of Oleic and linoleic glycerides decreased and the

percentage of saturated glycerides increased on heating the oil.

Thus it was shown that the heated oil in the synthetic and poor rice diets fed at 10 per cent level showed an adverse effect. It causes an increase in cholesterol level and liver weight and decrease in vitamin A content of the liver. It was also noted that heated oil aggravated the deficiency symptoms of poor rice diet. Thus it was inferred that fresh oil has better nutritive quality than the heated oil.

BIBLIOGRAPHY.

BIBLIOGRAPHY

1. Stare, F.G. EAT TO LIVE - A LONG HAPPY PRODUCTIVE LIFE. Wheat Flour Institute, West Jackson, B. Chicago 6. Illinois, 1952, p.309.

2. Basu, K.P. STUDIES ON PROTEIN, FAT AND MINERAL METABOLISM IN INDIANS. Indian Research Fund Association, Special Report No.15 June, 1946. p.17

3. FAO. of the United Nations. THIRD WORLD FOOD SURVEY. Basic Study No.11, Rome, 1963, p.42

4. Aykroyd, W.R., Gopalan, C. and Balasubramanian, S.C. THE NUTRITIVE VALUE OF INDIAN FOODS AND THE PLANNING OF SATISFACTORY DIETS, I.C.M.R., New Delhi, 1963, p.29

5. Subramanyan, V. SOME PROBLEMS AND PROCESSES RELATING TO THE UTILISATION OF OIL, OIL SEED MEALS AND OIL SEED PROTEINS. Indian oil Seeds Journal, Vol. I No.3 1957, p.

6. Sweethman, M.D. and Mackellar, FOOD AND SELECTION AND PREPARATION, Job Wiley and Sons, Inc., New York, 1954, pp. 446-450

7. Fitch, N.K. and Francis, C.A. FOODS AND PRINCIPLES OF COOKERY. Prentice - Hall, Inc., New York, 1955, pp. 131 - 134.

8. Raju, N.V. and Rajagopalan, R. NUTRITIVE VALUE OF HEATED VEGETABLE OILS, Nature, Vol. 116 1955, p. 513 - 514

9. Perkins, E.G. NUTRITIONAL AND CHEMICAL CHANGES OCCURRING IN HEATED FATS - A REVIEW. Food Technology, Vol. XIV, 1960. p.508

10. Rao, B.Y. NUTRITIONAL ASPECTS OF HEATED, OXIDISED AND POLYMERISED FATS AND OILS. Journ. Sc. Ind. Res., Vol. IX, No.9, 1960. pp.430 - 437

11. Lowe, B. Pradhan, S. and Kastelic. THE FREE FATTY ACID CONTENT AND SMOKE POINT OF SOME FATS, J.H.E., Vol. 1958, pp. 778 - 779.

12. McCollum, E.V. Orent Keiles, E. and Day, H.G. THE NEWER KNOWLEDGE OF NUTRITION. Fifth edition, The Macmillan Company, New York, 1944, pp. 6 - 20

13. McCollum and Davis, (1913) cited by McCollum, E.V., Orent Keiles and Day, H.G. THE NEWER KNOWLEDGE OF NUTRITION, Fifth edition, The Macmillan Company, New York, 1944, pp. 6 - 20

14. Burr, G.O., Burr, M.M., and Miller, E.S. (1932) cited by Basu, K.P. STUDIES ON PROTEIN, FAT AND MINERAL METABOLISM IN INDIANS. Indian Research Fund Association, 1946, p.17

15. Kundson, A. and Floody, R.J. (1940) cited by Basu, K.P. STUDIES ON PROTEIN, FAT AND MINERAL METABOLISM IN INDIANS. Indian Research Fund Association, 1946, p.17

16. Bloor, (1943) Cited by Lundberg, W.O. 'LIPIDS' in the CHEMISTRY OF FOOD AND FOOD PRODUCTS, Inter Science Publishers Inc., New York, Second Edition, Vol.I, 1951 p.104

17. West and Todd, THE TEXT BOOK OF BIOCHEMISTRY, 3rd editions The Macmillan and Company, New York, 1961, p.135.

18. Hawk, P.B. Oser, B.L., and Summerson, W.H. PRACTICAL PHYSIOLOGICAL CHEMISTRY, 12th edition, The Blaskiston Company, Toronto, 1948, p. 86 - 88 1947

19. Kleiner I.S. and Orten, J.M. HUMAN BIOCHEMISTRY, Fifth Edition, The C.V. Mosby Company, 1958, p. 82.

20. Mitchell, P.H. A TEXT BOOK OF BIOCHEMISTRY Second edition Mcgraw Hill book Company Inc., Toronto 1946 p- 65 - 69 46

21. Holman, R.T. ESSENTIAL BIOCHEMISTRY, IN VITAMINS, CHEMISTRY, PHYSIOLOGY, PATHOLOGY. Vol. II Press, New York, 1954, p. 268 - 269

22. Turpeinen, O., FURTHER STUDIES ON THE UNSATURATED FATTY ACIDS ESSENTIAL INNUTRITION, J.Nutrition, Vol. XV, 1938, p. 351

23. Smedly, Maclean, I. and Nunn, L.C.P., FAT DEFICIENCY OF RATS. THE EFFECT OF DOSES OF METHYL ARACHEDONATE AND LINOLEATE ON FAT [illegible] METABOLISM WITH ANOTE ON THE ESTIMATION OF ARACHIDONIC ACID. Biochem. J., XXXIII 1939, p.935

24. Martin, G.J., STUDIES ON FAT FREE DIETS. J. Nutrition, Vol. XVII, 1939, p. 127

25. Mackenzie, C.G., Mackenzie, J.B. and McCollum, E.V. GROWTH AND REPRODUCTION ON ALOW FAT DIET Biochem. J. Vol. XXXIII, 1939. p.935

26. Burr, G.O., Brown, J.B., Kass, J.A. and Lundberg, W.O. COMPARATIVE CURATIVE VALUES OF UNSATURATED FATTY ACIDS IN FAT DEFICIENCY, Proc. Soc. Exp. Biol. Med. Vol.XLIV, 1940, P.242.

27. Hume, E.M., Nunn, L.C.R., Maclean, I.M., and Smith, H.H. FAT DEFICIENCY DISEASE OF RATS - THE RELATIVE POTENCIES OF METHYL LINOEATE AND METHYL ARACHIDONATE WITH A NOTE ON THE ACTION OF METHYL-ESTERS OF FATY ACID FROM COD LIVER OIL. Biochem. J. Vol. XXXII, 1940, P. 2162

28. Burr, G.O., SIGNIFICANCE OF ESSENTIAL FATTY ACIDS? Fed. Proc. Vol. 224, 1942.

29. Decker, Arthur, B., Fillerup, Dorathy, L. and Mead James, F. CHRONIC ESSENTIAL FATTY ACID DEFICIENCY IN MICE. J.Nutrition, Vol. XLI, .1950 pp.507-21

30. Greenberg, Samueal, M., Calbert, Clarence, E. Savage Evelyn, E., and Deuel Harry, J. Jr. THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION J. Nutrition, Vol. XLI, 1950, p. 473 - 86

31. Deuel Harry, J. Jr. Greenberg Samuel M., Amisfeld Lille and Melnick Daniel, THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION. J.Nutrition, Col. XLV, 1951, pp. 49 .

32. Greenburg, Samuel, M., Calbert, Clarence, E. Deuel, Harry, J., Jr. And Brown J.B. THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION. J.Nutrition Vol. XLV, 1951, pp. 521-533

33. Fenton Paul, F. and Carr Claire, J. THE NUTRITION OF THE MOUSE. J.Nutrition Vol. XLV, 1951, pp. 225-33

34. Deuel, H.J. Jr., Alfin - Slater, R.B., 19Wells, A.F. Kryder, G.D. and After good, L. THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION. J. Nutrition, Vol. LV, 1955, pp.337 - 46

35. Evans, H.M., Lepkovsky and Murphy, A. Vital need of the Body for certain unsaturated fatty acids. J. Biol.Chem. Vol. CVI, 1934, p. 431

36 Mackenzie, C.G. Mackenzie, J.B. and McCollum, E.V. GROWTH AND REPRODUCTION LOW FAT DIETS. Biochem. J. Vol. XXXIII, 1939, p. 935

37. Scheer, B.T. Codie, J.F. and Deuel, H.J.Jr. THE EFFECTS OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION 3. Weight loss, mortality and recovery in young adults rats maintained on restricted calories. J.Nutrition. Vol. XXXIII, 1947, P.641.

38. Kummerow, S.A. Pan, H.P. and Hickman, H-, THE EFFECT OF DIETARY FAT ON THE REPRODUCTIVE PERFORMANCE AND MIXED FATTY ACID COMPOSITION OF FAT DEFICIENT RATS. J.Nutrition, Vol. XLVI, 1952, p459.

39. Forbes, E.B., Swift, R.W., Elliott, R.F., and James, W.H., RELATION OF FAT TO ECONOMY OF FOOD UTILIZATION 1. By the Growing albino rats, J.Nutrition Vol. XXXI, 1946, p.203.

40. Forbes, E.B., Swift, R.W., Elior, R.F. and James, W.H., RELATION OF FAT TO ECONOMY OF FOOD UTILIZATION 2, By the Mature albino rats. J. Nutrition, Vol. XXXI, 1946, p213.

41. William, W., Brush, Clark, H.and Swanson, P. DIETARY FAT AND THE NITROGEN MATOBOLISM OF RATS A FED PROTEIN FREE RATIONS. Federation Proc. Vol. VI, 1947, p. 423

42. Pearson, P.B. and Pansor, EFFECT OF FAT IN THE DIET OF RATS ON A THEIR GROWTH AND EXCRETION OF AMINO ACIDS. J.Nutrition, Vol. XXXVIII, 1949, p. 257

43. Evans, H.M. and Lepkovsky, S. , SPARING ACTION OF FAT ON THE ANTI NEURITIC VITAMIN B. , J. Biol.Chem. Vol. LXXXIII, 1929, p.269.

44. Kemmer, A.R., and Steenbock, H. , A STUDY OF SPARING ACTION OF FATS ON THE VITAMIN B CONTENT OF ANIMAL TISSUES. J. Biol.Chem., Vol. CIII 1933, p. 353

45. Mc Henry, E.W., VITAMIN B AND SYNTHESIS OF FAT FROM CARBOHYDRATE. Science Vol. LXXXVI, 1937, p. 250

46. Mc Henry E.W., , Vitamin B and FATTY LIVERS. J.Physiol. Vol. LXXXIX, 1937, p.287.

47. Quackenbush, F.W., Steenbock, H.,and Platz, B.R., THE NONSPECIFICITY OF THIAMINE IN FAT SYNTHESIS. J. Biol. Chem. Vol. CXLV, 1942, p.163.

48. Sarma, P.S. Shell, E. E. and Elvehem, C.A., THE BIOASSAY OF VITAMIN B_6 IN NATURAL MATERIALS. J.Nutrition Vol. XXXIII,, 1947, p.121

49. Williams, V.R., and Feiger, E.A. OLEIC ACID AS A GROWTH STIMULANT FOR LACTOBACILLUS CASEI. J. Biol. Chem., Vol. CLXVI, 1946, p. 335

50. Williams, V.R., and Geiger, E.A., FURTHER STUDIES ON LIPID STIMULATION OF LACTOBACILLUS CASEI, J. Biol. Chem., Vol. CLXI, 1946, Øp. 399.

51. DEUEL, HARRY, J.Jr. NON CALORIC FUNCTION OF FAT IN THE DIET. J.A.D.A., Vol. XXVI, 1950, p. 255 - 258

52. Rathmann, Dorothy, M. VEGETABLE OILS IN NUTRITION WITH SPECIAL REFERENCE TO UNSATURATED FATTY ACIDS . Canda, Starch Company Ltd,m Monheal, P.Q. Canada, 1957, pp 23-42.

53. Deuel, F.J. Jr. Alfin - Slater R.B., Wells, A.F. Kryder G.D. and Aftergood. L., THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION . J.N. Vol. LV., 1955, p.337 - 46

54. Hansen, A.E. SERUM LIPIDS IN EXZEMA AND IN OTHER PATHOLOGICAL CONDITIONS. American Journal of Dis. Chil., Vol. LIII, 1937, p. 933.

55. Harris, R.S. Holman, R.T., Hansen, A.E., and Emerson, G.A. ESSENTIAL FATTY ACIDS IN VITAMINS, Chemistry PHYSIOLOGY AND PATHOLOGY. Sebrell Witt and Harris R.S., New York, NY. , Academic Press Inc. Vol. II, 1954, p. 268 - 319

56. Anderson, R.J. and Lusk, G. Annual Calorimetery, B. THE INTER RELATION BETWEEN DIET AND BODY CONDITION AND THE ENERGY PRODUCTION DURING MECHANICAL WORK. J. Biol. Chem. Vol. XXXII, 1917, p.421.

57. Samuels, L.T., Bilmore, R.C. and Reinecke, R.M., THE EFFECT OF PREVIOUS DIET ON THE ABILITY OF ANIMALS TO DO WORK DURING SUBSEQUENT FASTING. J.Nutrition., Vol. XXXVI, 1948, p. 639.

58. Kramar, J. and Levine, Victor, E., INFLUENCE OF FATS AND FATTY ACIDS ON THE CAPILLARIES. J. NUTRITION Vol. L, 1953, p. 149 - 160 .

59. Alfin - Slater, Roslyn, B. and Bernick, S. CHANGES IN TISSUE LIPIDS AND TISSUE HISTOLOGY RESULTING FROM ESSENTIAL FATTY ACID DEFICIENCY IN RATS. Am. J. Cli. Nutrition, Vol. VI, 1958, p.613.

60. Macfarlane, R.G., Trevan, J.W. and Atwood, A. M.P. PARTICIPATION OF FAT SOLUBLE SUBSTANCE IN COAGULATION OF THE BLOOD. J. Physiol, Vol. IC, 1941, p.7

61. Rousel, G. PHOSPH LIPIDS AND BLOOD COAJULATI N. A.J. Clinical Nutriti n, Vol. VI, 1958, p. 683.

62. Burr, G.O. and Burr M.M. (1933) cited by Goldsmith, Grace A., in BIOCHEMISTRY AND PHYSIOLOGY OF NUTRITI N by Burne G.H. and Kidder G.W. Academic Press, Inc. Publishers, New York, 1953, Vol.II, p. 526 - 28

63. Goldsmith G.A. FATS IN HUMAN NUTRITI N IN BIOCHEMISTRY AND PHYSIOLOGY OF NUTRITI N. By Bourne G.H. and Kidder G.W., Academic Press. Inc. Publishers New York. 1953, Vol II 526 - 28

64. Whipple, Dorothy, V. , A SYNDROME PRODUCED IN THE DOGS BY INCLUSI N OF OXIDIZED FAT IN THE DIET. Proc. Expt. Biol. Med. Vol. 30.XXX, 1932, p. 31.

65. Morris H.P., Larsen, C.D., and Lip incott, S.W., cited by Perkins E.G. (1948) NUTRITIONAL AND CHEMICAL CHANGES OCCURING IN HEATED FATS A REVIEW. 1960 Food Tech. Vol. 14, p.509.

66. Crampton, E.W., Farmer, F. A. and Berryhill, F.M., THE EFFECT OF HEAT TREATMENT ON THE NUTRITIONAL VALUE OF SOME VEGETABLES OILS. J.Nutrition. Vol. XLII, 1951, p.

67. Goodman, A.M., and Blocks, PROBLEMS ENCOUNTERED IN THE COMMERCIAL UTILIZATION OF FRYING FATS. J.Am. Oil Chem. Soc. Vol. XXIX, 1952, pp. 616 - 619.

68. Kaunitz, H. Slanetz, C. A. and Jhonson R.E. ANTAGONISM OF FRESH FAT TO THE TOXCITY OF HEATED AND AERATED COTTONSEED OIL. J.Nutrition. Vol. LVIII, 1955, pp. 577 - 587

69. Kaunitz, H., Slanetz, C.A., Johnson, R.E., Knight, H.B., Saunders, D.H. and Swern, D. Biological effects of the polymeric residues isolated from autoxidized fats. J. Am. oil Chemists Soc., Vol 33. 1956, PG 30

70. Johnson, O.C., Sakuragi, T. and Kummerow, F.A. A COMPARATIVE STUDY OF THE NUTRITIVE VALUE OF THERMALLY OXIDIZED OILS. J.Am. Oil. Chem. Soc. Vol. XXXIII, 1956, pp. 433 - 435.

71. Johnson, O.C. Perkins, E., Sugai, M. and Kummerow, STUDIES ON THE NUTRITIONAL AND PHYSIOLOGICAL EFFECTS OF THE THERMALLY OXIDIZED OILS. J.Am. Oil Chem. Soc. Vol. XXXIV, 1957, p. 597.

72. Irwin, M.T., Weber, J., Steen Bock, H., and Godfrey, T.M. THE INFLUENCE OF HYDROGENATION AND OXIDATION OF FATS UPON THE RATE OF ABSORPTION. Am. J. Physiol. 1938, Vol. CXXIV p. 124, 800

73. Pavcek et al, P.L., and Small G.M. INACTIVATION OF BIOTIN BY RANCID FATS. J. Biol. 1946, Vol. CXLVI p.351, C

74. Holman, R.T., Spectro PHOTOMETRIC STUDIES OF THE OXIDATION OF FTS. IX COUPLED OXIDATION OF VITAMIN A ACETATE Arch. Biochem. 26, 1950, P. 85.

75. Kummerrow, F.A., Chu, T.K. and Randolph, P. EFFECTS OF AUTOXIDATION ON ANTI ACRODYNIC POTENCY OF FATS AND LINOLEIC ESTERS. J. Nutrition. Vol. XXXVI, 1948, p. 523

76. De N.K., Ranganathan, S. and Sundararajan, A.R., VITAMIN A AND CAROTENE CONTENT OF GHEE (CLARIFIED BUTTER) AND FORTYFIVED. MARGARINE. I.J.M.R. Vol. XXXIV 1946, p. 3

77. Alfin - Slater, Rosylin, B., AUERBACH, Sheila, and Aftergood, Lilla. NUTRITION EVALUATION OF SOME HEATED OILS. J. AM. Oil. Chemists Soc. Vol. XXXVI 1959, p. 638

78. Bernheim, F. Wilbur, K.M., and Kenaston, C.B., (1952) cited by Roy, B.Y. NUTRITIONAL ASPECTS OF HEATED, OXIDIZED AND POLYMERIZED FATS AND OILS JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, 1960, Vol. IX A. No. 9. p. 435.

79. Herisset, A. (1954) cited by ~~Ibid.~~ Roy, BY NUTRITIONAL ASPECTS OF HEATED, OXIDIZED AND POLYMERIZED FATS AND OILS., J. Scientific and Industrial Research, 1960, Vol IX A No 9, P 435

80. Farmer, Florance, A., Crampton, E.W. and Siddall, M.I. THE EFFECT OF HEATED LINSEED OIL ON REPRODUCTION AND LACTATION OF RATS. Vol. CXIII, Science 1951, p.408.

81. Blunt, K. and Fennely C.M., THE SMOKING TEMPERATURE OF EDIBLE OILS. M.J.He. Vol.7. 1915, p.535.

82. Lowe B., EXPERIMENTAL COOKERY FROM CHEMICAL AND PHYSICAL STAND POINT, 4th EDITION John Wiley and Sons, Inc; New York. 1955. pp.533-535 Vol.

83. Jacobs, M. B. THE CHEMICAL ANALYSIS OF FOODS AND FOOD PRODUCTS. 3rd edition, D. Van. Nostrand Company, Inc Toronto, 1958, PP. 375, 373, 700.

84. Rippon, W. P. A COMPARISON OF SEVERAL METHODS FOR ESTIMATING THE NUTRITIVE VALUE OF PROTIENS Vol XLII, 1939 P. 243.

85. Manson, B. D. Theophilues, Florence, Vrimodt Mollar. J., THE INFLUENCE OF BUTTER FAT IN THE ABSENCE OF PRESENCE OF CASEIN ON GROWTH IN YOUNG RATS ON A RICE DIET. I. J.M.R. Vol. XXXIII, 1955. p.319.

86. Devadas, R.P., ACRITICAL EVALUATION OF THE POOR RICE DIET OF SOUTH INDIA. Dissertation, Presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the graduate school of the Ohio State, University 1950, p.81 - 83.

87. Hepler, O.S., Mannual of Clinical Laboratory methods 4th Edition Charles C. Thomas, Publishers, Spring field, Illinois, U.S.A. 1958. pp 288-289.

88. Fulmer, E. and Manchester, T., THE EFFECT OF HEAT UPON THE PHYSICAL AND CHEMICAL CONSTANTS OF COTTONSEEDS OIL. J.Am. Chem. Soc. Vol XXX, 1908, p. 1477

89. Snedecor, W.G., STATISTICAL METHOD, Iowa, The Iowa State College Press, 1956, pp. 217 - 219.

90. Rice, E.E., Mone, P.E. and Poling C.E. THE EFFECT OF COMMERCIAL FRYING OPERATION ON NUTRITIVE VALUE VALUE OF FATS. Fed. Proc. 16, 1957, p.398.

APPENDIX.

APPENDIX I

QUESTIONNAIRE TO GET INFORMATION ABOUT THE COMMON OILS IN SELECTED HOUSES IN COIMBATORE.

1. Name of the head of the family:
2. Name of the homemaker:
3. Address:
4. Locality:
5. Income per month:
6. Religion:
7. Information about the members of the family :

Name	Age	Sex	Occupation	Income

IV. What are the oils used for shallow frying?

V. What are the oils used for deep fat frying?

VI. Will you heat the oil till the smoke comes before frying?

VII. How long will be frying time?

VIII. Will you use the fried oil again?

IX. Do you add fresh oil to the fried oil again?

APPENDIX II.

PROCEDURE FOR WIJS IODINE VALUE

Weighed accurately .1 to .5 grams of the oil into a flask of 250 ml. capacity with a glass stopper. Added 15 ml. of carbon tetrachloride to dissolve the oil and then 25 ml. of the wijs iodine solution with the aid of a safety pipette, allowing the pippette used to deliver the iodine solution to drain for a definite time. Placed the bottle in a dark place and allowed to stand for 30 minutes. At the end of this period, added 20 ml. of 15 per cent potassium iodide solution, stoppered the bottle and shook thoroughly and washed down the sides of the bottle and stopper with 100 ml. of recently boiled cooled water. Titrate with standard 0.1 N sodium thiosulphate solution adding the reagent with constant shaking until the yellow colour of the iodine has almost disappeared. Add 2 ml. of a 1 per cent starch solution and continue the titration. When the blue colour was almost disappeared, stopper the bottle and shake vigorously so that any iodine remaining in the organic layer will pass into the water layer. Completed the titration. Run two blank determinations on an equal portion of the wijs reagent, allowing the pipette drain for the same length of time as for the unknown. The number of millimetres of .1N sodium thiosulphate solution required by the blank less the quantity used in the determination gives the thiosulphate equivalent of the iodine absorbed by the oil. Calculated the grams of iodine absorbed by 100 g. of the fat or oil. This is Wij's iodine value.

<u>Preparation of Reagents:</u> <u>Iodine chloride solution:</u> Dissolve 13 grams of

iodine in 1 liter of glacial acetic acid and pass in sufficient washed and dried chlorine until the halogen content is almost but not quite double. This may be ascertained by thiosulphate liberations. An excess of iodine is permissible, but an excess of chlorine must be avoided.

APPENDIX III.

DETERMINATION OF THIOCYANOGEN VALUE.

Preparation of reagents: a) Lead thiocyanate. Dissolve 331 grams of finest C.P. Pb $(No_3)_2$ in 700 ml. of H_2o and filter. Slowly, add the $Pb(NO_3)_2$ solution to the KCNS solution with stirring. Continue stirring for 30 minutes and allow precipitate to settle. Decant supernatant liquid through filter paper on Buchner funnel using slight suction and wash the precipitate several times with water by decantation. Transfer precipitate to Buchner funnel using horn spoon and water and wash with water until washings give no test for nitrates. Place precipitate on a watch glass and dry to constant weight (Ca 7 days) in vacuum desicator over H_2SO_4. The dried $Pb(SCN)_2$ should be white in colour. Store in air tight brown bottle.

b) Thiocyanogen Solution: .2 N.: Prepare unhydrous acetic acid by boiling gently in litre flask with ground - in glass air condenser for about one hour, 500 ml of acetic acid (at least 99.5%) with 40 ml. of acetic anhydride. Attach a calcium chloride tube to end of condenser and allow the acid to cool to room temperature.

Solution 1. For the preparation of one litre of solution, suspend 50 grams of the lead thiocyanate in 500 ml. of acid. Two glass stoppered acid bottles of 2 liters capacity, which have previously been cleaned and dried, should be used for this purpose. Add the bromine solution to the lead thiocyanate suspension slowly, in small portions, and shake vigourously between each addition until the solution is completely decolourised. After all the bromine has been added, allow the precipitated lead bromide and the excess lead thiocyanate to settle and then filter the solution as rapidly as possible. Use a 15 cm. Buchner funnel and a qualitative filter paper together with two litre pressure flasks for the filteration. These should be cleaned and dried for one hour at 105° C. Filter the entire solution by suction into the flask. Transfer the funnel, containing the paper and the cake to the second flask and refilter the solution. It should be perfectly cleared for the second filteration. Store the solution in glass stoppered brown bottles.

procedure: Weigh accurately 0.1 to 0.3 gm. of the oil as above and transfer to a dry 125 ml. glass stoppered flask. Keep in dark for 24 hours. The storage place should be from 18 - 21° C in temperature. At the end of the 24 hours add 1 g. of dry powdered potassium iodide to the flask, and swirl the flask rapidly for 2 minutes. It is advisable to agitate the blank determination for 3 minutes. Then add 30 ml. of water and titerate the liberated iodine with 0.1 ml. sodium thiosulphate solution using starch as the indicator. At least three blanks should be run with the samples. The solutions should also be titrated at the beginning of the 24 hour period.

$$TV = \frac{\text{Blank titre value} - \text{titre value of the sample} \times N \text{ of } Na_2S_2O_3 \times 12.69}{\text{weight of the sample}},$$

in which

TV = Thiocyanogen value.
N = Normality.

This index makes it possible to calculate the composition of an oil composed of the glycerides of oleic, linoleic and a group of saturated acids. From the theoretical values of the thiocyanogen and iodine values of the glycerides the following equations may be set up.

	Theoretical iodine value.	Thiocyanogen Value
Linoleic glycerides (L)	173.20	90.59
Oleic glycerides (O)	86.01	86.01
Saturated glycerides (S)	0	0

Then placing,

$$L + O + S = 100$$

$$173.20\ L + 86.0\ O + S = IV \times 100$$

$$90.59\ L + 86.0\ O + S = TV \times 100.$$

From this formula the percentage of linoleic oleic and saturated glycerides can be obtained.

APPENDIX IV

SAMPLE CARD USED ON THE CAGE OF RATS

Exp. No.		Cage:		Diet	Card
Date	Weight gms.	Food given gms.	Food left gms.	Food eaten gms.	Remarks

APPENDIX. V

McCOLLUM DAVIS SALT MIXTURE (Gms) (1947).

Calcium lactate	—	35.5
$Ca(H_2PO_4)_2H_2O$	—	9.38
NaCl.	—	4.67.
$MgSO_4$ anhydrous	—	7.19
Fe Citrate	—	3.19 .

APPENDIX VI

VITAMIN MIXTURE (Hipron) Mgms. (in 100 Gms. of Sucrose) 1959.

Thiamine hydrochloride	10
Pyridoxine	10
Pantothemic acid	50
Nicotinamide	100
Inositol	400
Choline Chloride	4
Ribo-flavin (added last)	10

APPENDIX VII.

BLOOR'S METHOD FOR CHOLESTEROL ESTIMATION.

Colourimetric method:

(1) Add 1 ml. of blood dropwise to approximately 40 ml. of alcohol ether mixture in a glass stoppered 50 ml. volumetric flask with constant shaking in order to avoid clumping of the protein.

(2) Heat to boiling in a water bath rotating the bottle while heating to avoid loss of solution while boiling over.

(3) Cool the solution to room temperature dilute to volume with the alcohol ether mixture and mix.

(4) Place all of the solution in a fat free filter paper and allow all of it to filter into 60 ml. glass stoppered bottle. Stopper immediately and mix.

(5) Place 10 ml. of the filterate in each of two 50 ml. beakers and evaporate, just to dryness over a steam or sand bath.

(6) Add 2 ml. of chloropharm to each beaker while it is still warm and allowed to stand until coâled.

(7) Transfer the chloroform extract quantitatively to a glass stoppered 10 ml. cylinder.

(8 Wash the beaker twice with 2 ml. portions of chloroform and transfer each quantitatively to the cylinder. Make the extract upto 6 ml. with chloroform.

(9) <u>Standard:</u> Place 5 ml. of chlesterol standard solution (5 ml. =.5mg) measured at 20°C in a 10 ml. cylinder. Make upto 6 ml. with chloroform at room temperature.

(10) Place the unknown and standard in a 25°C water bath, and add 2 ml. of acetic anhydride and exactly .1 ml. of concentrated sulphuric acid to each.

(11) Stopper and micing by inverting.

(12) Allow the colour to develop in the dark for exactly 25 minutes.

(13) Read in the colour meter against the standard set at 20 mm. Dry the and prisms completely before using them.

(14) Calculations.

$$\frac{RS}{RU} \times 0.5 \times \frac{100}{.2} = \frac{5000}{RU} \text{ mg.}$$

APPENDIX

ESTIMATION OF VITAMIN A

Principle: Antimony trichloride in Chloroform gives a blue colour with vitamin A.

Reagents: Precaution: All reagents should be free from moisture.

1. Commercial cholerofom was washed three times with an equal volume of water, dried over anhydrous sodium sulphate distilled and stored over K_2CO_3 in amber bottles.

2. Antimony trichloride reagent: This is prepared by dissolving 125 grams of dry antimony trichloride C.P. in sufficient chloroform to make a final volume of 500 ml. This solution should be filtered if turbid. It is stable at room temperature for several days if protected from moisture.

3. Vitamin A Standard: 100 I.U./ml. This is prepared by dissolving the required amount of vitamin A concentrate in chloroform.

4. Procedure: Weigh 0.5 or 1 gram of the liver. Add 15 ml. of alcoholic potassium hydroxide solution. Saponify it for 15 minutes or until saponification is complete. Then add 100 ml. of glass distilled water, and transfer it to a separatory funnel. Then add to it 50 ml. of 1 : 1 ether and petroleum ether solution and shake vigourously. Allow the layers to separate and draw off the acqueous layer into another separatory funnel. Rinse the saponification flask with another 50 ml. of ether and petroleum ether mixture and extract the acqueous layer with this portion of ether. Allow the layers to separate and draw off the

acqueous layers into another separatory funnel and combine the ether layers. Extract again with 35 - 50 ml. of ether. Repeat two or more times and combine all the extracts in the separatory funnel and wash by pouring 50 to 100 ml. of water through the ether layer, without shaking. Draw off the acqueous layer and discard. Wash with 5 to 8, 50 ml. portions of water until free of alkali, testing with phenolphthalein. Take 20 grams of anhydrous Sodium Sulphate in a conical flask. Transfer the extract to the conical flask, shake and keep it aside. After 5 or 10 minutes transfer it to another flask and evaporate it to dryness. Add 5 ml. of chloroform.

The reading was taken as follows:

(1) Blank: 1 ml. of chloroform+2 drops of acetic anhydride+5 ml. of antimony tric loride.

(2) Standard vitamin A : 1 ml. of standard solution+2 drops of acetic anhydride and 5 ml. of antimony trichloride.

(3) Unknown: 1 ml. of unknown+2 drops of acetic anhydride+5 ml. of antimony trichloride.

APPENDIX XII

SERUM CHOLESTEROL LEVEL OF THE EXPERIMENTAL RATS

Group I	Group II	Group III	Group IV
63	57.7	59.8	51.5
66	52.3	64.4	56.6
64	54.1	62.2	57.7
66	54.2	63.6	51.4
61	4[illegible].9	63.4	51.4
62	52.5	63.9	56.9
62	50.9	61.8	56.2