ESSENTIALS OF FOOD AND NUTRITION

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VOLUME I

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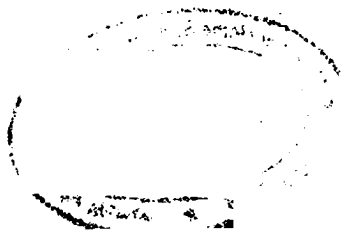

To

MY PARENTS,

Friend (late) R. S. PARTHASARATHY

and

Professor (late) S. N. CHAKRAVARTI

PREFACE

The purpose of writing this book is to provide a basic Text Book in Nutrition for undergraduate and post-graduate students of Nutrition and Dietetics, Food Science and Technology and Home Science and also a reference volume for students of Medicine and Public Health. During the last decade, the science of Food and Nutrition has grown progressively broader in scope and hence it has become difficult to cover all the aspects in one Volume. The book is, therefore, divided into two Volumes. Volume I deals with the Fundamental Aspects while Volume II deals with the Applied Aspects. The different topics have been covered in 49 Chapters in simple language so that the student can readily understand the subject. References for supplementary reading are appended to each Chapter. It is hoped that the students and teachers concerned with the discipline of Nutrition will find this book useful. The Report of the FAO/WHO *Ad Hoc* Expert Committee (1973) on "Energy and Protein Requirements" was received when the printing of the book was nearing completion. Hence, a summary of the report is given as a separate Chapter in Volume I. The material for Chapter "Applied Nutrition Programme" (Volume II) was kindly provided by Col. Barkat Narain. I am thankful to several colleagues in the Institute for kindly providing material for preparing the Chapter on "Food Processing, Preservation and Storage" (Volume II) and also to Mr. V. A. Daniel for ably assisting me in going through the manuscript before sending it to the press and in the correction of the proofs.

I wish to thank Dr. H. A. B. Parpia, Director, Central Food Technological Research Institute, Mysore for kindly granting permission to publish this book.

I also wish to express my gr[illegible] hanks to M/s. Ganesh & Co., Madras for their keen inte[illegible] encouragement and to Mr. P. M. Kuruvilla, Superint[illegible] Press, Mysore, for his kind co-operation and excelle[illegible]

CONTENTS

CHAPTER 1

HISTORY OF NUTRITION

Man has exhibited much thought and foresight in cultivating a variety of grains, fruits, vegetables, nuts and oilseeds and in rearing birds and animals for use as food. The selection of foods best suited for promoting good health has been found out by trial and error by continued use. Use of milks of different mammals as food for infants has been practised from very early times. The newer knowledge of nutrition has been built upon the observations made by several pioneers during the nineteenth century. The early history of nutrition may be conveniently discussed under the following heads: (1) Chemical nature of plant foods and animal tissues, (2) Observations on respiration and energy output in animals and human subjects, (3) Early studies on protein nutrition, (4) Feeding experiments with animals on purified diets leading to the discovery of vitamins and (5) Observations on the treatment of certain diseases in human beings by changing diet.

Important landmarks in the history of nutrition are given in Table 1.1.

Chemical nature of plant foods and animal tissues

A.Fr. de Fourcroy (1789) distinguished three kinds of animal products on the basis of their nitrogen content and laid the foundation for the study of the proteins by noting similarity of gluten of wheat and animal flesh and by showing that plant juices when heated gave a coagulum similar to animal proteins. He also discovered adipose tissue fat (Corpse wax) and described cholesterol which he obtained from bile. Sheele (1742-1786) prepared and described tartaric acid, citric acid and lactic acid. In 1747, Marggraf discovered milk sugar, and malt sugar was discovered by Prout in 1806. The latter also crystallised glucose from grape juice. In 1814, Chevreul discovered th- fats are composed of fatty acids and glycerol. Kirsc (1815) studied the acid hydrolysis of starch. Although of milk as it separates from sour milk, gelatin from water

Table 1.1 **Important landmarks in the history of nutrition**

I. *Chemical nature of plant foods and animal tissues:*

1789. A. Fr. de Fourcroy identified proteins and fats in plant and animal tissues.
1742-1786—Sheele described tartaric, citric and lactic acids.
1747—Marggraf discovered milk sugar.
1806—Prout discovered malt sugar and glucose.
1814—Chevreul described that fats are composed of fatty acids and glycerol.
1815—Kirschoff studied acid hydrolysis of starch.
1818—Beccaria prepared and studied gluten of wheat.
1820—Prout made the elementary analysis of proteins.
1822—Bracconnot hydrolysed proteins with acids.
1872—Rithausen wrote a book on the proteins of seeds.
1873-1878—Claude Bernard discovered glucose in blood and glycogen in liver.
1895—Baumann discovered iodine in thyroid gland.
1785-1850—Prout wrote a book 'Chemistry and the Function of Digestion'.
1895—Atwater published a book 'Chemistry and Economy of Food'.

II. *Observations on respiration and energy output in animals and human subjects:*

1770-1794—Lavoisier discovered that organic compounds were oxidised in the body to CO_2 and water, producing heat.
1842—Leibig suggested that it was not carbon and hydrogen which were oxidised but carbohydrates, fats and proteins.
1849—Regnault determined the respiratory quotient.
1852—Bidder and Schmidt published a book 'Digestive Juices and Metabolism' and found that the energy metabolism was constant in a fasting animal.
1862—Carl Voit determined the energy requirements of man using a human respiration calorimeter.
1892—Rubner built a calorimeter for studying energy metabolism in dogs. He established that 1 g. carbohydrate produced 4.1 KCal, 1 g. protein 4.1 KCal and 1 g. fat 9.2 KCal on oxidation in dogs.
1899—Atwater determined the energy requirements of human beings using a human respiration calorimeter and found the physiological calorific value of 1 g. carbohydrate as 4 KCal, 1 g. of protein as 4 KCal and 1 g. fat as 9 KCal.

Early studies on protein nutrition:

1890—Carl Voit and co-workers determined the protein requirements of adult man as 118 g./day.
Chittenden found the protein requirements to be 35-45 g/day.

1905-1912—Folin distinguished between two types of N metabolism (*i*) 'endogenous' i.e., metabolism of tissue proteins and (*ii*) 'exogenous' i.e., metabolism of dietary proteins.

1906—Hopkins
1912—Osborne and Mendel } They showed that zein (a protein from maize) did not support growth in mice as it was deficient in lysine and tryptophan and good growth occurred when these two amino acids were added to zein diet.

IV. *Feeding experiments with animals on purified diets leading to the discovery of vitamins:*

1881—Lunin found that mice did not survive on a diet based on casein, milk fat, cane sugar and inorganic salts for more than a few months.

1897—Eijkman discovered that addition of rice polishings extract cured polyneuritis in chicken fed on polished rice diet.

1905—Pekelharing found that addition of small amounts of milk to a synthetic diet helped to promote growth in mice.

1907—Holst and Frolich produced scurvy in guinea pigs by feeding them on a diet based on oats.

1906—Hopkins observed that addition of 2 ml. of milk per rat to a synthetic diet promoted growth. He called the growth factors present in milk 'accessory growth' factors.

1912—Funk coined the term 'Vitamine' for the growth factors and propounded the famous 'Vitamine' theory.

1913—McCollum and Davis and Osborne and Mendel independently showed that butter fat or egg yolk fat or cod liver oil added to a synthetic diet promoted good growth. McCollum and Davis designated the factor as 'Fat soluble A' and it is now known as vitamin A.

1916—McCollum and co-workers showed that addition of whey of milk to synthetic diet promoted good growth of rats. They called it 'Water soluble B'.

V. *Observations on the treatment of certain diseases in human beings by changing diet:*

1753—James Lind clearly established that scurvy among British sailors could be prevented by lime juice.

1820—Coindet used iodides for the treatment of goitre.

1824—Schutter used cod liver oil for the treatment of rickets.

1887—Takaki prevented the occurrence of beriberi among sailors in Japanese Navy by adding meat, vegetables, condensed milk and barley to polished rice diet.

1915—Goldberger established that addition of 200 g. of meat or 30 g. of brewers' yeast prevented the occurrence of pellagra in volunteers fed on a poor maize diet.

1935—Cicely Williams demonstrated that kwashiorkor in children is a disease caused by protein deficiency and can be cured by feeding milk.

meat had been cooked, egg white and flesh of animals were familiar examples of protein materials from time immemorial, no idea of their nature was gained till Beccaria (1818) prepared and studied the properties of the gluten of wheat. Prout (1820) made the first elementary analysis of proteins. Braconnot (1822) first hydrolysed proteins with acids. He discovered the amino acids, glycine and leucine. Mulder in 1838 coined the term 'protein'. In 1872, Rithausen wrote his book on the proteins of seeds. Claude Bernard (1873-1878) discovered glucose in blood and found that liver could store glucose as glycogen which he first prepared. Baumann (1895) discovered that iodine is present in thyroid gland. Prout (1785-1850) published in 1834 a book 'Chemistry, Meteorology and the Function of Digestion'. In this book, he set forth the modern views that several kinds of nutrients are essential. He distinguished them as saccharine group, oleaginic group and albuminous group and stated that during digestion these are dissolved. Atwater analysed American foods for carbohydrates, fats and proteins and published in 1895 a book 'Chemistry and Economy of Food'.

Observations on respiration and energy output in animals and human subjects

Lavoisier (1770-1794) made the great discovery regarding the nature of respiration. He believed that organic substances were oxidised in the body with the production of CO_2 and heat. A calorimeter devised by him was used to measure the heat production in guinea pigs. The studies of Leibig in 1842, showed that it was not carbon and hydrogen which were burnt in the body but carbohydrates, fats and proteins. Regnault (1849) first determined the respiratory quotient i.e., ratio of volume of CO_2 expired to O_2 consumed in animals. With Reisert, he studied O_2 consumption by animals of different sizes and found that small animals used far more O_2 per unit body weight than did large animals. It was 10 times greater in sparrows than in chicken. They rightly assigned this difference to the relative difference in the body surface between the large and small birds. Bidder and Schmidt (1852) published a book 'The Digestive Juices and Metabolism'. They described the results of studies on fasting animals, showing that energy

metabolism as represented by the amount of heat lost to the environment is constant, having the same value for animals with similar body volume, surface and temperature. Carl Voit determined the energy requirements of human adults. Atwater (1873-1900) studied the energy output of human subjects using the human respiration calorimeter and related the energy output to oxygen consumption.

Early studies on protein nutrition

Carl Voit and co-workers (1870-1890) carried out pioneering studies on protein metabolism and protein requirements. They found that when gelatin was substituted for meat protein in the diet of dogs, loss of body protein occurred. They showed that protein consumed in excess was not stored but oxidised to urea and excreted in urine. Voit set the protein requirements of adult humans at 118 g. per day. Chittenden (1901) carried out extensive studies on the protein requirements of adult man and found that active men could be maintained in good health on protein intakes of 36 to 45 g. per day. Folin (1905-1912) made extensive studies on protein metabolism. He distinguished two types of protein metabolism (*i*) endogenous i.e. metabolism of tissue proteins and (*ii*) exogenous i.e. metabolism of dietary proteins. The pioneering studies of Hopkins (1906) in England and Osborne and Mendel (1912) in U.S.A. showed that rats did not grow on a purified diet containing zein (a protein from maize) as the sole source of protein. Chemical analysis revealed that zein did not contain tryptophan and lysine. They showed that when these two amino acids were added to zein, rats grew well.

Feeding experiments with animals on purified diets leading to the discovery of vitamins

Lunin (1881) fed mice on a diet containing casein, milk fat, cane sugar and different inorganic salts. The animals survived for some months and finally lost body weight and died. Eijkman (1897) in Batavia reported that chicken fed on polished rice, developed polyneuritis and addition of rice polishings extract cured the disease. Pekelharing (1905) in Holland, found that mice did not survive on diet based on bread, rice flour, egg

albumin, casein, lard and a mixture of salts and addition of small amounts of milk to the above diet helped to promote growth and maintain the animals in good health. In 1907, Holst and Frolich produced scurvy in guinea pigs by feeding them on a diet based mainly on oats. Hopkins (1906) found that rats fed on purified mixture of casein, starch, cane sugar, lard and inorganic salts did not grow and died after some months. Addition of milk in small quantities (2 ml. per rat) promoted growth. He postulated the presence of some unknown growth factors in milk which are essential for the growth of rats and called them 'accessory factors of the diet', which are now known as 'vitamins'. Funk (1912) coined the name '*Vitamine*' for the accessory food factors and propounded the famous 'Vitamine' theory that natural foods contained distinct anti-beriberi, anti-scurvy, anti-rickets and anti-pellagra 'vitamines'. McCollum and Davis (1913) and Osborne and Mendel (1913) independently showed that albino rats fed on a diet containing bread, casein, lard or olive oil, starch and inorganic salts did not grow but when butter fat or egg yolk fat or cod liver oil was added, good growth occurred. McCollum and Davis designated the factor as 'Fat soluble A' and it is now known as vitamin A. McCollum and co-workers (1916) using purified diets showed that whey of milk contained a factor necessary for the growth of rats. They called it 'Water soluble B'.

Observations on the treatment of certain diseases in human beings by changing diet

Burnt sponge has been used from time immemorial in the treatment of goitre. Courtois (1811) discovered the presence of iodine in burnt sponge. Baumann (1896) discovered the presence of iodine in thyroid gland. Iodides were used in the treatment of goitre by Coindet in 1820. Cod liver oil has been used in the treatment of rickets from ancient times. Its use for the treatment of rickets was reported in 1824 by Schutter. Takaki (1887) reported the results of a remarkable investigation in which he showed that addition of meat, vegetables, condensed milk and barley to a ration based mainly on raw milled rice, helped to prevent the occurrence of beriberi among sailors in the Japanese Navy. James Lind (1753) clearly established that scurvy can be prevented or cured by the provision of fresh fruits or vegetables

among sailors in the British Navy. From 1795 onwards the British Navy introduced lime juice as part of the sailors' ration. Goldberger (1915) in U.S.A. found that addition of milk and eggs to a poor maize diet prevented the occurrence of pellagra among inmates in an institution. They also produced pellagra among human volunteers by feeding them on a poor diet consisting of maize, wheat flour, potato, salt pork and syrup for a period of 6 months and found that addition of 200 g. meat or 30 g. of brewers' yeast or 2 pints of milk can cure the disease. Cicely Williams demonstrated in 1935 that kwashiorkor in children is caused by protein deficiency and can be cured by feeding milk.

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CHAPTER 2

CARBOHYDRATES

Carbohydrates are widely distributed in plants in which they are formed from carbon dioxide of the atmosphere by photosynthesis. They may be broadly classified as follows:

I. Monosaccharides

1. Biose, $C_2H_4O_2$, Glycolic aldehyde.
2. Trioses, $C_3H_6O_3$ e.g., Glyceraldehyde and dihydroxy acetone.
3. Tetroses, $C_4H_8O_4$, e.g., Erythrose, threose.
4. Pentoses, $C_5H_{10}O_5$, e.g., Arabinose, xylose, ribose, deoxyribose,
5. Hexoses, $C_6H_{12}O_6$, e.g., Glucose, galactose, fructose, mannose.

II. Oligosaccharides

1. Disaccharides, $C_{12}H_{22}O_{11}$, e.g., Sucrose, lactose, maltose,
2. Trisaccharides, $C_{18}H_{32}O_{16}$, e.g., Raffinose.
3. Tetrasaccharides, $C_{24}H_{42}O_{21}$, e.g., Stachyose.

III. Polysaccharides

1. Pentosans e.g., Araban, xylan.
2. Hexosans, (*a*) Starch, dextrin and glycogen.
   (*b*) Cellulose, inulin, mannan, gallactan.

IV. Complex Polysaccharides

Hemicelluloses, gums, mucilages, pectins.

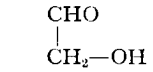
The chemical properties and uses of carbohydrates are briefly described below:

I. MONOSACCHARIDES

Biose ($C_2H_4O_2$)

Glycolic aldehyde can be prepared synthetically. It is a crystalline substance M.P. 95-97°C. It is easily soluble in water and has a sweet taste.

The chemical structure is given below:



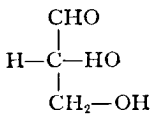
Glycolic aldehyde.

Trioses ($C_3H_6O_3$)

Two compounds of this category, i.e., glyceraldehyde and dihydroxy acetone occur in small amounts in animal and plant tissues in the form of phosphoric esters. These are derived from the break-down of glucose.

Glyceraldehyde

The chemical structure of D(+) glyceraldehyde is given below.

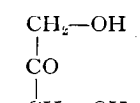


D (+)-Glyceraldehyde

The DL-glyceraldehyde has been prepared synthetically. It is a crystalline compound (MP 138°C) with a sweet taste and easily soluble in water. This compound has been taken arbitrarily as a reference compound for representing the configuration of sugars. In D-glyceraldehyde, the OH group of the CHOH is shown on the right side and in the L-glyceraldehyde on the left side. The optical rotation, dextro and levo is shown by (+) or (−) within brackets and not by the letters D and L. Glyceraldehyde phosphate is formed during the conversion of glucose to lactic acid in animal and plant cells.

Dihydroxy acetone

The chemical structure of dihydroxy acetone is given below.

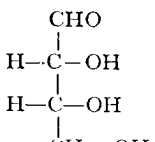


Di-hydroxy acetone

It is a white crystalline compound. It is readily soluble in water and has a sweet taste. Dihydroxy acetone phosphate is formed during the conversion of glucose into lactic acid in animal and plant cells.

Tetroses ($C_4H_8O_4$)

The chemical structure of D(-) erythrose is given below.



D(-) Erythrose

It occurs in small quantities in micro-organisms and plants. It is an intermediary product in the photosynthesis of glucose in plants.

PENTOSES

From the number of assymetric carbon atoms present, 4 isomers of aldopentoses are possible. These are arabinose, xylose, ribose, lyxose. Pentoses are not oxidised in the body to an appreciable extent.

L(+) *Arabinose:* It does not occur free in nature. The polysaccharide, Araban present in cherry gum or gum arabic is formed by the combination of a large number of arabinose molecules. Arabinose can be obtained from the hydrolysis of cherry gum or gum arabic.

L(+) *Xylose:* Xylose does not occur in free state. The polysaccharide, Xylan present in wood gum and straws, is formed by the combination of a large number of xylose molecules.

D(-) *Ribose:* It occurs in the nucleotides, adenylic acid and ribonucleic acid (RNA) present in the plant and animal tissues. It is synthesised in the tissues from glucose according to the pentose pathway.

D-2-Deoxyribose: It is present as a constituent of deoxy ribonucleic acids (DNA) present in the nucleus. It contains one oxygen atom less than ribose. The structures of ribose and deoxyribose are given in Fig. 2.1.

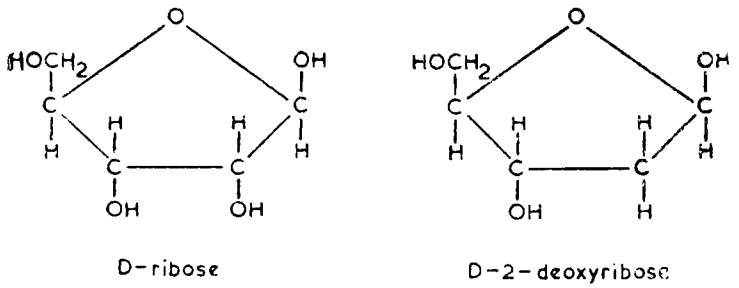


Fig. 2.1. **The structures of ribose and deoxyribose**

HEXOSES

Like other monosaccharides, hexoses can be divided into two main groups: (*i*) *Aldoses* or sugars containing an aldehyde group, e.g., glucose, galactose and mannose and (*ii*) *Ketoses*, or sugars with a ketone group, e.g., fructose and sorbose. The chemical structures of some important hexoses are given in Fig. 2.2

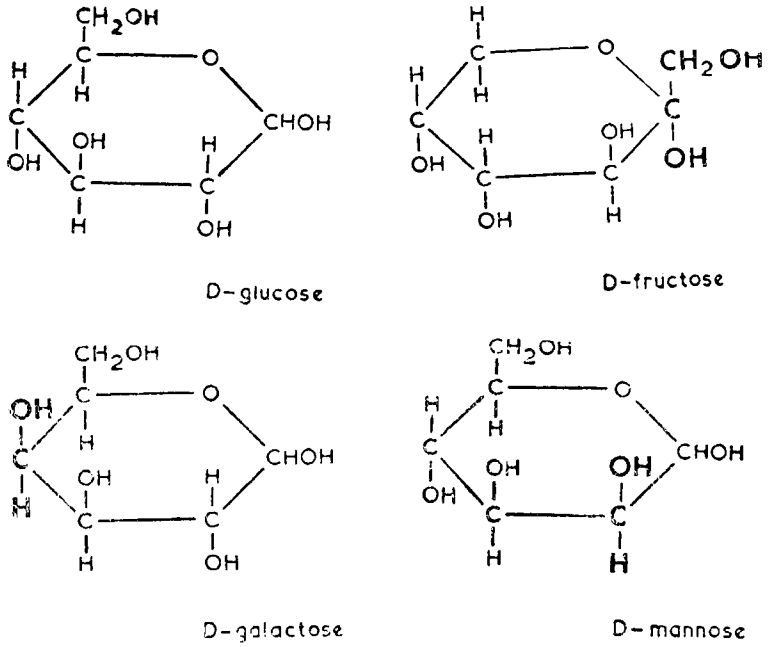


Fig. 2.2. **Structures of hexoses**

Glucose (Dextrose): Glucose occurs in the free state in many fruits (2-6 per cent) and in honey (30-40 per cent). It contains an aldehyde group. Glucose on reduction gives sorbitol. Glucose has been described as the 'Current carbohydrate coin of the body' as the body utilises carbohydrates mainly in the form of glucose. Normal human blood contains about 80-100 mg. of glucose per 100 ml. of blood. This level is maintained constant in healthy subjects. In persons suffering from the disease *diabetes mellitus* the concentration of glucose in blood may be very high (180 to 300 mg per 100 ml. of blood), depending on the severity of the disease. Glucose is not present in the urine of normal persons but in persons suffering from diabetes, glucose is excreted in the urine (upto 6 per cent).

Medicinal glucose (Glucose D, Dextrose): Glucose is manufactured on a large scale by the hydrolysis of starch by dilute hydrochloric acid or enzymes. Glucose (with or without added calcium salts and vitamin D) is used as a source of energy in the diets of medical and surgical patients as it is readily absorbed and utilised by the body.

Fructose (Levulose, fruit sugar): Fructose occurs in the free state along with glucose in many fruits (2-5 per cent) and in honey (30-40 per cent). Fructose is readily utilised by the body as a source of energy. Recent investigations have shown that fructose is better utilised as a source of energy by diabetic patients than glucose. It is twice as sweet as glucose. Fructose on reduction yields a mixture of sorbitol and mannitol.

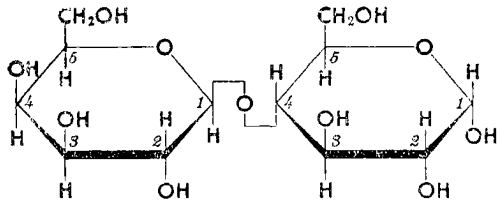
Galactose: It does not occur in the free state but is widely distributed in the combined state. Lactose, the sugar present in milk is a compound of glucose and galactose. It also occurs in cerebrosides present in brain and nervous tissues and hence its nutritional importance. Galactose on reduction yields the alcohol, dulcitol.

D(+) Mannose: This does not occur free in nature. It is found in the form of a polysaccharide, mannan occurring in ivory nuts, i.e., the seeds of Tagua palm, *Phytelephas macrocarpa* and in yeast polysaccharides. Mannose is a constituent of prosthetic polysaccharides of albumin, globulins and mucoids and also of the prosthetic polysaccharide of tubercle bacilli. Mannose on reduction gives mannitol.

Sugar alcohols. The important sugar alcohols are, sorbitol, mannitol and dulcitol. Of these, sorbitol has found use in diets for diabetic patients. D-sorbitol (D-glucitol) is an alcohol made commercially from glucose by hydrogenation. By this means, the aldehyde (CHO) group of glucose is reduced to an alcoholic group (CH_2OH). Sorbitol is found in small amounts in some fruits (such as rowan berries); it is chiefly the product made from glucose that finds use in diets for diabetics. About 90 per cent of the ingested sorbitol is absorbed and metabolised, probably after conversion to glucose as indicated by tracer studies. The rate of absorption from the gut is slow as compared with glucose and hence does not increase the blood sugar level. It is about 60 per cent as sweet as sucrose.

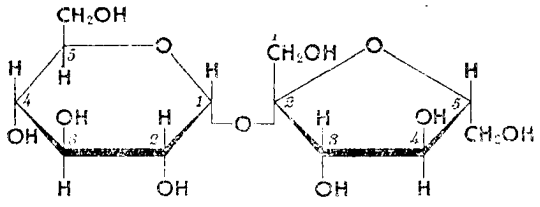
DISACCHARIDES

The disaccharides are formed by the condensation of two monosaccharides with the elimination of one molecule of water. The disaccharides of nutritional importance are (1) Sucrose,



Lactose (α form)
D-glucose 4-(β-D-galactoside)

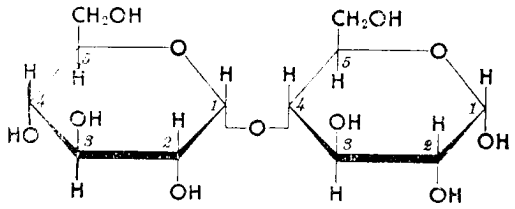
Fig. 2.3



Sucrose
α-D-glucopyranosyl-β-D-fructofuranoside

Fig. 2.4

(2) Maltose and (3) Lactose. The structures of these compounds are given in Figs. 2.3—2.5.



Maltose (α form)
D-glucose 4-(α-D-glucoside)

Fig. 2.5

Sucrose (*Cane sugar, beet sugar, saccharose*): Sucrose occurs in sugar cane (10-12 per cent) and beet root (12-18 per cent). It is manufactured on a large scale from sugar cane or beet root. It is also present in certain fruits, vegetables and honey.

Sucrose is formed by the condensation of one molecule of glucose and one molecule of fructose.

Sucrose is easily hydrolysed to glucose and fructose eithe by dilute mineral acids or by the enzyme *sucrase* present in the intestinal juice.

$$\text{Sucrose} + H_2O \xrightarrow{\text{Dilute HCl}} \text{Glucose} + \text{Fructose}$$

$$\text{Sucrose} + H_2O \xrightarrow{\text{Sucrase}} \text{Glucose} + \text{Fructose}$$

Maltose (*Malt sugar*): Maltose is present in malt. It is formed in cereal grains (Barley, jowar, ragi, etc.) during germination by the hydrolysis of starch by the enzyme, diastase (amylase).

$$\text{Starch} \xrightarrow{\text{amylase}} \text{Maltose}$$

It is also formed when starch present in the food is digested by salivary and pancreatic amylases. Maltose is formed by the condensation of two glucose molecules. It is hydrolysed to glucose by the enzyme *maltase* present in the intestinal juice.

$$\text{Maltose} + H_2O \xrightarrow{\text{Maltase}} \text{Glucose (two molecules).}$$

Lactose (*Milk sugar*): Lactose occurs in the milk of all mammals but has not so far been found in plant products. Cow's and

buffalo milks contain about 4 per cent while human milk contains about 7 per cent of lactose. Lactose is synthesised in the mammary gland by the glucose supplied by the blood. Lactose is formed by the condensation of one molecule of glucose and one molecule of galactose with the elimination of one molecule of water. Lactose is hydrolysed to glucose and galactose by the enzyme *lactase* present in the intestinal juice.

$$\text{Lactose} + H_2O \xrightarrow{\text{Lactase}} \text{Glucose} + \text{Galactose}$$

The relative sweetness of sugars and some artificial sweeteners taking the sweetness of sucrose as 100, is given in Table 2.1.

Table 2.1. **Relative sweetness of sugars**

Sugar	*Relative sweetness* (Sucrose, 100).
Lactose	16
Raffinose	22
Galactose	32
Rhamnose	32
Maltose	32
Xylose	40
Glucose	74
Sucrose	100
Invert sugar (1:1 glucose and fructose)	130
Fructose	173
Sodium cyclamate	3000
Saccharin	45000

POLYSACCHARIDES

STARCH

Starch occurs widely in the vegetable kingdom. The important sources of starch are (1) Cereals and millets (65-85 per cent) and (2) Roots and tubers (19-35 per cent). Starch occurs in the forms of granules which have characteristic shapes when seen under microscope. Starch is a polysaccharide formed in nature by the condensation of a large number (4000-15000) of glucose molecules.

Properties of Starch

It is a white, tasteless powder, insoluble in cold water. When starch is boiled with water, it forms a paste. Cereal starches differ from root and tuber starches in their physical properties. A starch paste (containing 5 per cent starch) made out of cereal starches sets to a *thick jelly* on cooling, whereas a starch paste (5 per cent starch) made out of tuber starches remains as a thick fluid and does not set to a thick jelly. A starch paste (5 per cent solution) made out of waxy cereal starches does not set to a jelly.

Starch is readily hydrolysed by hot dilute mineral acids to glucose. When cooked starch is acted upon by the enzyme, amylase present in saliva or pancreatic juice, it is hydrolysed to maltose. During the hydrolysis of starch by amylase, certain intermediate products called dextrins are formed.

$$\text{Starch} \longrightarrow \text{Dextrins} \longrightarrow \text{Maltose}$$

Starch when hydrolysed completely by dilute HCl yields glucose

$$\text{Starch} \xrightarrow{\text{Acids}} \text{Glucose}$$

Starch is used for the manufacture of glucose, dextrins, custard powder and sago.

Dextrins: Dextrins are polysaccharides formed by the partial hydrolysis of starch by means of enzymes or acids.

Chemistry of Starch

Starch consists of a mixture of two components namely amylose and amylopectin. These can be separated from one another.

Amylose: This is a long unbranched chain of glucose molecules linked together by 1 : 4 linkage, similar to that present in maltose. The molecular weights of amyloses range from 10^5 to 10^6. One molecule of amylose may contain 500 to 5000 glucose molecules. Amylose dissolves readily in water. The solution on keeping turns turbid due to the precipitation of amylose by a process known as retrogradation. Amylose is mainly responsible for the stiffening of cooked rice on standing. Amylose gives a *blue* colour with iodine.

Amylopectin: Amylopectin is insoluble in water. It has a larger molecular weight than amylose, the molecular weights ranging from 10^7 to 10^8. One molecule of amylopectin may contain 50,000

to 500,000 glucose molecules. Amylopectin has been shown to consist of branched chains, each chain consisting of 20 to 30 glucose units linked by 1:4 linkage. The different chains are linked to each other by 1:6 linkage. Amylopectin gives a *purple* colour with iodine.

The amylose and amylopectin contents of some starches are given in Table 2.2.

Table 2.2 **Amylose and amylopectin contents of cereal and root starches**

Source of Starch	Amylose (%)	Amylopectin (%)
Rice, (normal)	25	75
Rice glutinous	10-20	80-90
Wheat	24	76
Corn	28	72
Sorghum	22	78
Tapioca	17	83
Potato	22	78
Sago palm	27	73
Banana	21	79
Arrowroot	20	80
Sweet potato	18	82
Waxy rice, ,, Corn, ,, Sorghum	Nil.	97-99

Other Polysaccharides

Glycogen: Glycogen is the reserve carbohydrate found in the animal and human body. It is found mainly in the muscles (0.5 to 1 per cent) and liver (3 to 7 per cent). In a well nourished adult human being, the liver may contain about 200 g. and the muscle about 350g of glycogen. The liver contains enzymes which can convert glucose into glycogen and *vice versa*. Glycogen resembles starch in its chemical properties. It is formed by the condensation of a large number (5000-10000) of glucose molecules. It is a branched chain polysaccharide, resembling amylopectin. The chain length varies from 8 to 12 glucose units. The molecular weight of glycogen from different sources ranges from 10^5 to 10^8.

Cellulose: Cellulose is a polysaccharide made up of glucose. Its chemical structure differs from starch in the mode of linkage

between the glucose molecules. In starch, Alpha-glucose units are linked by 1:4 linkage. While in cellulose, Beta-glucose units are linked by 1:4 linkage. Due to this difference in the chemical structure, cellulose is not acted upon by amylases present in the digestive juices in the same way as starch. Cellulose is acted on by an enzyme called cellulase produced by some *moulds*. Pure forms of cellulose are cotton and paper pulp.

Fructosans: Fructosans are polysaccharides formed by combination of large number of fructose molecules. The well known example is *inulin* present in Jerusalem artichoke. Inulin on hydrolysis with dilute mineral acids yields fructose. Inulin when injected into the blood is readily excreted by the kidneys. The inulin molecule is small enough to be filtered through the renal glomerular membrane. Use is made of this property in the inulin clearance test of kidney function.

Galactans: Galactans are polysaccharides composed of galactose molecules. In addition, they contain D-mannonic acid and sulphuric acid in combination. They occur in large amounts in sea weeds. The well known poly-galactan is *agar-agar*. Agar-agar is insoluble in cold water, but soluble in hot water at concentrations of 1-4 per cent. The solution sets to a jelly on cooling. Agar-agar is used in media for culturing bacteria. Since it is not acted upon by digestive enzymes, it is used in medicine for relieving constipation in children.

Mannans: Mannans are polysaccharides composed of mannose. They occur in yeast, bacteria and some plants. The most abundant source of mannan is ivory nuts, i.e., seeds of Tagua palm *Phytelephas macrocarpa*.

Pentosans: Pentosans are polysaccharides formed by the combination of large number of pentoses. The most familiar examples are Xylan (composed of xylose) and Araban (composed of arabinose). They occur in grasses, straw, wood, gums and mucilages. They are not hydrolysed by the enzymes present in the digestive juices of human beings. The bacteria present in the large intestines decompose them.

Dextrans and Levans: Dextrans and levans are polysaccharides formed by the action of bacteria on glucose and fructose. Dextrans have high molecular weights and form rather viscous colloidal solutions in water. They have attracted considerable

clinical interest because of their possible use as plasma substitutes, since they form nontoxic solutions, with a high colloidal osmotic pressure, suitable for intravenous administration as a temporary replacement of plasma proteins lost from blood by haemorrhage. Dextrans so administered are slowly oxidised and gradually disappear from the circulation. Dextrans consist of D-glucose units joined in chains of 1:6 glycoside linkage with evidence of occasional cross-linking between chains by 1:4 linkage. Levans are composed of fructose units with 2:6 linkages between units, thus differing from inulin, which contains 1:2 linkages.

Pectins: Pectins are compounds formed by the combination of large number of galacturonic acids—anhydrogallacturonide residues, part of the carboxyl group existing as methyl esters. On hydrolysis, pectin yields mainly galacturonic acid and small amounts of galactose and arabinose. In the presence of sucrose and citric acid, pectin forms a gel. Pectin is used in food industry as an ingredient of jams and jellies. Pectin is also used in medicine for treating diarrhoea.

Hemicelluloses: Hemicelluloses are polysaccharides containing pentoses, hexoses and uronic acids. They are present in small amounts in all vegetables and in large amounts in corn cobs, grain hulls and straws. They are hydrolysed by hot dilute acids, but are not acted upon by the digestive juices in the human intestinal tract. They are acted upon by bacteria present in the large intestines.

Carbohydrate derivatives present in the mammalian tissues

A large number of carbohydrate derivatives occur as constituents of various proteins in the body.

Amino sugars: The two important amino sugars are D-glucosamine and D-galactosamine. The amino group is attached to the second carbon atom. Glucosamine occurs as a constituent of various mammalian polysaccharides and also of *Chitin*, the major polysaccharide of shells of insects and crustaceans. Galactosamine is found in the characteristic polysaccharide of cartilage-chondroitin sulphate.

Sialic acids: Sialic acids are a group of naturally occurring N- and O-acyl derivatives of a 9-carbon, 3-deoxy-5-amino sugar

acid called neuraminic acids. They are present in blood and various tissues of the body.

Muramic acid: The mucopeptides of bacterial cell walls contain another complex sugar derivative called *muramic acid.* This may be considered as a compound of N-acetylglucosamine and lactic acid.

Mucopolysaccharides: They are widely distributed throughout the animal body in the connective tissues. Some mucopolysaccharides and their constituents are given in Table 2.3.

Table 2.3 **Some Mucopolysaccharides and their Constituents**

Mucopolysaccharide	Constituents
Hyaluronic acid	D-Glucuronic acid; N-acetyl-D-glucosamine
Chondroitin	D-glucuronic acid; N-acetyl-D-galactosamine
Chondroitin sulphate A	D-Glucuronic acid: N-acetyl-D-galactosamine 4-sulphate
Chondroitin sulphate C	D-Glucuronic acid; N-acetyl-D-galactosamine 6-sulphate
Dermatan sulphate (formerly chondroitin sulphate B)	L-Iduronic acid; N-acetyl-D-Galactosamine 4-sulphate
Keratosulphate	D-Galactose 6-sulphate; N-acetyl-glucosamine 6-sulphate
Heparin	Glucuronic acid 2-sulphate, Glucosamine 6-O sulphate; N-sulphate

Hyaluronic acid: Hyaluronic acid is a polysaccharide composed of glucuronic acid and glucosamine. It forms a highly viscous solution in water and acts as a protective and lubricating factor. It is found in the synovial fluid of joints and in the vitreous humour of the eyes.

Mucus: Mucus is a secretion of mucous glands located in epithelial structures. Older literature refers to the presence in mucous of a polysaccharide of specific structure, which was termed mucoitin sulphate. Recent work does not provide any evidence for the presence of mucoitin sulphate. Mucous secretions contain a mixture of mucopolysaccharides and glycoproteins.

Chondroitin sulphate: Chondroitin is a polymer of D-glucuronic acid and N-acetyl-galactosamine. Chondroitin sulphate is widely distributed in connective tissue.

Heparin: Heparin is an anticoagulant produced in liver and found in blood. It is a sulphated polysaccharide and yields on hydrolysis, one molecule each of D-glucosamine, and D-glucuronic acid and two molecules of sulphuric acid. The molecular weight is in the range of 20,000.

Blood group polysaccharides: Blood-group specific polysaccharides are present in red blood cells. They are quite complex in structure and yield on hydrolysis glucosamine, galactosamine, galactose, fucose and N-acetylneuraminic acid.

Important sources of carbohydrates in the diet

The important sources of carbohydrates in the diets of children and adults are cereals, millets, roots, tubers, pulses, sugar and jaggery. The important sources of carbohydrates in the diets of infants are milk and sugar.

The carbohydrate contents of different groups of foods are given in Table 2.4.

Table 2.4 **Carbohydrate contents of some foods**

Name of food	Carbohydrate (g/100g)
Cereals and millets (Rice, jowar, etc.)	63-79
Pulses (Bengal gram, red gram, etc.)	56-60
Nuts and oilseeds	10-25
Roots and tubers (potato, tapioca, sweet potato, etc. on fresh basis)	22-39
Arrowroot flour	85-87
Cane sugar	99
Sago	87-89
Honey	79-80
Jaggery	94-95
Milk (fresh)	4- 5
Dried fruits (Raisins, dates, etc.)	67-77
Fresh fruits	10-25

The carbohydrate contents of individual foods are given in Table 1 A in the Appendix:

Functions of carbohydrates

Carbohydrates have a variety of functions in the anima l organisms: (1) they supply energy for body functions, (2) they are essential for the oxidation of fats, (3) they exert a sparing

action of proteins, (4) some of the carbohydrates are present in some tissue constituents (5) they add flavour to the diet and (6) starch which forms the main source of carbohydrates, in the average diets, has a bland taste and is non-irritant. It can be consumed in large amounts without any ill effects, thus providing the major portion of the energy requirements of the body.

The main function of carbohydrates is to supply energy for the body processes. Glucose is essential for life processes. A greater part of the energy in the diet, i.e., more than 50-80 per cent is supplied by carbohydrates. Even though fat yields twice as much energy as carbohydrates for unit weight, carbohydrate is essential for the oxidation of fats. The common expression that 'fat burns in the fire of carbohydrates' is used to emphasize the fact that in the absence of carbohydrates, fats cannot be oxidised by the body to yield energy. Recent studies have shown that oxaloacetic acid, a break down product of carbohydrate, is essential for the oxidation of acetate which is the breakdown product of fats. In the absence of oxaloacetic acid, acetate is converted into ketone bodies (acetoacetic acid, acetone etc.), which are toxic to the animal organism. Ketosis occurs in diabetes where the cells cannot utilise carbohydrates and in starvation where the cells must use fat stores in the body as a source of energy. Carbohydrates like sucrose are used to sweeten food preparations and drinks to make them more acceptable.

Digestion and absorption of carbohydrates

The first stage in the digestion of carbohydrates takes place in the mouth when the food is chewed. The saliva contains an alpha-amylase known as *Ptyalin*. This enzyme acts on starch splitting it into dextrin and maltose. Amylase acts best at neutral pH. As soon as the food reaches the stomach, it mixes with the gastric juice and amylase activity is inhibited. The acidity of the stomach contents may cause the hydrolysis of some of the sucrose present in the diet. The digestion of carbohydrates is mainly accomplished in the small intestines where they are subjected to the action of pancreatic amylase and intestinal amylase, sucrase, lactase, maltase and isomaltase (Oligo 1:6 glucosidase). Ultimately, starch is broken down to glucose, sucrose to glucose and fructose, maltose to glucose and lactose to glucose and galactose.

$$\text{Amylose} \xrightarrow[\text{Amylase}]{\text{Pancreatic}} \text{Maltose}$$

$$\text{Amylopectin} \xrightarrow[\text{Amylase}]{\text{Pancreatic}} \text{Maltose} + \text{Isomaltose}$$

$$\text{Glycogen} \xrightarrow[\text{Amylase}]{\text{Pancreatic}} \text{Maltose} + \text{Isomaltose}$$

$$\text{Maltose} \xrightarrow{\text{Maltase}} \text{Glucose}$$

$$\text{Isomaltose} \xrightarrow{\text{Isomaltase}} \text{Glucose}$$

$$\text{Sucrose} \xrightarrow{\text{Sucrase}} \text{Glucose} + \text{Fructose}$$

$$\text{Lactose} \xrightarrow{\text{Lactase}} \text{Glucose} + \text{Galactose}$$

The hexoses are absorbed through the walls of the intestines. The rates of absorption of the three hexoses present at 10 per cent solution by a 50 cm. length Jejunum in one hour are as follows: Galactose, 9.5g; glucose, 8 g; and fructose, 5 g.

The non-digestible polysaccharides present in the food, viz., cellulose, pentosans, galactans, fructosans are not acted upon by the enzymes present in the digestive juices. They are, however, acted upon by bacteria present in the large intestines, with the formation of CO_2, alcohols and organic acids.

Fate of absorbed carbohydrates

Glucose, galactose and fructose absorbed in the intestines pass through the portal circulation to the liver. In the liver, a part of glucose and the entire galactose and fructose are converted into glycogen. A part of the glucose passes into the general circulation and to the various tissues. A scheme of utilisation of glucose, galactose and fructose absorbed from the food is shown in Fig. 2.6. It is evident that (*a*) a part of the glucose enters the systemic circulation to different tissues for being oxidised and used as energy, (*b*) some of the glucose and the entire quantity of galactose and fructose pass to the liver for being converted into glycogen and (*c*) a small part may pass to adipose tissue for conversion to fat. All these processes occur at the same time.

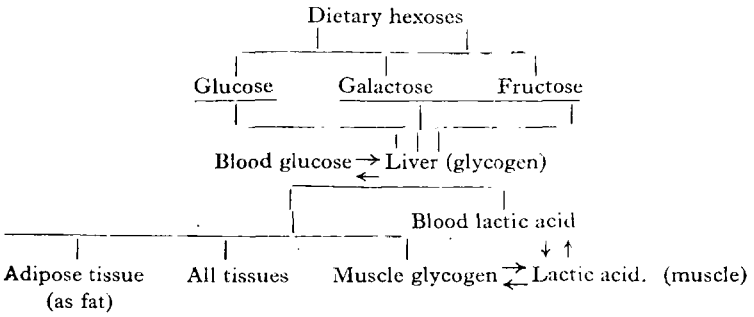


Fig. 2.6 Mode of utilisation of glucose and other hexoses in the body.

Formation of glycogen in liver

Fructose absorbed from the intestines is converted into glucose and glycogen. Fructose is converted into fructose-6-phosphate which, in turn, can be converted into glucose-6-phosphate. Galactose absorbed from the intestines is converted into galactose-1-phosphate which can be converted into glucose-1-phosphate. The steps involved in the conversion of glucose, fructose and galactose into glycogen in the liver are shown in Fig. 2.7.

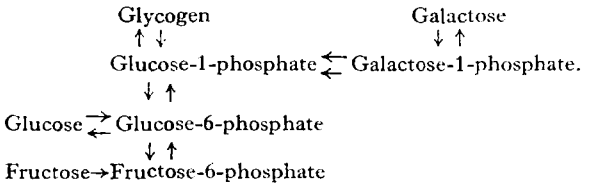


Fig. 2.7. Formation of glycogen from glucose, fructose and galactose.

Data regarding the quantity of glycogen and glucose present in the body are given in Table 2.5.

Table 2.5 **Carbohydrate content of a normal adult**

Body weight, 70 kg., liver weight, 1800g; muscle mass, 35kg., volume of blood and extracellular fluid, 21 litres (Soskin and Levine 1952)

Organ or tissue	Per cent	g.
Muscle glycogen	0.07	245
Liver glycogen	6.00	108
Blood and extra cellular fluid glucose	0.08	17
	Total	370

METABOLISM OF CARBOHYDRATES

The metabolism of glucose can be discussed under the following heads: (1) Glycolysis, (2) Kreb's tri-carboxylic acid cycle and (3) Other pathways of carbohydrate metabolism.

Glycolysis

The term glycolysis is used to the anaerobic phase of the carbohydrate metabolism whereby glucose or glycogen is converted to lactic acid. The sequence of reactions is frequently referred to Embden-Meyerhof pathway (Fig. 2.8). The conversion of glycogen to glucose is known as *glycogenolysis*, while the reverse process, i.e., conversion of glucose to glycogen is known as *glycogenesis*.

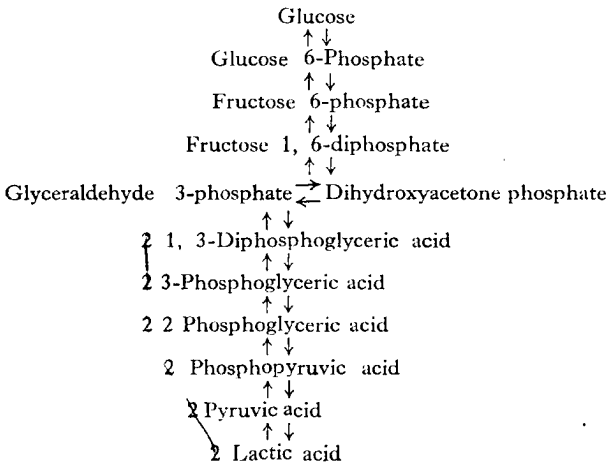


Fig. 2.8. The reactions of glycolysis

Oxygen is not required for glycolysis. Although two of the individual steps are oxidoreductions, viz., the oxidation of glyceraldehyde-3-phosphate to 1-3 Diphosphoglyceric acid and the reduction of pyruvic acid to lactic acid, the participation of DPN in both these reactions results in no net oxidation or reduction. The net yield of energy liberated in the overall process of glucose to lactate may be computed from a balance of molecules of ATP consumed and regenerated per mole of glucose degraded to lactate. It is evident that 2 Mols. of ATP are utilised and

4 Mols. of ATP are regenerated. Out of a total energy of 6,86,000 cal. per mole of glucose oxidised, only about 8 per cent of this, i.e., some 56,000 calories are produced per mole glucose converted to lactate. Glycolysis takes place mainly in muscle during muscular contraction and in almost all tissues.

Oxidation of carbohydrates—Kreb's tricarboxylic acid cycle

Under anaerobic conditions, pyruvic acid is converted into lactic acid but under aerobic conditions, pyruvic acid is oxidised through Kreb's tricarboxylic acid cycle to CO_2 and water. This scheme was originally discovered by Krebs and co-workers.

The different steps in the Kreb's cycle leading to the oxidation of acetate are shown in Fig. 2.9 and discussed below:

Pyruvic acid is oxidised to acetyl CoA

Pyruvic acid $\longrightarrow$ Acetyl CoA + CO_2

The acetyl group of Acetyl CoA is oxidised through Kreb's tricarboxylic acid cycle as outlined in Fig. 2.9.

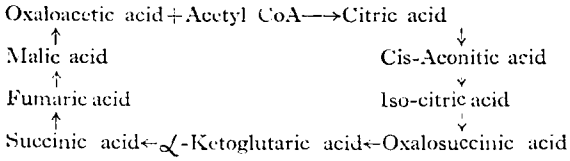


Fig. 2.9. Kreb's tricarboxylic acid cycle

(1) Acetyl CoA reacts with oxaloacetic acid leading to the formation of citric acid
(2) Citric acid is converted into Cis-aconitic acid
(3) Cis-aconitic acid is converted into iso-citric acid
(4) Isocitric acid is converted into oxalosuccinic acid
(5) Oxalosuccinic acid is oxidised to α-ketoglutaric acid
(6) α-ketoglutaric acid is oxidised to succinic acid
(7) Succinic acid is converted into fumaric acid
(8) Fumaric acid is converted into malic acid
(9) Malic acid is oxidised to oxaloacetic acid.

The oxaloacetic acid thus regenerated reacts again with another molecule of acetyl CoA and the cycle is repeated.

Alternate pathways of carbohydrate metabolism

The glycolytic pathway and the tricarboxylic acid cycle serve as the most important mechanisms whereby carbohydrates are oxidised to produce energy. However, alternate pathways for the metabolism of carbohydrates are known which serve not only to supplement the main ones, but also may provide a means for the oxidation or synthesis of metabolites which do not appear as an intermediate in the above pathways. The alternate pathways include (1) the hexose monophosphate shunt or the pentose phosphate pathway or phosphogluconate oxidative pathway, (2) the glucuronic acid cycle, (3) the Enter-Doudoroff pathway and (4) the glyoxylate pathway. The last two occur mainly in microorganisms and hence will not be described here.

(1) *The hexose monophosphate shunt or the pentose phosphate pathway:* The extent of the role this pathway plays in the total metabolism of carbohydrates in the animal body is not completely known. In liver, the pentose pathway may account for as much as 60 per cent of the total carbohydrate oxidation. It also operates quite actively in adipose tissue but is less important in other organs or may even not operate in other organs and tissues. By this pathway, ribose is formed from glucose.

(2) *The Glucuronic acid cycle:* Ascorbic acid can be synthesised from glucose by animals other than man, guinea pigs and anthropods by this pathway. This pathway is not of much importance in men and guinea pigs.

The regulation of glucose level in blood

In the fasting stage, the blood contains about 80 to 100 mg. of glucose/100 ml. After a meal, the glucose level steadily rises and may reach a level of 130-150 mg/100 ml. but rarely exceeds 180 mg/100 ml. in normal human subjects. If the level reaches above 180 mg/100 ml. (Renal threshold level), glucose is excreted in urine as in persons suffering from the disease, diabetes mellitus. The effect on the blood glucose level of taking 50 g. of glucose by mouth in the fasting state is known as the glucose tolerance test. This is described later in this chapter under diabetes mellitus. In the fasting state, the blood glucose level is maintained constant by the break down of glycogen in liver into glucose.

Various hormones play important roles in maintaining the blood glucose at a constant level. These are discussed below.

(1) *Insulin:* Insulin is a hormone secreted into the blood by the beta-cells of the islets of Langerhans of the pancreas. The normal stimulus to insulin secretion is ingestion of carbohydrates. Injection of insulin brings down the blood glucose level within a few minutes. Insulin increases the utilisation of glucose by the tissues. The bulk of current evidence favours the view that glucose is translocated from blood and extra cellular compartment to the intra cellular compartment, i.e. within the tissue cells. Synthesis of glycogen by liver and muscle and of fatty acids by liver and adipose tissue is enhanced; lactic and pyruvic acids are formed in greater quantities and a rise in the fasting respiratory quotient towards unity, indicating that large amounts of glucose have been utilised by the tissues for oxidation.

(2) *Adrenaline:* This is a hormone produced by the adrenal medulla. Injection of adrenaline produces a *rapid* but transitory rise in blood glucose by releasing glucose into circulation by the break-down of liver glycogen.

(3) *Glucagon:* This is a hormone produced by the alpha-cells of the pancreatic islets. Injection of glucagon causes an increase in blood glucose due to the break-down of liver glycogen.

(4) *Cortisone:* Cortisone and allied compounds produced by the *cortex* of adrenal gland increase the formation of liver glycogen from protein and fat (glycerol of fat) and hence increase the potential supply of blood glucose.

(5) *Hormones of the anterior pituitary:* Growth hormone of the anterior pituitary has been studied in relation to carbohydrate metabolism. Injection of growth hormone mobilises fat from the adipose tissue and also causes a rise in blood sugar level after a few days. In dogs, permanent state of diabetes mellitus can be produced by repeated injection of the growth hormone. Histological examination has revealed injury to the β-cells, thus affecting the production of insulin.

Houssay made the important discovery that in animals from which the anterior pituitary and pancreas have been removed, diabetes could not be induced by the injection of growth hormone. These animals (Houssay animals) are extremely sensitive to injections of insulin.

The regulatory mechanisms mentioned above are so balanced that they help to maintain the blood glucose level within normal range despite great variations in the rate of supply from the food and in the rate of utilisation by the tissues.

Hypoglycemia and Hyperglycemia: If the fasting blood glucose level falls below 80 mg/100 ml., the condition is known as hypoglycemia. If the level falls below 50 mg., convulsions may occur.

Hyperglycemia may be defined as a condition in which the fasting blood glucose increases over 180 mg/100 ml., the level at which glucose appears in the urine. Glucosuria is a condition in which glucose is excreted in urine.

Diabetes mellitus

Diabetes mellitus is a chronic disease characterised by a blood glucose level higher than 180 mg/100 ml. Numerous factors may be responsible for the disease. The most common factor is inadequate production and secretion of insulin by the beta-cells of the Islets of Langerhans of the pancreas. In many cases, the activity of insulin is inhibited by a number of factors, thus making the available insulin ineffective. A familial tendency to diabetes exists but the precise genetic factor and mode of inheritance are not known. The disease may appear at any age but about 80 per cent of the cases occur in adults over 50 years.

Insulin deficiency

Inadequate production and release of insulin from the β-cells of the Islets of Langerhans of pancreas will cause the following biochemical changes: (1) a reduction in the removal of glucose from blood and (2) increased breakdown of liver glycogen and release of glucose into blood stream. In support of this, histological changes have been observed in the beta-cells in the majority of diabetics after autopsy. The other metabolic consequences of insulin deficiency are as follows: Synthesis of protein is depressed and accelerated protein degradation takes place. With impairment of glucose utilisation in diabetes, the demand for energy is met by increased metabolism of fats and proteins. There is increased gluconeogenesis from protein with a greater excretion of nitrogen in the urine and a wasting of body tissues.

Much of the glucose produced is also wasted in the absence of insulin. Less of oxaloacetate will be available for combining with acetyl CoA obtained from fat oxidation. The excessive mobilisation of lipid from body stores leads to lipemia and may result in fatty liver and development of atherosclerosis. In addition, there is increased production of ketone bodies as acetyl CoA is not oxidised and ketosis occurs.

Antagonists to insulin

An insulin antagonist may be defined as a substance capable of interfering with the action of insulin. Such interference might occur in the synthesis of insulin in the pancreas or release into the portal circulation; an antagonist might increase the rate of degradation of insulin or modify or combine with circulating insulin so as to inhibit its biological activity. The action of several insulin antagonists is listed below:

(*i*) Injection of growth hormones of anterior pituitary can produce permanent diabetes in dogs. A majority of persons suffering from over-activity of the anterior pituitary have diabetes mellitus.

(*ii*) Increased insulin sensitivity is an important feature of Addison's disease and of hypopituitarism and this can be corrected by the administration of corticosteroids, whereas many patients with Cushing's syndrome are diabetic. The administration of cortisone to patients suffering from diabetes will usually raise further their blood glucose level.

(*iii*) Adrenaline increases blood glucose by causing the breakdown of liver glycogen to glucose.

(*iv*) Thyroid hormone secretion in excess will aggravate diabetes.

(*v*) An insulin antagonist associated with plasma albumin (the synalbumin antagonist) has been demonstrated in normal plasma. It has been reported to be present in excess amounts in severe diabetes.

(*vi*) An enzyme known as *insulinase* is produced by liver and is present in the blood and other tissues. This inactivates insulin. The insulinase activity may increase in diabetes.

Metabolism of carbohydrate, protein and fat in diabetes mellitus

In diabetes mellitus, carbohydrate metabolism is markedly disturbed; consequently, there is also disturbance in protein and fat metabolism.

Carbohydrate metabolism

The excretion of sugar in urine is due to hyperglycemia. Hyperglycemia is due to (*i*) decreased oxidation of glucose, (*ii*) decreased formation of glycogen in liver from dietary carbohydrates, (*iii*) increased formation of carbohydrates from certain amino acids and glycerol of fat (increased gluconeogenesis).

Oral glucose tolerance test

The patient starves overnight. After emptying the bladder, a sample of blood is taken to determine the fasting blood glucose level and 50g. glucose dissolved in about 200 ml. of water is then given by mouth. Thereafter, blood and urine specimens are collected at half-hourly intervals for two hours and their glucose contents estimated. In normal persons, the findings will be as follows:

(*i*) the fasting glucose level in blood does not exceed 120 mg/100 ml.

(*ii*) the level after the test dose does not exceed 180 mg/100 ml.

(*iii*) the level returns to the fasting level within two hours, and

(*iv*) glucose is not present in the urine collected during the test. In the diabetic subject, the fasting level will be over 130 mg/100 ml. After the glucose tolerance test, the glucose level in blood goes upto 300 mg/100 ml. or more and does not return to the fasting level in two hours.

Protein metabolism in diabetes mellitus

Protein metabolism is seriously affected. There is increased breakdown of tissue proteins for energy pu[illegible] non-

nitrogenous portion of 13 of the 21 known amino acids and glycerol are converted into glucose, while the non-nitrogenous portion of the remaining amino acids are converted into fatty acids or their break-down products. The formation of glucose from amino acids and glycerol of fat (neo-glucogenesis) is normally kept in check by insulin and is stimulated by diabetogenic hormone. Owing to the absence of insulin in diabetes mellitus, the latter hormone acts unchecked with the result that excessive amounts of glucose are formed from the oxidation products of amino acids. The evidences in support of the above view are: (*i*) Hyperglycemia and glucosuria persist in a severe case of diabetes even after fasting for a few days. As the glycogen stores will be used up on the first day of fast, the excess glucose present in blood and urine must therefore be derived from non-carbohydrate sources, e.g. proteins and glycerol of fats by gluconeogenesis and (*ii*) There will be negative nitrogen balance due to increased break-down of tissue proteins.

Fat metabolism

The danger to the diabetic's life arises from the excessive break-down of fats into the ketone bodies, e.g. acetoacetic acid, acetone and beta-hydroxy butyric acid. Since liver glycogen stores and the oxidation of glucose by the tissues are decreased due to deficiency of insulin, fat is oxidised in large amounts in the liver to ketone bodies. This ketogenesis is normally inhibited by insulin and stimulated by diabetogenic hormone. Owing to excessive ketogenesis, the level of ketone bodies in blood increases, as the tissues cannot oxidise the ketone bodies as soon as they are formed. There is a tendency to acedemia. Acetoacetic acid and acetone are toxic when present in excessive amounts. They stimulate breathing, causing deep respiration, and depress the higher centres causing loss of conciousness (Diabetic coma).

Treatment

The treatment of diabetic patients consists in controlling the diet and administration of hypoglycemic drugs or insulin.

Diets for diabetic patients

Diet control is by far the most important factor in the management of diabetes. The percentage of calories derived from carbohydrates should be approximately 40-45 per cent, from proteins 20-25 per cent and the remaining 35-40 per cent from fat. An approximate range of calorie requirements for the various groups will be as follows: (*i*) An obese middle aged or elderly diabetic 1400 to 1600 KCal (*ii*) An adult diabetic of normal body weight—1600-2000 KCal and (*iii*) An young active diabetic 2000-3000 KCal per day. In the case of obese subjects, the body weight should be reduced gradually while in the case of subject with normal body weight, the body weight should be maintained constant. In the case of diabetics who are underweight, the body weight should be increased to normal level.

Diets for diabetic patients providing varying amounts of calories are given in Table 2.6.

Carbohydrates: The types of carbohydrates included can be starch, sucrose and fructose. The intake of carbohydrates should be restricted to provide 40-45 per cent of the calorie requirements.

Proteins: The catabolism of proteins is increased in diabetes. Hence, the diet should include liberal amounts of proteins of high nutritive value, e.g., egg, fish, meat and milk proteins. Studies carried out in India have shown that (1) incorporation of certain legumes in the diet, e.g., black gram (*Phaseolus mungo*) and field bean (*Dolichos lablab*) tends to bring down the blood sugar level in diabetic patients and (2) addition of casein as calcium caseinate has been reported to bring down the blood glucose level to a significant extent. The protein intake of the subjects should be at least 2g/kg. body weight and should provide about 20-25 per cent of the calories in the diet. The protein intake should be adequate to make up protein loss due to increased catabolism of tissue proteins and maintain positive N balance.

Fat: Since the lipids and cholesterol in serum are increased in diabetes mellitus, dietary fat should consist mostly of vegetable oils rich in polyunsaturated fatty acids which will help to bring down the serum cholesterol level. The fat intake should provide about 35-40 per cent of the calories in the diet.

Table 2.6 **Diets for Diabetic adults (g/day/caput)**

Foodstuff	1400KCal		1600KCal		1800KCal		2000KCal		2200KCal		2400KCal	
	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV
Cereals	90	90	110	110	130	130	150	150	170	170	200	200
Pulses	90	60	90	60	90	60	90	100	100	70	100	70
Nuts rich in protein (groundnut, cashewnut etc.)	30	30	40	40	50	50	60	60	70	70	80	80
Cheese	50	–	50	–	50	–	50	–	50	–	50	–
Milk, full fat	900	500	900	500	900	500	900	500	900	500	900	500
Eggs	–	one	–	one	–	one	–	one	–	one	–	one
Meat and Fish	–	100	–	100	–	100	–	100	–	100	–	100
Vegetables, green leafy	200	200	200	200	200	200	200	200	200	200	200	200
Other Vegetables (excluding roots and tubers)	200	200	200	200	200	200	200	200	200	200	200	200
Fats rich in essential fatty acids (sesame, soybean, safflower, niger and cottonseed oils)	20	20	30	30	40	40	50	50	60	60	70	70
Butter or ghee	10	10	10	10	10	10	10	10	10	10	10	10
Proteins (g)	81	82	86	87	91	92	96	97	103	104	108	109
Fat (g)	70	75	80	85	90	95	100	105	110	115	120	125
Carbohydrates (g)	137	125	144	129	178	165	212	197	232	220	255	245

Insulin or oral hypoglycemic drugs

These must be administered under the advice of physicians. Administration of insulin helps the utilisation of carbohydrates and prevents the breakdown of proteins and excessive breakdown of fats. In the case of moderate and severe diabetes, it is essential to administer insulin according to the advice of the physician, in addition to the diet being controlled as suggested above.

Carbohydrate requirements

The body has a specific need for carbohydrates as a source of energy for the brain and other tissue cells, for the synthesis of lactose of milk (lactating women) and galactose and other sugars present in the cerebrosides, mucopolysaccharides etc. Carbohydrate is essential for the oxidation of fats and for the synthesis of certain non-essential amino acids. The percentage calories derived from carbohydrates in diets consumed by a vast majority of people in the developing countries is as high as 60-70 per cent while it is 40-50 per cent in Europe and 30-40 per cent in U.S.A. The carbohydrate calories should be at least 40 per cent in well balanced diets. The level of carbohydrate calories in the diet will also depend on the availability of fat and the economic condition of the people as fat is about twice as costly as cereals on equicalorie basis in the developing countries and the per caput production of fat per day is low (10-15g). The optimal levels of carbohydrates in the diet, taking into account the physiological needs for proteins and fats are given below:

Age group	Optimal level of carbohydrates (Calories as per cent of total calories)
Adults	50-70
Expectant and nursing mothers	40-60
Infants (1-12 months)	40-50
Preschool children (1-5 years)	40-60
Older children and Adolescents	50-70

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CHAPTER 3

LIPIDS

The term lipid is applied to a group of naturally occurring substances characterised by their insolubility in water, greasy feel and solubility in some organic solvents. They occur widely in the plant and animal kingdom, e.g., oils and fats.

CLASSIFICATION

A. Simple lipids

Oils and Fats: Esters of fatty acids and glycerol. Oils are liquids at 20°C while fats are solids at 20°C.

B. Compound lipids

The compound lipids contain, in addition to fatty acids and glycerol, some other organic compounds.

(1) *Phospholipids (Phosphotides):* These contain phosphoric acid and nitrogenous base in addition to glycerol and fatty acids. (Lecithin, Cephalins, Plasmalogens, Sphingomyelins).

(2) *Glycolipids:* Complex lipids containing carbohydrates in combination with fatty acids and glycerol (Cerebrosides, Gangliosides and Cytolipin).

(3) *Sulpholipids:* These contain sulphuric acid in combination with glycerol in addition to fatty acids and sugars.

C. Waxes

Esters of fatty acids and long-chain aliphatic alcohols.

D. Derived lipids

Fatty acids, alcohols, and sterols.

CHEMISTRY

Simple lipids

Simple lipids consist of esters of fatty acids with glycerol. The fatty acids commonly occurring in natural fats and oils contain *even number* of carbon atoms. Their chemical structures are given in Tables 3.1 and 3.2. Fatty acids are broadly divided into two main groups: (1) Saturated fatty acids and (2) Unsaturated fatty acids (containing one or more double bonds). Fats and oils are triglycerides as shown below where R_1 R_2 and R_3 represent fatty acids. The fatty acid contents of some common fats and oils are given in Table 3.3.

$$\begin{array}{l} CH_2\text{—}O\text{—}OOC\text{—}R_1 \\ | \\ CH\text{—}O\text{—}OOC\text{—}R_2 \\ | \\ CH_2\text{—}O\text{—}OOC\text{—}R_3 \end{array}$$

Table **3.1 Saturated Fatty Acids**

Molecular formula	Common name	Systematic name	Structural formula
$C_4H_8O_2$	n-Butyric	—	$CH_3(CH_2)_2COOH$
$C_6H_{12}O_2$	Caproic	n-Hexanoic	$CH_3(CH_2)_4COOH$
$C_8H_{16}O_2$	Caprylic	n-Octanoic	$CH_3(CH_2)_6COOH$
$C_{10}H_{20}O_2$	Capric	n-Decanoic	$CH_3(CH_2)_8COOH$
$C_{12}H_{24}O_2$	Lauric	n-Dodecanoic	$CH_3(CH_2)_{10}COOH$
$C_{14}H_{28}O_2$	Myristic	n-Tetradecanoic	$CH_3(CH_2)_{12}COOH$
$C_{16}H_{32}O_2$	Palmitic	n-Hexadecanoic	$CH_3(CH_2)_{14}COOH$
$C_{18}H_{36}O_2$	Stearic	n-Octadecanoic	$CH_3(CH_2)_{16}COOH$
$C_{20}H_{40}O_2$	Arachidic	n-Eicosanoic	$CH_3(CH_2)_{18}COOH$

Table **3.2 Unsaturated Fatty Acids**

Molecular formula	Common name	Systematic name	Number of double bonds
$C_{16}H_{30}O_2$	Palmitoleic	9-Hexadecenoic	1
$C_{18}H_{34}O_2$	Oleic	*cis*-9-Octadecenoic	1
$C_{18}H_{34}O_2$	Elaidic	*trans*-9-Octadecenoic	1
$C_{18}H_{34}O_2$	Vaccenic	11-Octadecenoic	1
$C_{18}H_{32}O_2$	Linoleic	*cis*, *cis*-9, 12-Octadecadienoic	2
$C_{18}H_{30}O_2$	Linolenic	9, 12, 15-Octadecatrienoic	3
$C_{18}H_{30}O_2$	γ-linolenic	6, 9, 12-Octadecatrienoic	3
$C_{18}H_{30}O_2$	Eleostearic	9, 11, 13-Octadecatrienoic	3
$C_{20}H_{32}O_2$	Arachidonic	5, 8, 11, 14-Eicosatetraenoic	4

Table 3.3 **Percentage composition of fatty acids in common oils and fats**

Fat	C_{4-12}	C_{14}	C_{16}	C_{18}	$C_{16:1}$	$C_{18:1}$*	$C_{18:2}$*	Poly-unsaturated
Butter fat	11	8	26	11	3	33	4	2
Coconut oil	63	18	9	2	—	8	1.6	—
Corn oil	—	—	8	2	1	30	56	2
Cottonseed oil	—	1	17	2	1	30	56	2
Egg fat	—	Trace	25	8	3	52	9	3
Groundnut oil	—	Trace	9	3	Trace	65	17	Trace
Olive oil	—	1	16	2	1	65	15	Trace
Ox fat	—	3	29	21	3	41	2	Trace
Pig fat	—	1	28	15	2	47	4	3
Rapeseed oil	—	—	2	—	—	28	15	59
Red palm oil	—	2	35	5	Trace	52	5	Trace
Safflower seed oil	—	—	6	4	—	38	51	—
Sesame oil	—	—	8	4	Trace	45	41	Trace
Soybean oil	—	Trace	10	2	Trace	29	51	6
Sunflower seed oil	—	—	—	—	Trace	14	73	1
Sardine oil	—	6.6	15.5	3.7	9.5	17.3	2.5	30.1

* $C_{18:1}$ = Oleic acid; * $C_{18:2}$ = Linoleic acid.

Properties of fats

(1) *Solubility:* Fats are soluble in ethyl ether, petroleum ether, acetone, hot alcohol and benzene. The quantity of fat present in food materials is usually determined by extraction with ethyl ether or petroleum ether.

(2) *Saponification value* (Saponification number): The saponification value is defined as the number of milligrams of potassium hydroxide required to saponify 1 g. of fat or oil.

(3) *Iodine value* (Iodine number) is a measure of the extent of unsaturated fatty acids present in fats and oils. It is defined as the number of grams of iodine absorbed by 100 g. of fat.

(4) *Reichert-Meissl value:* This is defined as the number of millilitres of 0.1N alkali (sodium or potassium hydroxide) required

to neutralise the *steam volatile water soluble* fatty acids present in 5 g. sample of fat. The test determines the amount of butyric acid and caproic acid which are readily soluble in water and a part of caprylic acid which is slightly soluble in water.

(5) *Thiocyanogen value:* The thiocyanogen number is the amount of thiocyanogen absorbed by one hundred grams of fat or oil. It has been found that thiocyanogen reacts with the unsaturated linkage in oleic acid, with only one of two unsaturated linkages in linoleic acid and two of the three unsaturated linkages in linolenic acid. When used in conjunction with iodine value, the thiocyanogen value can be used to estimate the quantity of oleic, linoleic and linolenic acids in a fat or oil. Data for some chemical constants for certain fats and oils are given in Table 3.4.

Sterols

The sterols comprise one of the important groups of lipids. They possess a cyclic structure i.e. cyclopentenophenanthrene ring with one secondary alcoholic group. In nature, they occur in the free state and as esters with fatty acids. Sterols are classified as follows : (*i*) Animal sterols e.g. cholesterol (*ii*) Plant sterols e.g. phytosterol and (*iii*) Mycosterols e.g. ergosterol.

Cholesterol: The chemical structure of cholesterol is given in Fig. 3.1. It occurs in all animal and human tissues. The white matter of the brain contains about 14 per cent cholesterol while the grey matter contains 6 per cent on dry basis. Large amounts of cholesterol are also present in sebum secreted by the sebaceous glands.

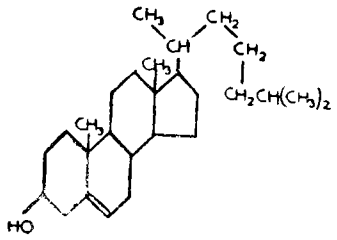


Fig. **3.1 Cholesterol**

Table 3.4 **Some Chemical Constants for certain Fats and Oils**

Name of Oil or fat	Refractive Index	Iodine Value	Saponification value	R.M. Value	Special characteristics
Arachis or groundnut oil	1.4628-1.4645	83-101	190-196	0.5	Contains arachidic acid
Beef tallow	1.4573-1.4587	38-44	193-200	0.5	
Butter fat (Cows)	1.459-1.462	26-38	220-232	23-25	Contains butyric acid
Butter fat (Buffalo)		30-36	222-234	20-24	,,
Castor Oil	1.4705-1.4720	82-90	177-187	0.2-2.3	
Coconut oil	1.4486-1.4492	8-9	255-260	6-8	
Codliver Oil	1.4700-1.4730	155-170	182-188	0.1-0.6	
Corn Oil		111-128	187-193	4.3	
Cottonseed Oil	1.4646-1.4653	105-112	191-195	0.7-0.9	Halphen reaction
Lard	1.4588-1.4620	53-65	195-196	0.5-0.8	
Linseed oil	1.4725-1.4748	175-200	187-195		
Mutton tallow		34-46	192-195		
Mustard Oil	1.4655-1.4670	115-126	174-180		
Nigerseed Oil		120-125	186-194		
Olive Oil	1.4605-1.4635	79-88	189-196	0.9-1.2	
Rape seed oil	1.4652-1.4659	97-105	170-177		
Rice Bran oil		97-104	185-194		
Safflower seed oil		138-144	187-192		
Sesame Oil	1.4650-1.4675	103-115	188-193		Budoin Test
Soybean Oil	1.4675-1.4682	130-136	190-193	0.6-0.8	
Sunflower seed oil	1.4669-1.4684	120-130	189-193		
Vanaspathi (Hydrogenated groundnut oil)		60-68	190-196		Contains sesame oil and answers Budoin Test

Compound Lipids

The important compound lipids present in animal and human tissues are given below. They occur in large amounts in nervous tissues and in small amounts in other tissues. The concentration of compound lipids in brain is given below:

Concentration of compound lipids in brain (g/100 g fresh tissue)

Constituent	Grey matter	White matter
Total lipids	4.5—7.6	13.6—22.4
Total phospholipids	3.2—4.4	6.0—8.9
Cholesterol	0.7—1.3	3.5—5.6
Glycolipids	0.4—1.8	3.8—7.2
Sphingomyelins	0.4—1.8	1.6—4.2

Phospholipids

The important members of this group are lecithin, cephalin, plasmalogens, phosphoglycerides and phosphoinositides.

Lecithin: Contains glycerol, phosphoric acid, choline and fatty acids.

Cephalin: Contains glycerol, phosphoric acid, ethanolamine and fatty acids.

Phosphatidyl serine: Contains glycerol, phosphoric acid, serine and fatty acids.

Plasmalogens: These differ from lecithin and cephalin in that one of the fatty acids is replaced by a α-β unsaturated ether. They are present in brain and heart.

Phosphoglycerides: They contain 3 molecules of glycerol and 2 molecules of phosphoric acid. An example is cardiolipin, originally isolated from heart muscle.

Phosphoinositides: These compounds contain 1 molecule of glycerol, 1 molecule of inositol, 2 molecules of fatty acids and 1-3 molecules of phosphoric acid. They are present in brain.

Sphingomyelins

They contain a complex amino alcohol called sphingosine, choline, fatty acid and phosphoric acid. They are present in brain and other tissues.

Glycolipids

The important members of this group are cerebrosides, gangliosides and cytolipins.

Cerebrosides: They contain hexoses (galactose or glucose) one fatty acid and the amino alcohol, sphingosine but no phosphoric acid or glycerol.

Gangliosides: These compounds contain sphingosine, fatty acids, hexoses (galactose or glucose) and neuraminic acid.

Cytolipins: These contain fatty acids, sphingosine, glucose and galactose.

Sulpholipids

These contain sphingosine, galactose, cerebronic acids, sulphuric acid and potassium.

Refined and Hydrogenated Fats

The crude oils and fats obtained by the expeller process or using village ghanis are widely consumed by the people. In addition, a fair quantity of the edible oils is sold in the form of refined and hydrogenated fats. The process of refining and hydrogenation is given below:

Refined Oils: The process of preparation of refined oil consists of the following steps: (1) alkali refining to remove free fatty acids, (2) bleaching with fullers earth or activated carbons to remove colouring matter and (3) deodourisation with super-heated steam. The refined oils thus obtained are free from odour.

Hydrogenated Oil: The refined oil obtained as described above is hydrogenated under optimal temperature and pressure in the presence of Nickel catalyst. During the process of hydrogenation, hydrogen is added to the unsaturated linkages. The changes in the unsaturated fatty acids taking place are as follows:

Oleic acid $\xrightarrow{\text{Hydrogenation}}$ Stearic acid.

Linoleic acid $\rightarrow$ { Oleic acid / Iso-oleic acid } $\rightarrow$ Stearic acid

Linolenic acid $\rightarrow$ { Linoleic acid / Iso-linoleic acid } $\rightarrow$ { Oleic acid / Iso-oleic acid }

The liquid fat becomes a solid fat and the unsaturated fatty acid contents decrease as a result of hydrogenation. *Vanaspathi* sold in India consists mostly of hydrogenated refined groundnut oil. Small amounts of refined cottonseed oil or soybean oil may be mixed with groundnut oil before hydrogenation. Since it is used as a substitute for ghee, Vanaspathi has been fortified with Vitamin-A. According to the Government Food Act, addition of sesame oil at 5 per cent level to Vanaspathi is obligatory. Vanaspathi containing sesame oil gives a positive Budoin's Test.

(Rancidity in Fats)

The development of off-flavours in fats is known as rancidity. There are three main types of rancidity—(*a*) Hydrolytic, (*b*) Oxidative and (*c*) Ketonic.

Hydrolytic rancidity: Hydrolysis of fats by lipase need not always produce off-flavours. In the case of butter fat and coconut oil, butyric acid and other low molecular weight fatty acids are set free by hydrolysis by lipase. The odours of these acids contribute largely to the smell of rancid butter. The higher fatty acids such as palmitic and stearic acids have little odour.

Oxidative rancidity: This is the common type of rancidity observed in all fats and oils. The oxidation takes place at the unsaturated linkage. Certain metals e.g. copper, hasten the onset of oxidative rancidity. The addition of oxygen to the unsaturated linkage results in the formation of a peroxide which, on decomposition, yields aldehydes and ketones having pronounced off odour.

Ketonic rancidity: This type is most frequently encountered as a result of action of such fungi as *Aspergillus niger* and blue-green mould, *Pencillium glaucum* on coconut or other oilseeds.

The tallowy odour developed may be due to aldehydes and ketones formed by the action of the enzymes present in the fungi on oil.

Tests for rancidity

The important tests for rancidity are (1) Determination of Peroxide Value, (2) Determination of Carbonyl compounds, and (3) Thiobarbituric acid test (TBA).

Peroxide value: The quantity of iodine liberated when rancid fats react with potassium iodide in acetic acid solution is a measure of the peroxide value.

Carbonyl compounds: The carbonyl compounds, i.e. aldehydes and ketones are measured colorimetrically by reaction with 2:4 dinitrophenyl hydrazine.

Thiobarbituric acid test: When rancid fats react with thiobarbituric acid, a red colour is produced. The compound which reacts with thiobarbituric acid has been identified as malonaldehyde.

Reversion

Many refined oils undergo change in flavour before the onset of rancidity. This process is known as 'reversion'. It has been observed that oils containing linolenic acid and other unsaturated fatty acids having three or more unsaturated linkages develop off flavour due to 'reversion'. This is due to the fact that such fatty acids undergo oxidation rapidly when exposed to air and decomposition products formed contribute to the off flavour.

Essential Fatty Acids

The term 'Essential fatty acid' (EFA) was introduced by Burr and Burr in 1930 for linoleic acid. They found it to be essential for the growth and health of young albino rats. Later they showed that linoleic acid was also effective in curing fat deficiency syndrome in rats. Turpeinen (1938) reported that arachidonic acid was highly effective in promoting growth in rats on a fat-free diet. The relative biological activities of the different essential fatty acids are given below:

Fatty acid	No. of double bonds	Biological activity (growth)
Linoleic acid (18c)	2	100
Linolenic acid (18c)	3	9
γ-Linolenic acid (18c)	3	100
Arachidonic acid (20c)	4	131
11, 14-Eicosadienoic acid (20c)	2	43

The essential fatty acid contents of some fats and oils are given in Tables 3.5 and 3.6. Data regarding the ratio between poly-unsaturated fatty acids and saturated fatty acids (P/S ratio) of some fats and oils are given in Table 3.7.

Table 3.5 **The relative amounts of essential fatty acids in vegetable fats as determined by analysis and by bioassay**

Vegetable fat or oil	Values by analysis		Total essential fatty acids by bioassay per cent
	Linoleic acid per cent	Linolenic acid per cent	
Almond	19.9	0	—
Avocado	10.3	0	—
Beechnut	38.0	2.9	30.1
Castor oil	3.6	0	3.8
Cocoa butter	21.1	0	2.2
Coconut	2.6	0	1.1
Corn	39.1	0	—
Cottonseed	50.4	0	48.5, 48.0
Hempseed	68.8	24.3	—
Kapokseed	31.3	0	26.9
Linseed	43.0	40.0	25.6
Niger	53.5	—	—
Olive	15.0	0	15.8
Palm	10.9	0	10.6
Palm kernel	0.7	0	1.5
Peanut	27.4	0	31.1
Poppyseed	62.2	—	—
Rapeseed	29.0	3.5	17.3
Rice bran oil	32·0	1.0	—
Safflower	78.0	0	78.8
Sesame	40.4	0	28.2
Soybean	58.8	8.1	62.4
Sunflower	68.0	0	55,65
Walnut	75.5	10.0	—
Wheat germ	52.2	3.5	52.9

Table 3.6 **The relative maximum amounts of essential fatty acids in hydrogenated fat, milk fat and animal fat as determined by analysis and by bioassay**

Fat	Values by analysis			
	Linoleic acid %	Linolenic acid %	Arachidonic acid %	Total essential fatty acids by bioassay %
Hydrogenated fats				
Coconut oil	0	0	0	0
Vanaspati (Groundnut oil)	4.9	—	—	—
Margarine oil	4.8	0	0	4.1
Shortening (non-selective)	6.8	0	0	13.2
Milk fat				
Milk fat (cow)	5.8	0	0.4	—
Milk fat (goat)	1.5	0	0	—
Milk fat (human)	7.9	5.4	0	—
Milk fat (buffalo)	1.8	0	0	—
Human and animal body fat				
Human body fat	11.0	0	1.0	—
Goose body fat	19.3	0	0	—
Hen body fat	21.3	0	0.6	—
Ox body fat	5.3	0	0.5	1.5
Pig body fat	15-26	0.5	—	—
Sheep body fat	5.0	—	—	—
Fish Oils				
Codliver oil	1.2	0.8	3.2	3.9
Herring oil	4.3	3.4	3.4	7.9
Sardine oil	2.5	1.3	2.5	—
Whale oil	2.0	0.8	4.1	6.4

Physiological and biochemical functions: Deficiency of essential fatty acids leads to cessation of growth and development of scaliness of the skin, and haemorrhages on the tail. Scales also develop on the dorsal and plantar surface of the feet and around the ears. Hair is lost from the face and around the back. Blood may appear in the urine. Essential fatty acid deficiency affects adversely (1) reproduction and lactation (2) integrity of the cell membranes and cells, (3) certain enzyme systems, (4) transport of cholesterol and (5) water balance.

Table 3.7 **The polyunsaturated and saturated fatty acid contents and the P/S ratio* of certain common food fats and oils (per cent of total fatty acids)**

Food	Poly-unsaturated fatty acids %	Saturated fatty acids %	Mono unsaturated fatty acids %	P/S* ratio
Butter	3	60	37	0.05
Lard	12	40	48	0.30
Coconut	2	92	6	0.02
Corn	55	12	33	4.58
Cottonseed	52	26	22	2.00
Linseed	83	10	7	8.3
Niger	54	9	37	6.00
Olive	5	9	86	0.55
Palm kernel	1	85	14	0.01
Peanut	31	19	50	1.63
Safflower	75	7	18	10.71
Sesame	43	14	43	3.07
Soybean	64	18	18	3.56
Sunflower	53	12	35	4.42
Egg fat	14	34	52	0.41
Beef fat	3	43	54	0.07
Lamb fat	3	59	38	0.05
Poultry fat (chicken and turkey)	22	30	48	0.73
,, Duck	31	26	43	1.19
Pork fat	10	39	51	0.26
Sardine oil	30	26	44	1.16

* Ratio between polyunsaturated and saturated fatty acids.

Effects of essential fatty acid deficiency in human beings: Phrynoderma: One of the common disorders of malnutrition observed in adults and children in India and many other developing countries is 'Phrynoderma' or 'toad skin'. This condition was first described by Nicholls in 1933 in Ceylon and subsequently by several workers in India. The condition is characterised by the presence of horny papular eruptions on the posterior and lateral aspects of the limbs, on the back and buttocks. Earlier workers have reported that phrynoderma could be cured by cod liver oil or vitamin A concentrates. Recent studies by Gopalan and associates have shown that phrynoderma is cured rapidly by the

administration of linseed or safflower seed oil rich in essential fatty acids (EFA) along with vitamins of the B_2-complex but not by vitamin A.

Deficiency in infants: Hansen and co-workers have reported that infants fed on a EFA deficient diet developed perianal irritation and changes in the skin within a few weeks. The skin changes appeared as dryness, thickening, and desquamation with oozing in the intertriginous folds. Supplementation of the diet with linoleic acid restored the skin to normal condition within 2 weeks.

Importance of fat in the diet. Fat has several important functions (*i*) It is a concentrated source of energy, yielding more than twice the energy supplied by carbohydrate per unit weight, (*ii*) Fats are essential for the absorption of vitamins A, D, E, K and especially carotenoids (provitamin A) present in foods of vegetable origin, (*iii*) Some animal fats e.g., fish liver oils, butter and ghee contain vitamin A and many vegetable fats contain vitamin E and red palm oil is a good source of carotene (Provitamin A). (*iv*) Fats contain essential fatty acids, viz., linoleic, linolenic and arachidonic acids which are essential for maintaining the tissues in normal health.[1] (*v*) Fats help to reduce the bulk of the diet as starchy foods, absorb lot of water during cooking, (*vi*) Fats improve the palatability of the diet and give a satiety value, i.e., a feeling of fullness in the stomach. (*vii*) Fats are essential for the utilisation of galactose present in lactose. (*viii*) Phosphatides and other complex lipids are essential constituents of nervous tissue, and (*ix*) Fats are deposited in the adipose tissue. This deposit serves a reserve source of energy during starvation. Further, adipose tissue functions like an insulating material against cold and physical injury.

The dietary fats should be a good source of essential fatty acids and hence at least 50 per cent of the dietary fat should be vegetable oils rich in EFA. The approximate optimal fat requirements for different age groups are as follows:

Adults, expectant and nursing mothers	10–20% of the total calories from fat
Children and adolescents (1–18 years)	15–20% ,,
Infants birth to 1 year	25–30% ,,

Digestion and Absorption of Fats

Fat is not digested in the stomach. The presence of fat in the diet delays the emptying of the food from the stomach. This may be responsible for the feeling of satiety after a meal rich in fat. In the duodenum, where pancreatic juice and bile are secreted, the digestion of the fat begins. Bile salts and small quantities of fatty acids and monoglycerides liberated by pancreatic digestion help in the emulsification of fat. Lipases present in the pancreatic and intestinal juices hydrolyse fats into fatty acids, diglycerides, monoglycerides and glycerol. Hydrolysis often stops at the monoglyceride stage.

The products of digestion pass into the cells of the mucous membrane of the intestines. Within the cells, synthesis of new triglycerides characteristic of the animal species takes place. The resynthesised triglycerides enter the lacteals of the small intestines as small particles known as chylomicrons. The chylomicrons pass through the lacteals and mesenteric lymph vessels, enter the thoracic duct and join the systemic circulation. A major part of the fat absorbed enters the circulation via the thoracic duct. A small part, consisting mostly of short chain fatty acids may reach the liver via the portal circulation.

The phosphatides are hydrolysed by the enzymes secreted by the intestines and absorbed. A degree of selectivity is exhibited by the intestinal mucosa in regard to the absorption of sterols. Of the dietary sterols, only cholesterol is readily absorbed. Phytosterols present in plant foods and vegetable fats and oils are not absorbed to any appreciable extent.

Lipids of the feces—Steatorrhea

Lipids present in the feces consist of (*i*) small amounts of unabsorbed food fat and (*ii*) lipids excreted in bile or pancreatic juice. When excessive amounts of lipids are present in the stools, the condition is known as *Steatorrhea*. Three important types of disturbances may lead to this condition (*i*) absence of bile, (*ii*) inadequate secretion of pancreatic juice and (*iii*) defective absorption by the intestinal mucosa. The steatorrhea due to bile insufficiency is usually recognisable by the presence of excessive amounts of digested and undigested fat. The lack of bile

pigment in the feces helps in classification. The steatorrhea which results from deficiency of pancreatic juice is characterised by the presence in the stools of excessive amounts of undigested fat. In diseases of the intestinal tract such as celiac disease in children or sprue in the adult, the steatorrhea is due to the failure of absorption of the hydrolysed fat. The lipids in the feces consist predominantly of fatty acids and their salts.

Transport of Lipids in the Blood

Chylomicrons are removed from the blood mainly by the liver and adipose tissues. In the liver, chylomicrons are broken down and resynthesised into triglycerides and phospholipids. Fat leaves the liver in two forms (*i*) in the form of minute particles (average diameter 70 mμ) known as secondary particles and (*ii*) as lipoproteins.

Normal human blood plasma in the post absorptive state contains about 500 mg. of total lipids per 100 ml. of which about one-quarter is triglycerides. There are 180 mg. or more of cholesterol of which about two-thirds is esterified with fatty acids. Phosphatides comprise about 160 mg. per 100 ml. Free fatty acids (non-esterified fatty acids—NEFA) are present to the extent of 8 to 20 mg. per 100 ml. These fatty acids have a high metabolic turnover ratio.

Plasma contains lipoproteins which are concerned in the transport of fat. Two groups of lipoproteins namely alpha and beta lipoproteins have been separated. They are synthesised in the liver. In plasma and serum, all lipid constituents including phosphatides and cholesterol exist mainly as alpha and beta lipoproteins. Alpha-lipoproteins contain about 30 per cent and beta-lipoproteins about 70 per cent of the blood lipids. The cholesterol of alpha-lipoproteins exchanges rapidly with chylomicron cholesterol indicating that the newly absorbed cholesterol may be conveyed to the liver by way of alpha-lipoproteins. An elevation of beta-lipoproteins in plasma indicates the possibility of development of atherosclerosis.

Non-esterified Fatty Acids (NEFA) in Blood

Lipid is mobilised from the adipose tissue in the form of free fatty acids. These are transported in lose combination with

serum albumin. The level of NEFA, in blood provides a rough index of rates of fat mobilisation. The fasting level of NEFA, in plasma is about 0.5 m-mole/l (i.e., about 128, mg fatty acid/litre). The rate of turnover of NEFA is high. The level of NEFA is very sensitive to the nutritional state. After a meal or test dose of glucose or injection of insulin, the level is reduced to 0.25 m-mole/l or less. In starvation, NEFA, rises slowly in plasma and may be 1-m-mole/l or even more as fat is mobilised and used as energy during starvation.

Hyperlipidemia

Hyperlipidemia is a non-specific term referring to conditions in which one or more lipid components of the serum is present in abnormally high amounts. They may be broadly grouped under two heads—(*i*) Hypertriglyceridemia with hypercholesterolemia and (*ii*) Hypercholesterolemia with normal triglyceride concentration.

Hypercholesterolemia

In this condition, the level of cholesterol in serum is very high. The normal levels of plasma cholesterol in children and adults lie between 120 and 160 mg. per 100 ml. Clinical experience has shown that atherosclerosis and coronary heart disease are minimal and intravascular thrombosis is rare when the blood cholesterol level is below 190 mg/100 ml. In countries where the blood cholesterol level is over 220 mg. per 100 ml. in middle-aged males, the incidence of coronary heart disease is high. Changes in total serum cholesterol usually reflect changes in the beta-lipoprotein concentration. The concentration of alpha-lipoproteins is quite constant and does not correlate with proness to atherosclerosis. The amount and kind of fat in the diet are the most important factors controlling the levels of cholesterol and beta-lipoproteins in blood. About 30 per cent of plasma cholesterol is in the alpha and 70 per cent in the beta-lipoprotein fractions. The cholesterol/phospholipids ratio is about 0.50 in alpha-lipoproteins and 1.34 in beta-lipoproteins.

Diet and serum cholesterol: Many factors influence the serum cholesterol level. The most important of these are:

(1) calorie intake, (2) cholesterol intake (3) fat intake and (4) quality of fat. Low calorie intakes tend to reduce serum cholesterol level while calorie excess tends to increase the same. The dietary intake of cholesterol in human adults consuming large amounts of fat from milk and foods of animal origin may vary from 500 to 1200 mg. per day. Data regarding the cholesterol content of foods are given in Table 3.8. The adult body synthesises, daily about 2000 mg. cholesterol. In a person with a tendency to hypercholesterolemia, dietary cholesterol intake should be restricted. The quantity of fat consumed influences serum cholesterol levels, the level being high in populations consuming excess of fat. The quality of fat has a profound influence on serum cholesterol level, saturated fats tending to increase and polyunsaturated fats tending to decrease serum cholesterol levels.

(*Atherosclerosis*) is a general term denoting a number of different processes which produce thickening of the arterial wall.

Table **3.8 Cholesterol content of foods**

Foods	Cholesterol mg/100 g	Foods	Cholesterol mg/100 g
Dairy Products			
Butter	280	Rabbit	50
Ghee	310	Veal	100
Cheese, Swiss Processed	145	Beef, liver	260
Cheese, American processed	160	Calf, liver	360
Cream, processed	140	Lamb, liver	610
Milk, whole (fresh)	11	Pork, liver	420
,, skimmed (fresh)	0.4	Brain	2000
,, powder (whole)	88	Heart	150
,, powder (skimmed)	0.4	Kidney	375
		Pancreas	250
Poultry products			
Egg, Hen (whole)	498	*Animal Fats*	
,, Yolk	1330	Lard and other animal fats	95
,, White	0		
,, whole (dried)	3·00	*Fish and Sea foods*	
,, Chicken, dark	60	Cod	50
,, light	90	Crab	145
Flesh foods:		Frog	40
Beef, round lean	95	Lobster	200
,, round medium fat	125	Mackerel	80
Duck	70	Oysters	200
Lamb	70	Sardine	70
Pigeon	110	Shrimps	150
Pork	60	Tuna	65

Marchand defined the term Atherosclerosis to designate the soft amorphous lipid accumulation in the intima. The lesions are usually scattered in distribution and are localised. They consist of internal plaques which are formed by connective tissue proliferation and the deposition of lipids. The lesion tends to enlarge encroaching upon the lumen, causing the formation of thrombus. Progression of the atherosclerotic process results in narrowing of the blood vessel with resultant intravascular thrombosis. The main pathological changes in advanced atherosclerosis are (*i*) hyalinised base on the plaque, (*ii*) presence of a large mass of amorphous cholesterol and lipids in the plaque, (*iii*) surface of the plaque composed of compact fibrous tissue, (*iv*) calcium deposition in the centre of the plaque, usually in the form of fine granules, (*v*) slight marginal vascularisation, (*vi*) fragmentation or frequent absence of internal elastic lamina and (*vii*) moderately thin, occasionally vascularised, media with loss of muscle fibres and increased fibrosis below the plaque. The important dietary factors contributing to the development of atherosclerosis are (1) consumption of large amounts of saturated fats, e.g., butter fat, coconut oil, etc., (2) consumption of large quantities of milk and animal foods rich in cholesterol and (3) excessive caloric intake, leading to obesity. These factors increase the lipid and cholesterol contents of blood, leading to the development of atherosclerosis. In addition, heredity and hormonal factors are also involved in the causation of atherosclerosis.

Storage of fat (Adipose tissue): Fat is stored in adipose tissues. In normal human subjects, about 10 to 15 per cent of the body weight consist of adipose tissue. It may increase upto 30 per cent in very obese persons. Fat, after absorption, is transported in the blood to adipose tissues in the form of chylomicrons and from liver as secondary particles and lipo-proteins. The composition of the adipose tissue fat is characteristic of each animal species and has very little relation to the composition of dietary fat. Excessive consumption of dietary fat influences to a considerable extent the composition of adipose tissue fat. The deposition and mobilisation of fat in the adipose tissue proceeds at rates which are dependent on the fat and energy content of the last meal and the immediate need for energy for physical and other activities. The main functions of adipose tissue fat are (*i*) to serve as a reservoir of energy especially during starvation, (*ii*) as an insulating

li, etc., it is possible to isolate prot

cipitation. By increasing th
monium sulphate, it is p
min from globulin.

The name 'Protein' was suggested by M[illegible]er in 1838 to the complex organic nitrogenous substances found in animal and plant tissues. Proteins constitute about three fourths of the animal body on moisture-free basis. They are essential for life processes. They play an important role in many biochemical, biophysical and physiological processes in the body. Most of the enzymes are proteins. There is hardly an important physiological function in which proteins do not participate. The important functions of dietary proteins are: (1) to replace the daily loss of body proteins, (2) to provide amino acids for the formation of tissue proteins during growth, (3) to provide the amino acids necessary for the formation of enzymes, blood proteins and certain hormones of protein nature and (4) to provide amino acids for the growth of foetus in pregnancy and for the production of milk proteins during lactation.

Chemical Composition

Proteins contain carbon, hydrogen, nitrogen and sulphur and some contain also phosphorus. Proteins are large molecules formed from the combination of a large number of simpler substances known as amino acids. The nitrogen content of proteins varies from about 14 to 20 per cent and in most of the proteins, the value is near about 16 per cent. This average figure of 16 per cent is used commonly for converting nitrogen content of foodstuffs or tissues into proteins by multiplying by the factor 6.25 (100/16). This factor, however, varies with different proteins as shown in Table 4.1.

Properties of Proteins

Amphoteric nature: Like amino acids, proteins are ampholites, i.e., they act as both acids and bases. Since proteins have electric charges, they migrate in an electric field, the direction of migration depending on the net charge on the molecule. For each protein,

are optically active and possess L-configuration. The amino acids naturally occurring in proteins have been broadly classified under the following heads: (1) Monoamino-monocarboxylic acids (2) Monoamino-dicarboxylic acids (3) Diamino-monocarboxylic acids (4) Sulphur containing amino acids and (5) Aromatic and heterocyclic amino acids.

1. *Monoamino-monocarboxylic acids:*

Glycine:—Amino-acetic acid
Alanine:—α-amino-propionic acid.
Valine:—α-amino-β-methyl-n-butyric acid.
Leucine:—α-amino-γ-methyl-n-valeric acid.
Isoleucine:—α-amino-β-methyl-n-valeric acid.
Norleucine:—α-amino-n-caproic acid.
Serine:—α-amino-β-hydroxy-propionic acid.
Threonine:—α-amino-β-hydroxy-n-butyric acid.

2. *Monoamino-dicarboxylic acids:*

Aspartic acid:—α-amino-succinic acid.
Glutamic acid:—α-amino-glutaric acid.

3. *Diamino-monocarboxylic acids:*

Arginine:—α-amino-δ-guanidino-n-valeric acid.
Lysine:—β,ε-diamino-n-caproic acid.

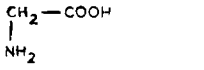
4. *Sulphur containing amino acids:*

Cystine:—di-(α-amino-β-thio-propionic acid).
Cysteine:—β-mercapto-α-amino propionic acid.
Methionine:—α-amino-γ-methylthio-n-butyric acid.

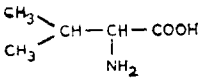
5. *Aromatic and heterocyclic amino acids:*

Phenylalanine:—α-amino-β-phenyl-propionic acid.
Tyrosine:—α-amino-β-parahydroxy phenyl-propionic acid.
Histidine:—α-amino-β-imidazole-propionic acid.
Tryptophan:—α-amino-β-indole-propionic acid.
Proline:—α-pyrrolidine-carboxylic acid.
Hydroxyproline:—γ-hydroxy-α-pyrrolidine-carboxylic acid.

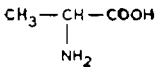
1. Monoamino-monocarboxylic acids



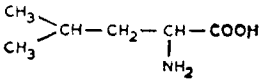
Glycine



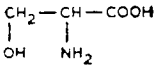
Valine



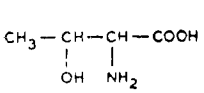
Alanine



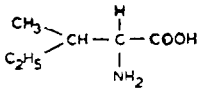
Leucine



Serine

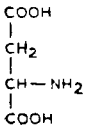


Threonine

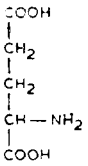


Isoleucine

2. Monoamino-dicarboxylic acids

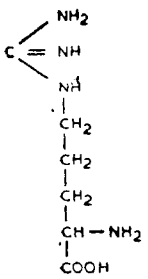


Aspartic acid

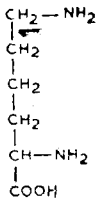


Glutamic acid

3. Diamino-monocarboxylic acids

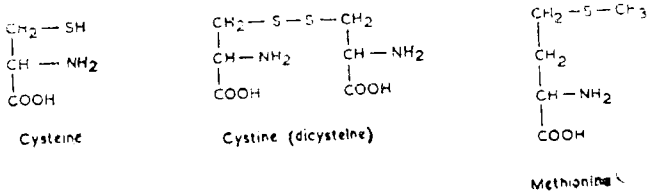


Arginine

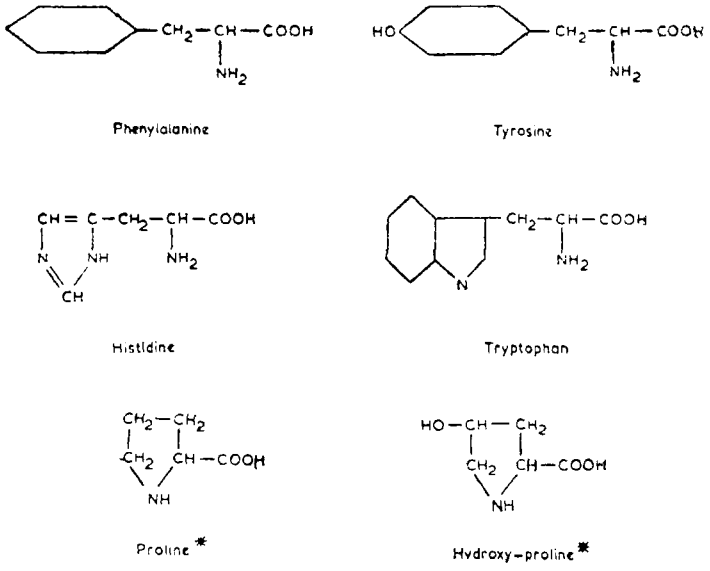


Lysine

4. Sulphur containing amino acids



5. Aromatic and heterocyclic amino acids



* Contains imino group

Fig. **4.1 Structures of amino acids**

Classification of Proteins

The classification of proteins according to the recommendations of the American Physiological Society and the American Society of Biological Chemists are given below:

I. Simple Proteins

Proteins which yield only amino acids or their derivatives on hydrolysis.

(*a*) *Albumins:* Soluble in pure water and coagulable by heat. Examples: egg albumin, serum albumin (blood).

(*b*) *Globulins:* Insoluble in pure water, but soluble in neutral salt solutions. Examples: serum globulin (blood) tuberin (potato,) arachin and conarachin (peanuts).

(*c*) *Glutelins:* Insoluble in all neutral solvents, but soluble in very dilute acids and alkalis. The best known protein of this group is the glutenin of wheat.

(*d*) *Prolamins:* Soluble in 70-80 per cent alcohol. Examples: gliadin (wheat) and zein (maize).

(*e*) *Fibrous proteins:* Proteins characteristic of the skeletal structures of animals and also of the external protective tissues, such as the skin, hair, etc. Examples: collagen, elastin and keratin.

(*f*) *Histones:* Soluble in water and insoluble in very dilute ammonia. On hydrolysis, they yield several amino acids, among which the basic ones predominate. The important members of this group are the thymus histones and the globin of haemoglobin.

(*g*) *Protamins:* Strongly basic proteins with low molecular weight, soluble in water, not coagulable by heat, and on hydrolysis yield large amounts of basic amino acids.

Example: Salmine from salmon sperm.

II. Conjugated Proteins

Proteins united to some other molecule otherwise than as a salt.

(*a*) *Nucleoproteins:* Compounds of proteins with nucleic acid.

(*b*) *Glycoproteins:* Protein molecule combined with a carbohydrate group. Example: mucins.

(*c*) *Phosphoproteins:* Proteins in which phosphorus is in union with the protein molecule. Examples: caseinogen (milk), ovovitelin (egg yolk).

(*d*) *Haemoglobins:* Protein molecule combined with haem. Example: Haemoglobin of blood.

(*e*) *Lecithoproteins:* Protein molecule combined with lecithin or related substances.

III. Derived Proteins

1. *Primary protein derivatives:* Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations.

(*a*) *Proteans:* Insoluble products which apparently result from the incipient action of very dilute acids or enzymes. Examples: Casein (Curdled milk), fibrin (coagulated fibrinogen).

(*b*) *Metaproteins:* Products resulting from the action of acids and alkalis whereby the molecule is sufficiently altered to form proteins soluble in weak acids and alkalis, but insoluble in neutral solvents.

(*c*) *Coagulated proteins:* Insoluble proteins which result from (1) the action of heat on protein solutions or (2) the action of alcohol on the protein. Example: Cooked egg albumin, or egg albumin precipitated by alcohol.

2. *Secondary protein derivatives:* Products of the further hydrolytic cleavage of the protein molecule.

(*a*) *Proteoses:* Soluble in water, not coagulable by heat, precipitated by saturating their solutions with ammonium sulphate.

(*b*) *Peptones:* Soluble in water, not coagulable by heat and not precipitated by saturating their solutions with ammonium sulphate. These represent a further stage of cleavage than the proteoses (the term 'peptone' is generally used to all protein

digestion products not coagulated by boiling and containing a mixture of proteoses, peptones and peptides.)

(c) *Peptides:* These are compounds containing two or more amino acids. An anhydride of two amino acids is called a di-peptide, one having three amino acids, a tri-peptide and one containing several amino acids, a poly-peptide. Peptides result from the further hydrolytic cleavage of the peptones.

Structure of Proteins

Proteins are large molecules formed by the combination of a number of amino acids. The molecular weights of some proteins are given in Table 4.2. The chemical nature of different amino acids has been discussed in the previous section. In 1920, Hofmeister and Emil Fisher proposed independently that the main type of linkage between the amino acids in the protein molecule is the *peptide bond.* The modern views on the structure of proteins are summarised below.

Table 4.2 **Molecular weights of some proteins**

Protein	Source	Mol. Wt.
Insulin	Bovine	5,700
Ribonuclease	Bovine pancreas	13,700
Lysozyme	Chicken egg white	14,400
Myoglobin	Human muscle	17,000
Papain	Papaya	21,000
Trypsinogen	Bovine pancreas	24,400
Trypsin	Bovine pancreas	24,000
Chymotrypsinogen	Bovine pancreas	24,700
α-chymotrypsin	Bovine pancreas	24,300
Ovalbumin	Hen's egg	40,000
Serum albumin	Human serum	66,500
Enolase	Rabbit muscle	67,000
Haemoglobin	Human erythrocytes	65,000
Lactic acid dehydrogenase	Bovine muscle	140,000
γ-Globulin	Human serum	160,000

Primary structure: The main mode of linkage of the amino acids in proteins is the peptide bond formed by the reaction

ot the α-carboxyl group of one amino acid with α-amino group of another as indicated below. This is sometimes called *primary structure* of proteins.

$$H_2N—CH_2—CO—NH—CH_2—COOH$$

glycyl—glycine

Secondary structure: If peptide bonds were the only structural linkage in proteins, the protein molecules would behave as randomly coiled long-chain polypeptides. However, the properties of globular proteins indicate a coiled structure in which peptide bonds are folded in a regular manner. Much of the folding is the result of linking of the carboxyl and amide groups of the peptide chain by means of hydrogen bonds. Such folding produced or maintained by hydrogen bonding is frequently called the *secondary structure* of the protein. Present evidence suggests that in many proteins, the hydrogen bonding produces a regular coiled arrangement, called α-helix.

Tertiary structure: If a globular protein consisted solely of a series of single helix, these molecules would be elongated structures with a large axial ratio (Length to cross section). However, physical measurements reveal that many proteins are spherical or nearly so. Hence, the helical arrangement must be periodically interrupted, permitting numerous bonds with additional folding. This special structure in three dimensions must be maintained by covalent or other bonds. The covalent disulphide bond involving two cystine residues is one way in which this is achieved. Many globular proteins, however, lack in disulphide bonds, yet they are stable globular molecules in solution. These additional types of bonds probably include hydrogen bonds, salt bonds and hydrophobic or nonpolar bonds. This has been called the *tertiary structure.*

Quarternary structure: In addition to the primary, secondary and tertiary structures some proteins may display a fourth level of organisation wherein several monometric units, each with appropriate primary, secondary and tertiary structures may combine. The association of similar or dissimilar sub-units confers on the protein a *quarternary structure.* Such a higher level of organisation of monometric units may be essential to the activity of protein enzymes.

Denaturation of proteins

Proteins occurring in animal and plant tissues are called native proteins. They possess many characteristic properties such as solubility, viscosity, optical rotation, sedimentation rate, electrophoretic mobility etc. Native proteins, when subjected to heat treatment, ultra-violet light, high pressures and ultrasonic vibrations or acted upon by acids, alkalis, heavy metal salts, urea and ethanol, undergo changes in their properties leading to denaturation of proteins. As a result of this, the protein molecules aggregate and precipitate. The term 'reversible denaturation' refers to only slight changes in the properties of the proteins which can be restored to the native state after suitable treatment. If the denaturation is severe, then the changes in the properties of the proteins are permanent and 'irreversible'. (Denaturation represents, the loss of native structure of the protein leading to the unfolding of the molecule.) As a result of this, more sulphydryl groups are formed from disulphide groups. There is also evidence that more of the phenolic group present in tyrosine and indole group present in tryptophan are exposed, as a result of unfolding of the protein molecule. If the protein is an enzyme or an antibody, denaturation is accompanied by a loss of activity. In general, denatured proteins are more rapidly hydrolysed by proteolytic enzymes than native proteins.

Estimation of amino acids

Hydrolysis of proteins

For the estimation of amino acids, the protein should be hydrolysed into amino acids. The hydrolysis can be effected by heating proteins with mineral acids or alkalis.

Acid hydrolysis: Hydrolysis of proteins by acids can be accomplished by heating proteins with 6N-HCl or 8N-H_2SO_4 at 15 lb pressure for 5 hrs. Acid hydrolysis especially in the presence of carbohydrates, results in almost complete destruction of tryptophan and partial destruction of some other amino acids, e.g., threonine, cystine etc.

Alkaline hydrolysis: Alkaline hydrolysis is accomplished by heating the protein with 5N-NaOH or 14 per cent $Ba(OH)_2$ (W/V) at 15 lb pressure for 5 hrs for liberating tryptophan. Barium ions are removed as sulphate after hydrolysis. Cystine is completely destroyed while arginine is partly destroyed during alkaline hydrolysis and the naturally occurring L-amino acids are converted into DL-amino acids which possess only half the biological activity of L-amino acids.

Methods for estimation of amino acids

The methods available for the estimation of amino acids are (1) Chemical (2) Microbiological (3) Enzymic and (4) Chromatographic methods.

Chemical methods: The chemical methods are based on specific colour reactions and are quite sensitive and accurate. They are widely used for the estimation of arginine, methionine, threonine, histidine, tryptophan, cystine, phenylalanine and tyrosine.

Microbiological methods: The microbiological methods are very sensitive and accurate and are widely used for the estimation of all essential amino acids. In the estimation of tryptophan obtained by alkaline hydrolysis, the value obtained should be doubled if L-tryptophan is used as a standard.

Enzymic methods: Certain amino acids can be estimated by the use of specific enzymes, e.g. arginase for the estimation of arginine and specific decarboxylases e.g. lysine decarboxylase for the estimation of lysine etc.

Chromatographic methods: The chromatographic methods include (1) Paper chromatography (2) Ion exchange chromatography and (3) Automatic amino acid analysers.

Paper chromatography: Partition chromatography on filter paper (uni-dimensional or two-dimensional) is widely used for the separation and estimation of amino acids. On spraying with ninhydrin, the spots develop colour which are eluted and estimated.

Ion exchange chromatography: Synthetic resins are used in the ion exchange chromatography for the separation of amino

acids. Sulphonated polystyrene resins bear many $-SO_3H$ groups. Moore and Stein (1951) described a procedure for the quantitative separation and estimation of amino acids.

Automatic amino acid analysers: An automatic amino acid analyser was first developed by Spackman, Stein and Moore (1958). Several models of automatic amino acid analysers are now available. The separation of amino acids is achieved by sulphonated polystyrene resin. The colour is developed by mixing with a ninhydrin reagent and recorded automatically and compared with that obtained with a mixture of standard amino acids.

ESTIMATION OF PROTEINS

Proteins are quantitatively estimated in foods and other biological materials by the following methods: (1) Kjeldahl method (2) Colorimetric methods and (3) Electrophoretic methods.

Kjeldahl method: The Kjeldahl method consists in digesting a known weight of the biological material with concentrated H_2SO_4 and potassium sulphate with copper sulphate, mercuric oxide or selenium dioxide as a catalyst. The amino groups of the amino acids and N present in the heterocyclic rings of histidine, tryptophan, proline and hydroxyproline are converted into ammonia and carbon is oxidised to CO_2. The ammonia present as sulphate is estimated after distillation, by titration with standard acid or colorimetrically using Nessler's reagent. The nitrogen content of the sample is calculated and converted into 'crude protein' content by multiplying by the conventional factor 6.25. The two defects of the Kjeldahl method are (1) the factor 6.25 is only approximate for some proteins and (2) the nitrogen of urea, creatinine and other N compounds (which have no nutritive value) present in animal foods, fish and milk in small amounts (2 to 7 per cent of the total N) also forms part of the total nitrogen i.e. crude protein.

Colorimetric methods: The colorimetric methods used for the estimation of proteins are based on the biuret reaction or Folin's phenol reagent. The methods are used for the determination of protein content of serum.

Electrophoretic methods: The electrophoretic methods are used for the separation and estimation of proteins in serum, tissues

and foodstuffs. In the case of tissues and foods, the proteins are extracted with suitable solvents before separating electrophoretically. The electrophoretic separation can be achieved using filter paper, agar gel and starch gel. The proteins after separation are treated with suitable dye (e.g. Amido Schwarz 10B) and the intensity of the colour of individual dye-protein complex bands are measured using a densitometer.

DIGESTION AND ABSORPTION

The digestion of protein takes place in the stomach and intestines. As a result of digestion, proteins are broken down to amino acids.

Gastric digestion: The proteolytic enzyme of gastric juice is *pepsin*. It is formed from its zymogen precursor, *pepsinogen*, which is elaborated and secreted by the chief cells of the gastric mucosa. The inactive *pepsinogen* is converted into the active pepsin by the HCl of gastric juice. Pepsin rapidly initiates hydrolysis of proteins. Studies with synthetic substrates have revealed that the peptide linkages most susceptible to this enzyme are those involving aromatic amino acids (Phenylalanine and tyrosine), tryptophan and leucine with aspartic and glutamic acids. Pepsin hydrolyses dietary proteins chiefly into a mixture of polypeptides. If gastric HCl production is inadequate to maintain the pH optimum of 2 to 3 necessary for peptic digestion, protein digestion in the stomach will be affected, as for example in *pernicious anaemia*. Pepsin has a strong clotting action on milk. In the young infant, in addition to pepsin, another milk clotting enzyme *rennin*, may be present in small amounts in the gastric juice.

Intestinal digestion: In the intestines, the products of gastric digestion are further acted upon by the enzymes present in the pancreatic and intestinal juices. The pancreas secretes a strongly alkaline fluid containing several inactive zymogen precursors of proteases, e.g. trypsinogen, two chymo-trypsinogens, two *procarboxy peptidases* and *proelastase*. An intestinal enzyme termed *enterokinase* converts trypsinogen to trypsin and chymotrypsinogen into active chymotrypsin. Procarboxypeptidase and proelastase are converted by trypsin to active carboxypeptidases and

elastase, respectively. The alkaline pancreatic juice neutralises the acidic chyle from the stomach and provides the slightly alkaline pH optimal for the hydrolytic action of the pancreatic enzymes. Trypsin acts on peptide linkages involving the carboxyl group of lysine and arginine. The chymotrypsins are most active towards peptide bonds involving phenylalanine, tyrosine and tryptophan. Thus, the action of these enzymes is additive resulting in more complete degradation of proteins into small peptides. The carboxypeptidases rapidly liberate carboxyl terminal amino acid residues. The mucosa of the intestines also contains enzymes that hydrolyse peptides into amino acids. It is not clear whether these enzymes are actually secreted into intestinal juice or they remain within the cells and participate in proteolysis as the peptides traverse the villi. Extracts of intestinal mucosa contain a group of amino peptidases which act on polypeptides and hydrolyse them to simpler peptides and amino acids. They also contain dipeptidases which act on dipeptides and hydrolyse them to amino acids.

Absorption of amino acids from the intestine: The major products of intestinal digestion of proteins are the amino acids and these are rapidly absorbed. Absorption of amino acids is confined chiefly to the small intestine and is an active, energy-requiring process which reflects a high degree of structural specificity. Impairment of absorption occurs with anoxia or in the presence of metabolic inhibitors or poisons. Experimental studies with isolated segments of rat intestine have demonstrated that the L-isomers of amino acids are more rapidly absorbed than the D-isomers and that, in general, the neutral and the more hydrophilic amino acids are more rapidly absorbed than the basic and the more hydrophobic amino acids. A role for the participation of pyridoxal and Mn in amino acid absorption has been suggested. The diminished rate of amino acid absorption from the intestine of vitamin B_6 deficient rats can be stimulated by administration of pyridoxal phosphate. Amino acids absorbed from the intestine enter the circulation almost wholly by way of the portal blood. Under special circumstances, traces of native proteins may also be absorbed in the intestinal mucosa and appear in the blood especially in the young mammal. This may be the basis for allergic reactions sometimes encountered in some infants towards some food proteins, e.g. milk proteins, egg white, etc.

PROTEIN AND AMINO ACID METABOLISM

The metabolism of a protein essentially is the metabolism of amino acids. Food proteins after digestion are absorbed as amino acids into the portal blood stream and pass on to the liver. The liver draws a certain proportion of amino acids for its own needs. Non-essential and semi-essential amino acids synthesised in the liver are added to the blood amino acids. The amino acids derived from the hydrolysis of tissue proteins are also passed on to the blood stream. The blood amino acids derived from food proteins, synthesis in liver and hydrolysis of tissue proteins, undergo metabolism. The metabolism of proteins is indicated in Fig. 4.1 and is discussed under the following heads: (1) Dynamic aspects of protein metabolism (2) Nitrogen balance, (3) Nitrogen metabolism in starvation, (4) Oxidation of amino acids and formation of urea, (5) Biosynthesis of non-essential and semi-essential amino acids and (6) Synthesis of blood and tissue proteins.

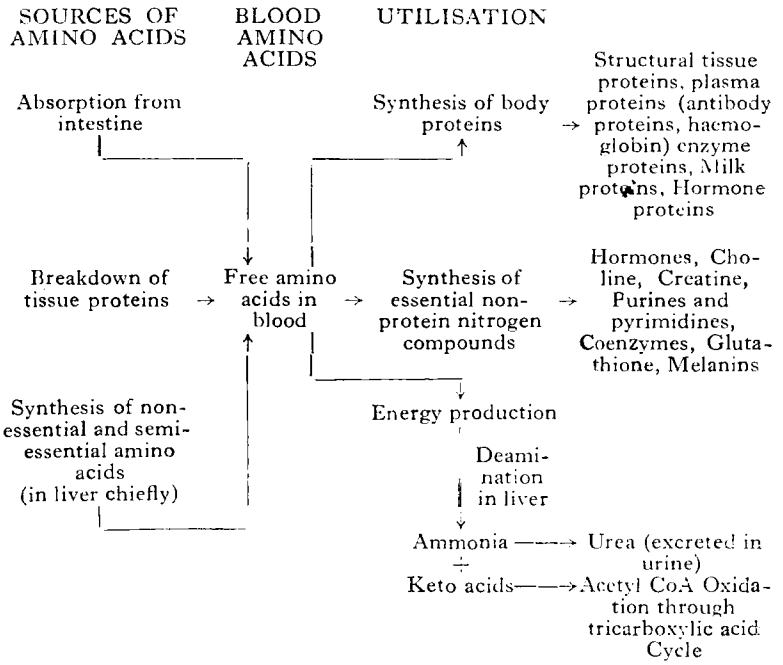


Fig. **4.1 Protein Metabolism**

Dynamic aspects of protein metabolism: The classical theory of protein metabolism as proposed by Folin (1905) has held the field for many years. Folin found that under widely varying intakes of nitrogen, the quantity of creatinine and neutral sulphur excreted in urine remained constant whereas the quantity of urea excreted varied with dietary nitrogen intake. Folin thought that creatinine and neutral sulphur were derived from the 'wear and tear' of body tissues and were an index of *endogenous* protein metabolism. Urea, on the other hand, was believed to be derived solely from the catabolism of dietary proteins and was referred to as the exogenous nitrogen of protein metabolism. Several studies made with isotopic nitrogen by Schoenheimer and Rittenberg gave results different from the views of Folin. The main conclusions from the latter studies are (1) when amino acids containing isotopic nitrogen are fed to an animal in N equilibrium, only a part of the isotopic nitrogen appears in the urine and fairly large amounts (about half) are found in the tissue proteins. In other words, the dietary nitrogen had replaced the catabolised tissue proteins whose nitrogen is found in the urine and (2) it was also found that dietary proteins containing isotopic nitrogen was constantly and rapidly being incorporated into plasma proteins and into the proteins of liver, kidney and intestinal tract as well as into the proteins of haemoglobin. According to the new theory, protein metabolism is conceived as a dynamic mechanism in which breakdown and resynthesis of tissue proteins proceed hand in hand, a part of the tissue proteins being broken down continuously and replaced by the formation of new tissue proteins from the dietary proteins. Even in the starving animal, a part of the amino acids derived from the breakdown of tissue proteins, is used for the resynthesis of tissue proteins.

Nitrogen balance: When the nitrogen intake equals the nitrogen lost from the body, the body is in a state of *nitrogen equilibrium.* If the nitrogen lost from the body is less than that of nitrogen intake, the body is in *positive* nitrogen balance. The nitrogen retained is in the form of new tissue proteins as for example during growth, pregnancy, lactation and convalescence. If the nitrogen lost from the body is higher than nitrogen intake, the body is in *negative* nitrogen balance. Negative nitrogen balances are seen in undernutrition, malnutrition, fevers, injury, after

surgery and in starvation. Under these conditions, the body proteins are slowly but steadily depleted.

Nitrogen metabolism on protein free diet: Many investigators have attempted to determine nitrogen loss in urine by placing subjects on a protein free diet providing adequate calories and other nutrients and measuring the minimum loss of endogenous urinary nitrogen. The loss of nitrogen in urine follows the pattern shown in Fig. 4.2. There are two characteristics of the curve;

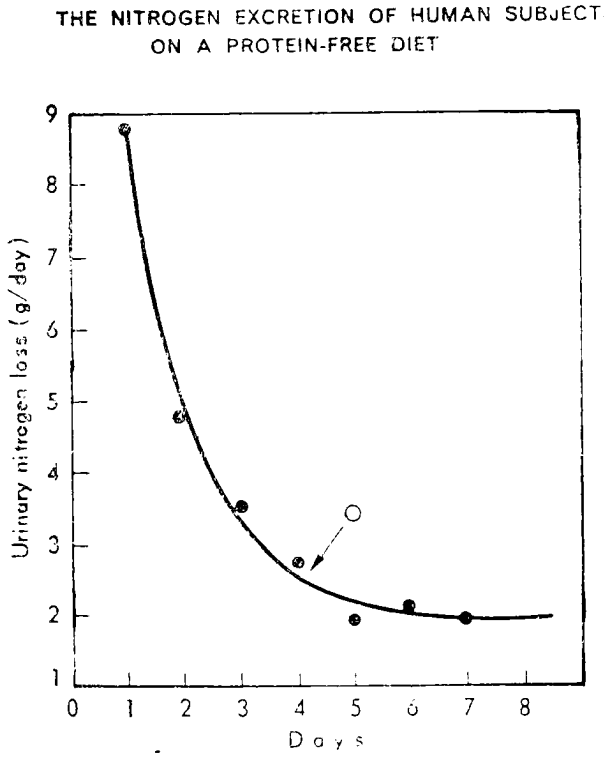


Fig. 4.2

(*a*) the nitrogen output in urine falls steeply in the first 2 or 3 days and afterwards the fall is very slight. There is no 'fixed' endogenous loss and most of the investigators have chosen a point in the latter part of the curve and (*b*) between the initial rapid fall and

the slower decline, there is a fairly sharp inflection which in adults occur after 4-6 days on a protein free diet. The evidence from animal experiments suggests that during the first few days, the major losses of proteins are from organs with a rapid turnover notably the liver, gastro-intestinal mucosa, pancreas and muscle. These organs gain and lose proteins very rapidly in response to changes in protein intake and the fraction so gained or lost has been termed 'Labile body protein'. Loss of labile protein is rapid during the first few days on a protein free diet, while losses of tissue proteins from liver, gastro-intestinal mucosa, pancreas, muscle and skin continue at a steady rate. The point of inflection in the curve thus seems to distinguish two distinct types of response to protein depletion, the second phase representing loss of tissue proteins. The FAO/WHO Committee on protein requirements (1965) suggested that the point of inflection provides the best estimate of obligatory loss of nitrogen in urine before depletion of tissue protein supervenes and termed this loss as 'Basal' urinary nitrogen loss. There is evidence that in a wide range of mammals, the endogenous nitrogen output in urine is related to the basal energy metabolism of the animal and amounts approximately to 2 mg nitrogen per basal calorie. This figure has been used by the FAO/WHO Committee (1965) to compute the endogenous N loss in urine for adults and children. Recently, however, lower values have been reported for infants and children.

Table **4.3 Loss of protein from some tissues during a 7-day fast in adult rats**

Values are expressed in grams per rat with an original body surface of 310 sq.cm. (200 g body weight).

Organs or tissue	Protein content (g)		Protein lost (g) ($a-b$)	Proportion of total protein lost (%)	Protein loss from different organs (%)
	(a) Fed rats	(b) Fasted rats			
Muscle, skin and skeleton	28.9	26.5	2.4	62	8
Liver ...	1.53	0.92	0.61	16	40
Alimentary tract, pancreas and spleen ...	1.81	1.30	0.51	14	28
Drawn blood ...	1.19	0.95	0.24	6	20

Nitrogen metabolism in starvation: The results of studies of Addis and co-workers (1936) in rats shown in Table 4.3, indicate that during 7 days starvation, the adult rat lost about 12 per cent of the body protein; the maximum loss occurred in muscle, skin and skeleton, followed by liver, alimentary tract, pancreas, spleen and blood. In complete starvation, tissue proteins (particularly muscle proteins) is broken down to amino acids on a much larger scale than in a subject receiving a protein free diet. As mentioned above, some of these amino acids are used for the synthesis of essential nitrogenous constituents. Since no food energy is available, most of the liberated amino acids are deaminated and the residues are utilised for energy purposes to maintain the blood sugar level. In starvation, therefore, N-loss in urine is greater than on a protein-free diet, i.e. 8-10 g. N per day as compared with 3 to 4 g. N per day on a protein free diet in adult human subjects. The bulk of the protein lost is derived from muscle and skin, though liver and alimentary tract also lose proteins.

Oxidation of amino acids and formation of urea: The amino acids not utilised for the formation of tissue proteins are oxidised mainly in the liver by the enzymes, amino acid oxidases.

$$\underset{\text{Amino acid}}{\begin{matrix}NH_2\\ |\\ R.CH.COOH\end{matrix}} \xrightarrow{\text{Oxidation}} \underset{\text{Keto acid}}{R.CO.COOH} + NH_3$$

The ammonia thus formed is converted into urea in the liver and the non-nitrogenous residues are further oxidised to yield energy as explained below.

Urea formation: The mechanism of formation of urea was first demonstrated by Krebs and Henseleit (1932) according to the following cycle:

(1) $\text{Ornithine} + NH_3 + CO_2 \longrightarrow \text{Citruline}$.

(2) $\text{Citruline} + NH_3 \longrightarrow \text{Arginine}$.

(3) $\text{Arginine} \xrightarrow[\text{by arginase}]{\text{hydrolysis}} \text{Ornithine} + \text{Urea}$.

Oxidation of non-nitrogenous residues: The non-nitrogenous residues of amino acids are oxidised to yield energy. Studies

with diabetic animals have shown that a majority of them are glycogenic, some are both glycogenic and ketogenic and one is ketogenic. The classification of amino acids according to the above categories is given below.

Glycogenic	Ketogenic	Glycogenic and ketogenic.
Alanine, Hydroxyproline	Leucine	Isoleucine
Arginine, Methionine		Lysine
Aspartic acid, Proline		Phenylalanine
Cystine, Serine		Tyrosine.
Glutamic acid, Threonine		
Glycine, Tryptophan		
Histidine, Valine.		

The glycogenic non-nitrogenous residues are oxidised in the same way as glucose while the ketogenic non-nitrogenous residues are oxidised in the same way as fatty acids.

Biosynthesis of non-essential and semi-essential amino acids: The non-essential amino acids are by definition those amino acids which need not be provided in the diet since they can be synthesised in adequate amounts in the body. The carbon residues of non-essential amino acids are formed in the body during the catabolism of tissue proteins. Some of these can also be synthesised from the products of carbohydrate metabolism, e.g. pyruvic acid, oxaloacetic acid and α-ketoglutaric acid.

The amino acids are formed from the corresponding keto-acids by the process of transamination as explained below:

Glutamic acid + Oxaloacetic acid $\longrightarrow$ α-ketoglutaric acid + Aspartic acid.

Glutamic acid + Pyruvic acid $\longrightarrow$ α-ketoglutaric acid + Alanine.

The semi-essential amino acids are synthesised from the corresponding essential amino acids, e.g. cystine from methionine and tyrosine from phenylalanine.

Synthesis of tissue and blood proteins: As mentioned earlier, the synthesis of tissue proteins from the dietary amino acids supplied through the circulation is proceeding constantly in all the tissues. The rate of synthesis varies with the tissues. The plasma proteins are synthesised in the liver. The mechanism of biosynthesis of proteins is discussed in Chapter 5.

NUTRITIVE VALUE OF PROTEINS

The nutritive value of a protein depends on its content of essential amino acids. For assessing the nutritive value of a protein, it is essential to know (1) the essential amino acid content of the protein and (2) its ability to meet the protein needs of the body for maintenance, growth, pregnancy, lactation, etc. During recent years, a large amount of work has been carried out on the various aspects of the nutritive value of the proteins. Data regarding the contents of essential amino acids in some food proteins are given in Table 4A in the appendix.

The nutritional significance of amino acids

The pioneering investigations of Hopkins (1906) in England and of Osborne and Mendel (1912) in America showed that young rats fed on a synthetic diet, containing zein (a protein contained in maize) as the only source of protein failed to grow. Chemical analysis of zein showed that it did not contain tryptophan or lysine. When these two amino acids were added to the zein diet, animals grew normally and remained in good health. Gelatin, like zein, was found to be an incomplete protein. It lacks tryptophan, and is highly deficient in histidine and cystine. A diet containing 18 per cent gelatin did not promote growth nor could maintain the body weight of rats. When, however, the lacking amino acids, tryptophan, histidine and cystine were added to the gelatin diet, normal growth occurred.

The classical investigations of W. C. Rose and co-workers in U.S.A. during 1930-35, have shown that out of the 19 known amino acids, 10 are essential for growth and maintenance of rats in the sense that they must be provided in the diet. It is well known that casein when fed at 18 per cent level of protein in the diet allows very good growth in young rats. Rose and co-workers (1935) attempted to grow young rats by substituting casein by an amino acid mixture similar to that of the amino acid composition of casein as was known before 1935. At that time, the amino acid—threonine—was not known. They found at first the amino acid mixture did not promote growth. Further studies led to the isolation of threonine from the hydrolysates of casein. This investigation led to the discovery of the essential nature of

threonine. Later, they found that with the addition of threonine to the amino acid mixture, the diet promoted equally good growth as the casein diet. This was the first successful attempt at growing rats on diets containing pure amino acids instead of proteins.

Essential amino acids: Next, they proceeded to study the importance of each amino acid for growth. They omitted one amino acid at a time from the mixture and studied the effect of absence of each amino acid on growth. Thus, they were able to show that out of 19 known amino acids, only 10 (including arginine) are essential in the sense that they are necessary for promoting growth and health of albino rats and must be present in the diet. In another remarkable experiment, they found that rats could grow well on diets containing only the 10 essential amino acids (Rose and co-workers, 1936). The other amino acids are non-essential in the sense that rats are able to synthesise them in their body. The list of essential, semi-essential and non-essential amino acids is given in Table 4.4.

Table 4.4 **Nutritional classification of amino acids**

Essential (Indispensable)	Semi-essential	Non-essential (dispensable)
Histidine	Arginine	Glutamic acid
Lysine	Tyrosine	Aspartic acid
Tryptophan	Cystine	Alanine
Phenylalanine	Glycine	Proline
Methionine	Serine	Hydroxyproline
Threonine		
Leucine		
Isoleucine		
Valine		

Arginine and glycine are essential for chicks and turkeys. Serine will spare or replace glycine. Tyrosine will spare but not completely replace phenylalanine. Cystine will spare but not completely replace methionine. An essential or indispensable amino acid is defined as one which is necessary for the growth and health of a growing animal and which *cannot be synthesised in adequate amounts* in the body and must, therefore, be supplied by the protein in the diet. In rats deprived of valine, grave nutritional defects develop which have been the subject of study by

Epstein and Rose (1939). The animals became very sensitive to touch and showed severe muscular incoordination (e.g. staggering gait and circus movements) together with loss of appetite, emaciation and eventually death.

Amino acids essential for the growth of infants and for maintenance in adult human: Studies on infants and human adults showed that arginine is not essential for infants and both arginine and histidine are not essential for adults (Table 4.7). Infants require 9 essential amino acids while adults need only 8 amino acids.

Nutritional classification of proteins: It was pointed out earlier that proteins differ in their amino acid make up and that proteins lacking in one or more of the essential amino acids, e.g. gelatin or zein, cannot be utilised to meet the protein requirements of the body. The reason is, all the tissue proteins (excepting that of the connective tissue) contain in their molecule varying proportions of certain essential amino acids not contained in zein or gelatin.

From the amino acids absorbed into the blood stream after the process of digestion, the body will utilise a portion of the amino acids required for the repair and growth of various tissues and to meet other physiological needs. The fraction of the amino acids not required will be discarded. These discarded amino acids will be deaminated in the course of metabolism and the nitrogen excreted in urine. The nutritive value of a protein will be high if the amino acid make up is very similar to that of the body proteins and will be low if it lacks partially or completely any one of the 10 essential amino acids or if its amino acid composition is very much different from that of the body proteins. Proteins may be broadly divided into three groups as regards their nutritive value.

Group	Limiting essential amino acid
I. Complete proteins—e.g. Egg proteins: Promote good growth in rats and other animals.	Nil
II. Partially complete proteins—e.g. Wheat proteins: Promote moderate growth.	Lysine and Threonine
III. Incomplete proteins: e.g. Gelatin or Zein; Do not promote growth.	Completely lacking in one or more essential amino acids

Methods for the determination of the nutritive value of proteins

A knowledge of the amino acid content of proteins can only help to interpret nutritional differences among proteins in terms of their amino acid make up. Quantitative data regarding the relative digestibility coefficient and nutritive value of proteins, i.e. suitability to meet the protein requirements of the body can be obtained only through experiments on animals or human beings.

A large number of methods have been proposed by various workers for the evaluation of protein quality (Table 4.5). Only

Table **4.5 Methods Available for the Evaluation of Protein Quality**

1. *Methods Based on Growth and Body Weight Changes*
   - Protein efficiency ratio
   - Net protein ratio
   - Gross protein value
   - Rat repletion method
   - Nitrogen growth index
   - Slope ratio method
2. *Methods Based on Carcass Nitrogen Analysis*
   - Nitrogen retention method
   - Net protein utilisation
   - Rat repletion methods
3. *Methods Based on Nitrogen Balance*
   - Nitrogen balance
   - Digestibility coefficient; biological value and net utilisation of dietary proteins.
   - Nitrogen balance index
   - Egg replacement method
4. *Methods Based on Regeneration of Blood and Liver Constituents*
   - Liver protein regeneration
   - Blood protein regeneration
   - Regeneration of liver enzymes
5. *Determination of Availability of Amino Acids*
   - Chemical methods
   - Enzymatic methods
   - Microbiological methods
   - Animal assays
6. *Miscellaneous Methods*
   - Plasma amino acid levels
   - Microbiological methods
7. *Chemical Scoring Methods*
   - Chemical score
   - Essential amino acid index
   - Simplified chemical score

a few of them measure the overall nutritive value of the protein, e.g. protein efficiency ratio (PER), net protein utilisation (NPU) and biological value (BV). Most of the other methods measure the protein needs of depleted animals or the protein needs for certain specific functions such as regeneration of liver proteins and blood proteins. During recent years, only three methods, i.e. PER, NPR and NPU have been widely used as the most suitable methods for the evaluation of quality of dietary proteins.

Digestibility coefficient: Dietary proteins are hydrolysed to amino acids during digestion. The digestion begins in the stomach by the action of pepsin and later completed in the intestines by trypsin, erepsin and other enzymes. Proteins differ in their digestibility. In the same period of say, 1 to 2 hours, certain proteins e.g. proteins of milk and eggs, are easily digested and converted into amino acids, while certain other proteins, e.g. proteins of pulses, are only partly digested to amino acids. Since only amino acids are absorbed into the blood stream from the intestines, unhydrolysed portion of the protein is wasted in the feces. The term *digestibility coefficient* of protein refers to the percentage of the ingested protein absorbed into the blood stream after the process of digestion is complete.

When an animal is fed on N-free diet, certain amount of nitrogen is excreted in the feces. This is derived mainly from the digestive juices. This is called *endogenous fecal N*. When a protein food is given, the N found in feces consists both of endogenous N and food N lost in digestion. To find out N lost in digestion, endogenous fecal N should also be determined. For the determination of the digestibility coefficient, the following data are required: (1) Food nitrogen intake (I_n), (2) Total fecal nitrogen excreted (F_n) and (3) Endogenous fecal nitrogen (F_e). The digestibility coefficient can be calculated using the following formula:

$$\text{Digestibility coefficient} = 100 \times \frac{\text{N intake} - (\text{N in feces} - \text{endogenous fecal N})}{\text{Nitrogen intake.}}$$

$$= 100 \times \frac{I_n - (F_n - F_e)}{I_n}$$

Where $F_n - F_e$ is the food nitrogen lost in digestion.

The digestibility coefficients of proteins are influenced by several factors, the most important of these being (1) the presence of indigestible carbohydrates like cellulose and hemicelluloses and (2) the presence of proteolytic enzyme inhibitors.

Protein Efficiency Ratio (PER): This method developed by Osborne, Mendel and Ferry in 1919 is based on the growth of young rats. The diets usually contain 10 per cent of the protein to be tested and are complete in all other dietary essentials. Groups of albino rats (21 days old) are fed for a period of 4 weeks on different diets. Records of the gain in body weight and protein intake of the rats are maintained. The protein efficiency ratio (PER) is calculated using the following formula:

$$\text{PER} = \frac{\text{Gain in body wt. (g)}}{\text{Protein intake (g)}} = \text{g ain in weight per g. of protein consumed}$$

Chapman and co-workers (1959) and A.O.A.C. (1960) have described standardized procedures using rats aged 21-28 days for an experimental period of 28 days. Hegsted and Worcester (1947) made a critical study of the growth method. They observed a high correlation between gain in weight and PER for a number of proteins and concluded that little additional information is gained by the calculation of PER. However, the Rutger University collaborative study (1950) demonstrated clearly that the variation between laboratories was reduced by approximately 50 per cent by taking food consumption into account and calculating the PER. The A.O.A.C. method was subjected to collaborative assay in eleven laboratories.

Biological value: A method for determining the biological value of proteins was developed by Mitchell in 1925. It measures the quantity of dietary proteins utilised by the animal for meeting its protein needs for maintenance and growth. Groups of albino rats (28 days old) are fed successively on the following diets for a period of 10 days: (1) protein free diet and (2) diet containing 10 per cent protein to be tested. Urine and feces are collected by keeping the rats in metabolism cages. Records of food intake are maintained. The diet, urine and feces are analysed for nitrogen.

$$\text{Biological value} = \frac{(\text{Nitrogen digested} - \text{Nitrogen lost in metabolism}) \times 100}{\text{Nitrogen digested.}}$$

Nitrogen digested = N intake (I_n) - N in feces on the protein diet (F_n)- N in feces on protein free diet (F_e).

i.e. Nitrogen digested = $I_n - (F_n - F_e)$

Nitrogen lost in metabolism = N in urine on protein diet (U_n)- N in urine on protein free diet (U_e) = $U_n - U_e$

$$\text{Biological value} = \frac{I_n - (F_n - F_e) - (U_n - U_e)}{I_n - (F_n - F_e)} \times 100$$

Similar experiments can be done with human subjects.

Example Boy aged 10 years

N-intake on wheat diet 6.0g (I_n)
Fecal N on wheat diet 1.9g (F_n)
Fecal N on protein free diet 1.0g (F_e)
Urinary N on wheat diet 3.8g (U_n)
Urinary N on protein free diet 1.8g (U_e)
Biological value of the proteins of wheat diet

$$= 100 \times \frac{6.0 - (1.9-1.0) - (3.8-1.8)}{6.0 - (1.9-1.0)}$$

$$= \frac{3.1 \times 100}{5.1} = 61.$$

$$\text{Digestibility coefficient} = \frac{6.0-(1.9-1.0)}{6.0} \times 100 = 85.$$

Net protein utilisation: Mitchell (1922) introduced the term 'Net Utilisation of Dietary Protein' which is a product of digestibility coefficient and biological value divided by 100.

$$\text{N.P.U.} = \frac{\text{Digestibility coefficient} \times \text{Biological value}}{100}$$

Miller and Bender (1955) developed a direct method of estimating 'Net Protein Utilisation' (NPU) of proteins. The method is briefly as follows: Groups of albino rats 28 days old are used. One group is fed on a non-protein diet while the other groups are fed on the test diets containing different proteins at 10 per cent level for a period of 10 days. The food intakes of the animals are measured. The animals are killed at the end of 10 days and the body nitrogen determined by Kjeldahl method on a sample of

dried and powdered carcass. The NPU is calculated according to the following formula:

$$\text{NPU} = \frac{\text{Body N of the test group} - \text{Body N of the non-protein group} + \text{N consumed by non-protein group}}{\text{N consumed by test group}} \times 100$$

The main advantage of determining NPU of a food or diet is that it helps in the calculation of the net available protein (which is the same as Reference Protein) of the diet. A comparative statement of the PER, NPU and Chemical Score of some dietary proteins is given in Table 4.6. It will be seen that there is good correlation between the values for PER, NPU and Chemical score.

Table **4.6 PER, BV, NPU and Chemical score of some Proteins**

Food	PER	BV	NPU	Chemical score	Limiting amino acids
Animal foods					
Egg ...	4.5	96	91	100	Nil
Milk, cow's ...	3.0	84	75	65	Sulphur Amino acids
Liver ...	2.9	77	65	66	,,
Meat ...	2.8	80	76	70	,,
Fish ...	3.0	85	72	60	Tryptophan
Cereals					
Rice ...	2.0	64	57	60	Lysine, threonine
Wheat ...	1.7	58	47	42	Lysine, threonine
Pulses					
Bengal gram ...	1.7	58	47	44	Sulphur amino acids
Peas, dried ...	1.6	56	45	42	,,
Nuts and Oilseeds					
Groundnut ...	1.7	54	45	44	Lysine, sulphur amino acids and threonine
Soybean ...	2.0	64	54	57	S. Amino acids
Cottonseed ...	2.1	63	53	55	,, and lysine
Coconut ...	2.5	67	56	52	,, lysine and threonine
Sesame ...	1.7	60	51	40	Lysine

Net protein ratio (NPR): This method introduced by Bender and Doell (1957) is a modification of the PER method. In this

method, an allowance is made for the protein requirements for maintenance. The method consists in feeding a group of weanling rats on a diet containing 10 per cent of the test protein and another comparable control group on a non-protein diet for a period of 10 days. The NPR is calculated by adding the loss in weight of the control group to the gain in weight of the test group and dividing the total weight (g) by the quantity (g) of protein consumed by the test group according to the following formula:

$$\text{NPR} = \frac{\text{Gain in weight (g) of the test group + loss in weight (g) of the non-protein group}}{\text{Protein intake (g)}}$$

NPR values have been reported to correlate closely with NPU values.

Data regarding the PER, BV and NPU of some dietary proteins determined using albino rats are given in Table 4.6.

Chemical score: Since egg proteins contain all essential amino acids in adequate amounts and possess the highest nutritive value among dietary proteins, Block and Mitchell (1946) assigned a chemical score of 100 to egg proteins. Since the nutritive value of the proteins will depend on the essential amino acid most limiting in the dietary proteins, they evolved a chemical score (based on the most limiting essential amino acid) which can serve as an index of the nutritive value of the proteins. The chemical score is the ratio between the content of the most limiting amino acid in the test protein to the content of the same amino acid in egg protein expressed as a percentage. The chemical scores of some proteins are given in Table 4.6.

Amino acid imbalance: Certain amino acids when present in excessive amounts affect the growth and health of experimental animals. Three types of amino acid imbalance have been observed: (1) Amino acid imbalance, (2) Amino acid antagonism and (3) Amino acid 'toxicity'.

Amino acid imbalance: The term amino acid 'imbalance' is applied to an amino acid pattern containing excessive amounts of certain essential amino acids or inadequate amounts of certain essential amino acids. The mixture causes a *depression* in growth which is completely prevented by a small supplement of the limiting amino acids in the diet.

Example:	Gain in weight (g) of the rats (2 weeks)
Fibrin 6%	33
,, +amino acid mixture minus histidine	2
,, amino acid mixture+histidine	33

Histidine is the limiting amino acid in fibrin. Addition of other amino acids (except histidine) aggravates histidine deficiency and produces imbalance with great depression of growth. Addition of histidine along with other amino acids overcomes the growth depression.

Amino acid 'antagonism': The term 'antagonism' is applied to those changes in the amino acid pattern of a diet that cause a growth depression which is largely or completely prevented by the addition of small amounts of a *structurally similar amino acids or amino acids not originally limiting in the diet.*

The antagonism caused by the addition of leucine is overcome by the addition of structurally similar amino acids—isoleucine and valine.

Example:	Gain in weight (g) of the rats (2 weeks)
Casein 9%	36
,, + Leucine 3%	4
,, +Leucine 3%+Isoleucine 1.3%	16
,, ,, ,, +Valine 1.2%	31

Amino acid toxicity: Two of the essential amino acids namely methionine and tyrosine have been found to produce toxic symptoms and depression in growth in rats when added in excessive amounts to a diet containing casein. The toxic effects of tyrosine are overcome to some extent by the incorporation of threonine.

Example I:

Casein%	L-Tyrosine%	L-Threonine%	Weight gain (g) of albino rats (2 weeks)
6	...	...	33
6	3	...	7
6	3	0.2	11
6	3	0.8	19

Example II:

	Growth (g) in albino rats (4 week.)
Casein 18%	78
,, +0.4% DL-methionine	85
,, +1.0% DL-methionine	25
,, +2.5% DL-methionine	3

PROTEIN REQUIREMENTS

A considerable amount of work has been carried out during recent years on the amino acid and protein requirements of infants, children and other age groups. Nutrition Expert Groups in different countries have made recommendations regarding protein requirements. The question of protein requirements was recently examined by Swaminathan and Parpia (1971). A brief summary of the available information is given below:

Amino acid requirements

Data regarding the essential amino acid requirements of infants, children and adults are given in Table 4.7. The quantity of egg

Table **4.7 The average minimal daily amino acid requirements of humans and the quantities of Egg and Milk proteins required to provide the same**

Amino Acid	Infant[1] (mg/kg)	Children[2] (mg/kg)	Adults[3] Female (mg/kg)	Male
Histidine ...	34	—	—	—
Isoleucine ...	119	30	8	10
Leucine ...	150	45	11	16
Lysine ...	103	60	9	11
Methionine ...	45	27	6	16,3
Cystine ...	—	—	4	12
Total sulphur amino acids ...	—	—	10	16,15
Phenylalanine ...	90	27	4	16,4
Tyrosine ...	—	—	16	16
Total aromatic amino acids	—	—	20	16,20
Threonine ...	87	35	6	7
Tryptophan ...	22	9	3	4
Valine ...	105	33	12	11
Protein required to meet amino acid needs ...	g/kg	g/kg	g/kg	g/kg
Human milk ...	1.6	—	—	—
Cow's milk ...	2.0	0.9	0.28	0.43
Egg ...	1.6	0.9	0.18	0.26

[1] Holt and Snyderman (1965)
[2] Nakagawa *et al* (1962)
[3] 'Evaluation of Protein Nutrition', Pub 711-Food and Nutrition Board, National Research Council, National Academy of Sciences, U.S.A.

protein and cow's milk protein (g/kg/day) required to meet the amino acid needs are as follows: Infants, 1.6 and 2.0 g; Children aged (10-12 yrs.), 0.9 and 0.9 g; Adults—Women 0.18 and 0.28 g; Men—0.26 and 0.43 g respectively.

Calculation of protein requirements by factorial method:

The nitrogen requirements have been calculated by a factorial method suggested by different Expert Groups as described below:

$$R=U+F+S+G$$

where R=N requirements, U=Loss of endogenous N in urine, F=Loss of endogenous N in feces, S=Loss of N through skin i.e. sweat and integumental losses and G=N required for growth. The recommendations of the different Expert Groups are given in Table 4.8 and briefly discussed below:

Nitrogen for maintenance: The FAO/WHO Expert Group (1965) calculated the nitrogen requirements for maintenance using the following values (1) Endogenous urinary loss as 2 mg. per basal KCal. (2) 20 mg. N per kg as endogenous fecal loss and (3) 20 mg N/kg as cutaneous and other losses. The ICMR Expert Group (1968) used the following figures for calculating the N requirements for maintenance. (*i*) Endogenous urinary N loss as 1.5 mg/basal calorie (*ii*) Endogenous fecal N loss as 20 mg/kg body weight and (*iii*) the cutaneous N losses as 20 mg/kg body weight. The Food and Nutrition Board, N.R.C., U.S.A. (1968) calculated N requirements for maintenance as follows: (*i*) Loss in urine as 2.0 mg. N per basal calorie (*ii*) The endogenous fecal loss 0.4 mg. N/basal KCal and (*iii*) The cutaneous N losses as 0.8 mg. N/basal calorie. The total N losses amounted to 3.2 mg. N/basal KCal. The U.K. Expert Panel (1969) used the following figures for calculating the endogenous N losses; (*i*) Endogenous urinary loss as 2.0 mg. N/basal KCal (*ii*) Endogenous fecal loss as 0.57 mg/basal KCal and (*iii*) Cutaneous losses as 0.08 mg. N/basal KCal, the total losses amounting to 2.65 mg. N/basal calorie. It is evident that the Food and Nutrition Board, N.R.C., U.S.A. and U.K. Expert Panel, related the endogenous N losses from feces and skin to basal calorie and thus improved on the procedure suggested by FAO/WHO Expert Group. Swaminathan and Parpia (1972) used the following figures for calculating N requirements for

Table 4.8 **Estimates of endogenous losses of nitrogen in human subjects**

No.	Authors	Age group	Urine N (mg)/ basal calorie	Skin (Sweat + Integumental losses)	Feces	Total N (mg)/ per basal calorie
(1)	FAO/WHO Expert Group	All age groups	2.0	20 mg/kg body wt.	20 mg/kg body wt.	...
	Recalculated per basal calorie	Child (2-3 yrs)	2.0	0.4 mg/basal calorie	0.4 mg/basal calorie	2.8
		Adult	2.0	0.8 mg/basal calorie	0.8 mg/basal calorie	3.6
(2)	Food and Nutrition Board NRC, U.S.A.	All age groups	2.0	0.8 mg/basal calorie	0.4 mg/basal calorie	3.2
(3)	Expert Panel, U.K.	All age groups	2.0	0.08 mg/basal calorie	0.57 mg/basal calorie	2.65
(4)	I.C.M.R. Nutrition Expert Group	All age groups	1.5	20 mg/kg body wt.	20 mg/kg body wt.	...
	Recalculated per basal calorie	Child (2-3 yrs)	1.5	0.4 mg/basal calorie	0.4 mg/basal calorie	2.3
		Adult	1.5	0.8 mg/basal calorie	0.8 mg/basal calorie	3.1
(5)	Swaminathan (1971)	All age groups	2.0 (Urine + Sweat per basal calorie)	0.1 mg/basal calorie (integumental losses)	0.6 mg/basal calorie	2.7

maintenance (*i*) Endogenous urinary N loss+sweat loss, 2.0 mg/basal calorie (*ii*) Integumental N losses (0.1 mg/basal calorie) and (*iii*) Endogenous fecal N losses (0.6 mg/basal calorie) amounting to a total N loss of 2.7 mg/basal calorie.

Nitrogen for growth: For calculating the N requirements for growth, the figures given in the N.R.C. Publication (1959) for infants from birth to 12 months have been used. For other age groups, i.e. 1-18 years, the figures suggested by the FAO/WHO Expert Group (1965) have been used.

'Reference' protein requirements

The FAO/WHO Committee (1965) recommended that the protein requirements are best expressed in terms of a 'reference' protein having NPU of 100. They adopted amino acid pattern of egg proteins as the 'reference' pattern as egg proteins have $NPU_{st.}$ of 100 and possess the highest nutritive value among dietary proteins. The FAO/WHO Committee recommended an additional allowance of 6 g. of 'reference' protein for pregnant women and 15 g. for lactating women. The above allowances will work out to about 0.2 g and 0.4 g/kg body weight/day. The nitrogen requirements obtained by the factorial method are converted into 'reference' protein requirements by multiplying by the factor 6.25. To this, 20-30% excess is added as suggested by the different Expert Groups to allow for individual variations.

Protein requirements in terms of dietary proteins

The protein requirements in terms of dietary proteins are calculated as follows:

$$\text{Dietary protein requirements} = \frac{\text{'Reference' protein requirements (g)}}{\text{NPU of dietary proteins}} \times 100$$

Available evidence would indicate that the NPU of diets consumed in U.S.A. and Europe will range from 60-70 and of poor cereal diets consumed in India from 45-55 (average 50).

Comparison of protein allowances of different Expert Groups

The recommended protein allowances of different Expert Groups are given in Tables 16.1-16.3 in Chapter 16 in this Volume. They are briefly discussed below:

Infants (Birth to 12 months): The protein allowances of ICMR Expert Group range from 1.5 to 2.3 g/kg body weight depending on age of the infant, as compared with 20 g/day/infant of U.K. Expert Panel and 1.8-2.2 g/kg of N.R.C., U.S.A. There is good agreement between the three recommendations.

Preschool children (1-5 years): The protein allowances on ICMR Expert Group range from 17-22 g/day/child depending on age, as compared with 30-40 g of U.K. Expert Panel and 25-30 g of the N.R.C., U.S.A. It is evident that the ICMR Expert Group recommendations are lower than those of the other two Expert Groups.

Older children (6-12 years): The protein allowances of ICMR Expert Group range from 22 to 41 g depending on age, as compared with 45-63 g of U.K. Expert Panel and 30-45 g of the N.R.C., U.S.A.

Adolescents (13-18 years): The protein allowances of ICMR Expert Group range from 50-60 g depending on age and sex as compared with 58-75 g of U.K. Expert Panel and 50-60 g of the N.R.C., U.S.A.

Adults: The protein allowances for adults are as follows: ICMR Expert Group 45 and 55 g for women and men; U.K. Expert Panel 55-63 g (women) and 68-90 g (men); and N.R.C., U.S.A. 55 g (women) and 65 g (men).

Pregnant and nursing mothers: The protein allowances of various Expert Groups for pregnant and lactating women are as follows: ICMR Expert Group 55 and 65 g; U.K. Expert Panel, 60 and 68 g; and N.R.C., U.S.A., 65 and 75 g.

Protein requirements in terms of Net Dietary Protein Calories

Platt, Miller and Payne (1961) suggested that the protein requirements are best expressed in terms of net dietary protein calories per cent (NDPCal per cent). They reported that a NDPCal per cent of 8 is optimal for the growth of infants and NDPCal per cent of 4.0 for N maintenance in adults. They determined the NDPCal per cent of human milk and found it to be 8.7, thus confirming that the NPU values obtained in rats are applicable to human beings. The protein needs of children of

the age group 1-18 years can be met by diets having NDPCal per cent greater than 4.0 but less than 8.0.

NDPCal per cent can be calculated as follows:

NDPCal per cent=Protein calories %×NPU÷100.

Important dietary sources of proteins

The important sources of proteins in the diets of low income groups in the developing countries are cereals and legumes. Meat, fish, eggs and milk are important sources of proteins in the diets of people in Western Countries. Oilseeds and oilseed meals are also rich potential sources of proteins in the developing countries. The protein contents of some important groups of foods are given in Table 4.9.

Table 4.9 **Protein content of important foods**

Foods	Protein (g/100g)
Cereals	6-14
Legumes	18-24
Nuts and oilseeds	18-40
Oilseed meals	45-55
Egg, hen (fresh)	12-13
Milk, fresh	3.5
Milk powder, full cream	26-27
Milk powder, skimmed	35-38
Fish	18-20
Meat and liver	18-22

Protein content of net available food supplies in some countries

The total protein contents of net available daily food supplies per caput in the developing countries range from 38-54 g as compared with 82-120 g in the developed countries. The availability of animal proteins is low (4-8 g) in the developing countries while it is high (30-75 g) in the developed countries (Table 4.10).

Table **4.10 Protein contents of net food supplies in some countries (1963-65)* (caput day)**

Country	Proteins		
	Animal	Vegetable	Total
Europe			
Italy	32.6	49.6	82.2
U.K.	53.1	36.0	89.1
North America			
Canada	62.4	31.5	93.9
U.S.A.	65.6	26.7	92.3
South America			
Argentina	47.9	37.0	84.9
Brazil	18.7	52.6	71.3
Near East			
U.A.R.	12.6	71.5	84.1
Far East			
Ceylon	8.3	36.8	45.1
India	5.7	48.2	53.9
Indonesia	4.5	33.7	38.2
Japan	23.6	54.8	78.4
Pakistan	5.5	45.2	50.7
Philippines	15.7	32.8	48.5
Africa			
Ghana	11.1	35.7	46.8
Uganda	10.9	47.5	58.4
Oceania			
Australia	60.0	30.2	90.2
New Zealand	75.3	44.9	120.2

* FAO Production Year Book (1966), Vol. 20.

Mutual and amino acid supplementation of proteins

There are two ways of improving the nutritive value of dietary proteins (1) by blending two or more proteins so that the excess of essential amino acids present in one protein makes up the deficiencies of the same amino acids in another protein and (2) by supplementation of the dietary proteins with limiting essential amino acids. During recent years, L-lysine and Dl-methionine

are being manufactured on a large scale at low cost in U.S.A. and several other countries and are being used to improve the nutritive value of proteins of animal feeds. There is considerable scope for the fortification of proteins of human foods with limiting amino acids.

Mutual supplementation

Improvement of cereal proteins: Cereals, in general, are limiting in lysine and threonine while legumes, milk, meat and fish are good sources of the above two amino acids. Hence, the proteins of legumes, milk, meat and fish supplement effectively cereal proteins. The results of some of these studies are given in Table 4.11.

Table **4.11 Mutual supplementation between proteins**

Basic Protein source		PER
Cereals and Millets:		
Italian Millet	...	0.80
,, ,, + Chickpea	...	2.20
Kaffir corn	...	1.61
,, ,, + Chickpea	...	1.91
Maize, meal, white	...	0.32
,, ,, + Fish flour	...	1.31
,, ,, + Soybean flour	...	1.66
,, ,, + Skim milk solids	...	1.84
Pearl millet	...	1.60
,, ,, + Chickpea	...	2.16
Rice	...	1.54
,, + Fish flour	...	2.50
Wheat flour, white	...	0.71
,, ,, + Fish flour	...	2.12
,, ,, + Soybean flour	...	1.86
,, ,, + Skim milk powder	...	2.16
Oilseeds:		
Soybean meal	...	1.73
Sesame meal	...	1.72
Soybean + Sesame	...	2.12

Soybean and sesame proteins: Soybean proteins are good sources of lysine but are deficient in methionine while sesame

proteins are good sources of methionine but are deficient in lysine. Hence, the proteins of sesame supplement effectively those of soybean (Table 4.11).

Improvements of protein quality in poor cereal diets by supplementation with legume and milk proteins: The proteins of poor diets based on different cereals are limiting in lysine and threonine. Their quality can be improved effectively by the incorporation of legumes, soybean or milk proteins in the diets.

Amino acid supplementation of proteins

Improvement of protein quality in cereals and cereal diets by supplementation with lysine and threonine: Studies carried out by several workers have shown that supplementation with lysine alone or a mixture of lysine and threonine brings about a marked increase in the PER of cereal proteins or proteins of poor cereal diets (Table 4.12).

Improvement of the quality of soybean and cow's milk proteins by supplementation with methionine: The proteins of soybean

Table 4.12 **Amino acid supplementation of proteins of some cereals and cereal diets**

Protein source	Protein Efficiency Ratio		
	No supplement	+Lysine	+Lysine and threonine
Cereals and Millets:			
Wheat	0.93	1.45	2.00
Rice	1.50	1.91	2.61
Sorghum (kaffir corn)	0.95	2.44	2.92
Pearl Millet	1.70	3.40	3.53
Ragi	0.95	2.08	3.12
Poor Cereal diets:			
Wheat diet	1.73	2.58	2.70
Rice diet	2.52	2.71	3.46
Sorghum diet	1.99	2.71	2.80
Pearl Millet diet	2.18	3.16	3.22
Ragi diet	2.05	2.37	2.76

and cow's milk are deficient in methionine. Supplementation with methionine helps to increase the PER of soybean proteins from 2.0 to 2.9 and of cow's milk proteins from 3.0 to 4.0.

Improvement of the quality of sesame and sunflower seed proteins by supplementation with lysine: Supplementation with the limiting amino acid—lysine—brings about an increase in the PER of sesame proteins from 1.7 to 2.9 and of sunflower seed proteins from 1.2 to 1.8.

PROTEIN-CALORIE MALNUTRITION IN CHILDREN

Protein-calorie malnutrition (kwashiorkor and marasmus) is widely prevalent among weaned infants and preschool children in India and other developing countries. The diets consumed by weaned infants and preschool children in these countries are lacking in proteins, calories, certain vitamins and minerals. The word 'kwashiorkor' was given to this disease by people of Gold Coast in Africa, and was first introduced by Dr Cicely Williams in 1935. The term 'kwashiorkor' means 'the disease which the child gets when the next baby is born' i.e., 'sickness of the deposed child'. She discovered that the disease could be cured by feeding milk.

KWASHIORKOR

The important clinical signs and symptoms of kwashiorkor are as follows:

Growth failure: This is manifested by decreased body length and low body weight in spite of retention of water in the body (oedema) and presence of subcutaneous fat in some children. This growth retardation is primarily due to the general quantitative lack of proteins.

Mental changes: Several workers have stressed on the constant finding of mental changes described as apathy and peevishness. In advanced cases, children tend to live in an inert, listless condition and show no interest in the surroundings.

Oedema: Oedema occurs at first in the feet and lower legs and then may involve the hands, the thighs and face. The oedema is mainly due to lowered serum albumin and probably also

due to high sodium and low potassium levels in serum. There is also some evidence that the normal diuretic-antidiuretic hormonal control of urine secretion is upset.

Muscle wasting: Muscle wasting is a constant feature of kwashiorkor and a reduction in the circumference of the upper arm is usually evident. It is less affected by oedema than in the forearm or leg.

Moon-face: The full well-rounded face, known as moon-face, is often present in kwashiorkor.

Liver changes: Liver is slightly enlarged and fatty infiltration of liver is usually present.

Gastro-intestinal tract: Loss of appetite and vomiting are common. Diarrhoea is present in most cases.

Skin and hair changes: The characteristic skin changes of kwashiorkor are known as the 'crazy pavement' dermatosis. This is most marked on the buttocks, backs of thighs and axille. These lesions consist of dark hyperpigmental brownish black areas of skin.

Anaemia: Anaemia is invariably present. It is due to the deficiency of iron and folic acid. Anaemia may be aggravated by parasitic infection which prevents the absorption of nutrients.

Vitamin deficiency: Signs and symptoms of vitamin A deficiency such as xerophthalmia and keratomalacia are widely prevalent. Angular stomatitis and glossitis due to deficiency of riboflavin may be present.

Biochemical changes

Several biochemical changes have been reported in children suffering from kwashiorkor.

Serum albumin: The serum albumin content is usually low ranging from 0.7 to 2.2 g/100 ml. serum compared with normal values of 3.5-4.0 g. The serum albumin level is a good index of the severity of the disease and the rise in serum albumin level during treatment is a reliable index of the rate of recovery.

Enzymes in serum and digestive juices: Low levels of choline esterase, alkaline phosphatase, amylase and lipase have been

reported in kwashiorkor. In experimental animals fed on protein deficient diet, reduction in the lipase, amylase and protease activities of pancreas has been reported.

Lipid metabolism: Lipid metabolism is affected as there is fatty infiltration in liver.

Carbohydrate metabolism: Low levels of blood glucose are usually found in severe cases.

Electrolyte and water balance: A deficiency of potassium occurs as a result of diarrhoea. The loss in body potassium may be as high as 40 per cent. A deficiency of magnesium also arises due to the same causes. Serum sodium levels are usually normal. This may be due to adequate intake of salt in the diets usually consumed by these children. There is retention of water in the body (oedema) due to low serum albumin levels.

Treatment

The main principles of treatment are to ensure (*a*) an acceptable and readily digestible diet (liquid diet initially for a week) rich in proteins, calories and supplying all other dietary essentials in required amounts and (*b*) treatment of any bacterial and parasitic infections present. The photograph of a child before and after treatment is shown in Plate I.

Diet: The diet usually consists of skim milk powder (re-constituted), sugar, cooked cereals and ripe banana. Fat is introduced in the diet from the 2nd week of treatment. Vegetable protein mixtures along with sugar, cereals and ripe banana have proved useful in the treatment of mild to moderate cases of kwashiorkor.

Calories: The calorie intake should be 140-150 KCal per kg body weight. This can be achieved by the addition of sugar and fat to the diet. The rate of recovery with calorie intakes of 140-150 KCal/kg is faster than that observed with intakes of 100 KCal/kg.

Proteins: The proteins present in the diet should be of high nutritive value. Milk proteins are commonly used, as skim milk powder is readily available at low cost. Rapid recovery has been

reported on protein intakes of 3-5 g/kg body weight in the form of milk proteins.

Vitamins: Vitamin A deficiency is commonly present in children suffering from kwashiorkor. In severe vitamin A deficiency, parenteral administration of 50,000 I.U. of vitamin A (oil soluble) and in milder cases, oral administration of 50,000 I.U. of vitamin A will be adequate to effect a cure.

Anaemia: Administration of iron salts and folic acid is essential to cure the anaemia.

Electrolytes: Potassium chloride (3-4 g) and magnesium chloride (0.5 to 1.0 g) should be added to the diet daily. The treatment should be continued for a period of 4-6 weeks till the subjects have fully recovered and regained normal weight.

NUTRITIONAL MARASMUS

Nutritional marasmus is principally due to the consumption of diets markedly deficient in both proteins and calories. It is seen most commonly in weaned infants of about 1 year of age in contrast to kwashiorkor which occurs more often among children of the age group 2-4 years. Nutritional marasmus usually is precipitated by diarrhoeal diseases.

Clinical features: The two constant features of nutritional marasmus are growth retardation and severe wasting of muscle and subcutaneous fat (Plate II).

Growth retardation: This is usually very severe. Loss of weight is much more marked than decrease in height. The child is usually below 60 per cent of the standard weight.

Wasting of muscle and of subcutaneous fat: The subject is severely emaciated. The muscles are wasted. The arms are thin and the skin is loose. Subcutaneous fat is practically absent.

Other changes: The skin is dry and atrophic. The subject shows signs of dehydration. Eye lesions due to vitamin A deficiency and anaemia may be present.

Biochemical changes: There is slight lowering of serum albumin. Vitamin A content of serum is low. The important differences in the clinical and biochemical features between marasmus and kwashiorkor are given in Table 4.13.

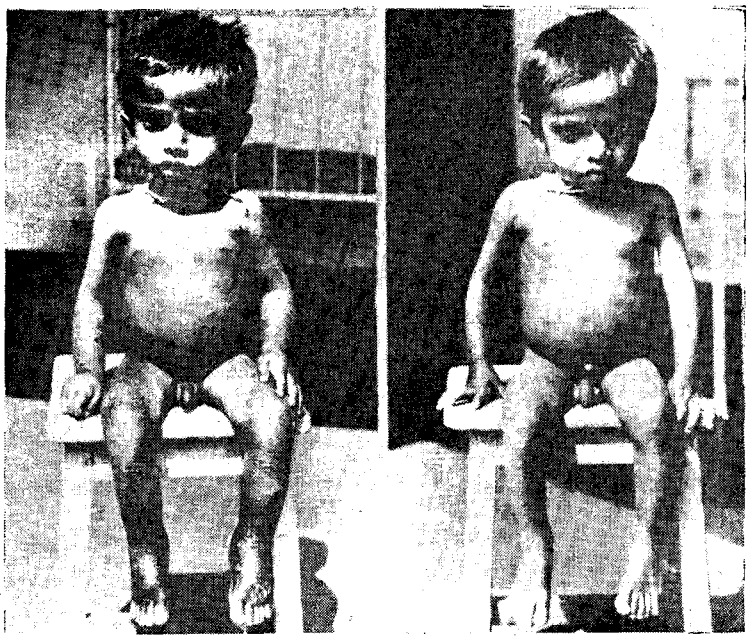


Plate I: *Left:* A child suffering from kwashiorkor showing oedema of legs, hands and crazy pavement dermatosis.

Right: Same child after treatment with processed protein food

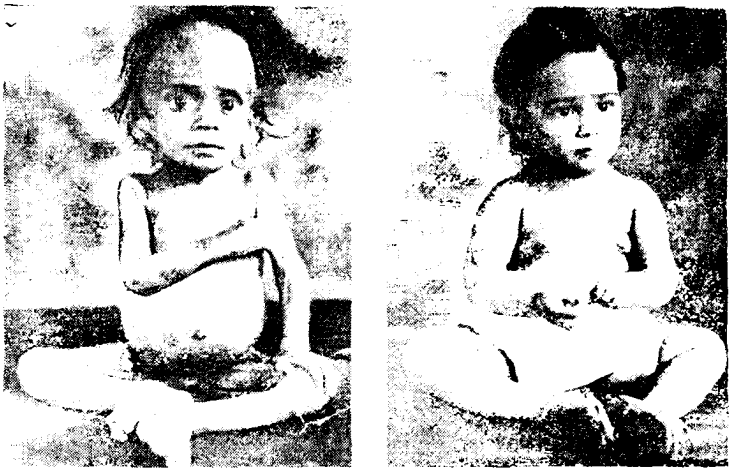


Plate II: Left: A child suffering from marasmus
Right: Same child after treatment with protein enriched cereal food

Table **4.13 Salient features of marasmus and kwashiorkor**

Features	Marasmus	Kwashiorkor
Age of maximum incidence	... 6-18 months	12-48 months
Loss of body weight	... + + +	+ to + +
Emaciation (Loss of muscle and subcutaneous fat)	... + + + +	+ to + +
Oedema	... Absent	+ to + +
Fatty infiltration of liver	... 0 to +	+ + +
Skin changes	... +	+ + +
Serum albumin	... Slightly less	Markedly less
Serum enzymes	... Slightly lowered	Markedly lowered
Serum lipids		
Triglycerides	... Normal	Normal
Cholesterol	... Normal	Lowered
Non-esterified fatty acids	... Elevated	Elevated
Blood sugar	... Normal	Slightly lowered
Response to adrenaline	... Exaggerated	Lowered
Blood Urea	... Normal	Lowered
Increase in body wt. after high protein and high calorie therapy during the first 4 weeks	... Slow	Satisfactory

Treatment: The diet for the treatment of marasmus is similar to that used for kwashiorkor. The photograph of a child before and after treatment is shown in Plate II. The clinical improvement and the increase in body weight are quite slow as compared with those obtained with kwashiorkor.

MARASMIC-KWASHIORKOR

In countries where the incidence of protein-calorie malnutrition (PCM) is high, a large number of cases show signs and symptoms of marasmus and kwashiorkor. These intermediate forms are called 'Marasmic-kwashiorkor'. In addition, the inter-relationship between the two major syndromes are such that changing circumstances may result in a transition from one clinical picture to another. A child with early kwashiorkor can develop nutritional marasmus by severe infective diarrhoea and ill-advised prolonged under-feeding. Conversely, an infant with nutritional

marasmus may develop kwashiorkor if it is fed on protein deficient carbohydrate rich foods along with adequate common salt.

Treatment: The diet in the treatment of marasmic-kwashiorkor is the same as that used in the treatment of kwashiorkor.

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CHAPTER 5

NUCLEIC ACIDS

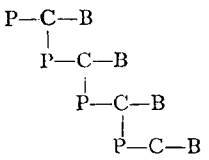
Nucleic acids are important constituents of cells. They are broadly divided into two groups, depending on the pentose (deoxyribose or ribose) present in them. The deoxyribonucleic acids (DNA) are found in the nucleus while the ribonucleic acids (RNA) are found in cytoplasm. Recent studies have shown that they play an important part in the synthesis of proteins, in the division of cells and in determining the activity of viruses and bacteriophages.

CHEMISTRY

Nucleic acids contain the following substances: (1) purine and pyrimidine bases, (2) ribose or deoxyribose and (3) phosphorie acid as indicated below:

DNA (Deoxyribonucleic acid)		RNA (Ribonucleic acid)
Phosphoric acid		Phosphoric acid
Deoxyribose		Ribose
Guanine, Adenine	Purine bases	Guanine, Adenine
Cytosine, Thymine	Pyrimidine bases	Cytosine, Uracil

If we designate phosphoric acid as P, the carbohydrate as C and the base as B, the combination of the three in nucleic acids is as follows:



P—C—B is known as nucleotide. When the phosphoric acid is removed, the compound C—B is known as nucleoside. The nucleotide found in muscle and other tissues which plays an important role in phosphorylation reactions is known as adenylic acid.

Molecular size: From the viscosity measurements and sedimentation rates, the molecular weight of DNA has been found to be of the order of 6 million (6×10^6) or higher. Molecular weights reported for RNA's range from 10,000 to 30,000 for a soluble RNA to 2 million (2×10^6) for tobacco mosaic virus RNA.

Molecular architecture of DNA: Application of X-ray diffraction analysis and electron microscopic studies has helped in unravelling the structure of DNA. Watson and Crick proposed that DNA is composed of two chains of polynucleotides which exist in a double helical structure. The main chain of each strand consists of deoxyribose nucleotide residues, joined by 3′-5′ phospho-diester bridges (Fig 5.1). The two chains are held together in part by hydrogen bonding, each amino group being joined to a keto group i.e. adenine to thymine, thymine to adenine, guanine to cytosine etc. In each case, the pairing involves a pyrimidine and a purine base.

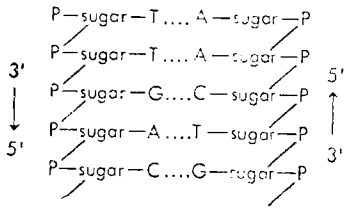


Fig. **5.1. Two dimensional depiction of a segment of the double polynucleotide chain in DNA**

. . . H bonds between bases A—T and G—C.

———Covalent bonding.

Structure of ribonucleic acids: Several types of ribonucleic acids have been found in the cytoplasm of the cells. The internucleotide linkages in them are indicated in Fig 5.2.

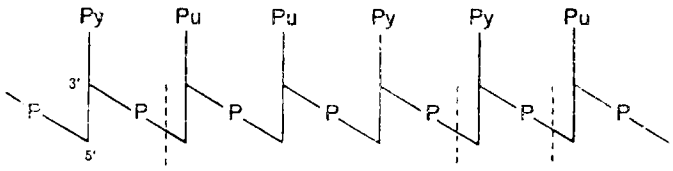


Fig. **5.2. Schematic representation of linkages in RNA**

P—Phosphoric acid
P_y —Pyrimidine base (Cytosine and Uracil)
P_u —Purine bases (Guanine and Adenine)
3′ and 5′ are hydroxyl groups in the ribose linked to the phosphoric acid.

Viruses and bacteriophages: There is evidence that viruses are composed of DNA or RNA and bacteriophages are composed of DNA.

The structure of gene: Data regarding the chemical structure of gene have been provided by a variety of studies which are summarised below. The data indicate that the activity of genes is due to DNA in most of the cases. (1) Mutation of genes is produced by ultraviolet light. The action spectrum (those wavelengths of light which cause this effect) which is effective in producing mutations is in close agreement with the absorption spectra of DNA. (2) The transforming substances of bacteria which carry heredity information consist of DNA. (3) The DNA content of a set of chromosomes is constant for each species. All these evidences have led to the view that the genetic material consists of DNA in all cases except for certain plant and animal tissues where RNA serves this function.

Nucleo-proteins: DNA is found in the nuclei of cells as parts of the chromosome structure. Basic proteins, either histones or protamines, are associated with DNA, undoubtedly as a result of ionic linkages between anionic phosphate groups of DNA and cationic groups of the basic amino acids of histone and protamine.

SYNTHESIS OF PROTEINS

The synthesis of proteins takes place within each cell from the free amino acids available to it from the blood. The free amino acids in blood are increased as a result of absorption of amino acids through the intestines from the dietary proteins, by the hydrolysis of tissue proteins by the cathepsins present in the tissues and by synthesis from non-amino acid precursors by transamination. Protein breakdown and synthesis proceed simultaneously at all times resulting in a continuous turnover, more rapidly in some tissues than in others. The most recent evidence concerning the mechanism of protein synthesis indicates the participation of three different types of RNA as well as DNA. The available evidence is briefly summarised below:

Activation of amino acids: There is evidence that the first step in protein synthesis consists of activation of the amino acids. This occurs through reaction with ATP in the presence of activating enzymes, each one of which is specific for one amino acid. The result of this reaction is the formation of amino acid adenylates, in which a mixed anhydride is formed between the carboxyl group of the amino acid and the phosphoric acid of adenylic acid.

Transfer to soluble RNA: In cytoplasm, there are a number of relatively low molecular weight (25,000-35,000) soluble ribonucleic acids (S-RNA). Each S-RNA is specific for or has specific sites for a particular amino acid which it accepts from the activated amino acid adenylate. In this process, the amino acid is transferred from the activated complex to the ribose moiety of the terminal adenylic acid of S-RNA forming an ester bond at either the 2′ or 3′ hydroxyl positions. An amino acid acyl-S-RNA is thus formed. The amino acid is transferred to ribosomal RNA, releasing S-RNA which again can accept another amino acid adenylate. S-RNA therefore acts as a carrier between the activated complex and ribosomal RNA. S-RNA is referred to as the transfer RNA and the ribosomal RNA as the 'acceptor' RNA.

Role of Messenger RNA (M-RNA): Recently, it has been found that a ribonucleic acid known as messenger RNA, is synthesised in the nucleus in direct contact with DNA. After formation, the M-RNA diffuses from the nucleus into the cytoplasm where it combines in some manner with ribosomal RNA (acceptor RNA) and imparts to it a specificity which determines the amino acid sequence of protein synthesised. The M-RNA has a high turnover rate. With the help of M-RNA, the DNA present in the nucleus controls the nature of the proteins which are synthesised in the cytoplasm.

The nucleic acid code (*Genetic code*): In recent years, intensive studies have been made by several workers on the mechanism by which the sequence of amino acids in proteins is controlled. Some 20 amino acids may occur in a protein chain and the sequence of the amino acids is controlled by a sequence of nucleotides which contains only 4 different purine and pyrimidine bases. A sequence of only 2 bases arranged in all possible combinations from the four which occur in nucleic acids will yield only 16 different combinations, a number insufficient to be specific for each of

the possible amino acids. Therefore, a sequence of at least 3 bases which would allow 64 possible combinations is the minimum possible. Such a code is known as a triplet code. Evidence for the existence of a triplet code has also been obtained by experiments involving deletion, addition and mutation of bases, one or more at a time, from natural and synthetic polynucleotides and observing the effect on amino acid incorporation into a polypeptide. It has been found that 60 to 62 of the 64 triplets do indeed code for the 20 amino acids required in the synthesis of proteins. Consequently, there may be more than one code word or 'Codon' for a given amino acid. This is referred to as 'degeneracy' of the code. Two of the 64 triplets do not code for any amino acid. Rather, they seem to function as information to terminate a polypeptide chain at that point. As a result of this function, these triplets are termed 'Chain-terminating triplets'.

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CHAPTER 6

THE COMPOSITION AND FUNCTIONS OF TISSUES AND ORGANS

The human body contains several vital organs and tissues which carry on the life processes. The composition and functions of various tissues and organs are briefly outlined below:

MUSCLE

Muscular tissue constitutes about 40-50 per cent of the body by weight and is therefore the largest single tissue component of the body. Three major categories of muscles are known (*i*) skeletal muscle—striated voluntary (*ii*) cardiac muscle (striated) and (*iii*) plain smooth muscle (involuntary e.g. muscles of stomach, intestines, bladder, uterus etc.).

SKELETAL MUSCLE

Skeletal muscle accounts for the production of a greater part of the energy in the body. Muscle contains about 75 per cent water, 18 to 20 per cent proteins, 1-2 per cent glycogen and about 5 per cent of other organic and inorganic compounds. Three major categories of cellular organization are known in muscle (*i*) *myofibrils*, responsible for contractile activity (*ii*) *sarcoplasm*, containing glycolytic and other enzymes and (*iii*) *mitochondria*, containing the enzymes for respiration and oxidative phosphorylation. The important proteins present in muscle are discussed below:

Myoglobin: It is a water soluble protein present in sarcoplasm which differs from haemoglobin in molecular weight and amino acid composition. It is a haem-protein which combines reversibly with oxygen. Myoglobin can be released from muscles after crushing or other physical injury and because of its relatively small molecular weight, it is filterable through the renal glomeruli and may appear in urine.

Proteins of myofibril: Myosin was first obtained in a crystalline form by Szent-Gyorgyi. In view of its role as a myofibrillar

structure protein, considerable interest attaches to the establishment of its molecular dimensions. It has also adenosine triphosphatase activity.

Actin: This has the striking property of existing in two forms: globular (G) and fibrous or polymerised (F).

Actomyosin: It is formed when solutions of myosin and actin are mixed in the ratio of 3:1.

Other compounds: Muscle contains other important compounds such as creatine, phosphocreatine, adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) which play an important part in energy metabolism in muscle.

Biochemistry of muscular activity: The sequence of chemical changes during muscular activity is as follows: (1) Breakdown of ATP to ADP, phosphate and energy for initial muscular work (2) Reaction of creatine phosphate with ADP to give ATP and creatine (3) Breakdown of muscle glycogen to lactic acid (anerobic phase) and (4) Oxidation of lactic acid to CO_2 and water (aerobic phase) through the tricarboxylic acid cycle. Acetyl COA and ketone bodies derived from oxidation of fatty acids are also oxidised to CO_2 and water through the tricarboxylic acid cycle.

LIVER

Liver is an important organ which takes part in several metabolic functions. It stores glycogen, vitamin A and vitamin B_{12} and synthesises plasma proteins, fibrinogen, prothrombin and heparin. It secretes bile which is essential for the absorption of fat and fat soluble vitamins. It plays an important part in carbohydrate metabolism especially synthesis of glycogen from glucose, fructose and galactose and regulation of blood sugar level and also in the metabolism of fats and proteins. It takes part in detoxication of organic compounds such as drugs, food preservatives etc.

Diet and Liver Function

Diets deficient in protein and choline and high fat diets affect the structure and functions of liver.

(*a*) *Protein deficiency:* This results in loss of liver proteins. Cytoplasmic vaculation and periportal fatty infiltration are also observed.

(*b*) *Choline deficiency:* This causes fatty liver (centrilobular fatty infiltration).

(*c*) *High fat diets:* Diets rich in fats also cause fatty liver.

(*d*) *Toxic agents:* Chloroform, carbontetrachloride etc., cause cirrhosis of the liver.

Liver Function Tests

Liver function tests are useful as diagnostic aids to assess the function of the liver in certain diseases.

(*i*) *Galactose tolerance test:* Galactose absorbed from the intestines is normally converted into glycogen in the liver and subsequently released as glucose for utilisation by tissues. In a liverless animal, galactose is not utilised and is excreted in urine. If liver function is impaired, only a part of the galactose may be utilised. The test consists in administering orally 40 g galactose and determining blood galactose at 0.5, 1.0, 1.5 and 2.0 hr. intervals. In normal subjects, the blood galactose rises very slightly. If liver is damaged, a rise in blood galactose takes place. The sum of the four galactose values (at the intervals stated above) in mg per 100 ml. blood gives the galactose index. The normal average is 68 mg. and the normal maximum is 160 mg. Higher values indicate liver damage.

(*ii*) *Hippuric acid test:* When sodium benzoate is taken by mouth, it is conjugated in the liver with glycine to form hippuric acid. The test consists in administering orally 6 g of Na benzoate to an adult and estimating the hippuric acid excreted in the urine in the course of 4 hrs. In normal persons, 3 to 3.5 g of benzoic acid is excreted as hippuric acid. A value below 2.7 g is taken as an indication of liver damage.

(*iii*) *Bromosulphalein test:* Normal liver cells secrete this dye from the blood into the bile. The test consists in injecting 5 mg of the dye (bromosulphalein) per kg body weight and determining the concentration of the dye in blood after 5 minutes and 45 minutes. In normal subjects, the dye concentration in blood falls

to 85 per cent of initial value in 5 minutes and 5 per cent of the initial value after 45 minutes. A value exceeding 10 per cent after 45 minutes indicates liver damage.

(*iv*) *Blood coagulation:* In liver disease, the formation of prothrombin and Factor VII are reduced and the blood coagulation time is increased. If administration of vitamin K does not decrease the clotting time, it indicates liver damage.

(*v*) *Serum albumin level:* Serum albumin is synthesised in liver. If liver is damaged, serum albumin level is lowered. Feeding a high protein diet fails to increase the serum albumin level if there is liver damage.

KIDNEY

The kidney is one of the vital organs which plays an important role in the regulation of water and electrolyte balance and in the excretion of waste products. The formation of urine is briefly as follows:

(*i*) *Glomerular filtration:* The renal glomeruli filters about 170 litres of the protein free fluid from the blood in an adult (Table 6.1).

(*ii*) *Tubular reabsorption:* About 99 per cent of water is reabsorbed by the tubules so that only about 1 per cent i.e. 1.5 litres of water is excreted as urine. Of the crystalloid ions filtered, nearly all sodium and chloride are reabsorbed by the tubules. Glucose is completely reabsorbed by the tubules in the normal human subjects so long the plasma glucose level does not exceed 180 mg/100 ml. About one third of urea is reabsorbed (Table 6.1).

Kidney Function Tests

The kidney function tests are used to measure the efficiency of the kidney in excreting waste products and also to measure glomerular filtration or renal blood flow. Some of the tests are briefly described below.

Urea clearance test: The urea clearance test aims to obtain a measure of the efficiency with which kidney excretes urea. It shows the response of the kidney to a test dose of urea. The

Table **6.1 Glomerular filtration and tubular reabsorption in kidneys of an adult man**

Constituent	Concentration in plasma (mg. per 100 ml)	Total in 170 litres of glomerular filtrate	Total excreted in 24 hrs. urine	Total reabsorbed in tubules
Water	—	170 L.	1.5 L.	168.5 L.
Glucose	100	170 g.	none	170 g.
HCO_3	150	255 g.	0.1 g.	255 g.
Na^+	330	560 g.	5 g.	555 g.
Cl^-	365	620 g.	9 g.	611 g.
K^+	17	29 g.	2.2 g.	26.8 g.
Phosphate (as P)	3	5.1 g.	1.2 g.	3.9 g.
Ca^{++}	10	17 g.	0.2 g.	16.8 g.
Urea	30	51 g.	30 g.	21 g.
HSO_4 (as S)	3	3.4 g.	2.7 g.	0.7 g.

patient is administered orally 15 g urea in 500 ml. water. Blood samples are collected at 0, 0.5, 1.0, 1.5 and 2.0 hr. intervals. Urine is completely collected after 2 hours. The percentage of urea administered which is excreted in urine is calculated.

Inulin clearance test: The soluble polysaccharide inulin is filtered from the glomeruli in the same concentration as in plasma. In the tubules, inulin is not reabsorbed. A test dose of inulin is administered by injection and the plasma levels are determined at intervals. Urine is collected after 2 hrs and inulin content of urine determined. The inulin clearance rate can be calculated.

Creatinine clearance test: Creatinine is filtered from glomerulus and is not reabsorbed by the tubules. The creatinine clearance test is carried out in the same way as the inulin clearance test.

Measurement of renal blood flow: This can be determined by using the following compounds (*i*) diodrast and (*ii*) para amino hippuric acid (PAH).

ORGANS OF DIGESTION

The food, we ingest, contains proteins, fats, carbohydrates, vitamins and minerals. The complex carbohydrates, fats and proteins are broken down to simpler compounds during digestion.

Mouth and salivary glands: When food is taken, there is flow of saliva. The saliva is secreted by three pairs of glands as well as by the small glands of the mucus membranes of the mouth. It is a colourless cloudy liquid, slimy in character. Its specific gravity is about 1.007. It is almost neutral in reaction (pH 6.4-7.0). Its approximate composition is given in Table 6.2.

Table **6.2 Percentage composition of saliva**

Water	99.27	Na	0.03
Solids	0.73	K	0.08
Organic solids	0.50	Ca	0.006
Inorganic solids	0.23	CNS	0.01
Mucin	0.30	Mg	0.00_1
Cl	0.03	Enzymes:	Ptyalin (α-amylase)
P	0.02		Lysozyme

The main functions of saliva are to moisten the food and to facilitate its mastication and deglutition. Ptyalin (α-amylase) acts on cooked starch at pH 6.0 and hydrolyses it to dextrin, maltose and isomaltose. The digestion of starch may continue for about 10-20 minutes till the stomach contents become more acidic. Another important enzyme present in saliva is lysozyme. This enzyme is present in most tissues and body fluids. It is a polysaccharidase, hydrolysing certain complex polysaccharides present in the cell wall of many different species of bacteria.

STOMACH

Stomach acts as a temporary reservoir of food. During the period, food stays in the stomach, it is acted upon by the gastric juice secreted by the stomach. The approximate composition of gastric juice is given in Table 6.3.

Table **6.3 Percentage composition of gastric juice**

HCl-0.45	Total solids-0.55
Other chlorides-0.107	Organic solids-0.41
Na-0.05	I(including pepsin)
K-0.028	norganic solids-0.14
Ca-0.02	Sp. gravity-1.007

The most important enzyme present in gastric juice is pepsin. Pepsin hydrolyses proteins to peptones and peptides. Free tyrosine is also a frequent product of peptic digestion. Pepsin acts on peptide linkages at the points where tyrosine and phenylalanine are present.

Normal gastric response to certain foods: When different foods are taken, the period for which the food stays in the stomach and the quantity of gastric juice secreted varies with the food. If water is taken, it leaves the stomach almost immediately. (Table 6.4).

Table **6.4 Evacuation time and highest total acidity attained with some foods**[1]

Food (100g portions unless otherwise stated)	Average highest total acidity (ml 0.1 N alkali to neutralise 100 ml juice)	Average evacuation time (hours and minutes)
Bread	80	2:40
Egg	80	2:40
Fish	130	2:50
Lamb (meat)	135	3:00
Milk, Cow's 400 ml	100	2:30
Nuts (25 to 50g)	100	3:30
Vegetables (cooked)	75	2:15

[1]Hawk, Rehfuss and Bergeim (1926) *Am. J. Med. Sci.* **171**, 359.

Fractional gastric analysis: The fractional method of analysis of the gastric contents in response to a test meal yields results which are helpful in the diagnosis of certain diseases of the stomach, viz. pyloric obstruction, cancer of the stomach, peptic and duodenal ulcers. The method consists of the following steps: (*i*) [illegible] of stomach tube (*ii*) [illegible] gastric juice (*iii*) Feeding of the test meal (*iv*) [illegible] of samples of gastric contents at 15 minutes [illegible] the stomach is [illegible] (*v*) Analysis of gastric contents for the following constituents: (*a*) free HCl (*b*) total acidity (*c*) peptic activity (activity of pepsin) (*d*) presence of lactic acid (*e*) presence of blood (*f*) presence of bile

(*g*) presence of starch (if the test meal does not contain starch). and (*h*) mucus.

Test meals: Three types of test meals are commonly used: (*i*) Evald test meal of toast and black tea (*ii*) Oat meal gruel and (*iii*) Dilute alcohol (7 per cent). Evald test meal consists of 35 g of toast without butter and 250 ml of black tea. Oat meal gruel is practically colourless and does not block the tube. It is prepared by boiling about 2 table spoons of breakfast oatmeal with $1\frac{1}{4}$ pint of water, straining through muslin cloth and adding sugar to taste. Salt should not be added, because chloride estimations would thereby be rendered valueless. Dilute alcohol (7 per cent) 50 or 100 ml has been extensively used.

Histamine test of gastric function: Histamine is a powerful stimulant for the secretion of gastric juice. 0.5 mg of histamine is injected subcutaneously (The test should not be conducted on subjects having blood pressure less than 110 mm). The gastric contents are continuously removed by a stomach tube at 10 minutes intervals for 50 minutes. The volume and acidity of the samples are determined. The results are compared with the average values obtained for normal subjects. In true achlorhydria, HCl is absent in the gastric contents even after injection of histamine.

Interpretation of gastric analysis

Gastric residuum

If it is foul smelling and contains food residues, organic acids and blood, it is indicative of carcinoma of the stomach.

Fractional gastric analysis

Free HCl: Normal range (Isochlorhydria) 10 ml to 60 ml 0.1 NHCl per 100 ml.

Achlorhydria: Complete absence of HCl e.g. as in pernicious anaemia.

Hypochlorhydria: Free HCl less than 10 ml of 0.1 NHCl per 100 ml.

Hyperchlorhydria: Free HCl greater than 60 ml of 0.1 NHCl usually in peptic and duodenal ulcers.

Pancreas

The pancreas has dual functions (*i*) it secretes the pancreatic juice which contains proteases, amylase and lipase for the digestion of food and (*ii*) the islets of Langerhans present in the pancreas secrete insulin—a hormone essential in carbohydrate metabolism.

Composition of pancreatic juice. The juice has the following composition:

Water 98.2%	Organic solids 0.6%
Total solids 1.2%	(enzymes, mucin etc.)
(including $NaHCO_3$)	

Pure pancreatic juice is a colourless transparent viscous fluid which has an alkaline reaction (pH 8.4) due to the presence of $NaHCO_3$ (0.3—0.65 per cent). In man, it is estimated that about 500 to 1200 ml are secreted in 24 hrs.

Functions of pancreatic juice: $NaHCO_3$ is present in such concentration that even after it neutralises the acid from an equal amount of gastric juice, the intestinal contents are still slightly alkaline.

Proteases: Proteolytic enzymes present are trypsin, chymotrypsin and carboxy peptidases A and B. These enzymes are secreted in the juice in the *Zymogen* or inactive form i.e. trypsinogen, chymotrypsinogen and procarboxy peptidase. Trypsinogen is converted into active trypsin by the action of enterokinase, an enzyme secreted into the intestinal lumen by the duodenal mucosa. Trypsin activates chymotrypsinogen to chymotrypsin and procarboxy peptidase to carboxypeptidase. Trypsin splits peptide bonds at the site of combination of arginine or lysine while chymotrypsin attacks peptide bonds at the site of combination of phenylalanine or tyrosine. Carboxypeptidases hydrolyse peptides to amino acids.

Lipase: This enzyme is the main fat splitting enzyme in the gastrointestinal tract. It hydrolyses fats into a mixture of diglycerides, monoglycerides and fatty acids.

Amylase: Its action is similar to that of salivary amylase. It converts starch into maltose.

Intestines

The digestion of food by the pancreatic enzymes actually takes place in the small intestines. The last stages of digestion are effected by enzymes present in the intestinal mucosa. For a long time, the concept prevailed that these mucosal enzymes were secreted in the intestinal juice. During recent years, it has been demonstrated that they act on the end products of pancreatic digestion when they pass through the intestinal mucosa. A large number of digestive enzymes are present in the intestinal mucosa viz. amylase, maltase, sucrase, lactase and isomaltase, peptidases, lipases, lecithinase, lysolecithinase, cholesterol esterase, alkaline phosphatase, nucleotidase and nucleosidase. The intestinal mucosa also contains many synthetic enzymes.

Intestinal absorption: The absorbing area in the small intestines is very large because of the numerous villi present. The mucosal surface in the small intestines of an adult human has been estimated to be 75,000 sq. cm. The villi are covered by a single layer of mucosal cells and each villi has a capillary net work, the venous side of which empties into the portal vein as well as the central lacteal which empties into the lymphatic system. In the normal man at rest, the blood-flow through small intestinal capillaries is about 1 litre per min. and during digestion the blood-flow is still more rapid. Intestinal absorption may be active or passive. Active absorption is a rapid and specific process which has the following characteristics: (*a*) it occurs against a concentration gradient and (*b*) it is dependent on metabolically obtained energy. Passive absorption lacks the above two characteristics.

Central Nervous System

The central nervous system consists of the brain which gives rise to 12 pairs of cranial nerves and the spinal cord from which 31 pairs of spinal nerves emerge. The cerebral cortex with its appendages is concerned with the highest functions of association, memory etc. The spinal cord, the brain stem and cerebellum i.e. the greater part of the brain without the cerebral cortex, are chiefly concerned with reflex functions of an instructive type and with conduction to and from the cerebral cortex.

Chemical composition: Probably in no other tissue of the body is the relationship between structure, chemical composition and

function, more complex than in the nervous system. The approximate composition of brain tissue is as follows. Moisture 77 to 78 per cent; Protein (N×6.25) 8-9 per cent; Lipids 10-12 per cent and soluble constituents 1.8 to 2 per cent.

Proteins: Most of the proteins appear as complexes with lipids, carbohydrates and other constituents. Very little is known regarding the nature of the proteins.

Lipids: Large amount of information is available on the chemical nature of lipids present in the nervous tissues. Lipids present in nervous tissue can be broadly classified into three groups (*i*) phospholipids (*ii*) glycolipids and (*iii*) cholesterol. Nervous tissue is exceptional among body tissues in that it contains neither cholesterol esters nor triglycerides, except in certain degenerative diseases. The total lipids of the white matter contain about 50 per cent phospholipids, 25 per cent glycolipids and 25 per cent cholesterol.

Inorganic salts: Sodium, potassium and chloride are the main inorganic constituents present in the nervous tissues.

Free amino acids: The amount of free α-amino acids in brain is considerably higher than in blood plasma. Six of the amino acids account for over 75 per cent of the total free amino acids. These are glutamic acid, N-acetylaspartic acid, glutamine, γ-amino butyric acid, aspartic acid and glutathione.

The blood-brain barrier: The rate of entry of many substances from blood to the brain is controlled by a membrane. The exact nature of this membrane is not known. This barrier controls the passage of certain nutrients and drugs from the blood to the brain.

Malnutrition and brain function: Pyridoxine deficiency causes seizures in experimental animals and during this condition the concentration of γ-aminobutyric acid in the brain increases. Research on experimental animals has shown that protein malnutrition *per se* is capable of producing physical, biochemical and functional changes, in the central nervous system. This subject has been dealt with in detail in Chapter 14 in Volume II.

Bone

Bone consists of both organic and inorganic substances. Bone can be regarded as a connective tissue made rigid by orderly

deposition of mineral crystals. Bone fibres consist of collagen, encrusted with crystalline calcium phosphate, the fibres being held together by amorphous cementing substance (ground substance) of mucopolysaccharides. Collagen and ground substance together comprise the bone matrix. It is important to remember that both of them are being continuously formed and broken down.

Chemical composition: Bone consists of 35 per cent mineral salts (mostly calcium phosphate) 20 per cent organic matrix of which 95 per cent is collagen and 5 per cent ground substance and 45 per cent water. The mineral salts consist chiefly of hydroxyapatite $3\ Ca_3\ (PO_4)_2$, $Ca\ (OH)_2$ but they also contain calcium carbonate and citrate and small amounts of magnesium, sodium, potassium, chloride and fluoride.

Bone metabolism, growth and replacement: The calcified mass of adult bone has three types of surfaces: (*i*) A surface on which there is no change, (*ii*) A surface on which bone is being formed and (*iii*) A surface in which bone is being resorbed.

Bone formation and bone resorption go on continuously and simultaneously throughout life, though the rates change with age and vary with different parts of the skeleton. The dynamic aspects of bone formation and resorption are under the control of two types of cells. Bone formation is controlled by single nuclear cells called *osteoblasts* and bone resorption by multinuclear giant cells called *osteoclasts.* The function of osteoblast is to lay down a calcifiable collagen fibril in a suitable ground substance.

Role of vitamins A, C and D

Vitamin A: In vitamin A deficiency, the growth of skeleton is impaired. Rats given excessive amounts of vitamin A develop multiple fractures of the long bones and bone deformities have been observed in children receiving excessive amounts of vitamin A.

Vitamin C: Vitamin C is essential for normal bone formation. In vitamin C deficiency, the ground substance is not formed.

Vitamin D: Vitamin D promotes the absorption of calcium from the intestinal tract. The vitamin also acts in a more direct manner on the bones promoting calcification. Administration of vitamin D causes degeneration of the growing cartilage cells of the epiphysis; this is followed by invasion of the older cartilage

zone by blood vessels which bring the necessary calcium salts and thus permit ossification to proceed.

Epithelial and Connective Tissues

Epithelial tissue: The major portions of hair, nails, feathers and the epidermal layer of the skin is made up of proteins called *keratins*. As a class, the keratins are characterised by their extreme insolubility in the usual protein solvents. They are rich in sulphur amino acids (cystine) and are not acted upon by digestive enzymes. The keratin molecule is considered to consist of closely packed polypeptide chains which are held together by disulphide bonds of cystine. Keratins have been grouped broadly into two classes, (*i*) eukeratins and (*ii*) pseudo-keratins. The eukeratins are found in hair, nails, feather and horn. They contain, in addition to other amino acids, the amino acids, histidine, lysine and arginine in the approximate ratio of 1:4:12 with large amounts of cystine. The pseudo-keratins, found chiefly in the skin, contain less cystine and do not have the same histidine-lysine-arginine ratio as eukeratins. The composition of the hair is influenced by age and sex. Human hair contains more cystine than wool and feathers.

Connective tissue

White fibrous tissue: The principal solid constituent of white fibrous connective tissue is collagen. Collagen is also found in bone, cartilage, ligament and in lesser amounts in skin. Collagens from various sources have different amino acid composition. The amino acid composition of collagen differs markedly from that of keratin. It has a very low cystine content and does not contain tryptophan. The concentrations of glycine is highest followed by proline and hydroxyproline. Despite the difference in the composition of collagen from various sources, glycine always represents about one third of all amino acids and a second third of the molecule is composed of proline and hydroxyproline. Gelatin is formed when collagen is boiled in water or very dilute acids.

Yellow elastic tissue (*Elastin*)*:* The principal solid constituent of the elastic tissue is elastin, a protein similar in properties to

collagen. Elastin differs from collagen in containing less hydroxyproline but more of leucine and isoleucine. It is also very rich in glycine.

Cartilage: The principal solid constituents of the matrix of cartilaginous tissue are collagen, chondromucoid and chondro-albuminoid.

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Best, C. H. and Taylor, N. B. (1961). The Physiological Basis of Medical Practice, Balliere, London.

CHAPTER 7

ENDOCRINE GLANDS

The endocrine glands secrete certain active principles known as hormones which regulate the physiological and biochemical processes in the body. The term 'hormone' was first used for these compounds by Bayliss and Starling in 1892. Hormones produced by endocrine glands are secreted into the blood and transported to various organs and tissues where they exert their effects. The major endocrine glands indicating the hormones produced by them are given in Table 7.1.

Table **7.1 Major Endocrine Glands**

Endocrine gland and hormone	Endocrine gland and hormone
Adenohypophysis:	*Corpus luteum:*
Prolactin	Progesterone
Adrenocorticotropin (ACTH)	Relaxin
Thyrotropin (TSH)	*Ovary:*
Somatotropin (growth hormone)	Estrone and estradiol
Luteinizing or interstitial cell-stimulating hormone (LH or ICSH)	*Placenta:*
	Estrogens
	Progesterone
Follicle-stimulating hormone (FSH)	Gonadotropin
	Relaxin
Neurohypophysis:	*Pancreas*
Oxytocin;	Insulin
Vasopressin (antidiuretic hormone)	Glucagon
	Parathyroids
Adrenal medulla	Parathormone
Epinephrine	*Testis:*
Norepinephrine	Testosterone
Adrenal cortex:	*Pars Intermedia:*
Adrenal cortical steroids	Melanocyte-stimulating hormone (MSH)
Aldosterone	
Corticosterone: 17-hydroxycorticosterone	*Thyroid*
	Thyroxine and triiodothyronine

Adrenal Glands

The adrenal glands in mammals are located near the upper pole of the kidneys. Each gland consists of two parts (1) Cortex and (2) Medulla.

Adrenal cortex:

The adrenal cortex is essential to life. It participates in responses to stresses of various kinds and regulates the metabolism of carbohydrates, protein, fat, water and minerals. The important hormones secreted by the glands are listed below:

Group	Name of compound	Mean daily secretion in adults
Glucocorticoid	Cortisol	15-30 mg
	Corticosterone	2-5 mg
Mineralo-corticoid	Aldosterone	5-150 μg
	11-deoxy corticosterone	traces
Androgen	Dehydroepiandrosterone	15-30 mg
Progestin	Progesterone	0.4-0.8 mg
Oestrogen	Oestradiol	trace

Glucocorticoid: Corticosterone is of very little importance in man. It has a weak effect on protein metabolism favouring conversion of protein to carbohydrate and fat.

Cortisol: Cortisol has physiological actions, in daily doses of 25 mg to maintain in good health persons suffering from Addison's disease (adrenal cortex insufficiency).

Carbohydrate metabolism: Cortisol deficiency leads to hypoglycemia, increased sensitivity to insulin and a marked decrease in liver glycogen. Cortisol in excess produces hyperglycemia, glucosuria and increased resistance to insulin and an increase in liver glycogen. Cortisol promotes gluconeogenesis.

Protein metabolism: Cortisol promotes catabolism of tissue proteins. Excess cortisol causes negative nitrogen balance due to excessive breakdown of tissue proteins.

Lipid metabolism: Cortisol in excess causes redistribution of fat in the adipose tissues.

Electrolyte and water metabolism: The effect of cortisol on electrolyte and water excretion is qualitatively similar to that of aldosterone, though it is very much less potent. Thus, cortisol promotes retention of Na-ion (and usually of cl-ion) and excretion of K-ion by the kidney. In adrenal cortex insufficiency, there is reduced diuretic response to ingested water so that water taken is not excreted for 12 hrs or more, leading to water retention in the body. Excess of cortisol may cause oedema and hypertension.

Mineralocorticoids: 11-deoxy corticosterone promotes retention of NaCl.

Aldosterone: It promotes retention of Na-ion and chloride ion and excretion of K-ion. It has very little effect on water retention.

Disorders of adrenocortical function: Chronic insufficiency leads to the development of the disease known as Addison's disease. Over activity of the cortex leads to a condition known as Cushing's syndrome.

Adrenal medulla

The active principles of adrenal medulla are (1) adrenaline and (2) noradrenaline. The ratio between adrenaline and noradrenaline produced by the adrenal medulla in man is 4:1.

Effects on metabolism

Blood pressure: They increase blood pressure, noradrenaline being more potent in this respect than adrenaline.

Blood sugar: Adrenaline raises blood sugar, sometimes high enough to produce glucosuria. The hyperglycemia is due to excessive breakdown of glycogen in liver and muscle. Noradrenaline is only one fifth as potent as adrenaline in this respect.

Blood lactate: This is increased as a result of rapid glycogenolysis in skeletal muscle.

Energy metabolism: Adrenaline increases energy metabolism. The same dose of noradrenaline has no effect.

Adipose tissue: Adrenaline acts on adipose tissue and causes release of free fatty acids into the circulation.

Pancreas

The islets of Langerhans in the pancreas contain two types of cells, (*i*) α-cells and (*ii*) β-cells.

Functions of α-cells: The α-cells secrete a hormone called *glucagon* which on injection causes hyperglycemia. It is a polypeptide containing 29 amino acid residues. It acts like adrenaline, to stimulate the activity of liver phosphorylase, thus promoting hepatic glycogenolysis.

Functions of β-cells: The β-cells secrete the hormone *insulin*. Insulin is a polypeptide containing 51 amino acid residues.

Effects of insulin on carbohydrate metabolism

Insulin increases the withdrawal of glucose from blood and tissue fluids in three ways. (*a*) It increases the deposition of glycogen in the liver and in the muscles, (*b*) It increases the rate of complete oxidation of glucose in tissues and (*c*) It increases the rate of conversion of glucose into fatty acids in the liver and adipose tissues and prevents the breakdown of adipose tissue fat.

It decreases the rate of addition of glucose to the body fluids in two ways. (*a*) It depresses neoglucogenesis (new glucose formation) from proteins and glycerol of fat and (*b*) It depresses the rate of conversion of glycogen in the liver to glucose.

Control of insulin activity by other hormones

Anterior pituitary: The anterior pituitary hormone which has an opposing action on insulin is identical with or closely associated with growth hormone. Attempts to separate the growth promoting activity from the diabetogenic activity in preparations of growth hormone have been unsuccessful. Growth promoting hormones produce hyperglycemia and ketosis when administered to hypophysectimised animals.

Adrenal cortex: Glucocorticoid produces hyperglycemia, glucosuria, increase in liver glycogen and increased resistance to insulin.

Adrenal medulla: Adrenaline causes hyperglycemia by excessive breakdown of glycogen in liver and muscle.

Effects of insulin insufficiency

Decreased production of insulin by the β-cells leads to the development of diabetes mellitus.

THE HYPOPHYSIS (PITUITARY GLAND)

The hypophysis exerts a profound influence by regulating a large number of endocrine activity in the animal and human organisms. It consists of anterior and posterior lobes. The anterior portion is called *adenohypophysis* while the posterior portion is called *neurohypophysis*.

Hormones of posterior pituitary (Neurohypophysis): Neurohypophysis secretes the following hormones.

(*i*) Antidiuretic hormone (vasopressin) (*ii*) Oxytocin. The antidiuretic hormone (ADH) is also known as vasopressin. It controls the excretion of water by the kidney, by accelerating water reabsorption by the tubules. The oxytocin secreted by the gland causes secretion of milk from the lactating breast. It may also have a physiological role in parturition. Antidiuretic hormone and oxytocin are peptides. They have been prepared synthetically.

Pars intermedia: Pars intermedia secretes hormones which possess melanocyte stimulating activity. Injection of the hormone was reported to intensify skin pigmentation.

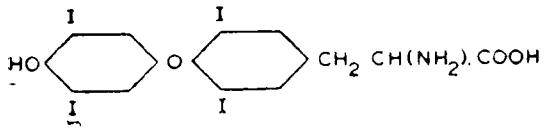
Anterior pituitary: The functions of anterior pituitary are numerous. It controls the growth of the organism and the growth, development and activity of adrenal cortex, thyroid, testis, ovary and breast. Six different hormones have been isolated from the gland. (1) Growth hormone (Somatotrophin, Somatotrophic hormone (STH) probably identical with diabetogenic hormone). (2) Corticotropin (Adrenocorticotropic hormone (ACTH). (3) Thyrotrophin (Thyrotropic hormone, thyroid stimulating hormone (TSH). [illegible] (5) Interstitial [illegible] (ICSH or LH) and (6) Prolactin, [illegible] luteotrophin).

Growth hormone: The functions of growth hormone are as follows: (1) It promotes the growth of skeleton, muscles, viscera and the body as a whole, causing increased retention of nitrogen

and minerals. (2) *Adipose tissue:* Large doses of growth hormone mobilises fat from adipose tissues, causes a rise in the free fatty acids in blood and causes fatty infiltration of liver. (3) *Protein metabolism:* It accelerates protein synthesis. (4) *Diabetogenic action of growth hormone:* When growth hormone si njected into animals, it produces a condition resembling diabetes mellitus. The effects of the hormone are as follows: (*i*) increase in blood sugar level, and development of glucosuria, (*ii*) mobilisation of fat from adipose tissue and increased oxidation of fatty acids and production of ketone bodies, (*iii*) increase in the conversion of glycogen into glucose in the liver and (*iv*) damage to the β-cells, thus decreasing insulin production.

THYROID

The thyroid glands weigh about 15-25 g in normal adults. It has a remarkable capacity to absorb iodine. The hormone *thyroxine* is synthesised in the glands and is bound to a protein called thyroglobulin. The chemical structure of thyroxine is given below.



The thyroid hormones (over 90 per cent in the form of thyroxine) are carried in the blood bound to certain plasma proteins.

Action of thyroid hormones: The actions of the hormones are as follows: (1) Hypothyroidism produces *cretinism* in infants and children and *myxoedema* in adults. (2) Hyperthyroidism causes a condition known as *Grave's disease, exophthalmic goitre* or *thyrotoxicosis*. (3) *Energy metabolism:* Administration of thyroxine increases the energy metabolism. The basal metabolic rate is reduced in hypothyroidism and increased in hyperthyroidism. (4) *Protein metabolism:* Excess thyroxine causes breakdown of proteins and increases neoglucogenesis. (5) *Carbohydrate metabolism:* Thyroxine increases intestinal absorption of glucose

and increased oxidation of glucose by tissues. (6) *Lipid metabolism:* In hypothyroidism, the levels of cholesterol and free fatty acids increase in the blood and (7) *Water and mineral metabolism:* When thyroxine is given to a patient suffering from myxoedema, water and NaCl are excreted in large quantities through the kidneys. In hyperthyroidism, there is increased excretion of Ca and P in urine.

Parathyroid Glands

The parathyroid glands secrete a hormone which is essential for the maintenance of the level of calcium in blood and in extracellular fluid within normal limits in spite of large variations in the intake and excretion of calcium and deposition of calcium in bone. Parathyroid activity is apparently regulated by the calcium content of blood. When the level of calcium in blood falls, the parathyroid activity increases and when the level rises, parathyroid activity is reduced.

Nature of parathyroid hormone: The parathyroid hormone isolated from extracts of bovine parathyroid is a polypeptide with a molecular weight of 8500. Its amino acid composition is known. The purified hormone acts equally well as the crude extract in mobilising calcium from bone and increasing the excretion of calcium and phosphorus in urine.

Action of parathyroid hormone: (*i*) It acts directly on the bone, mobilising calcium and thus raising the blood calcium level and (*ii*) It increases the excretion of phosphate in urine.

Abnormalities of parathyroid function

Hypoparathyroidism: Hypoparathyroidism is generally due to damage to parathyroid glands during the operation of thyroid glands. The accompanying symptoms are those of tetany. This is due to a lowering of the serum calcium levels to 4-7 mg/100 ml. The treatment consists of (1) administration of parathyroid hormone and (2) increase of serum calcium levels by large intake of calcium salts and vitamin D.

Hyperparathyroidism: This condition is generally caused by tumour of one of the parathyroid glands. The symptoms of hyperparathyroidism are due to hypercalcemia, increased rarefaction of bone and renal stones. The blood calcium levels may increase to 11 to 22 mg/100 ml. Removal of the tumour causes the

blood calcium levels to fall to normal limits and calcification of the rarefied bone.

Gonads (Sex Hormones)

The term *androgens* and *oestrogens* are used to denote the hormones secreted by the testis and ovary respectively.

Hormones of testis: The active hormone of the testis is testosterone. This hormone is converted in the liver into androsterone and dehydroepiandrosterone both of which appear in the urine conjugated with glucuronic acid or sulphuric acid. Testosterone is about 10 times more active than androsterone.

Action of testosterone and other androgens: (1) Naturally occurring testosterone is responsible for the development of the accessory sex organs and the secondary sexual characters at puberty. (2) Like pituitary growth hormone, it causes nitrogen retention in the body and increased synthesis and deposition of protein in certain tissues, especially skeletal muscle and (3) It also causes retention of calcium and other minerals.

Hormones of ovary and corpus luteum

The natural hormones of ovary and corpus luteum include (1) oestrogens and (2) progestins.

Oestrogens: The natural oestrogens are ovarian hormones which control the female sexual cycle. Oestradiol-17β is the hormone secreted by ovary.

Action: (1) Oestrogens are responsible for the growth of the uterus, female sex organs and breasts which occur at puberty, and (2) They influence the menstrual cycle and play an important role in pregnancy and parturition.

Progestins: The active hormone from the corpus luteum is *progesterone.* This hormone influences—(*i*) the premenstrual growth phase in the non-pregnant human uterus (*ii*) the growth of alveolar tissue in the breast (*iii*) the development of the placenta during pregnancy and the embedding of the fertilised ovum in the uterus and (*iv*) inhibition of ovulation during pregnancy.

REFERENCE

Best, C. H. and Taylor, N. B. (1961). The Physiological Basis of Medical Practice, Balliere, London.

CHAPTER 8

ENERGY VALUE OF FOODS AND ENERGY REQUIREMENTS

Lavoisier is rightly considered as the pioneer in studies on energy metabolism. He, along with Laplace, carried out, during the 18th century, experiments with guinea pigs and found a relationship between heat produced and CO_2 output. Later, Lavoisier also measured the oxygen consumed by men and found that exercise increased the O_2 consumption. In 1866, Pettenkofer and Voit built the first human respiration calorimeter. They measured the heat output, N excretion in urine and CO_2 output in a fasting man for 24 hours. The work was further continued by Rubner in Berlin, Zuntz in Switzerland and Johanson in Sweden. During 1892-1902, Atwater of U.S.A. did pioneering work and established clearly, the relations between food consumed, heat output, O_2 consumed and CO_2 output by conducting experiments with human subjects using an improved type of human respiration calorimeter. Among other scientists who have made extensive studies on energy metabolism, mention may be made of H. Armsby, F. Benedict and E. F. DuBois.

The energy yielding food factors: The human body utilises the potential energy contained in foods for maintaining life and doing work. The energy yielding food factors are: (1) carbohydrates (2) fats and (3) proteins. Within the body, these nutrients are oxidised in the cells with the help of catalysts such as enzymes, co-enzymes (containing certain vitamins) and hormones. The process is one of continuous utilization of oxygen and production of CO_2, water and heat:

$$\text{Carbohydrates and Fats} \xrightarrow[\text{Oxidation}]{O_2} CO_2 + \text{water} + \text{heat}$$

$$\text{Proteins} \xrightarrow[\text{Oxidation}]{O_2} CO_2 + \text{water} + \text{urea.}$$

The energy value of food

The energy or calorific value of foods depends on the quantity of carbohydrates, fats and proteins present in them. This can

be determined by oxidising a known weight of food in an instrument called bomb calorimeter and measuring the heat produced.

Energy units: The energy value of foods can be expressed in terms of kilo calories (KCal) or mega joules (MJ). The Unit *Kilo Calorie* has been in use till now. The International Union of Nutritional Sciences has suggested the use of Mega-Joule (MJ) as the energy unit in place of KCal.

Kilo calorie: One kilogram calorie is the quantity of heat required to raise the temperature of 1 kg. of water through 1°C. It is one thousand times the small calorie used in physics.

Mega Joule: One kilo calorie equals 4.186 kilo joules. Hence, 1,000 kilo calorie equals 4.186×10^3 kilo joules or 4.186 mega joules.

Determination of energy value using bomb calorimeter

The energy value of foods is usually determined using the instrument called bomb calorimeter (Fig. 8.1). It consists of a heavy steel bomb, with a platinum or gold-plated copper lining and a cover held tightly in place by means of a strong screw collar. A weighed amount of sample, usually pressed into pellet form, is placed in a capsule within the bomb which is then closed except for the oxygen valve, charged with oxygen to a pressure of about 300 pounds to the square inch. The oxygen valve is then closed and the bomb immersed in a weighed amount of water. The water is constantly stirred and its temperature taken at intervals of one minute by means of a differential thermometer, capable of being read to one-thousandth of a degree. After the temperature of the water has been determined, the sample is ignited by means of an electric fuse and on account of the large amount of oxygen present, it undergoes rapid and complete combustion. The heat liberated is absorbed by the water in which the bomb is immersed and the resulting rise in temperature is accurately determined. The thermometer readings are also continued through an 'after period', in order that the 'radiation correction' may be calculated and the observed rise of temperature corrected accordingly. This corrected rise, multiplied by the total heat capacity of the apparatus and the water in which it is immersed, gives the total heat liberated in

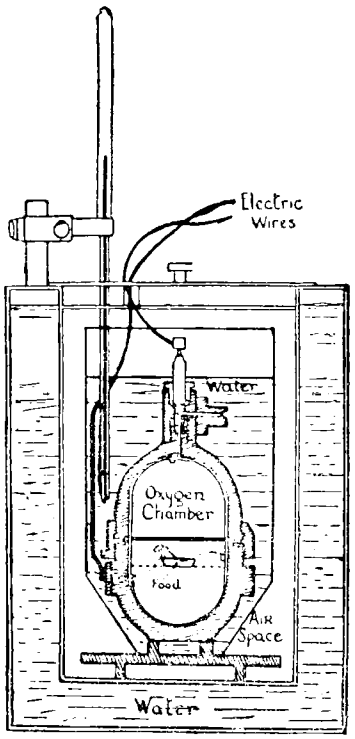


Fig. 8.1. The Bomb Calorimeter

the bomb. From this, the heat arising from accessory combustions (the oxidation of the iron wire used as a fuse etc.) must be deducted to obtain the number of calories arising from the combustion of the sample.

Example: Wt. of wheat taken=2 g.
Wt. of water in the outside vessel=3,000 g.
Water equivalent of the calorimeter=500 g.

Initial temperature of water=24°C
Final temperature of water=26°C
Rise in temperature=2°C
Heat gained by water and calorimeter=3500×2=7,000 small calories or 7 KCal.
2 g. wheat produces 7 KCal.

1 g. wheat produces 3.5 KCal.
Calorific value of 1 g. of wheat=3.5 KCal.

Calorific value of carbohydrates, fats and proteins

The average calorific values of pure carbohydrates, fats and proteins determined with the bomb calorimeter are given below:

	Calories
1 g. carbohydrate	4.1
1 g. fat	9.45
1 g. protein	5.65

Physiological energy value of foods

In the bomb calorimeter, carbohydrates and fats are completely oxidised to CO_2 and water. Protein is oxidised to CO_2, water and nitrogen. Another important error in the use of bomb calorimeter for determining the calorific value of foods of vegetable origin is that the fibre present in foods is burnt and yields energy, while it is not utilised by human beings. But, in the utilisation of carbohydrates, fats and proteins in the body, a certain percentage is lost in digestion and the nitrogen in proteins is excreted mainly as urea which contains some energy value.

Loss in digestion: The average losses of carbohydrates, fats and proteins during digestion in human beings on diets consumed in Western countries have been estimated to be as 2 per cent for carbohydrates, 5 per cent for fats and 8 per cent for proteins. Food energy available after loss in digestion is shown, in Table 8.1.

Table **8.1 Calculation of physiological energy value of carbohydrates fats and proteins from gross energy value**

Nutrient	Gross energy value (KCal/g.)	Loss of food energy in digestion (%)	Energy available after digestion (KCal/g)	Loss of food energy in metabolism (KCal)	Physiological energy value of foods (KCal/g)
Carbohydrates	4.1	2	4.0	nil	4.0
Fats	9.45	5	9.0	nil	9.0
Proteins	5.65	8	5.2	1.2	4.0

Loss of energy in metabolism due to incomplete oxidation: There is no loss in metabolism in the case of carbohydrates and fats. But in the case of proteins, a part of the energy is lost as urea due to incomplete oxidation. This loss has been estimated as 1.2 cal. per gram of protein oxidised. The physiological energy values of carbohydrates, fats and proteins are 4, 9 and 4 after making allowances for losses of food energy in digestion and metabolism (Table 8.1). These values are known as Atwater-Bryant factors. Rubner used dogs for determining digestibility coefficients and found somewhat lower losses in digestibility of the nutrients. The physiological energy values according to Rubner are slightly higher (Table 8.2).

Table 8.2 **Physiological energy value of carbohydrates, fats and proteins** (KCal/g)

	Rubner	*Atwater-Bryant*
Carbohydrate	4.1	4.0
Fat	9.3	9.0
Protein	4.1	4.0

The Atwater-Bryant values are used widely in all countries for calculating energy value of foodstuffs from their chemical composition.

Calculation of calorific value of foodstuffs from proximate composition

The calorific values of foodstuffs given in food composition tables (Table 1A in Appendix) are calculated from their contents of carbohydrates, fats and proteins using the physiological energy values of 4 KCal per gram of carbohydrate and protein and 9 KCal per gram of fat.

Benedict's oxy-calorimeter

Another apparatus used for the determination of the energy value of foods is the oxy-calorimeter, devised by Benedict and co-workers. This instrument measures the volume of oxygen required to burn a known weight of the food. The apparatus consists of a combustion chamber, in which the weighed sample

is burnt, a soda lime container for absorption of carbon dioxide, a spirometer for measuring the oxygen used and a motor-blower unit for circulating the gas mixture. Using this instrument, the amount of oxygen consumed in burning 1 g. of pure carbohydrate, fat or protein can be determined. The factors for converting litres of oxygen to calories are given by Benedict and Fox in Industry and Engineering Chemistry—Volume 17, page 912 (1925).

Relation between oxygen required and calorific value

It is of importance to know the heat produced when 1 litre of oxygen is used for the oxidation of carbohydrates, fats or proteins. This can be calculated from the data obtained by the oxy-calorimeter and bomb calorimeter as explained below:

1 g. carbohydrate requires about 0.8 lit. of O_2 for complete oxidation and yields 4.1 KCal.
1 g. fat ,, ,, 2.2 ,, ,, ,, ,, 9.5 KCal
1 g. protein ,, ,, 1.2 ,, ,, ,, ,, 5.5 KCal
0.8 litres of O_2 oxidises 1g. carbohydrate and produces 4 KCal heat.
1 litre of ,, ,, 1.25 g ,, ,, 5 KCal heat.
2.2 ,, ,, ,, 1 g. fat and produces 9.5 KCal heat.
1 ,, ,, 0.49 g. fat and produces 4.5 KCal heat
1.2 ,, ,, ,, 1 g. of protein ,, 5.5 ,,
1 ,, ,, ,, 0.83 g. protein ,, 4.6 ,,

One litre of oxygen oxidising carbohydrates, fat or protein produces nearly the same amount of heat i.e. 4.5 to 5 KCal. This is an important conclusion as this is the basis for indirect determination of energy requirements.

Direct calorimetry

The relation between energy output and oxygen consumed has been determined using the human respiration calorimeter.

Reliable data regarding the energy output and oxygen consumed in human beings could be obtained only after Atwater-Rosa-Benedict perfected the human respiration calorimeter. This equipment (Fig. 8.2) consists of an air-tight copper chamber insulated by wooden walls with air space in between. A folding bed, chair and table are provided in the chamber. A man can comfortably stay in the calorimeter for a few days and

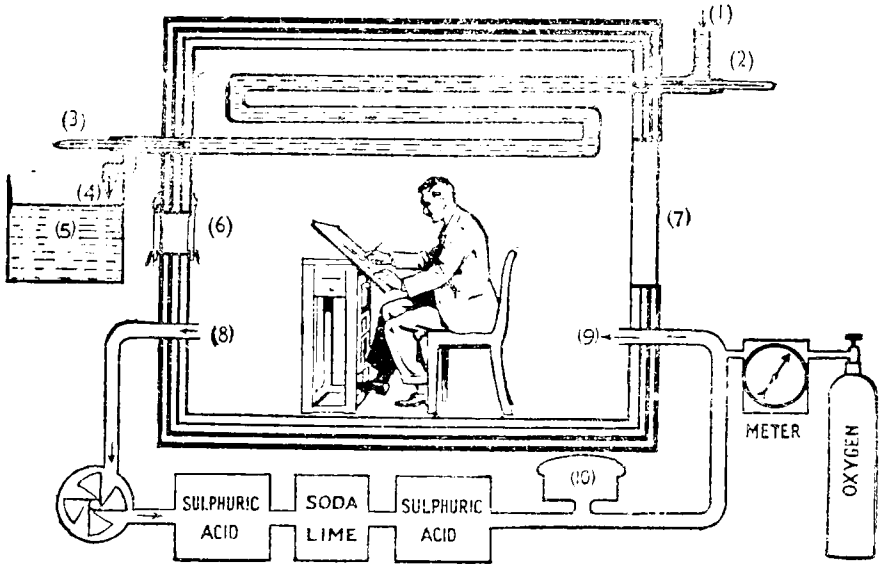


Fig. **8.2 The Atwater-Benedict Respiration Calorimeter (From Bell G.H. Davidson J.N. and Scarborough H. (1968) Text book of Physiology and Biochemistry, 7th ed. Edinburgh, Livingstone**

do some work such as reading, writing etc. A small opening is provided at the two ends for passing food and drinks and removing the excreta. The chamber is ventilated by a current of air, the CO_2 and water given off are removed by soda lime and sulphuric acid respectively. Oxygen utilized by the subject is replaced by introducing known amounts of oxygen through gas metre into the chamber with the air current. From the above, the quantity of oxygen consumed and CO_2 produced can be calculated. The heat produced is measured accurately by circulating a current of water through copper pipes and measuring the quantity of water that has been circulated through the chamber and also the difference between the temperature of the water entering and leaving the chamber.

Example: Adult weighing 65 kgs
Amount of heat output in 24 hours—2400 KCal
Amount of oxygen consumed in 24 hours—500 litres
Heat output per litre of oxygen consumed-4.8 KCal.

Relation between respiratory quotient and energy output: The term respiratory quotient refers to the ratio between the volume of CO_2 given out and the volume of O_2 consumed by the human subjects.

$$\text{Respiratory quotient (R.Q.)} = \frac{\text{Vol. of } CO_2 \text{ produced}}{\text{Vol. of } O_2 \text{ consumed}}$$

When only carbohydrate is oxidised, the R.Q. is 1.0 and when only fat is oxidised, the R.Q. is 0.7 and when only protein is oxidised, the R.Q. is 0.82. The R.Q. at the post-absorptive state when basal metabolism is determined, is about 0.82 as at this stage the body derives energy from the oxidation of carbohydrate (50 per cent), fat (40 per cent) and protein (about 10 per cent) present in the body. After an average meal containing 10 per cent protein, 20 per cent fat and 70 per cent carbohydrate, the R.Q. is about 0.82. The R.Q. is lowered to about 0.7 in diabetes mellitus as the body oxidises large amounts of fat. Data regarding the quantities of carbohydrates and fats oxidised per litre of O_2 with the corresponding non-protein R.Q. and calories produced are given in Table 8.3.

Table 8.3 **Non-protein respiratory quotient, quantities of carbohydrates and fats oxidised and heat produced per litre of O_2 consumed.**
(Data per litre of O_2 consumed)

Non-protein RQ	Carbohydrate g.	Fat g.	Calories KCal
1.00	1.232	0	5.047
0.95	1.010	0.091	4.995
0.90	0.793	0.180	4.924
0.85	0.580	0.267	4.862
0.80	0.375	0.350	4.801
0.75	0.173	0.433	4.739
0.70	0	0.502	4.686

Calculation of amounts of proteins, fats and carbohydrates oxidised in the body

From the quantity of nitrogen excreted in urine and the quantity of CO_2 produced and O_2 consumed in 24 hours, it is possible to calculate the amounts of proteins, fats and

carbohydrates oxidised in the body. The following example will illustrate the principles involved in such a calculation:

Example: An adult excretes in 24 hours 8 g. N (equivalent to 50 g proteins) in urine and uses 400 litres of O_2 and gives out 320 litres of CO_2. It is known that for 1 g of urinary N, 6.25g protein would have been oxidised and this will require 5.92 litres of O_2 and will produce 4.754 litres of CO_2. So, 8 g N in urine will need 47.4 litres of O_2 which produce 38.0 litres of CO_2. After deducting these from the total O_2 consumed and CO_2 produced, the O_2 utilised and CO_2 produced in the oxidation of fats and carbohydrates can be obtained.

O_2 consumed in the oxidation of fats and carbohydrates will be 400-47.4=352.6 litres; CO_2 produced in the oxidation of fats and carbohydrates will be 320.0-38.0=282.0 litres.

$$\text{Non-protein R.Q.} = \frac{282.0}{352.6} = 0.80$$

It will be seen from Table 8.3 that for R.Q. of 0.80, 1 litre of O_2 consumed would have oxidised 0.375 g carbohydrate and 0.350 g fat, 352.6 litres O_2 used would have oxidised 352.6×0.375 g carbohydrates and 352.6×0.350 g of fat.

Therefore, the quantities of nutrients oxidised and energy produced in 24 hours are as follows:

		Energy (KCal)
Protein	$=50g \times 4.1$	205.0
Carbohydrates	$=132.1g \times 4.1$	543.3
Fat	$=123.4g \times 9.3$	1147.6
	Total energy (KCal)	1895.9

Specific dynamic action of food: Rubner observed that carbohydrates, fats and proteins fed to a fasting dog, stimulated the energy metabolism over the basal level to varying extents. For example, he found that in a fasting dog requiring 400 KCal, feeding of 100 g of carbohydrates produces 425 KCal, 44.4 g of fat produces 416 KCal and 100 g of protein produces 520 KCal of heat. (Table 8.4). The extra heat produced is obtained by the oxidation of the tissue constituents and the animal will be in negative energy balance. This stimulating effect of carbohydrates, fats and proteins on energy metabolism is called

Table: **8.4 Energy output in a fasting dog fed carbohydrates, fats, proteins and their blends** (SDA of food)

Sl. No.	Nature of Food	Energy value of Food (KCal)	Energy output (KCal)	Extra energy produced due to SDA of Food (KCal)	(%)
1.	Fasting	—	400	—	—
2.	+ 100g of carbohydrates	400	425	25	6.2
3.	+ 44.4g of fat	400	416	16	4.0
4.	+ 100g of protein (casein)	400	520	120	30.0
5.	+ carbohydrate 62.5g + fat 10g+protein 10g	400	432	32	8.0

Specific Dynamic Action (S.D.A.) The S.D.A. of proteins is the highest (about 30 per cent) while that of carbohydrates and fats is only 6 per cent and 4 per cent respectively. The S.D.A. of a mixed diet containing 62.5 g carbohydrates, 10 g fat and 10 g protein is about 8 per cent.

A considerable amount of work has been carried out by Lusk and several other workers on the causes of the high S.D.A. of proteins. When different amino acids were fed, alanine, glycine and phenylalaline were found to produce high S.D.A. According to Krebs, two main factors are responsible for the high S.D.A. of proteins. (1) The energy required for deamination of amino acids which again is derived by the oxidation of other metabolites and (2) The energy required for the synthesis of urea which is obtained by the oxidation of other metabolites present in the tissues.

DETERMINATION OF ENERGY METABOLISM BASED ON OXYGEN CONSUMPTION (INDIRECT METHOD)

Since the calorific value of one litre of oxygen consumed in the oxidation of nutrients has been well established and tables indicating the calorific value of one litre of oxygen for varying R. Q. are available (Table 8.3), the energy metabolism can be easily calculated if oxygen consumption and CO_2 output are known for a fixed period. Various types of instruments are available for determining energy metabolism at rest (Basal metabolism) and energy metabolism during work.

BASAL METABOLISM

Definition: The energy metabolism of a subject at complete physical and mental rest and having normal body temperature and in the post-absorptive state (i.e., 12 hours after the intake of last meal) is known as *Basal Metabolism.*

Determination of basal metabolism

Basal metabolism is usually determined using the apparatus of Benedict and Roth (Fig. 8.3). The apparatus is a closed circuit system in which the subject breathes in oxygen from a metal cylinder of about 6-litre capacity and CO_2 produced is absorbed by soda-lime present in the tower. The oxygen cylinder floats on water present in an outer tank. The subject wears a nose clip and breathes through a mouthpiece the oxygen present in the cylinder for a period of 6 minutes. The volume of O_2 used is recorded on a graph paper attached to a revolving drum by a pen attached to it. Since the subject is in the

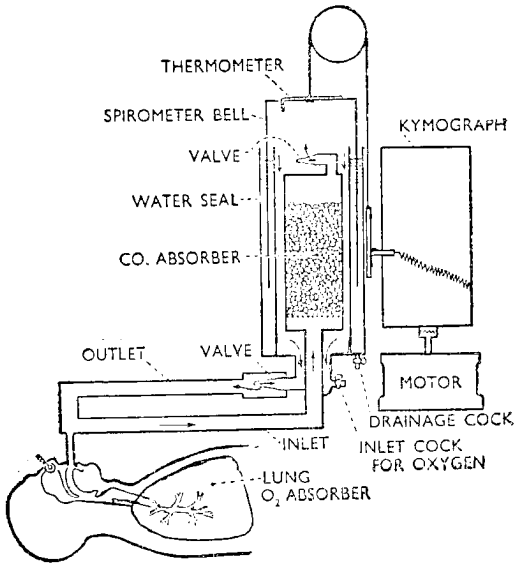


Fig. **8.3 Benedict-Roth Apparatus**

post-absorptive state, R.Q. is assumed to be 0.82 and the calorific value of one litre of O_2 consumed is taken as 4.8 KCal (Table 8.3).

Example: Subject: Adult male, 50 kg. body weight.

Oxygen consumed in 6 minutes=1,100 ml.

Heat produced in 6 minutes=$4.8 \times 1.1 = 5.28$ KCal

Heat produced in 24 hours=$\frac{5.2 \times 60 \times 24}{6} = 1,267$ KCal

The basal metabolism of the individual for 24 hours=1,267 KCal.

Standards for basal metabolism

Studies carried out by various workers have shown that basal metabolism is most closely related to the body surface area and less directly related to either to the weight or height of the individual. The body surface area can be calculated according to the formula of DuBois and DuBois given below:

$$A = W^{0.425} \times H^{0.725} + 71.84$$

where A=body surface area in square centimeters.

H=height in centimeters.

W=weight in kilograms.

$$\text{Log.}A = (\text{Log. } W \times 0.425) + (\text{Log. } H \times 0.725) + 1.8564.$$

The body surface area of human subjects for various heights and weights calculated according to the above formula is given in Table 8.5.

Table 8.5 Surface area in square meters for different heights and weights (DuBois-DuBois)

Height in centimeters	Weight in kilograms								
	40	45	50	55	60	65	70	75	80
180	—	—	—	—	1.77	1.83	1.89	1.95	2.00
175	—	—	—	1.67	1.73	1.79	1.85	1.91	1.96
170	—	—	1.57	1.63	1.69	1.75	1.81	1.86	1.91
165	—	—	1.54	1.60	1.66	1.72	1.78	1.83	1.88
160	—	1.44	1.50	1.56	1.62	1.68	1.73	1.78	—
155	1.33	1.40	1.46	1.52	1.58	1.64	1.69	—	—
150	1.30	1.36	1.42	1.48	1.54	1.60	1.65	—	—
145	1.27	1.33	1.39	1.45	1.51	1.56	—	—	—
140	1.24	1.30	1.36	1.42	1.47	—	—	—	—

The important standards for basal metabolic rates i.e., basal metabolism per square meter body surface per hour are those of (*i*) Harris and Benedict (1919); (*ii*) Aub and DuBois (1935); (*iii*) Fleisch (1951); (*iv*) Robertson and Reid (1952); (*v*) Boothby (1952), and (*vi*) Boothby, Berkson and Dunn (1936). The standards of Fleisch (1951) are given in Table 8.6.

Table **8.6 Standards for basal metabolic rates (KCal./sq.m./hr.) (Fleisch, 1951)**

Age (years)	1	3	5	7	9	11	13	15	17	19	20
Male ...	53.0	51.3	49.3	47.3	45.2	43.0	42.3	41.8	40.8	39.2	38.6
Female ...	53.0	51.2	48.4	45.4	42.8	42.0	40.3	37.9	36.3	35.5	35.3

Age (years)	25	30	35	40	45	50	55	60	65	70	75	80
Male	37.5	36.8	36.5	36.3	36.2	35.8	35.4	34.9	34.4	33.8	33.2	33.0
Female	35.2	35.1	35.0	34.9	34.5	33.9	33.3	32.7	32.2	31.7	31.3	30.9

Factors affecting the basal metabolic rate (B.M.R.)

The factors affecting the basal metabolism are briefly discussed below:

Body size: The B.M.R. is closely related to the body surface area. This has been shown from experiments with human subjects and also in different animals (Table 8.7). Basal metabolism is less directly related either to the height or weight of the individuals.

Table **8.7 Basal metabolism in relation to body weight and body surface area in men and some animals**

Species	Body weight Kg.	Body surface area (m^2)	Basal metabolism (KCal./day)		
			Total	Per m^2 body surface	Per kg. body wt.
Rat	0.300	0.0384	26.3	686	8.77
Monkey, rhesus	3.22	0.257	156	608	48.5
Dog	14.9	0.652	542	831	36.4
Man	65.0	1.83	1697	910	25.6
Woman	57.2	1.63	1347	828	23.6
Pig	186	2.67	2647	993	14.2
Cattle	366	4.56	5678	1245	15.6

Age: The B.M.R. is higher in infants and young children than in adults (Table 8.6).

Sex: Females have slightly lower B.M.R. than males (Table 8.6).

Body composition: The B.M.R. is directly related to the lean body mass. Persons with well-developed muscles will have a higher B.M.R. than obese persons whose higher body weight is due to adipose tissue.

Climate: In persons living in tropical climates, the B.M.R. is about 10 per cent less than those living in temperate zones. The exact causes for the lower B.M.R. are not known.

S.D.A. of food: Food has a stimulating effect on B.M.R. If a person in post-absorptive state is given food, the B.M.R. has been found to increase by about 8 per cent. This is known as the Specific Dynamic Action of food and has been discussed in an earlier section.

Undernutrition and starvation: Prolonged undernutrition or starvation causes a reduction of about 10-20 per cent in the B.M.R.

Sleep: The B.M.R. in sleep is about 5 per cent less than in the basal metabolic state.

Fever: Fever increases the B.M.R. For every 1°F rise in body temperature, B.M.R. increases by about 7 per cent. A person with high fever of 105°F (about 7°F above normal) would have an increase of 50 per cent in the B.M.R.

Physical activities: If an individual takes physical exercise about half-an-hour before the determination of B.M.R., appreciable increase in the B.M.R. is observed.

Fear and nervous tension: Fear and nervous tension during the test increase the B.M.R.

Thyroid: Hypothyroidism decreases B.M.R. upto 30 per cent and hyperthyroidism may cause an increase in B.M.R. upto 100 per cent depending on the severity of the condition.

Adrenaline: Injection of 1 mg. of adrenaline increases the B.M.R. by about 20 per cent for a few hours.

Anterior pituitary: The anterior pituitary influences B.M.R. through its thyrotropic hormone, the B.M.R. being low in hypo-activity and high in hyper-activity of the glands.

Other diseased conditions: An increase in B.M.R. has been observed in splenomedullary and lymphatic leukemia.

Determination of energy metabolism during work

The energy metabolism is profoundly influenced by physical work. The energy metabolism during work can be determined using one of the following equipments: (1) Douglas bag. (2) Max-Planck respirometer. (3) Kofranyi-Michaelis respirometer and (4) Integrating motor pneumotachygraph developed by Wolff. The general principles underlying the above methods are as follows:

(1) measuring the volume of expired air during work for fixed periods of 5 to 10 minutes (2) collection of a sample of expired air for the analysis of O_2 and CO_2 contents (3) calculation of O_2 consumption, CO_2 output and R.Q. and (4) calculation of the energy output from the R.Q. and O_2 consumption.

Douglas bag: The Douglas bag is made of rubber and usually of 100 litres capacity. The subject breathes into the bag for 5 to 6 minutes. The air in the bag is then measured using as gas meter, and a sample taken for the analysis of O_2 and CO_2. The apparatus is suitable for use in the laboratory but not in the field.

Max-Planck respirometer: This instrument is portable and can measure directly the volume of expired air and pass on simultaneously a small quantity into a rubber bladder attached to it. (Fig 8.4). The air is subsequently analysed for CO_2 and oxygen. It has been extensively used in the determination of the energy requirements for various types of work.

Kofranyi-Michaelis respirometer: This is similar in principle to the Max-Planck respirometer and has been commonly used in studies on energy requirements of workers.

Integrating motor pneumotachygraph: This instrument has been designed by Wolff (1958). It is not used so commonly as other instruments mentioned above.

Energy requirements for various types of activities

The energy requirements of various types of physical activities in adult men and women have been determined by several workers. The results of studies carried out in Sweden by

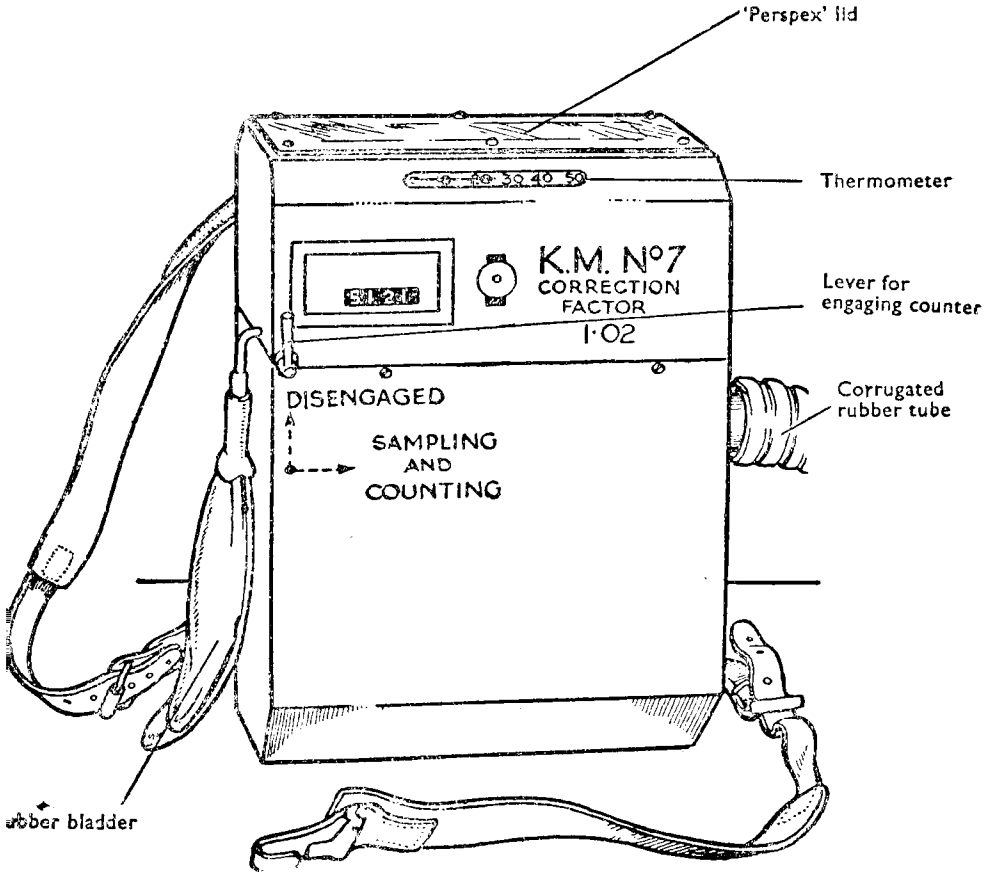


Fig. **8.4 The Max—Planck Respirometer**

Christensen (1953) on workers in steel industry are given in Table 8.8. Data reported by Durnin and Passmore (1959) are given in Table 8.9. It is evident from the tables that the energy required for light work is only 150 KCal/hour and may increase to 750 KCal/hour for very heavy work for men of body weight of 65 kg.

Influence of varying body weights on energy requirements for work: The energy required for walking at a particular speed will depend on the body weight of the individual (Table 8.10). The results presented in Table 8.10 indicate that for walking at the rate of 2 miles per hour, an individual with body weight of 200 lbs. will

Table 8.8 **Mean calorie requirements for various types of activities for adult man**

Grade of work	Calorie requirements (KCal/hr)	
	Mean	Range
Sleep	60	—
Basal metabolism	65	—
Sitting at rest	75	—
Standing	85	—
Very light work	120	100-140
Light work	225	150–290
Moderate work	375	300–440
Heavy work	525	450–590
Very heavy work	675	600–750

Table 8.9: **Energy expenditure in relation to intensity of muscular work (Durnin and Passmore, 1955)**

I. Light work—150-294 KCal/hour:
Light industry, carpentry, military drill, domestic work, gymnastic exercises, brick laying, plastering, painting, agricultural work (mechanised) and driving a truck.

II. Moderate work—300–444 KCal/hour:
Agricultural work (non-mechanised); route march; tennis; cycling (6 miles per hour).

III. Heavy work—450-594 KCal/hour:
Coal mining, football, hockey.

IV. Very heavy work—over 600 KCal/hour:
Lumber work, running, swimming, hill climbing.

require twice as much energy as an individual weighing 80 lbs and one-half times as much energy as an individual weighing 120 lbs. These results are of great importance in calculating the energy requirements of persons of varying body weights.

Table 8.10 **Influence of body weight on energy requirements for walking (Passmore and Durnin, 1953)**

Walking speed (miles /hour)	Body weight of individuals (lb.)						
	80	100	120	140	160	180	200
	KCal/hour						
2.0	114	132	156	174	192	210	228
3.0	162	186	216	240	264	288	318
4.0	210	246	282	312	348	384	420

The activity increment—Relation between basal metabolism and total energy requirements: The basal metabolism is related directly to the body surface area and approximately to body weight. The data presented in Table 8.10 indicate that the energy required for work (walking) is related to the body weight of the individual. The extra energy required for work, over the basal metabolism (expressed as a per cent of the basal metabolism) has been termed 'activity increment' by Mitchell (1962).

Children: Bedele (1923) determined the daily calorie requirements of children of 1-18 years living in a boarding home. The activity increment, i.e., extra energy required over basal metabolism (expressed as a percentage of basal metabolism) was 106-107 per cent. Maroney and Johnston (1937) found average extra calorie intakes for work over basal metabolism (activity increment) of 67 per cent for girls (aged 5-14 years) and 74 per cent for boys of same age-group (Table 8.11).

Adults: Bricker and co-workers (1945) found the extra energy required for work over basal metabolism (activity increment) was 100 per cent for young women of 19-23 years. Passmore and co-workers (1952) reported that extra calorie requirement over basal metabolism for very light work was 73 per cent and for very hard work, 195 per cent for young men aged 19-25 years (Table 8.11).

Table **8.11 Calorie requirements expressed as percentage of basal metabolism**

Age group (years)	Sex	Activity	Total calorie requirements as % of B.M.	Activity increment %	Reference
1–18	Boys	Light	207	107	Bedale (1923)
,,	Girls	,,	206	106	,,
19–22	Young men	Very light	173	73	Passmore *et al.* (1952)
,,	,,	Hard physical work	295	195	,,
19–23	Young women	Light	200	100	Bricker *et al.* (1945)
5–14	Boys	Light	174	74	Maroney and Johnston (1937)
5–14	Girls	,,	167	67	,,

Factorial method for calculation of the daily energy requirements of an adult for varying degrees of physical activity

The daily calorie requirements of an adult man (65 kgs.) for varying degrees of physical activity (1) sedentary. (2) light work, (3) moderate work and (4) heavy work can be calculated from the data given in Table 8.5. To this, 8 per cent extra calories are added to meet the needs of S.D.A. of food. The total calorie requirements have been expressed as a per cent of basal metabolism (Table 8.12). The results indicate that 'activity increment' for the various types of physical activities are as follows: sedentary 53 per cent, light work 100 per cent, moderate work 160 per cent and heavy work 220 per cent.

Table 8.12: **Energy expenditure* per day (KCal) for various types of activities (adult male 65 kgs.—25 years)**

	Sedentary work	Light work	Moderate work	Heavy work
Sleep 8 hours	480	480	480	480
Work 6 hours	720	1350	2250	3150
Recreation 4 hours	480	480	480	480
Walking 1 hour	225	225	225	225
Household work and gardening—4 hours	480	480	480	480
	2385	3015	3915	4815
SDA of food (8%)	190	240	312	384
Total calorie requirements (KCal)	2575	3255	4227	5199
,, ,, ,, as per cent of basal metabolism	153	200	260	320
Calorie requirements for work over basal metabolism (KCal)	950	1630	2602	3574
,, as per cent of basal metabolism (KCal) (Activity increment)	53	100	160	220

* Basal metabolism = 1,625 KCal.

Recommended allowances for calories

Recommended allowances for calorie requirements have been made by various Expert Committees from time to time. Some of these are (1) F.A.O. Second Committee on Calorie requirements (1957), (2) Food and Nutrition Board, N.R.C., U.S.A. (1968), (3) Expert Panel, U.K. Department of Health and Social Security (1969), and (4) Nutrition Expert Group, I.C.M.R., India (1968) (See Chapter 16).

F.A.O. Second Committee Allowances

The Committee was of the opinion that there could be no single calorie allowances which will be applicable to adult men and women of varying ages, body size, levels of activity, climate etc., all of which influence energy requirements. In view of the above,

the Committee recommended calorie allowances for a 'Reference Man' and 'Reference Woman' during light work as indicated below:

	Age (years)	Environmental (temperature)	Body Wt. (kg)	Nature of work	KCal/ day
Reference Man	25	10°C	65	light	3,200
Reference Woman	25	10°C	55	light	2,300

The basis of the calculation of energy requirements of 'Reference Man' and 'Reference Woman' is given in Tables 8.13 and 8.14 respectively.

Table **8.13 The energy expenditure of a 'Reference Man' (Weight 65 kg. Age 25 years. Mean annual environmental temperature, 10°C.)**

Type of activity		KCal/day
A. 8 hrs. Working activities : mostly standing (overall rate, 2.5 KCal./min.)		1200
B. 8 hrs. Non-occupational activities:		
1 hr. washing, dressing, etc. at 3 KCal./min.		180
1½ hrs. walking at about 6 Km/hr. at 5.3 KCal./min.		480
4 hrs. sitting activities at 1.54 KCal./min.		370
1½ hrs. active recreations and/ or domestic work at 5.2 KCal./min.		470
C. 8 hrs. rest in bed at basal metabolic rate		500
	Total	3,200

Table **8.14 The energy expenditure of a 'Reference Woman' (Weight 55 Kg. Age 25 years. Mean annual environmental temperature, 10°C.)**

	Type of activity	KCal/day
A.	8 hrs. Working activities in the home or in industry : mostly standing (overall rate 1.83 KCal./min.)	880
B.	Non-occupational activities:	
	1 hr. washing, dressing etc. at 2.5 KCal./min	150
	1 hr. walking at about 5 km./hr. at 3.6 KCal./min.	220
	5 hrs. sitting activities at 1.41 KCal./min.	420
	1 hr. active recreation and/or heavier domestic work at 3.5 KCal./min.	210
C.	8 hrs. rest in bed at basal metabolic rate.	420
	Total	2,300

The F.A.O. Committee considered the influence of the following on the calorie requirements: (1) physical activity (2) body size and weight (3) age and (4) climate and environment.

Physical activity: The F.A.O. Committee was of the opinion that the energy requirements of men of varying degrees of activity will range from 2,400 (sedentary) to 4,000 KCal (hard work). Similarly, the Committee was also of the opinion that the energy requirements of women of varying degrees of activity will range from 1,700 (sedentary) to 2,900 (hard work) calories per day. The above ranges of calorie allowances will meet the needs of the majority of the population according to the Committee. The calorie allowances of 'Reference Man' and 'Reference Woman', i.e., 3,200 and 2,300 KCal will represent the mean of the lowest and highest calorie allowances.

Body size and weight: The calorie needs will be influenced by body weight as the basal metabolism is closely related to the body weight and the food energy required for doing the same work for

a heavier person is more than that required for a lighter person (Table 8.10). The F.A.O. Committee suggested a formula for calculating the energy needs of individuals of varying body weights:

For men E=815+36.6 W
For women E=580+31.1 W
Where E=energy required, and W=body weight.

Example: The energy needs of a man of body weight of 55 kgs ('Indian Reference Man') and woman of body weight of 45 kgs ('Indian Reference Woman') will be as follows:

Indian Reference Man E=815+36.6×55=2,830 KCal.
Indian Reference Woman E=580+31.1×45=1,980 KCal.

These values agree well with those given for 'Indian Reference Man' and 'Indian Reference Woman' in Tables 8.17 and 8.18.

Age: As physical activities decline with advancing age, calorie requirements also correspondingly become less. The calorie allowances of adults of varying ages are given in Table 8.15.

Table **8.15 Effect of age on calorie requirements**

Age (years) (Ref. man or woman)	Man (65 kg)	Woman (55 kg)	Percentage of requirement of Ref. man or woman
25	3200	2300	100
30-39	3104	2230	97
40-49	3010	2160	94
50-59	2770	1990	86
60-69	2530	1820	79
Over 70	2210	1590	69

Climate: The energy requirements for 'Reference man' and 'Reference woman' are given for a mean working temperature of 10°C as in Western countries. The F.A.O. Committee recommended that for every 10°C rise of external temperature above the reference temperature of 10°C, a reduction of 5 per cent, and for every 10°C fall of external temperature below reference temperature, an increase of 3 per cent in energy output should be made.

Example	Environmental temperature	Increase or decrease (%)	KCal/day
Male, 65 kg. (Ref. Man)	10°C	—	3200
	20°C	— 5%	3040
	30°C	—10%	2880
	0°C	+ 3%	3296

Hence, for individuals living in tropical countries such as India, a reduction of 10 per cent in the calorie requirements will have to be made in the F.A.O. Committee recommendations.

Infants and children: The calorie allowances for infants and children of the F.A.O. Committee are given in Table 8.16. The allowances for children are for those of standard weight. Adjustments will have to be made for variations in body weight as in the case of adults.

Table **8.16: Calorie allowances for infants and children (FAO Committee, 1957)**

Age-group		KCal/day
Infants:	1–3 months	120 KCal/kg.
	4–9 ,,	110 ,, ,,
	10–12 ,,	100 ,, ,,
Children:	1–3 years	1300
	4–6 ,,	1700
	7–9 ,,	2100
	10–12 ,,	2500
Adolescents:	13–15 Boys	3100
	,, Girls	2600
	16–19 Boys	3600
	,, Girls	2400

Pregnancy and lactation: The F.A.O. Committee recommended an allowance of 40,000 KCal for the entire period of pregnancy. Since the maximum increase in the weight of the foetus occurs during the last 4 months of pregnancy, an extra daily allowance of 300 KCal per day for the last 4 months of pregnancy will meet the extra calorie needs. The average milk production

during the first 6 months of lactation will be about 850 ml. with a calorific value of 600 KCal. To provide 600 KCal in the form of human milk, about 1,000 KCal will have to be provided in the diet. Hence, the Committee recommended an extra allowance of 1,000 KCal per day during the first 6 months of lactation.

Recommended allowances of Food and Nutrition Board, U.S.A.

The recommended calorie allowances of Food and Nutrition Board, N.R.C., U.S.A. (1968) are given in Table 16.2 in Chapter 16. They agree fairly well with the F.A.O. Committee recommendations.

Recommended allowances of Expert Panel, U.K.

The recommended allowances of Expert Panel, U.K. (1969) are given in Table 16.3 in Chapter 16. They differ slightly in some respects from the F.A.O. Committee recommendations.

Recommended calorie allowances for persons in tropical countries

Recommended allowances for Indians were suggested by Patwardhan (1960). Like the F.A.O. Committee, Patwardhan recommended allowances for 'Indian Reference Man' and 'Indian Reference Woman' which are given below:

	Body wt. (kg)	Height (cm)	Mean environmental temp.	Age (yrs)	Nature of work	KCal/day
Indian Ref. Man	55	168	20°C	25	Moderate activity	2780
Indian Ref. Woman	45	154	20°C	22	,, ,,	2080

The basis of calculation of energy allowances for 'Indian Reference Man' and 'Indian Reference Woman' is given in Tables 8.17 and 8.18.

Table 8.17 **Calorie requirements of an Indian Reference Man**

A.	8 hours in bed at B.M. R. = 35.5 × 1.62 × 8 ...		460
B.	8 ,, off work:		
	1 hour personal necessities, such as dressing, undressing, washing, shaving, bathing etc. at 3.0 Cal./kg./hr. ...	165	
	2 hours walking at 3 m.p.h. at 4.0 Cal/kg/hr.	440	
	4 hours sitting and standing activities at 1.7 Cal/kg/hr.	374	
	1 hour recreation at 4.4 Cal/kg/hr.	241	
			1220
C_1.	8 hours sedentary occupation overall rate at 1.7 Cal/kg/hr.		750
C_2.	8 hours light or industrial work overall rate at 2.5 Cal/kg/hr.		1000
C_3.	8 hours heavy work overall rate 5 Cal/kg/hr.		2200
	$A+B+C_1$ = total net calories for light and sedentary work		2430
	$A+B+C_2$ = ,, ,, ,, ,, ,, industrial work (moderate activity of an Indian Reference Man)		2780
	$A+B+C_3$ = total net calories for heavy work		3880

Table 8.18 **Calorie requirements of an Indian Reference Woman**

A.	8 hours in bed at B.M.R. = 31.6 × 1.40 × 8		354
B.	8 hours off work:		
	1 hour personal necessities 3.0 Cal/kg/hr.	135	
	1½ hours walking at 3 m.p.h. at 4.0 Cal/kg/hr.	270	
	5½ hours sitting and standing activity 1.7 Cal/kg/hr.	421	
			826
C_1.	8 hours sedentary occupation overall rate 1.7 Cal/kg/hr.		610
C_2.	8 hours light industrial work overall rate 2.5 Cal/kg/hr.		900
C_3.	8 hours heavy work overall rate 5.0 Cal/kg/hr.		1800
	$A+B+C_1$ = total net calories for sedentary work		1790
	$A+B+C_2$ = ,, ,, ,, ,, light industrial occupation (moderate activity of an Indian Reference Woman)		2060
	$A+B+C_3$ = total net calories for heavy work		2880

Table 8.19 **Recommended calorie allowances for Indians**

Particulars	Patwardhan (1958)	Nutrition Expert Group, ICMR (1968)
Man (55 kg) :		
Sedentary work	2,400	2,400
Moderate work	2,800	2,800
Heavy work	3,900	3,900
Woman (45 kg) :		
Sedentary work	2,000	1,900
Moderate work	2,300	2,200
Heavy work	3,000	3,000
Latter half of pregnancy	2,300	+300
Lactation	2,700	+700
Infants:		
Under 1 year: 0-6 months	120/kg.	120/kg.
7-12 ,,	100/kg.	100/kg.
Children:		
1-3 years	1200	1200
4-6 ,,	1500	1500
7-9 ,,	1800	1800
10-12 ,,	2100	2100
13-15 ,, (Boys)	2500	2500
(Girls)	2100	2300
16-19 ,, (Boys)	3150	3000
(Girls)	2100	2200

The Nutrition Expert Group of the I.C.M.R. (1968) adopted the recommendations of Patwardhan with slight modifications. The recommended calorie allowances of Patwardhan (1960) and Nutrition Expert Group of the I.C.M.R. (1968) are given in Table 8.19 and are briefly discussed below.

Adults: The I.C.M.R. Expert Group allowances are about 15-20 per cent less than the F.A.O. Committee allowances. This is due to the lower body weight of Indians and the tropical climate prevalent in India.

Children: The I.C.M.R. Expert Group allowances are about 10-20 per cent less than the F.A.O. Committee allowances. This again is due to the lower body weight of Indian children and the tropical climate in India.

Pregnancy and lactation: The I.C.M.R. Expert Group additional allowances during pregnancy (latter half) and lactation are 300 and 700 KCal. These compare favourably with the F.A.O. Committee allowances.

Infants: The I.C.M.R. Group allowances of 120 KCal and 100 KCal per kg. for the age-group of 0-6 months and 7-12 months are the same as the F.A.O. Committee allowances.

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CHAPTER 9

VITAMINS

During the nineteenth century, scientists found that the main constituents present in different foods were carbohydrates, fats, proteins, minerals and water. At the beginning of the 20th century, feeding experiments were conducted on mice by some physiologists with synthetic diets based on blends of pure carbohydrates, proteins, fats and mineral salts. The results showed that animals would not grow on such synthetic diets. It was evident that natural foods contained certain unknown substances which are essential for the growth of animals. These were called 'accessory food factors' by Hopkins (1912) and 'Vitamine' by Funk in 1912. Other lines of investigations which helped to focus attention of scientists regarding the existence of vitamins in foods were (1) cure of scurvy, beriberi and rickets in human beings by changes in the diet and (2) production of scurvy, beriberi (polyneuritis) and rickets in experimental animals by feeding on faulty diets. A brief account of the above developments leading to the discovery of the different vitamins is given below and presented in Table 9.1.

Cure of scurvy, beriberi and rickets in human beings

During the sixteenth century, there were several records of successful treatment of scurvy in sailors by means of lemons and oranges. The most important of these are (1) The observations of Jacques Cartier in 1535 that scurvy could be cured by administering a decoction of pine needles (2) The report of Rousseus (1564) that citrus fruits prevented and cured scurvy in Dutch seamen and (3) The observations of Capt. James Lancaster in 1591 that scurvy could be prevented by lemon juice. All these investigations were summarised by Capt. James Lind in his famous book 'Treatise of the scurvy' published in 1753. Lind pointed out in his book from the available evidence that scurvy was caused by consuming stored, salted or dry foodstuffs and the disease could be prevented or cured by the consumption of fresh vegetables, fruits and herbs.

Table 9.1 **Discovery of Vitamins**

Year	Investigator	Nature of discovery
I. *Cure of scurvy, beriberi and rickets in man by certain foods:*		
1601	Lancaster	Cured scurvy in mariners by oranges and lemons
1882	Takaki	Prevented the occurrence of beriberi among Japanese sailors by replacing rice by wheat bread and adding milk and vegetable.
1865	Trousseau	Cured rickets in children by means of cod liver oil.
II. *Production of deficiency diseases in experimental animals by feeding on faulty diets:*		
1838	Guerin	Produced rickets in puppies by feeding on diets free from animal fat.
1890–97	Eijkman	Produced experimental beriberi (Polyneuritis) in fowls by feeding them on washed polished rice.
1901	Grijns	Produced polyneuritis in fowls by feeding on polished rice diet and postulated that polished rice lacked some accessory food factor.
1907-12	Holst and Frolich	Produced scurvy in guinea pigs by feeding them on cereal diets.
III. *Feeding of animals on purified diets:*		
1881	Lunin	Diets based on starch, protein, fat and minerals inadequate for promoting growth of mice
1905	Pekelharing	Small amounts of milk added to purified diets suffice to promote good growth.
1909	Stepp	Bread and milk powder extracted with alcohol and ether not adequate for promoting growth of rats.
1912	Hopkins	Convincing quantitative evidence for the presence of accessory food factors in milk and the beneficial effects of small amounts of milk in promoting good growth of rats on purified diets.
1912	Funk	Propounded the 'Vitamine' theory,
1915	McCollum	Evidence for the presence of at least two types of accessory food factors (1) fat soluble and (2) water soluble.

In 1882, Takaki, a Japanese naval surgeon, showed that beriberi could be prevented by replacing polished rice in the diet by wheat bread, vegetables and milk. In 1907, Braddon pointed out that beriberi occurred among the Chinese and others who ate white rice whereas Malays who ate homepounded rice and the Tamils who ate parboiled rice, were free from the disease. At about the same time, Fraser and Stanton (1909) and Fletcher (1907) showed that the bran, removed from rice during milling, contained some substances which will prevent or cure beriberi in human beings.

Rickets was recognised as a definite disease by medical writers in the last half of the seventeenth century. In 1782, Robert Darley wrote to Times Percival an account of his highly successful use of cod liver oil in the treatment of rickets. In 1865, Trousseau was teaching that cod liver oil was the well known and perfect cure for rickets. In 1889, Cheadle stated in his book 'Artificial feeding of infants' that 'rickets is produced as certainly by a rachitic diet as scurvy is by a scorbutic diet'.

Production of beriberi (polyneuritis), scurvy and rickets in experimental animals: Guerin (1838) produced rickets in puppies by feeding them on diets devoid of animal fats to support his theory that rickets was caused by faulty diet. In 1890 Eijkman, in the Dutch East Indies, made the important discovery of experimental beriberi (polyneuritis) in fowls by feeding them on polished rice diet. Eijkman's collaborator, Grijns stated in 1901 that beriberi was due solely to a dietary deficiency and was not caused by any positive agent or toxin. In 1907, Holst and Frolich in Norway produced experimental scurvy in guinea-pigs by feeding them on a cereal diet.

Feeding experiments with animals using synthetic diets: During the later part of the 19th century, feeding experiments on mice were carried out by some physiologists using purified diets. Lunin (1881) showed that animals failed to thrive when fed on a 'synthetic' diet comprising of the then known constituents of food i.e. protein, carbohydrate, fat, mineral salts and water. He concluded that 'a natural food such as milk must therefore contain besides these known principal ingredients small quantities of other and unknown substances essential to life'. Similar conclusions were reported later by certain other workers, notably Socin (1891). Stepp (1909) reported that the unknown substances present in

natural foods must be effective in very minute amounts for he had observed that small supplements of natural foods added to the artificial synthetic ration were sufficient to afford protection. A more detailed and thorough investigation by the great English Biochemist F. G. Hopkins (1912) showed that (1) albino rats fed on a 'synthetic' diet containing purified protein, fat, carbohydrate, mineral salts and water failed to grow and (2) that addition of very small quantities of milk to the diet rendered the diet adequate and promoted good growth of rats.

The concept of vitamins: Hopkins (1912) called the substances occurring in natural foods and essential for growth as 'accessory food factors'. In 1912, Casimir Funk, a Polish Biochemist, who was working on the anti-beriberi factor coined the word 'Vitamine' for this factor and propounded the famous 'Vitamine' theory. According to Funk, natural foods contained distinct anti-beriberi, anti-scurvy, anti-rickets and anti-pellagra 'vitamines'.

Differentiation of fat soluble and water soluble vitamins: Convincing evidence indicating the presence of more than one vitamin was given in 1915 by McCollum and Davis from America. They reported that two accessory food factors were needed to promote growth of rats on synthetic diets (1) one, soluble in fats and found in butter and (2) the other, soluble in water and found in the whey of milk. These were named fat-soluble 'A' and water soluble 'B' respectively. At the suggestion of Drummond (1920) 'water-soluble B' was renamed as 'vitamin B', and the terminal 'e' of the word 'vitamine' was omitted. Similarly, 'fat soluble A' was renamed 'vitamin—A'. Since the anti-scurvy factor had different chemical properties from either 'vitamin B' or 'vitamin A', it was named 'vitamin C'.

Classification of vitamins

Vitamins may be defined as organic compounds occurring in small quantities in the different natural foods and necessary for the growth and maintenance of good health in human beings and certain experimental animals. If vitamins are not present in sufficient quantities in the diet, vitamin deficiency diseases occur. So far about 15 different vitamins have been isolated in a pure state from natural foods. Almost all of them are essential for human beings. Vitamins may be classified broadly into two groups:

(*a*) fat soluble vitamins i.e. vitamins soluble in fats and fat solvents but insoluble in water and (*b*) water soluble vitamins i.e. vitamins soluble in water but insoluble in fats or fat solvents.

(*a*) *Fat soluble vitamins:*

(1) Vitamin A and Carotene (Provitamin A)
(2) Vitamin D
Vitamin D_2 (Calciferol, artificial vitamin D) and
Vitamin D_3 (Irradiated dehydrocholesterol, natural vitamin D)
(3) Vitamin E.
and (4) Vitamin K.

(*b*) *Water soluble vitamins*

I. *Vitamin B complex*
1. Vitamin B_1 (thiamin, aneurin)
2. Riboflavin
3. Nicotinic acid and Nicotinamide (Niacin and Niacinamide)
4. Pyridoxin (vitamin B_6)
5. Pantothenic acid
6. Folic acid
7. Biotin
8. Choline
9. p-amino benzoic acid
10. Inositol and
11. Vitamin B_{12}

II. Vitamin C (ascorbic acid or Civitamic acid)

III. Vitamin P. (Bioflavanoids)

I. FAT SOLUBLE VITAMINS

Vitamin A

Vitamin A occurs only in foods of animal origin. Vitamin A activity is also possessed by carotenoids found in plants. Hence carotenoids are called provitamin A.

History

McCollum and Davis (1917) and Osborne and Mendel (1913) showed that a fat soluble factor present in butter was essential for the growth of rats on synthetic diet. The former

group of workers called the factor as 'fat soluble A'. Steenbock (1919) discovered the vitamin A activity of carotenoids. Later he (1920) found that vitamin A was present in the unsaponifiable fraction of fish liver oils. Moore (1930) made the important discovery that when carotene was fed to vitamin A deficient rats, vitamin A was found in the liver. In 1931, Karrer obtained a highly concentrated vitamin A preparation and determined the structure of the vitamin. In 1937, Kuhn and Morris announced a method of synthesis of vitamin A. Holmes and Corbet (1937) obtained vitamin A in a crystalline form.

Chemistry and Properties of Vitamin A

The chemical structure of Vitamin A is given in Fig 9.1

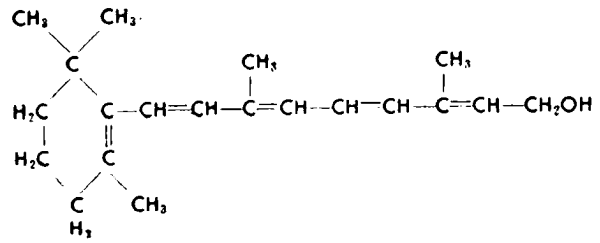


Fig. 9.1 Vitamin A

Vitamin A contains a β-ionone ring. The side chain contains two isoprene units, 4 double bonds and one alcoholic group. Due to the presence of 4 double bonds, vitamin A is destroyed easily by oxidation. Due to the presence of alcoholic group, it forms esters such as palmitate, acetate, succinate etc. The oxidised products of vitamin A have no vitamin A activity. Vitamin A is stable to heat (100°C) for short periods in the absence of oxygen but in the presence of oxygen it is slowly destroyed. Vitamin A is slowly destroyed when its solutions are exposed to light. It is for these reasons pharmaceutical preparations containing vitamin A are packed in brown bottles.

Vitamin A_2: While vitamin A occurs in the livers of marine fish, egg and butter, vitamin A_2 is present in the livers of fresh water fish. Vitamin A_2 differs from vitamin A in having one more double bond in the β-ionone ring. The biological activity of vitamin A_2 in the rat is only about 30 per cent of that of vitamin A.

Vitamin A aldehyde (Retinene, Retinal): The classical studies of Wald (1935) and Morton (1944) have shown that vitamin A is oxidised to vitamin A aldehyde in the retina of eyes. The role of retinal (vitamin A aldehyde) in vision in dim light is discussed later.

Vitamin A acid (Retinoic acid): Retinoic acid (Vitamin A acid) was first synthesised by Arens and Van Dorp (1946) who showed that when given in oil it was only 10 per cent as active as retinol in supporting the growth of vitamin A deficient rats but when administered orally as the Na salt, its biopotency was equal to that of retinol. It does not support reproduction in female rats. It cannot be converted into retinol and hence does not take part in the visual cycle and does not prevent the eye lesions and blindness in animals fed on vitamin A deficient diet. Biological activities of vitamin A (retinol), vitamin A aldehyde, vitamin A_2 and vitamin A acid are given in Table 9.2.

Table **9.2 Biological activity of vitamin A and its derivatives for promoting growth of rats**

	Biological activity
Vitamin A alcohol (Retinol) ...	100
Vitamin A alcohol esters ...	100
Vitamin A aldehyde (Retinal) ...	100
Vitamin A_2 ...	30
Vitamin A acid ...	50

Absorption and storage of vitamin A: Vitamin A alcohol or esters are almost completely absorbed in normal human subjects. Vitamin A esters are hydrolysed in the lumen of the intestine to free vitamin A alcohol prior to its absorption through the mucosal membrane. Vitamin A alcohol (Retinol) is absorbed through the mucosal membrane and is esterified into vitamin A palmitate. Vitamin A palmitate passes via the lymphatic system to the blood stream and then to the liver. It is stored in the Kupffer cells of liver. When vitamin A is mobilised from the liver, the stored vitamin A ester is hydrolysed to free alcohol prior to its release in the blood stream and vitamin A alcohol is transported to the different tissues by the blood. Liver can store large amounts (2 to 3 lakhs I.U.) of vitamin A, when the subject is fed on diets rich in vitamin A.

Physiological and biochemical functions

The important functions of vitamin A are as follows:

Vitamin A and vision: Vitamin A plays a critical role in vision in dim light. The vitamin A visual cycle is shown in Fig. 9.2.

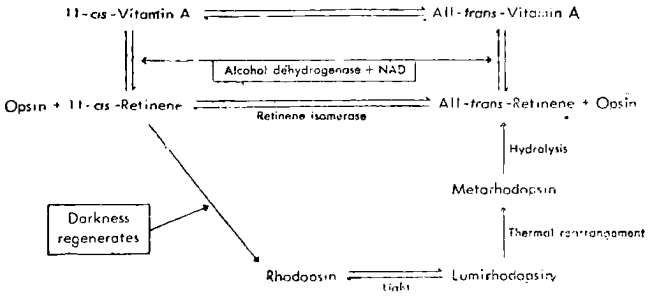


Fig. 9.2 Vitamin A visual cycle

According to Wald (1935) the characteristic pigments of the retinal rods and cones are rhodopsin and iodopsin. They differ only in respect of the protein moieties (called scotopsin and photopsin respectively). The specific pigment common to both is a cis-isomer of retinene (vitamin A aldehyde). Vitamin A alcohol is oxidised to vitamin A aldehyde in the epithelium of the rods by alcohol dehydrogenase in the presence of NAD. The protein opsin (scotopsin) reacts with retinene to form rhodopsin. Light initiates a series of photochemical changes in rhodopsin, beginning with bleaching of the purple pigment and ending with the formation of all-trans-retinene and its isomerisation. The resynthesis of rhodopsin is isomer specific, requiring the 11-cis-isomer of retinene. Morton and coworkers (1953) demonstrated the regeneration of rhodopsin with all-trans vitamin A alcohol, phosphate buffer, magnesium ions and excised rat retinas. It is evident that the retina is able to isomerise all-trans-retinene to cis-trans retinene.

Vitamin A and epithelial tissues: Vitamin A is essential for the integrity of the mucous secreting cells of epithelial tissues. In vitamin A deficiency, the epithelial tissues are keratinised. The tissues affected are salivary glands, respiratory tract, eyes, skin and sex organs.

Vitamin A and nerves: Mellanby (1926) made the important observation in experimental animals that vitamin A deficiency causes degeneration of the myeline sheath.

Vitamin A and bone: Mellanby (1934) found that vitamin A is essential for normal bone formation. Excess of vitamin A, however, is toxic and causes brittleness of bone and hence bone fractures.

Vitamin A and protein deficiency: The absorption and mobilisation of vitamin A is impaired in protein malnutrition. When protein is fed, vitamin A is mobilised from liver.

Mucopolysaccharides and sulphate metabolism: Vitamin A takes part in the incorporation of inorganic sulphate in mucopolysaccharides and in the synthesis of mucopolysaccharides.

Vitamin A and mucoprotein synthesis: Vitamin A is essential for the synthesis of mucoproteins and glycoproteins.

Vitamin A and reproduction: In vitamin A deficiency, reproduction does not take place through (*i*) infertility in the male and (2) failure of the female to conceive or resorption or abortion of the foetus if conceived.

Effects of vitamin A deficiency

The important deficiency states due to lack of vitamin A in the diet are (*i*) Night blindness (*ii*) Xerosis conjunctivae (*iii*) Xerosis cornea (*iv*) Bitot's spots and (*v*) Keratomalacia.

Night blindness: The role of vitamin A in dim vision has been discussed earlier. In early stages of vitamin A deficiency, the individual cannot see well in dim light. Difficulty in reading or driving the car in dim light is experienced. In advanced deficiency, the subject cannot see objects in dim light. Night blindness is fairly common in regions where the vitamin A intake is inadequate.

Xerosis conjunctivae: The conjunctiva is dry, thickened, wrinkled and pigmented. This is due to the keratinisation of the epithelial cells. The pigmentation gives the conjunctiva a smoky appearance. This condition is extremely common among all age groups in India and other developing countries where the vitamin A intake is low.

Xerosis cornea: When dryness spreads to cornea, it takes on a dull, hazy, lustreless appearance. This is due to the keratinisation of the epithelial tissue over the cornea.

Plate III: Bitot's Spot

Plate IV: Keratomalacia
(Late with necrosis of cornea)

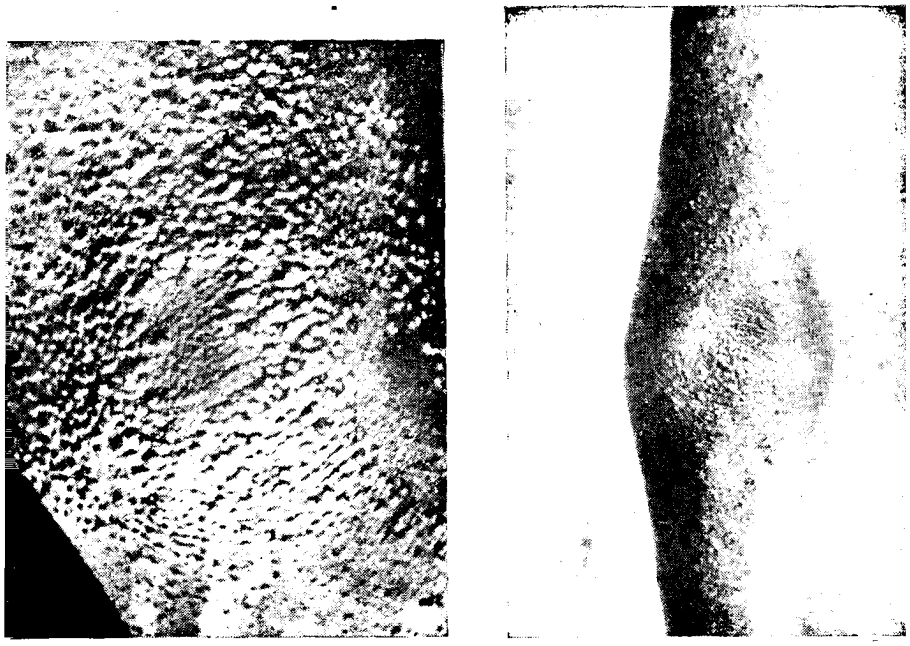


Plate V: Phrynoderma: The extensor aspects of the extremities are involved,. The horny summits of the keratinized follicles may be seen clearly in the magnified picture on the left.

Bitot's spots: Bitot described this condition in 1863. Greyish or glistening white plaques formed of desquamated thickened conjunctival epithelium, usually triangular in shape and firmly adhering to the conjunctiva are frequently found in children having other signs of vitamin A deficiency. (Plate III).

Keratomalacia: When xerosis of the conjunctivae and cornea is not treated it may develop into the condition known as 'keratomalacia'. Metaplasia and degeneration of the corneal epithelium occur, producing opacities and the cornea becomes vascularized, oedematous and infiltrated with leucocytes. Necrosis, leading to ulceration and bacterial invasion brings about the destruction of the cornea. Blindness often results from corneal scarring or perforation (Plate IV).

Follicular hyperkeratosis: (Phrynoderma) Wolbach and Howe (1925) showed that in the skin of vitamin A deficient animals, follicular keratosis developed. There is hyperkeratinisation of epithelium lining the follicle. The sebaceous glands are absent or present only in remnants. The sweat glands and their ducts show changes similar to those seen in pilosebaceous follicles. The skin becomes rough and dry and papules of varying sizes arising at the site of pilosebaceous follicles are observed. (Plate V). Earlier workers showed that this condition responds to treatment with vitamin A. More recent studies, however, indicate that the above condition can be cured by the administration of essential fatty acids and pyridoxin (Gopalan, 1947).

Vitamin A and urinary calculus: Studies with animals have shown that vitamin A deficiency predisposes the animal to the development of urinary calculus, due to degeneration of the epithelial cells of the urinary tract. There is no association, however, between the incidence of vitamin A deficiency and urinary stones in human beings.

Diagnosis of vitamin A deficiency in human subjects

Two methods are used for the diagnosis of vitamin A deficiency in human beings.

Dark adaptation test: In early stages of vitamin A deficiency, the individual cannot see objects in dim light. This principle is made use of in the dark adaptation test. The test is carried out in

one of the following two ways (*i*) In the first method, the subject is kept in a dark room for some time and asked to identify an object which is dimly illuminated, the intensity of light being increased till the subject is able to see the object (*ii*) In the second method, the visual purple is bleached by exposure to bright light for some time and the time required for the visual purple to be regenerated is measured by the ability of the subject in seeing a dimly illuminated object. Several instruments are available for this purpose. Wald's method of measuring 'final rod threshold' as modified by Dow and Steven (1941) has proved to be quite reliable. The studies carried out under the Medical Research Council, U.K. (1949) on experimental vitamin A deficiency in human subjects, showed that dark adaptation test is a sensitive measure of early vitamin A deficiency.

Serum vitamin A content: In vitamin A deficiency, serum levels of vitamin A are low. Studies carried out by Gopalan and co-workers (1960) and Pereira and co-workers (1966) in India have shown that serum vitamin A levels are reliable index of vitamin A deficiency in children. The levels of vitamin A in serum (I.U./100 ml) indicative of various degrees of deficiency, are as follows: (*i*) Normal children 80 I.U. and above (*ii*) children having xerophthalmia and Bitot's spots less than 45 I.U. and (*iii*) subclinical state between 45 and 80 I.U.

Methods of assay: The methods used for the assay of vitamin A are as follows: (*i*) The absorption spectrum of vitamin A in the ultra-violet shows a sharp band at the wave length 380 mμ. with an extinction coefficient of $E\ \frac{1\%}{1cm} = 1780$. Vitamin A can be estimated spectrophotometrically by this method. (*ii*) Vitamin A gives a blue colour with antimony trichloride in chloroform solution. The colour can be measured in a colorimeter or spectrophotometer at 620 mμ. and (*iii*) Biological methods of assay are based on the growth of rats fed on vitamin A deficient diet or cure of vitamin A deficiency in rats.

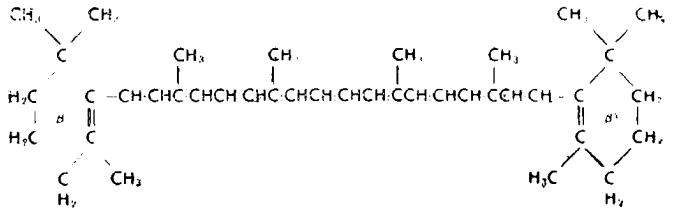
Carotenoids (Provitamin A)

Carotene was first isolated from carrot by Wackenroder in 1831. The first evidence regarding the vitamin A activity of carotenoids was produced by Steenbock (1919). Moore (1930) showed that

when large amounts of carotene are fed to vitamin A deficient rats, vitamin A is found in large amounts in liver, indicating the conversion of carotene to vitamin A in the animal body.

Chemistry and properties

A large number of carotenoid pigments occur in nature. The chemical structure of β-carotene is given in Fig. 9.3.



β-Carotene

Fig. 9.3

It contains 2 β-ionone rings designated as β_1 and β_2 bridged by a chain of 4 isoprene ($CH_2=\underset{\underset{CH}{|}}{C}-CH=CH_2$) units. α- and γ-carotenes differ from β-carotene in containing only one β-ionone ring. Some other carotenoids i.e. cryptoxanthin and xanthophyll differ from β-carotene in having hydroxyl group in one or both the β-ionone rings. At least one β-ionone ring similar to that present in β-carotene is essential for vitamin A activity. Lycopene (with broken β-ionone rings) or xanthophyll (having hydroxyl groups in the β-ionone rings) possess no vitamin A activity. The presence of conjugated double bonds in the carotenoid bridge makes possible a large number of cis-trans isomers. β-carotene has been widely used as a food colour. A related group of products, apocarotenals, have vitamin A activity. Of these β-apo-8'-carotenal is used as orange colour in foods.

Absorption and conversion into vitamin A

The absorption of carotenoids from the intestines varies considerably depending on whether the carotene is administered in the pure form or in foodstuffs and the quantity of fat present in the diet. β-carotene dissolved in oil is absorbed to the extent of

70-90 per cent. The carotenoids present in the vegetables are absorbed to a lower extent (25 to 50 per cent) depending on the quantity of fat in the diet and the extent to which the vegetables have been homogenised. Data regarding the percentage absorption of carotene by human subjects are given in Table 9.3. It must be realised that measurement of the absorption of carotene by the analysis of food and feces for their carotene contents is made difficult and uncertain by technical complications. The state of carotene in the diet and in the feces will be widely different. Some of the carotene may be destroyed by bacterial action. The results obtained are approximate and indicate the factors influencing the absorption of carotenes.

Table **9.3 Availability of carotene in human subjects from various sources**

No. of Subject	Source of Carotene	Intake mg/day	Absorption (percentage of intake)
Infants:			
3 Infants	Carotene in oil	0.4—2.6	55 (30-70)
1 Infant	Carrot meal	3.8	50
Children:			
4 children	Carotene in oil	8—15	52 (30-75)
1 child	Carrot puree	8	30
1 child	Spinach, cooked, minced	5	30
Adults:			
5 adults	Carotene in oil or fat	3.1—3.6	73 (69-82)
2 adults	Carrots, canned sliced	2.2	24 (16, 31)
4 adults	Carrots, cooked, puree	3.0	25 (6-36)
4 adults	,, ,, + fat	3.0	33 (0-60)
2 adults	Carrots, cooked, homogenized	3.0	56 (46, 63)
3 adults	Spinach, canned, puree	2.5	41 (28-62)
2 adults	Spinach, canned, homogenized	4.0	43 (35, 47)
3 adults	Cabbage, dried, outer leaves	1.9	41 (13-60)
2 adults	,, ,, ,,	3.8	28 (28, 27)

Conversion of carotene to vitamin A: Moore (1930) first demonstrated that when large quantities of β-carotene were fed to vitamin A deficient rats, large quantities of vitamin A were deposited in the liver. He suggested that the conversion of β-carotene

to vitamin A takes place in the liver. But attempts to convert carotene to vitamin A by incubation with liver slices yielded negative results. Deuel and co-workers (1947) administered carotene dissolved in oil intraperitonially or intravenously to vitamin A deficient rats and found carotene administered in the above manner did not promote growth and did not cause any increase in liver vitamin A. They suggested that carotene orally administered is probably converted into vitamin A in the intestinal wall. In later studies, they administered carotene (1800 μg) orally in oil in a single dose to vitamin A deficient rats and killed them after 3 hrs. for determination of vitamin A in the intestine and liver. The intestines contained a total of 13.5 μg. carotene and 40.3 I.U. of vitamin A whereas the liver contained only 1.6 μg. carotene and 22.8 I.U. of vitamin A. This experiment showed definitely that intestine is the site of conversion of carotene to vitamin A. These results were confirmed later by studies of Morton and co-workers (1948).

Biological activity of isomers of β-carotene: The biological activity of β-carotene and its isomers have been determined by feeding the isomers dissolved in oil to vitamin A deficient animals and observing the increase in the growth rates. The relative biological activity of different carotenoids is given in Table 9.4. In careful experiments 0.6 μg. of carotene dissolved in oil has been found to possess the biological potency of 0.3 μg of vitamin A (one International Unit).

Table **9.4 Relative biopotency of carotenoids**

β-Carotene (all-trans)	100
∝-Carotene (all-trans)	53
γ-Carotene (all-trans)	27, 45
Cryptoxanthin (all-trans)	57
Neo-β-Carotene U (mono-cis)	38
Neo-β-Carotene B (di-cis)	53
Lycopene	0
Xanthophyll	0
β-Apo-8'-Carotenal	68

Method of assay

β-Carotene occurs in leafy vegetables and carrots along with α and γ-carotenes, and other carotenoid pigments such as cryptoxanthin or lycopene. The procedure involves the extraction of the pigments by using suitable solvent mixtures (Phasic separation). Mixtures of hexane and acetone or hexane and methyl alcohol permit separation of carotenes from xanthophyll, chlorophyll and other pigments. The carotenes are separated chromatographically and estimated using a spectrophotometer at 436 mμ.

Dietary sources

Vitamin A is present only in fish liver oil and foods of animal origin, such as liver, eggs, butter milk and fatty fish. All plant foods contain only carotenoids. Vitamin A and carotene contents of foods are given in Table 1A in appendix. A summary of the data is given in Tables 9.5 and 9.6.

Table **9.5 Vitamin A (Retinol) content of foodstuffs**

Source	Vitamin A content μg/100g
Rich sources	
Halibut liver oil	600,000-1000,000
Cod liver oil	10,000-100,000
Cod liver oil B.P.	18,000
Shark liver oil	9,000-16,000
Shark liver oil (Indian Pharmacopea)	6660
Liver (sheep, goat, ox or pig)	6,000-10,000
Good sources	
Butter	720-1200
Ghee (clarified butter fat)	600-700
Egg (hen), whole	300-400
Egg (yolk)	600-800
Milk powder (full cream)	400-450
Cheese (fatty type)	200-400
Fair sources	
Milk, cow or buffalo (whole)	50-60
Milk, human	60-70
Fatty fish	30-40

Table 9.6 **Carotene content of foods**

Source	Carotene content μg/100g	Vitamin A equivalent (μg)*
Red palm oil	25,000-33,000	4167-5500
Green leafy vegetables		
Amaranth leaves	1,600-7,000	266-1166
Cabbage	1300	217
Coriander leaves	7,000-8,000	1166-1333
Curry leaves	8000	1333
Drumstick leaves	7700	1283
Fenugreek leaves	2700	450
Radish leaves	4500	750
Mint	1800	300
Spinach	3600	600
Other vegetables		
Carrot	1,300-2,600	217-434
Pumpkin, yellow	600-720	100-120
Fruits		
Jack fruit	320	54
Mango, ripe	3000	500
Orange	210	35
Tomato ripe	190	32

*Vitamin A equivalent calculated on the assumption that 6 μg. carotene in the diet will have the same biological activity as 1 μg. of vitamin A alcohol as suggested by the FAO/WHO Expert Group on Requirements of Vitamins (FAO Report Ser. No. 41).

Vitamin A requirements

The vitamin A requirements for the different age groups recommended by the FAO/WHO Expert group on Vitamin Requirements and I.C.M.R. Expert groups are given in Table 9.7.

Table 9.7 **Recommended intakes of vitamin A or carotene (μg day)**

Age	I.C.M.R. Expert Group		FAO Expert Group	
	Vitamin A Retinol (μg)	Carotene[1] (μg)	Vitamin A Retinol (μg)	As Carotene[2] (μg)
Upto 1 year	300-400	1200-1600	300	1800
1–3 years	250	1000	250	1500
4–6 ,,	300	1200	300	1800
7–9 ,,	400	1600	400	2400
10–12 ,,	600	2400	575	3450
13–15 ,,	750	3000	725	4350
Adolescents	750	3000	750	4500
Adults (men and women)	750	3000	750	4500
Pregnancy	750	3000	750	4500
Lactation	1150	4600	1200	7200

[1] Vitamin A equivalent calculated on the assumption that 4 μg Carotene in the diet will have the same biological activity as 1 μg vitamin A alcohol (retinol).

[2] Vitamin A equivalent calculated on the assumption that 6 μg carotene in the diet will have the same biological activity as 1 μg vitamin A alcohol (retinol).

Treatment and prevention of vitamin A deficiency

Mild to moderate cases of vitamin A deficiency can be treated by the daily oral dose of 10,000 μg of fat soluble vitamin A for a period of 10 days. In severe cases, larger doses of 50,000 μg. of fat soluble vitamin A will have to be administered daily for a few weeks. Studies carried out by Swaminathan and co-workers at Hyderabad (1970) have shown that a single massive dose of 100,000 μg. of fat soluble vitamin A will help to prevent vitamin A deficiency in children for about a year.

Toxic effects of excess of vitamin A

Toxic effects of large doses of water soluble vitamin A have been reported in children by several workers. In all these cases the children have been receiving 30,000 to 150,000 μg. of water soluble vitamin A for several months. The characteristic signs and symptoms observed were anorexia, headache, a dry itching skin and swelling over the long bones due to bony exostoses. The

liver was enlarged. The serum vitamin A level was very high. Rapid recovery of the subjects followed on withdrawal of the vitamin.

Polar bear liver poisoning: The toxic effects in humans and dogs due to consumption of polar bear livers have been reported. Rodahl and Moore (1943) found by chemical estimation that polar bear livers are unusually rich in vitamin A containing 600,000 μg. vitamin A per 100 g. Consumption of about 500 g of liver would provide toxic doses (3000,000 μg) of vitamin A.

VITAMIN D

History

Mellanby (1919) discovered that cod liver oil can cure or prevent experimentally produced rickets in dogs. McCollum and co-workers (1922) established experimentally that the antirachitic vitamin was different from vitamin A and called the factor vitamin D. Steenbock and associates (1924) and independently Hess (1924) found that antirachitic activity could be induced in some foods by irradiation with ultra-violet light. Later work by several workers showed that the sterol fraction of oils when irradiated with ultra-violet light developed antirachitic activity, though the sterols by themselves, were inactive. Reerink and Van Wijk (1931) isolated a crystalline vitamin D preparation from activated ergosterol. Schenck (1937) obtained a crystalline vitamin D preparation from activated 7-dehydrocholesterol.

Chemistry and properties

The chemical structures of vitamin D_2 (Ergocalciferol) and Vitamin D_3 (cholecalciferol) are given in Figs. 9.4 and 9.5 as compared with ergosterol and 7-dehydrocholesterol from which they are prepared.

The conversion of ergosterol and 7-dehydrocholesterol to vitamin D_2 and D_3 results in the opening of ring B, resulting in one more unsaturated linkage. The sequence of the formation of vitamin D_2 from ergosterol is given below:

Ergosterol→Lumisterol→Tachysterol→Calciferol→ {→Toxisterol; →Suprasterols}

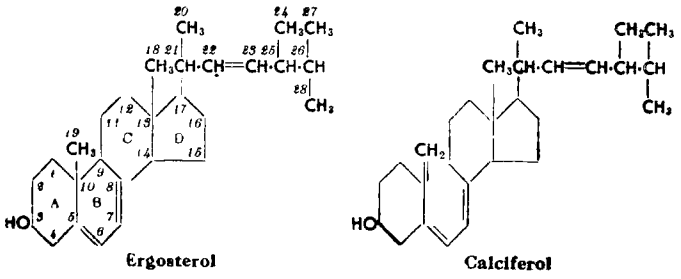


Fig. 9.4

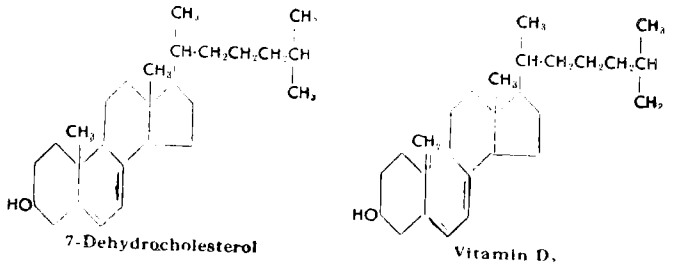


Fig. 9.5

Toxisterol, as the name implies is toxic and the other compounds have no vitamin D activity. Vitamin D_3 was found to be identical with the natural vitamin D occurring in fish liver oils.

Vitamin D is fairly heat stable. It is soluble in fats and fat solvents and insoluble in water.

Absorption and storage

Vitamin D can be administered orally or by injection. Orally administered vitamin D is absorbed from the small intestines. Fat helps in the absorption and bile is essential for absorption. Vitamin D passes to the general circulation via lymph and distributed throughout the body. Small amounts of vitamin D are found in blood and other tissues.

Physiological and biochemical functions

The different functions of vitamin D are as follows:

(1) Vitamin D promotes the absorption of calcium and phosphate from the small intestines. When vitamin D is absent,

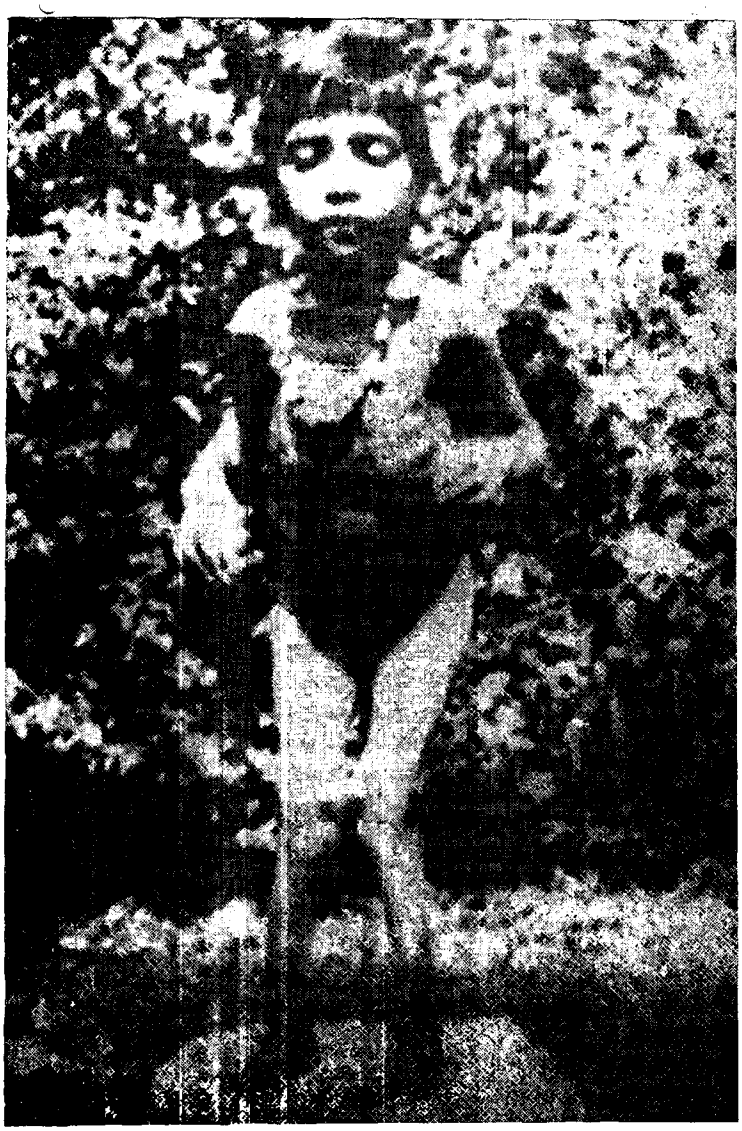


Plate VI: A child suffering from rickets showing marked bony deformities.

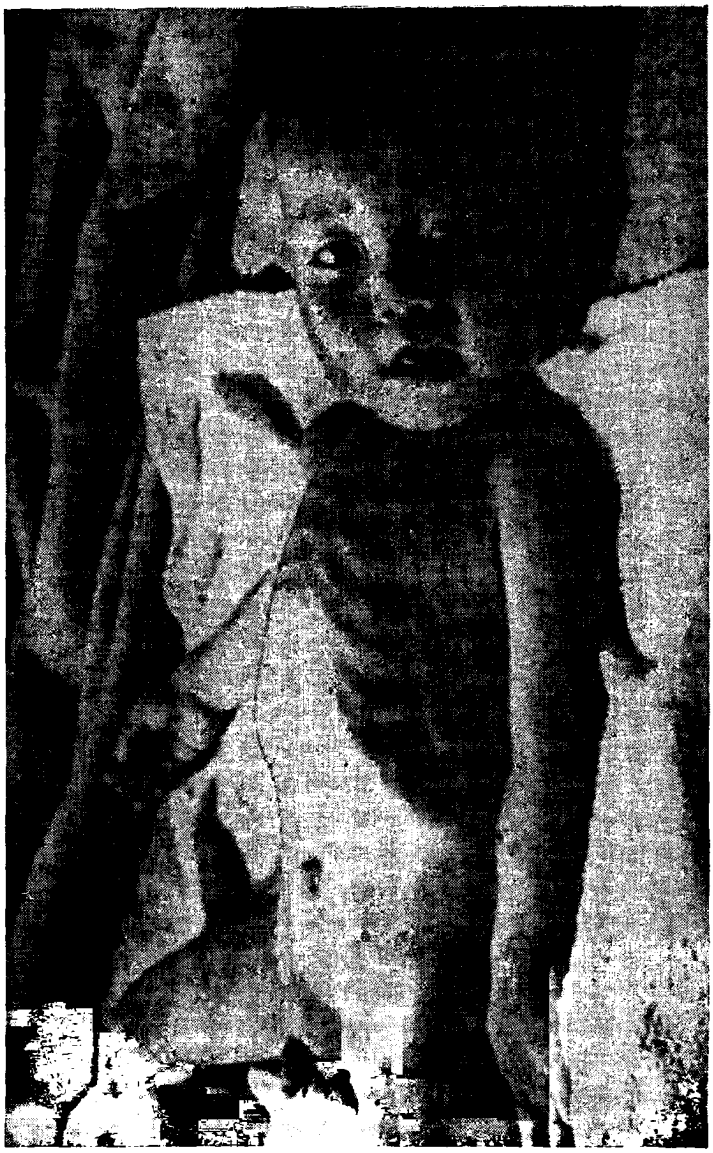


Plate VII: A child with rickets showing signs of rickety rosary

absorption of calcium is very low and a greater part of the ingested calcium is lost in the feces. (2) The vitamin also acts in some more direct manner on the bones directly promoting calcification. Administration of vitamin D to a rachitic animal causes degeneration of the growing cartilage cells of the epiphysis. This is followed by invasion of the older cartilage zone by blood vessels which bring the necessary calcium and phosphate for the ossification of the bone. (3) Recent studies have shown that vitamin D is hydroxylated at position 25 into 25-hydroxy vitamin D (25-HCC) in the kidney. This hydroxylated form is more potent than vitamin D, in calcification of the bone and hence appears to be the active form of vitamin D (1-25HCC). This form also is more active in the intestinal absorption of calcium than vitamin D. It is believed that vitamin D helps to elaborate an active calcium binding protein in the intestines which helps the absorption of calcium from the intestines. A calcium dependent A.T.P. ase has also been demonstrated in microville of intestines when vitamin D is administered. The above system appears to be involved in the intestinal absorption of calcium (Deluca 1971).

Effects of vitamin D deficiency

Vitamin D deficiency causes the diseases, rickets in children and osteomalacia in adults.

Rickets: This disease was widely prevalent among children in all the countries of the world till recently. It is characterised by bone deformities. The earliest bony lesion is often craniotabes —small round unossified areas in the membranous bones of the skull. Another early sign is the 'beading' at the costochondal junctions of the ribs, known as 'rickety rosary'. Later features are 'bossing' of the frontal and parietal bones and delayed closure of the anterior fontanelle and deformities of the chest such as undue prominence of the sternum known as 'pigeon chest' (Plates VI and VII). Since the bone is soft due to non-deposition of calcium salts they are easily bent by the weight of the body. If the rickets continue during the second and third year of life, serious bone deformities, such as bow legs, knock knees and deformities of the spine and pelvis develop. There is extension and widening of the epiphyses at the growing points.

Osteomalacia: Osteomalacia may be called adult rickets. It occurs generally in pregnant women of the low income groups who

consume a diet devoid of vitamin D and low in calcium and who live indoors for social reasons and observe 'purdha' (covering their body completely with a loose gown, including the face). Osteomalacia has been reported from India, Pakistan, China and Middle east countries. The changes in the blood are essentially similar to those in rickets. Due to lack of vitamin D, there is a progressive decalcification of the bone and the bone becomes soft. Bone deformities due to the weight of the body occur in pelvis, legs, ribs, sacrum and lower lumbar vertebrae (Plate VIII). Due to the deformity of the pelvis, normal delivery of the baby becomes difficult.

Methods of assay

The methods used for the assay of vitamin D are as follows:

(*i*) *Chemical methods:* The method involves separation of vitamin D by column chromatography from other interfering substances and determination spectrophotometrically. This method can be used only for pharmaceutical preparations containing pure vitamin D.

(*ii*) *Biological methods:* Biological methods are accurate for the assay of preparations containing small amounts of vitamin D. The international unit of vitamin D activity is the potency of 0.025 micrograms of ergocalciferol (Vitamin D_2). Three types of biological methods are used (*a*) Growth method (*b*) Bone ash method and (*c*) Line test.

(*a*) *Growth method:* Graded amounts of standard vitamin D and unknown are given to vitamin D deficient young rats. The growth rates are proportional to vitamin D present. By comparing the growth rates of rats on diets containing graded amounts of the standard and the unknown, the vitamin D potency can be calculated.

(*b*) *Bone ash method:* Graded amounts of standard vitamin D and unknown are given to vitamin D deficient rats and the ash content of tibia or radii is determined. The increase in ash content is proportional to vitamin D content.

(*c*) *Line test:* Graded amounts of standard vitamin D and unknown food are given to vitamin D deficient rats. The proximate end of the tibia or the distal end of the radii or ulnae are taken, cleaned from adhering tissue and longitudinal medium section is

Plate VIII: A case of advanced Osteomalacia showing marked deformities affecting the spine, pelvis and lower extremities.

made. The section is immersed in a 2 per cent aqueous solution of silver nitrate for one minute whereby the newly deposited calcium phosphate is converted into silver phosphate. After washing with water, the section is exposed to light when the silver phosphate develops a black stain. The width of the line of silver phosphate is an index of cure effected by vitamin D.

Dietary sources: Vitamin D is present only in some foods of animal origin. It is not present in foods of vegetable origin. The important sources of vitamin D are fish liver oils, fatty fish, eggs, butter, cheese, full fat milk powder and milk. The vitamin D content of some foods is given in Table 9.8.

Table 9.8 **Vitamin D content of certain foods**

Food	Vitamin D	
	μg/100g	I.U./100g
Fish Liver Oils		
Halibut liver oil	500—10,000	20,000—400,000
Cod liver oil	200—750	8,000—30,000
Cod liver oil B.P.	200	8,000
Shark liver oil	30—100	1,200—4,000
Other Foods		
Fat, fish (sardine, salmon, herring)	5—30	200—1,200
Egg, hen, whole	1.25—1.5	50—60
Egg, yolk	3—4	120—160
Butter	0.5—1.5	20—60
Ghee (clarified butter fat)	0.5—1.5	20—60
Milk powder, full fat	0.4—0.6	15—25
Milk, fresh, whole	0.05—0.1	2—4

1 microgram of vitamin D = 40 I.U.

Vitamin D, sunlight and ultra-violet light

Since the incidence of rickets was less common in children in the tropics as compared with those in the temperate climate, Palm (1890) was of the view that the abundant sunshine in the tropics was probably responsible for the low incidence of rickets. In 1919, Huldschinsky demonstrated on the basis of X-ray studies that exposure to ultra-violet light from mercury vapour lamp for short periods daily can cure rickets in children. Hess and Longer

(1921) confirmed that exposure of children suffering from rickets to sunlight had a curative effect. The studies of Chick and co-workers (1923) under controlled conditions proved conclusively that rickets can be cured in children either by giving cod liver oil or by exposure daily to ultra-violet light from mercury vapour lamp for short periods. The beneficial effect of sunlight is obviously due to the ultra-violet radiations present in it.

Requirements

The daily requirements of vitamin D for infants and preschool children have been estimated to be about 10 μg (400 I.U.). For older children and adults lesser amounts 5 μg (200 I.U.) may be adequate while expectant and nursing mothers will need about 10 μg (400 I.U.). In tropical climate, half the above amounts will be adequate if the subjects are exposed to direct sunlight for some hours daily.

Treatment and prevention of vitamin D deficiency

For the treatment of rickets and osteomalacia about 1000 to 5000 I.U. of vitamin D (25 to 125 μg) should be administered orally daily for about a month. Alternately, a single massive oral dose of vitamin D, 2500 μg (100,000 I.U.) can be given if a doctor is not readily accessible to the patient. The daily administration of small doses is safer than a single massive dose. In addition to the vitamin, the subject should receive adequate quantities of calcium and phosphates. The subject should be encouraged to spend some time daily in the sunshine. The earliest evidence of healing in rickets and osteomalacia is provided by radiological examination. Occasionally, cases of rickets that are resistant to treatment with vitamin D are encountered. The exact cause is not known. One of the possible causes may be loss of phosphate due to lack of reabsorption by renal tubules. (Franconi syndrome).

Prevention

Since milk is a poor source of vitamin D, infants, young children and expectant and nursing mothers should be given additional source of vitamin D such as shark liver oil or cod liver oil. Alternately, a single massive oral dose of vitamin D should be administered to all infants and children (once annually) in a hospital in the locality. A supplement of vitamin D is also essential

in tropical and sub-tropical countries, if the child is not exposed to sunshine by the mother during the first year of life.

Toxic effects of excess vitamin D

Vitamin D in excess produces toxic symptoms. The earliest sign is loss of appetite. Nausea and vomiting are frequently associated. Intense thirst and polyuria soon follow. The affected child will be dull and weak in appearance and wasting of muscles is observed. If the over-dosage is continued, calcification of soft tissues such as arteries, kidneys and lungs may take place, leading to death. The diagnosis of vitamin D toxicity has to be made early if the child is to be saved.

VITAMIN E

History

Vitamin E is essential for normal reproduction in several species of animals and also in man. Evans and Bishop (1922) found that for normal reproduction in rats, a fat-soluble factor present in crude vegetable oils was essential. This was termed vitamin E. In 1936, Evans and co-workers isolated two different compounds (α- and β-tocopherols) possessing vitamin E activity. They were given the name tocopherol (GK. tocos-child birth, phero-to bear, ol-an alcohol). Later, Fernholtz (1937) and Evans and co-workers (1939) elucidated the chemical structure of tocopherols. Tocopherols were synthesised independently by three groups of workers. Three more tocopherols namely Delta, Eta and Zeta tocopherols have been isolated by later workers.

Chemistry and properties

The chemical structure of α-tocopherol is given in Fig. 9.6.

They are derivatives of 6-hydroxy chroman with a phytyl side chain. α-tocopherol has 3 methyl groups in 5, 7, 8 positions while β -and γ-tocopherols contain 2 methyl groups in 5-8 and 7-8 positions respectively. Delta tocopherol has one methyl group in the 8 position, Eta tocopherol, in 7 position and Zeta tocopherol in the 5 and 7 positions. Tocopherols are soluble in fat solvents and insoluble in water. They are slowly destroyed in an alkaline medium and the vitamin activity is destroyed by oxidation.

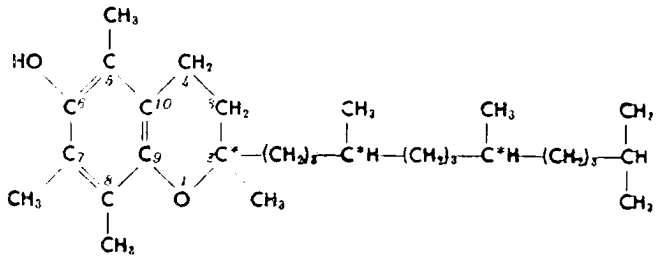


Fig. 9.6

Absorption and storage

Tocopherol, like other fat soluble vitamins, is absorbed to a greater extent from oil solutions. Bile is necessary for its absorption. The human serum contains about 0.9 to 1.2 mg per 100ml. Liver stores one-half to one-fourth as much tocopherol as the skeletal muscles, body fat and visceral organs.

Physiological and biochemical functions

The various functions of vitamin E are:

(*i*) Vitamin E prevents peroxidation of polyunsaturated fatty acids in tissues and membranes. In vitamin E deficiency, polyunsaturated fatty acids undergo peroxidation and yellow and brown pigments are formed. (*ii*) Vitamin E also protects erythrocytes from haemolysis by oxidising agents; e.g. dialuric acid and hydrogen peroxide. (*iii*) In vitamin E deficiency, there is degeneration of cellular and sub-cellular membranes which are known to be rich in polyunsaturated fatty acids (*iv*) Vitamin E offers definite protection to liver injury due to carbontetrachloride poisoning (*v*) Demyelination, gliosis and distortion of the axon pattern in the spinal cord, giving rise to hypalgesia and progressive paresis, occur in vitamin E deficient rats (*vi*) Vitamin E along with an activator present in microsomal supernatant is able to prevent the respiratory decline in isolated mitochondria and (*vii*) The requirements and functions of vitamin E are dependent on the quantity of polyunsaturated fatty acids present in the tissues and selenium status of the animal. Vitamin E appears

to increase the activity of selenium compounds present in traces in animal tissues.

Effects of deficiency in animals

Vitamin E deficiency in animals causes several disorders such as reproductive failure, liver necrosis, muscular dystrophy etc. They are briefly discussed below.

Reproductive failure: In the female rat fed on vitamin E deficient diets, foetal death and resorption take place. The primary metabolic defect which causes death and subsequent resorption of the embryo is not known. There are degenerative changes in the uterus. However, the vascular system of the embryo also undergoes degeneration. In the male rat irreversible testicular degeneration occurs in vitamin E deficiency.

Liver necrosis: Necrosis of the liver produced in rats fed on yeast is prevented by vitamin E.

Erythrocyte haemolysis: In vitamin E deficient animals, the erythrocytes readily undergo haemolysis when treated with hydrogen peroxide. This is prevented by vitamin E.

Anaemia in the monkey: Monkeys fed on vitamin E deficient diets develop anaemia. This is due to lack of haematopoiesis in the bone marrow rather than from excessive red cell destruction. Administration of vitamin E cures the anaemia.

Muscular dystrophy: Rabbits, guinea pigs, monkeys and chicks fed on vitamin E deficient diets develop muscular dystrophy which is cured and prevented by vitamin E.

Yellow fat, ceroid and lipofuscins: Peroxidation of unsaturated fatty acids and formation of yellow to brown pigments occur in animals fed vitamin E deficient diets containing fats rich in polyunsaturated fatty acids. The colour is due to a fat soluble yellow pigment and a dark brown fat insoluble pigment. The latter pigment known as ceroid has been found to occur in the uterine muscle, skeletal muscle and sex glands in vitamin E deficient rats.

Methods of assay

The different methods of assay are briefly discussed below:

Chemical method: Ferric chloride dipyridyl method:

Vitamin E reduces ferric chloride to ferrous chloride. The ferrous chloride formed reacts with α-α-dipyridyl reagent to give a red colour (Emmerie and Engel 1939).

Biological method (Emerson-Evans Resorption-gestation method): The bio-assay is based on the ability of tocopherols to prevent foetal death and resorption when administered to vitamin E deficient female rats during the first half of pregnancy. The international unit is the activity of one mg of Dl-α-tocopheryl acetate.

Relative biological potency of tocopherols: The relative biological activity of different natural tocopherols and synthetic Dl-tocopherol is given in Table 9.9.

Table 9.9 **Relative biological potency of tocopherols**

Substance	Relative biological potency (International units[1] per gram)	Relative potency[2]
Synthetic:		
DL-α-tocopherol	1100	110
DL-α-tocopheryl acetate	1000	100
Natural:		
D-α-tocopherol	1350	135
D-α-tocopheryl acetate	1240	124
D-β-tocopherol	400	40
D-γ-tocopherol	80	8
D-δ-(delta)-tocopherol	10	1

[1] One international unit = the activity of 1 mg of synthetic Dl-α-tocopherol.

[2] The relative potency of synthetic Dl-tocopherol acetate as 100.

Dietary sources

The tocopherol contents of foods are given in Table 9.10.

Table 9.10 **Vitamin E (Tocopherol) contents of some common foods (Values per 100g)**

Foodstuffs	Vitamin E (mg) Total	Alpha
Cereals		
Barley	3.2	...
Corn meal, yellow	1·7	0.8
Oat meal	2.1	1.9
Rice, brown	2.4	1.2
Rice, polished	0.6	0.4
Wheat, white flour (80% extraction)	1·2	...
Wheat, whole wheat flour	2.2	...
Oils and fats		
Coconut oil	8.3	3.6
Corn oil	91.0	10.0
Cottonseed oil	81.0	47.0
Linseed oil (crude)	113.0	...
Margarine	54.0	28.0
Mustard oil	32.0	8.6
Olive oil	30.0	...
Palm oil	56.0	30.0
Peanut oil	22.0	11.0
Pecan, refined	42.0	20.0
Poppyseed, crude	44.0	...
Rapeseed oil	56.0	15.1
Rice bran	91.0	58.0
Safflower, crude	80.0	...
Soy bean oil	118.0	15.9
Sunflower seed, crude	70.0	...
Wheat germ	255.0	142.8
Lard	2.7	...

Foodstuffs	Vitamin E (mg) Total	Alpha
Vegetables and fruits :		
Cabbage	0.1	0.1
Carrots	0.5	0.5
Onions	0.3	0.2
Peas, green	2.1	0.1
Tomatoes	0.4	0.3
Apples	0.7	0.7
Banana	0.4	0.4
Grape fruit	0.3	0.3
Oranges	0.2	0.2
Nuts, oilseeds and legumes :		
Peanut	9.3	4.6
Navy bean	3.6	0.1
Egg, meat and fish :		
Bacon	0.5	0.4
Beef-steak	0.6	0.4
Beef-liver	1.4	1.4
Lamb-chops	0.8	0.6
Pork, chops	0.7	0.6
Haddock (fish)	0.4	0.4
Chicken	0.3	0.2
Egg, whole	2.0	1.2
Dairy products :		
Butter	2.4	...
Cheese	1.0	...
Ice cream	0.3	...
Milk, whole fluid	0.1	...
Milk, evaporated	0.3	...

The cereal germ oils i.e. wheat germ oil and corn germ oil are the richest natural sources. Vegetable oils and fats are good sources. Cereals and animal foods are fair sources of tocopherol. Vegetables and fruits are poor sources. Since α-tocopherol has the highest biological activity and gamma and delta tocopherols have very little biological activity, the alpha-tocopherol content should be taken for calculating the human requirements.

Requirements

Requirements of tocopherol for human subjects of various age-groups are given in Table 16.2 in Chapter 16. Estimates of requirements for preventing deficiency states have been made in a few cases. Studies with experimental animals and children suffering from kwashiorkor showed that increased haemolysis of the R.B.C. and also megaloblastic anaemia occurred due to vitamin E deficiency. Studies on the vitamin E requirements of infants showed that the amount of tocopherol necessary to prevent the haemolysis of R.B.C. was within the range of 2 to 10 mg per infant per day. Human milk contains fair amounts (2 to 5 mg alpha-tocopherol per litre), and would meet the needs of infants. Cow's milk is a poor source and hence will not meet the vitamin E requirements. It has been observed that in male subjects, vitamin E deficiency developed in diets containing 25 per cent calories from fat and providing 3 mg of vitamin E per day. As the intake of fat and polyunsaturated fatty acids (PUFA) increased, the requirements for vitamin E also increased. The adult needs about 10 mg vitamin E when the intake of PUFA is about 7 g and 30 mg vitamin E when the intake of PUFA is high (over 35 g). The requirements of vitamin E during pregnancy and lactation should be greater than those of normal subjects. Estimates of vitamin E content of diets of varying composition have yielded values ranging from 2 to 66 mg. Since vitamin E deficiency has not so far been reported in the general population, it may be assumed that diets commonly consumed by normal healthy persons all over the world provide adequate amounts of vitamin E.

Therapeutic uses and toxicity

Vitamin E has been used in daily doses of 500-1000 mg for the treatment of diverse conditions such as habitual abortion, sterility, muscular dystrophy, diabetes, ischaemic heart disease, skin disorders and anaemia in infants. In cases, where vitamin E was involved in any of the above conditions, beneficial effects have been reported. Administration of 3-4 g of tocopherols daily over long periods has not produced any toxic effects in human beings.

VITAMIN K

History

Dam and Schonheyder (1934) found that chicks fed on purified diets containing all vitamins known at that time developed haemorragic condition which was cured by lucerne (alfalfa) and dessicated fish meal. The active principle was fat soluble. Dam (1935) named it Vitamin K (Koagulations-Vitamin). Dam, Karrer and co-workers (1939) isolated pure vitamin K_1. In the same year, Doisy and co-workers isolated pure vitamin K_2.

Chemistry and properties

The synthesis of vitamin K_1 was accomplished by three groups of workers in 1939. Doisy and co-workers elucidated the structure of vitamin K_2 in 1940. The chemical structure of vitamin K_1 is given in Fig. 9.7.

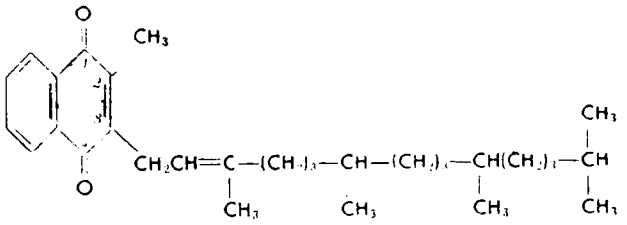


Fig. 9.7 Vitamin K

In 1939, Ansbacher and Fernholt discovered that 2-methyl 1-4-naphthoquinone had vitamin K activity. Another compound called Phthiocol (2-methyl-3-hydroxy 1:4 napthoquinone) isolated from tubercular bacilli was found to possess vitamin K activity. Vitamin K_1 (phyloquinone) is 2-methyl-3-phytyl 1:4 naphthoquinone while vitamin K_2 (menaquinone-6) is 2-methyl-3-difarnesyl-1:4 naphthoquinone.

Absorption and storage

Vitamin K is absorbed along with fat in the diet. Bile is essential for its absorption. The absorbed vitamin passes through the lymphatic system to the general circulation. Liver stores appreciable amounts. All tissues contain small amounts of vitamin K. The feces contain large amounts of vitamin K produced by the bacterial flora. For example, *E. coli* contain large amounts of vitamin K.

Physiological and biochemical functions

Vitamin K is essential for blood coagulation. Vitamin K administration leads to an increase in the prothrombin levels. In vitamin K deficiency, prothrombin levels are reduced. The exact mechanism of action of vitamin K in increasing prothrombin levels in blood is not fully known. Several factors such as proconvertin and serum prothrombin conversion factor (SPCA) have been postulated in the formation of prothrombin. It has also been postulated that proconvertin deficiency occurs in patients suffering from vitamin K deficiency resulting in a low prothrombin level. Another view is that vitamin K activates an inactive prothrombin precursor in the liver or involved in the synthesis of prothrombin in the liver.

Effects of deficiency

Vitamin K deficiency leads to a lowering of prothrombin level and increased clotting time of blood. This may lead to haemorrhagic conditions. Vitamin K deficiency can occur in the following ways (*i*) Inadequate intake of vitamin K and (*ii*) Inadequate intestinal absorption.

Inadequate intake of vitamin K (Haemorrhagic disease of the new born): Inadequate intake of vitamin K by the mother may cause the haemorrhagic disease of the new born. Several cases have been reported in the literature. All these infants have a low prothrombin level and they recover rapidly when vitamin K is administered by injection.

Inadequate intestinal absorption (Disease of liver and intestine): Inadequate intestinal absorption of vitamin K may result from (*a*) lack of bile in the intestine due to defective secretion of bile salts as in liver disorders (*b*) Pyloric or intestinal obstruction and (*c*) poor absorption due to diarrhoea or dysentery.

Methods of Assay

The different methods for the assay of vitamin K are briefly discussed below.

A. Physico-chemical methods

(*i*) *Colorimetric methods*

(*a*) *Irreverre-Sullivan reaction:* Vitamin K gives a deep cobalt

blue colour with sodium di-ethyl, di-thio-carbomate in alkaline solution. The test is sensitive to 35 μg vitamin K per ml.

(*b*) *Craven's test:* Vitamin K is reacted with ethyleyano-acetate and on the addition of strong ammonium hydroxide a blue violet colour develops.

(*ii*) *Fluorimetric methods:* Qualitative and quantitative tests based on the formation of fluorescent reaction products have been developed by some workers.

(*iii*) *Spectrophotometric method:* This method is more accurate and sensitive than the colorimetric and flourimetric methods.

B. Biological methods

Methods based on the coagulation of blood can be carried out prophylactically or curatively. The latter is to be preferred when a quantitative assay is desired. The principle of the curative test is the determination of the coagulability of the blood of vitamin K deficient animals before and after ingestion of a test dose of the sample to be tested and comparison of the results with those obtained with known amounts of pure vitamin K. Chicks are better suited for animal assays than laboratory mammals because it is easier to exclude coprophagy with chicks than for example with rats. Further, the requirements of chicks for vitamin K are about 5 times as much as those of rats i.e. 0.5 and 0.1 mg. vitamin K_1 per kg of diet respectively.

Biological activity standards: The relative biological activity of vitamins K_1 and K_2 and 2-methyl 1:4 naphthoquinone is given below:

Compound	Relative Vitamin K activity (%)
2-methyl 1 : 4 naphthoquinone (menadione)	200
Vitamin K_1 (Phylloquinone)	100
Vitamin K_2 (menaquinone-6)	80

It is evident that 2-methyl 1:4 naphthoquinone is about twice as potent (weight for weight) than vitamin K_1. This may be due to the smaller molecular weight of the former. No internationally accepted standard of vitamin K activity has been

adopted so far. Several workers have reported vitamin K potency of foodstuffs in their own units. Vitamin K equivalents of different units given in the literature are given below:

Vitamin K_1 equivalent of biological units of vitamin K activity

Unit	Vitamin K_1 equivalent (μg)
Thayer-Doisy unit	1.0
Ansbacher unit	2.0
Thayer unit (1938)	2.0
Dam units	0.1
Dann unit (1938)	4.0
Dann unit (1939)	1.6

Detection of vitamin K deficiency

In vitamin K deficiency, the prothrombin content of blood is markedly decreased and the blood clotting time is considerably prolonged. The principal defect in vitamin K deficiency is the occurrence of haemorrhage in addition to a prolonged bleeding time. Low prothrombin levels are observed in diseases of the liver and intestines. The tests for the diagnosis of vitamin K deficiency are based on the determination of coagulation time of blood or prothrombin time. They are briefly discussed below.

Coagulation time of blood: About 1 ml of fresh blood is drawn in a tube containing thromboplastin. The tube is shaken and the clotting time is observed. The determination is also carried out with the blood of a normal person. This test has been recommended for routine clinical use as it is easy to carry out and is very reliable.

Prothrombin time determination: The basic prothrombin time is the clotting time obtained when an excess of thromboplastin and an optimal amount of calcium are added. The prothrombin time of normal adult human plasma is 12 ± 0.5 seconds. It is prolonged most commonly by a lack of prothrombin Factors V, VI, VII and X. The important causes of a prolonged prothrombin time, are lack of vitamin K or the presence of vitamin K antagonists and diseases of the liver.

Dietary sources

Vitamin K_1 occurs in plant foods while vitamin K_2 occurs in microorganisms. The best sources of vitamin K_1 are the green leafy vegetables e.g. alfalfa, spinach, cabbage, kale etc. Good sources are cauliflower, soybean, wheat bran, wheat germ etc. Carrots and potatoes are fair sources. Milk, meat and fish are poor sources. The vitamin K contents of foods are given in Table 9.11.

Table **9.11 Important sources of Vitamin K (μg/100g)**

Foodstuffs	Vitamin K (μg/100g)	Foodstuffs	Vitamin K (μg/100g)
Rich sources:		*Fair sources*	
Alfalfa	425–850	Wheat (whole)	36
Cabbage	250	Wheat (germ)	37
Cauliflower	275	Tomato (green)	49
Spinach	334	Tomato (ripe)	24
Liver	115–230	Potatoes	20
Good sources:		*Poor sources*	
Soybean	190	Corn	10
Peas	110	Carrots	10
Wheat bran	80	Mushrooms	7
Oats	75	Strawberry	13
		Cow's milk	6
		Human milk	2

Requirements

The exact requirements for vitamin K are not known. The average diet apparently contains adequate amounts of vitamin K as vitamin K deficiency has not been reported in healthy individuals except in new born infants fed on mothers' milk when the mothers' diet has a low vitamin K content. Mother's milk contains on an average 15 μg vitamin K_1/litre while cow's milk contains 60 μg/litre. Vitamin K deficiency occurs more often in breast-fed infants.

Therapeutic and prophylactic uses

Vitamin K_1 can be administered orally or parenterally. 2-methyl 1:4 naphthoquinone given to premature infants has been reported to cause toxic effects characterised by hyperbilirubinaemia and kernicterus. *Vitamin K_1 does not have these toxic effects and is safe for use.* For the treatment or prevention of haemorrhage in the new born, 1mg vitamin K_1 must be administered intramuscularly and repeated if necessary after 24 hrs. For the treatment of adults suffering from biliary obstruction and fistula or malabsorption syndrome, vitamin K_1 or menadione can be administered in doses of 10-20 mg intramuscularly.

Toxic effects

Vitamin K_1 does not produce any toxic effects in doses (10-20mg) normally used for the treatment of subjects suffering from disorders of liver or intestines. Vitamin K_1 analogues i.e. menadione and water soluble forms of menadione, however, when administered to premature infants have been found to produce toxic effects. The toxicity of menadione has been attributed to increased breakdown of red blood cells and inhibition of glucuronide formation.

II. WATER SOLUBLE VITAMINS

Ascorbic Acid (Vitamin C)

History

Much of the early pioneering work on the isolation of vitamin C was carried out by Zilva during 1917-27. He obtained highly potent concentrates and noted that they had strong reducing properties. Szent-Gyorgy (1928) isolated from cabbages, adrenal glands and oranges, an acid with intense reducing properties. He called it 'hexuronic acid' but he was not aware that it might be identical with vitamin C. In 1932, Waugh and King isolated vitamin C in a crystalline state from lemon juice. Soon after, Szent-Gyorgy confirmed that the hexuronic acid isolated by him years ago was identical with vitamin C. Vitamin C was named ascorbic acid due to its antiscorbutic properties.

Chemistry and properties

The chemical structure of ascorbic acid was established by Haworth and co-workers in 1933. Synthesis of vitamin C was reported independently in 1933 by Haworth and co-workers in England and Reichstein and co-workers in Switzerland. The chemical structures of L-Ascorbic acid and dehydro L-Ascorbic acid are given in Fig. 9.8.

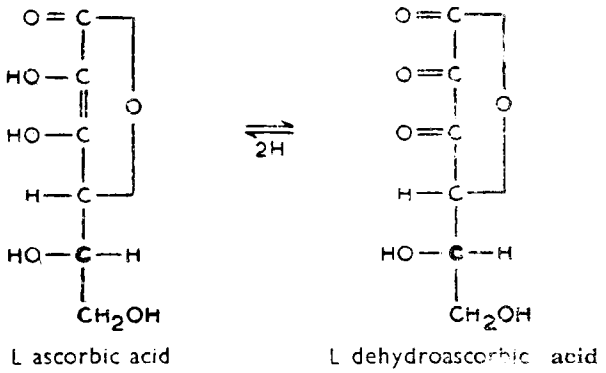


Fig. 9.8

It is of interest to note that D-ascorbic acid does not possess any antiscorbutic activities while dehydro L-ascorbic acid possesses almost the same vitamin activity as the L-ascorbic acid. Ascorbic acid has strong reducing properties. It is highly soluble in water (30 g/100 ml), slightly soluble in alcohol and is insoluble in ether, petroleum ether and benzene. Ascorbic acid in solution rapidly undergoes oxidation to dehydro-ascorbic acid. The oxidation is catalysed by copper and silver ions. The oxidation is faster at higher temperatures e.g., during cooking of foods.

Absorption and storage

Ascorbic acid is rapidly absorbed from the intestines. It passes through the portal vein to the general circulation and to all tissues. Ascorbic acid is not stored in the body to any appreciable extent. Each organ or tissue has an optimal saturation level of ascorbic acid. Excessive intakes of ascorbic acid do not increase the saturation level but are excreted in the urine.

Physiological and biochemical functions

Ascorbic acid is essential for (*i*) formation of collagen and intercellular cement substance (*ii*) metabolism of tyrosine (*iii*) absorption of iron and incorporation of plasma iron in ferritin (*iv*) Hydroxylation of aromatic nuclei (*v*) Bone formation (*vi*) Adrenal cortex function and (*vii*) Electron transport.

Formation of collagen and intercellular cement substance: Studies carried out by a large number of workers have shown that ascorbic acid is essential for the hydroxylation of proline into hydroxyproline and this takes place after a peptide containing proline is formed. Since collagen is formed even in ascorbic acid deficiency, there may be more than one mechanism for the formation of collagen. Ascorbic acid is also concerned in the formation of intercellular cement substances in the capillaries, teeth, bones etc. In ascorbic acid deficiency these are not formed fully.

Metabolism of tyrosine: When tyrosine is fed to scorbutic guinea pigs in quantities of 0.5 g, the animal excretes homogentisic acid, p-hydroxy phenylpyruvic acid and p-hydroxy phenyl lactic acid. When ascorbic acid is administered, the excretion of the above metabolites ceases. This indicates that ascorbic acid is involved in the metabolism of tyrosine.

Absorption of iron and incorporation of plasma iron in ferritin: Ascorbic acid helps to reduce the ferric iron to ferrous state in the intestines and thus helps in the absorption of iron. Recent studies have also shown that for the incorporation of plasma iron in the tissues as ferritin, ascorbic acid and ATP are required.

Hydroxylation of aromatic nuclei: Ascorbic acid plays an important part in (*i*) the hydroxylation of deoxycorticosterone (*ii*) hydroxylation of tryptophan to 5-hydroxytryptophan and phenylalanine to tyrosine etc. Staudinger (1961) suggested that the ascorbic acid dependent electron transfer in liver microsomes is generally coupled with hydroxylation.

Bone formation: The fundamental pathological changes in ascorbic acid deficiency are the defective formation of bone matrix and ground substance. Calcification is not affected. Osteoblasts that were invading the area of provisional calcification, change histologically into fibroblasts. The matrix they produce is not a gel but a loose connective tissue. It does not ossify as it is

abnormal matrix. These changes account for the X-ray pattern of bones of scorbutic infants. The 'white' line observed in the scorbutic bone is the piled up zone of provisional calcification.

Adrenal cortex: McCarrison (1919) made the important observation that hypertrophy of adrenal cortex glands occurs in scorbutic guineapigs. There is evidence that ascorbic acid is involved in the synthesis of cortical hormones.

Electron transport: It is generally believed that ascorbic acid may be involved in the electron transport in some of the reactions mentioned above.

Effects of deficiency in humans

Severe ascorbic acid deficiency results in the development of the disease, scurvy.

Scurvy in adults

The disease is characterised by general weakness, spongy bleeding gums, loose teeth with resorbed dentine, swollen tender joints and haemorrhages in various tissues.

General weakness: The first symptoms reported in experimentally induced scurvy are weakness, easy fatigue and listlessness. These are followed quickly by shortness of breath, pain in bones, joints and muscles of the extremities.

Swollen and tender joints and haemorrhages in various tissues: Haemorrhages deep in muscle occur particularly in calf, thigh and forearm, causing pain in surrounding tissues. Haemorrhages may occur in joints, causing swelling and pain in joints.

Bleeding gums and loose teeth: As ascorbic acid deficiency advances, the gums become swollen, blue-red, spongy and very friable. They may become infected by bacteria. The teeth loosen in the alveolar bone.

Infantile scurvy

Infantile scurvy differs in some respects from adult scurvy. The prescorbutic infant becomes anorexic and listless for a few days before other signs develop. With the onset of the disease, the infant line with legs drawn up on the abdomen. It cries when touched, especilly when its legs or arms are moved or lifted.

Extremely tender swellings may be felt at the ends of the long bones due to haemorrhages under the periosteum of the shaft. The sternum may sink slightly inward. Purpura occurs in the skin. If teeth are erupted, the gums are swollen with blue-red lesions. Dyspnea, cyanosis, convulsions and death follow if treatment with ascorbic acid is delayed.

Effects of deficiency on wound healing

Ascorbic acid deficiency delays healing of wounds. This is due to the fact that in ascorbic acid deficiency collagen formation is affected. The rapid healing of a wound requires the formation of strong connective tissue in the scar.

Methods of assay

Methods available for the assay of ascorbic acid may be broadly grouped under two heads (*i*) Physico-chemical and (*ii*) Biological.

Physico-chemical methods

These methods are widely used for determination of ascorbic acid in foodstuffs and animal tissues.

(*a*) *Dye method:* The method was first suggested by Tillmanns in 1932 and was made quantitative for the assay of ascorbic acid by Harris and Ray (1933). The method is based on the reducing action of ascorbic acid on the dye 2:6-dichlorophenol indophenol in acid medium to a colourless compound. The titration is carried out after extracting the vitamin in metaphosphoric acid or oxalic acid solution in order to stabilise the vitamin and prevent destruction by copper ions and ascorbic acid oxidase and to precipitate proteins and to liberate protein bound ascorbic acid. A modification of this method is based on the addition of an excess of the dye and measurement of the unreacted dye. In the presence of interfering turbidity of pigments, the dye can be extracted with xylene and the colour of the xylene extract read in a colorimeter.

(*b*) *2:4-dinitrophenyl hydrazine method:* Ascorbic acid is oxidised to dehydro ascorbic acid which is reacted with 2:4-dinitrophenyl hydrazine to yield a red colour. The colour is measured photometrically.

(*c*) *Chromatographic method:* In order to remove reductones and other interfering substances, a paper chromatographic method has been described by some workers. Colour is developed using 2:4-dinitrophenyl hydrazine reagent.

(*d*) *Spectrophotometric method:* The determination of the intensity of the characteristic absorption spectrum in water solution at 265 mμ before and after oxidation of the vitamin to dehydro-ascorbic acid has been used for the assay of ascorbic acid.

(*e*) *Polarographic method:* Determination of the oxidation potential of ascorbic acid in acid solution of fruits and vegetables using a polarograph has yielded satisfactory results.

Biological method

It is based on the cure of scurvy in guinea pigs. Groups of scorbutic guinea pigs are given the unknown food and also known amounts of standard ascorbic acid. From the data obtained, the ascorbic acid content of the unknown can be calculated.

Dietary sources

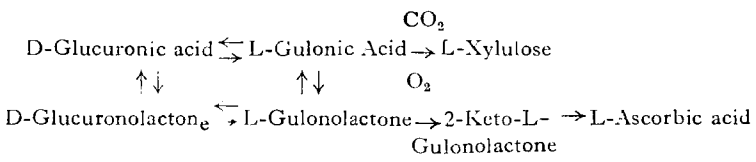
The vitamin C contents of common foodstuffs are given in Table 1.A in appendix. A few important sources are given in Table 9.12.

Table **9.12 Ascorbic acid contents of foods**

	Ascorbic acid mg/100g		Ascorbic acid mg/100g
Fruits:		*Green leafy vegetables :*	
Rich sources :		Amaranth leaves	173
Amla (Indian gooseberry)	700	Brussels sprouts	72
Guava	300	Cabbage	124
		Coriander leaves	135
		Drumstick leaves	220
Good sources :		Ipomoea leaves	137
Lime juice	63	Spinach	48
Orange	68	Radish leaves	65
Pineapple	63		
Mango, ripe	24	*Fair sources :*	
Papaya, ripe	46	Apple	2-8
Cashew fruit	60	Banana (Plantain ripe)	2-6
Tomato, ripe	32	Jackfruit	10

Biosynthesis of ascorbic acid in animal tissues

Certain animal species e.g., albino rat does not require an extraneous source of ascorbic acid. They are able to synthesise the same in their tissues. The pathway of the synthesis of ascorbic acid in rat tissues is given below:



Requirements

Studies carried out by several groups of workers have shown that the minimal daily intake of ascorbic acid in an adult to prevent scurvy is about 10 mg. The recommended daily allowance for ascorbic acid for adults is 20 mg in the United Kingdom and 30 mg in Canada and Australia, 60 mg in USA and 50 mg in India. The recommended allowances of the Nutrition Expert Group of the ICMR, India are given in Table 9.12.

Table **9.12 Ascorbic acid requirements**

Subject		I.C.M.R. (1968) Ascorbic acid mg/day	F.A.O. (1970) Ascorbic acid mg/day
Men		50	30
Women		50	30
,,	Pregnancy	50	50
,,	Lactation	80	50
Infants	0- 6 months		
	7-12 months	30	20
Children	1 year		
	2 years		
	3 years	30-50	20
	4- 6 ,,		
	7- 9 ,,		
	10-12 ,,		
Adolescents	13-15 Boys		
	Girls		
	16-18 Boys	50	30
	Girls		

Detection of sub-clinical deficiency

Methods have been developed by several workers for the detection of sub-clinical deficiency of ascorbic acid. These are briefly discussed below:

Ascorbic acid content of white blood cells: Several workers have shown that the ascorbic acid content of white blood cells and platelets is a good index of the tissue levels of ascorbic acid. The levels of ascorbic acid in normal persons range from 25-38 mg per 100 g. After feeding individuals on vitamin C deficient diet for 121 days, the value becomes zero. Determination of ascorbic acid content of white blood cells thus offers a highly sensitive index of the ascorbic acid nutrition of the individuals.

Plasma or serum ascorbic acid levels: The serum or plasma ascorbic acid levels were used for a long time as an index of ascorbic acid nutrition of humans. The normal serum values vary over a wide range from 0.5 to 2.2 mg per cent. In studies of population groups it has been found that when the daily dietary intake ranged from 75-100 mg ascorbic acid the plasma levels were 1.0-1.4 mg/100 ml and on dietary intakes of only 15-25 mg per day, the plasma levels were 0.1-0.3 mg/100 ml. The levels of ascorbic acid in plasma fall to zero after the subject is fed on vitamin C deficient-diet for 41 days while signs of scurvy usually appear after deprivation of vitamin C for about 130 days.

Urinary excretion: The urinary excretion of ascorbic acid falls when the dietary intake is low. Since the tissues are not saturated, a greater part of the test dose of ascorbic acid given to subjects habitually receiving low levels of ascorbic acid from the diet will be retained by the tissues and only a small part will be excreted in urine. In contrast, persons, whose daily intakes of ascorbic acid are high, will excrete daily larger amounts of ascorbic acid in urine and will also excrete a greater part of the test dose. Ralli and Sherry (1941) found that the test can be carried out in urine collected during a period of 3 hours after the administration of a test dose of 100 mg ascorbic acid. A normal saturated person excretes 50 per cent of the test dose and a person on low ascorbic acid diet about 15 per cent.

Intradermal dye test: The rate of decolouration of a small quantity of the dye 2:6-dichlorophenol indophenol injected intra-

dermally has been suggested by some workers as an index of the ascorbic acid nutrition status of the individual. In persons saturated with ascorbic acid, the dye is decolourised in 5 minutes while in deficient persons, the time taken is 10 minutes or more.

Skin capillary fragility test: One of the earliest signs of ascorbic acid deficiency is the fragility of capillaries. Assessment of the capillary fragility by means of blood pressure instrument or applying negative pressure through a cup can be used as a measure of ascorbic acid deficiency. The number of petechiae (haemorrhagic spots) that have appeared in a small area are counted. It is low in normal persons (less than 10 petechiae) and high in deficient persons (10-20 petechiae).

Therapeutic uses

For the treatment of adults suffering from scurvy, ascorbic acid is administered in doses of 100 mg (3-5 times a day) for a period of 10 days, followed by 100 mg twice daily for a week. In patients critically ill with scurvy, 1000 mg can be given daily by intravenous drip or in divided doses of 100 mg each intramuscularly. For infants and young children suffering from scurvy a daily dose of 10-25 mg (3 times a day) is adequate. Scorbutic signs subside within 24 hours after treatment and rapid recovery occurs within a week of treatment.

Ascorbic acid is used widely without adequate scientific basis, in the treatment of a large number of diseases such as common cold etc.

Toxic effects

Intakes of ascorbic acid in daily doses of 100-200 mg over long periods have not produced any harmful effects in human beings.

THIAMINE (VITAMIN B_1)

History

Takaki (1885) prevented the occurrence of beriberi in the Japanese navy by changing the diet. In 1897, Eijkmann produced polyneuritis in fowls by feeding them a diet consisting of washed polished rice and showed that the birds recovered when they were

administered extracts of rice polishings. Jansen and Donath (1926) isolated vitamin B_1 in crystalline form from rice polishings. In 1931, Windaus and co-workers isolated vitamin B_1 in crystalline state from yeast and established its emperial formula. Williams and co-workers (1934) and Peters and co-workers (1933, 1935) made improvements in the methods of isolation of vitamin B_1.

Chemistry and properties

Chemical structure: The chemical structure of thiamine was established by Williams and co-workers in 1936 and the synthesis accomplished independently by four groups of workers (Williams and Cline, 1936; Grewe, 1937, Andersag and Westphal, 1937; Todd and Bergel, 1937). The chemical structure of thiamine is given in Fig. 9.9.

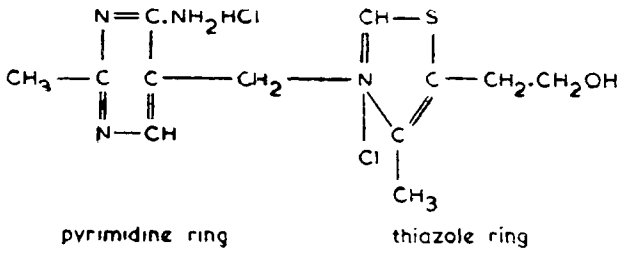
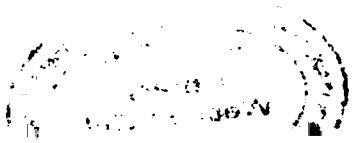


Fig. **9.9 Thiamine Hydrochloride**

Thiamine contains a pyrimidine ring and thiazole ring. Lohmann and Schuster (1937) isolated thiamine pyrophosphate (co-carboxylase) from yeast.

Properties: Thiamine is readily soluble in water (100 g in 100 ml. water) and slightly soluble in alcohol. It is stable in acid medium. It is destroyed when a neutral solution of thiamine is autoclaved at 120°C for 30 minutes. It is destroyed even at room temperature in an alkaline medium. When thiamine is dissolved in sodium bisulphite solution at pH 4.8-5.0, the molecule undergoes cleavage quantitatively into the pyrimidine half and thiazole half. When thiamine is oxidised with potassium ferricyanide in alkaline solution it is converted into a compound called thiochrome which has a strong fluorescence in ultraviolet light.



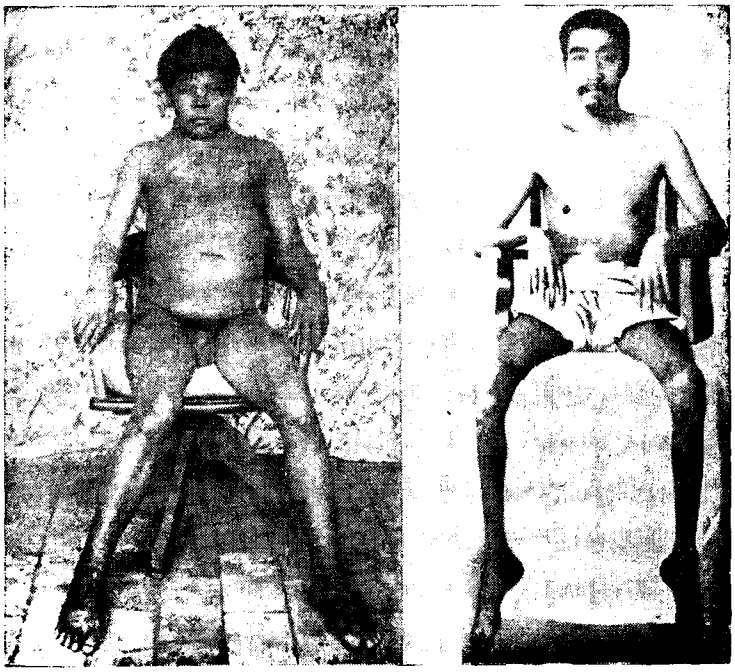


Plate IX: Left : A case of wet beri-beri
Right : A case of dry beri-beri

Methods of assay

The methods available for the assay of thiamine in foodstuffs may be grouped under three heads (1) Physico-chemical (2) Biological and (3) Microbiological.

1. Physico-chemical methods

(*i*) *Thiochrome method:* The method was first described by Jansen in 1936. Several modifications of the method have been published by different workers (Wang and Harris 1939; Swaminathan 1942; A.O.A.C. 1951; American Association of Vitamin Chemists, 1966). The method is highly sensitive and is widely used for the assay of thiamine in foodstuffs and animal tissues. The method involves the following steps: extraction of the vitamin after preliminary hydrolysis with phosphatase to hydrolyse thiamine pyrophosphate to thiamine (in the case of animal tissues) and also with proteolytic enzymes to liberate thiamine bound to proteins; oxidation of the vitamin by potassium ferricyanide in alkaline medium to thiochrome; extraction with isobutanol and determination of the fluorescence in a fluorimeter.

(*ii*) *Colorimetric method:* (Prebluda and McCollum 1936). The method is based on the red colour formed by the reaction of thiamine with diazotized p-amino acetophenone and measuring the colour photometrically. The method is less sensitive and less specific than the thiochrome method.

2. Biological methods

(*i*) *Rat growth method:* Rats are fed on thiamine deficient diet till they develop signs of thiamine deficiency. They are then grouped. Some groups receive graded doses of unknown while others receive graded doses of pure thiamine. From the data obtained the thiamine potency of the food is calculated.

(*ii*) *Bradycardia method:* The method was evolved by Birch and Harris (1934). It is based on a measure of the decline in the heart rate in thiamine deficient rats using an electrocordiograph and its cure by the administration of thiamine from the food to be tested and also with known amounts of standard thiamine.

(*iii*) *Cure of polyneuritis in birds or animals:* The curative test can be carried out with pigeons or rats. Pigeons or rats fed on

thiamine deficient diet develop paralysis and convulsions. The minimum quantities of the food extract and pure thiamine required for the cure are determined.

3. Microbiological methods

The microbiological methods are based on the growth of *lactobacillus L-viridescens* or yeast—*kloeckera brevis*.

(*i*) *Lactobacillus L-viridescens assay:* The method is highly sensitive. Neither the thiazole nor pyrimidine moiety nor their phosphorylated forms are active singly or in combination. Thiamine, thiamine monophosphate and thiamine pyrophosphate are equally active. It can estimate very small quantities —5 mμg of thiamine.

(*ii*) *Kloeckera brevis assay:* The method is more sensitive than *L-viridescens* assay method. The pyrimidine and thiazole moieties are not active singly or in combination. On molar basis, thiamine pyrophosphate is about 70 per cent as active as thiamine and hence must be hydrolysed to thiamine before assay. The method can estimate 0.5 to 2.5 mμg of thiamine.

Assessment of the thiamine nutrition status

Sub-clinical thiamine deficiency in human subjects can be assessed by one of the following methods:

Erythrocyte transketolase method: This method was developed by Brinn (1965). The enzyme, transketolase requires thiamine pyrophosphate for its activity. It is essential for the metabolism of pentose phosphate sugars. In the assay, haemolysed erythrocytes are incubated with ribose-5-phosphate both with and without added thiamine pyrophosphate (TPP). In thiamine deficiency, the enzyme activity is reduced and a small part of the added pentose-phosphate disappears. When thiamine pyrophosphate is added, the transketolase activity is increased. The per cent increase in transketolase activity due to added thiamine pyrophosphate (TPP) is an index of the degree of thiamine deficiency as indicated below:

Thiamine status	Increase due to added TPP (%)
Normal subjects	0-15
Marginally deficient	15-25
Moderate deficiency	>25

Normal individuals have an erythrocyte transketolase activity of 850-1000 μg hexose/ml hydrolysate/hr.

Urinary excretion test: Excretion of thiamine is related to the intake. Hence, determination of thiamine in urine in a fixed period of 6 hrs. or expressing it as thiamine (μg) excreted per gram of creatinine excreted has been found to be reliable index of the thiamine nutrition status of individuals (I.C.N.N.D. 1963).

Dietary sources

Whole cereals, pulses (legumes), oilseeds and nuts are good sources of thiamine. Rice polishings, wheat germ and dried yeast are rich sources. Meat, fish, eggs, milk, vegetables and fruits are fair sources. The thiamine contents of different foods (Swaminathan 1946) are given in appendix (Table 1 A). A short summary of the data is given in Table 9.13.

Table **9.13 Thiamine content of foods**

Foodstuff	Thiamine (mg/100g)	Foodstuff	Thiamine (mg/100g)
Rich sources		*Fair sources*	
Dried yeast	3-6	*Milled cereals*	
Rice polishings (from raw paddy)	2-3	Raw milled rice, wheat flour and pearled barley	0.07-0.12
Wheat germ	1.5-2.5	Vegetables	0.04-0.15
Good sources		Fruits	0.02-0.06
Whole cereals (whole wheat, millets, oats etc)	0.4-0.6	Milk	0.05
Legumes (chickpea, pigeon pea etc)	0.45-0.6	Meat and fish	0.11-0.18
Oilseeds and nuts (soybean, peanut, sesame seeds, cashewnut)	0.65-1.1		
Liver	0.3-0.4		

It is evident that the most important sources of thiamine in the diet are whole cereals, legumes, nuts and oilseeds, egg, milk, meat and fish.

Requirements

Thiamine is essential for carbohydrate metabolism. Since carbohydrates supply more than 70 per cent of energy in the diets of low income groups, it is reasonable to relate the thiamine requirements to calorie intake. The FAO/WHO Expert Group on vitamin requirements (1967) reviewed the available evidence and concluded that 0.33 mg of thiamine per 100 KCal represents the requirements and recommended 0.4 mg/1000 KCal to take care of individual variations. The literature on thiamine needs in maternal and child nutrition suggests an increased need for thiamine during pregnancy. The N.R.C., U.S.A. recommended an additional allowance of 0.2 mg/day during pregnancy. Since milk secreted by lactating women contains about 0.13 mg, an additional allowance of 0.5 mg/day has been recommended by N.R.C., U.S.A. The Nutrition Expert Group (ICMR) have made similar recommendations which are given in Table 9.14.

Table 9.14 **Thiamine requirements (Nutrition Expert Group, ICMR)**

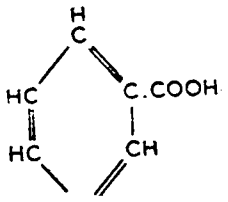
Group	Particulars	Thiamine (mg/day)
Men	Sedentary work	1.2
	Moderate work	1.4
	Heavy work	2.0
Women	Sedentary work	1.0
	Moderate work	1.1
	Heavy work	1.5
Pregnancy	Second half of pregnancy	+ 0.2
Lactation		+ 0.4
Infants :	0-6 months	0.3
	7-12 ,,	0.4
Children	1 year, 2 years, 3 years	0.6
	4-6 years	0.8
	7-9 years	0.9
	10-12 years	1.0
Adolescents :	13-15 years : Boys	1.3
	Girls	1.1
	16-18 years : Boys	1.5
	Girls	1.1

pellagra in human beings made the important discovery that pellagra can be cured by the administration of dried yeast or liver to the subjects. Later, they showed in 1928 that boiled yeast extract can cure pellagra. The pellagra preventive factor was defined as a heat stable vitamin of the B-complex and was named vitamin B_2. Another important step which helped rapid progress in the identification of the pellagra preventive vitamin was the observation that the diet producing pellagra in human beings will produce a disease called 'black tongue' in dogs. Ruffin and Smith (1934) found that a crude extract of liver was effective in curing human pellagra. In 1937, Elvehjem and co-workers isolated nicotinic acid from liver and made the important discovery that it cured black tongue in dogs. Later in 1937, three groups of workers (Spies *et al.*, Fouts *et al*, and Smith *et al.*) almost simultaneously but independently reported that nicotinic acid was effective in curing pellagra in human beings.

It may be of interest to mention that nicotinic acid was isolated from rice polishings and yeast by Funk in England and Suziki and co-workers in Japan as early as 1912 while attempting to isolate th anti-beri beri vitamin. The studies of Warburg and co-work (1934-35) and of von Euler and co-workers (1935-36) showed nicotinamide was a component of coenzyme I and II involv alcoholic fermentation in yeast and hydrogen transporting en in the tissues. But none of these studies gave any clue th be one of the vitamins.

Chemistry and properties

The chemical structures of nic are given in Figs. 9.11 and 9.12.



Nicotinic acid is sparingly soluble in water (1 g in 60 ml water at 25°C). It is freely soluble in alkali hydroxides and carbonates forming salts. It is stable to heat and not destroyed by autoclaving at 120°C for 20 minutes in acid or alkaline medium. Nicotinamide is highly soluble in water (100 g in 100 ml water). When heated or autoclaved in a strong alkaline or acid solutions, it is converted into nicotinic acid. It is stable to heat and not destroyed by autoclaving at 120°C for 20 minutes. Nicotinamide exists in human and animal tissues mostly as a constituent of coenzymes I and II. Coenzyme I (also known as DPN and now called NAD) is nicotinamide adenine dinucleotide while coenzyme II (also known as TPN and now called NADP) is nicotinamide adenine dinucleotide phosphate.

Absorption and storage: Nicotinic acid and nicotinamide are rapidly absorbed from the intestines through the portal vein into the general circulation. It is passed on to all tissues in the body where it is converted into NAD and NADP. Excess nicotinic acid is not stored in the body but is excreted in urine mostly in the form of N-methyl nicotinamide, N-methyl-6-pyridone-3
rboxamide and also in the free form. When large quantities
icotinic acid is administered, it is also converted into trigonel-
(N-methylnicotinic acid) and nicotinuric acid (a compound of
nic acid and glycine).

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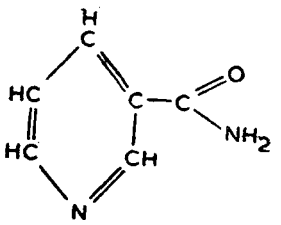


Fig. 9.12 **Nicotinamide**

Plate X: Left : Pellagrous dermatitis in forearms and arms

Right : Pellagrous dermatitis on face and neck (casal's necklace).

Effects of deficiency (Pellagra)

Nicotinic acid deficiency causes the disease, pellagra in human beings. The disease is characterised by three D'S-dermatitis, diarrhoea and dementia (mental symptoms). In addition, glossitis and stomatitis are usually present.

Digestive system: Glossitis and diarrhoea are the two outstanding symptoms. The glossitis and stomatitis vary from a mild redness, soreness and smoothness of the tongue and mouth to an extreme inflammation with fiery red mucosa and tongue ulceration and secondary infection of the tongue and buccal mucosa. Nausea and vomiting are seen in most cases. The diarrhoea may range from a few to several loose stools a day with blood and mucus.

Skin: The dermatitis is the most characteristic of the disease. It is symmetrical in distribution. In early stages, a bright red erythema resembling sunburn occurs over the exposed parts of the body. The commonest sites are the back of the fingers and hands, the forearms, dorsum of the feet and ankles and the neck. In the beginning, the skin is red and slightly swollen. The lesion may worsen by the formation of vesicles and bullae with cracking of the skin. Secondary infection is always present. With improvement, the skin becomes dry, less red and the surface desquamates. The dermatitis is precipitated by exposure to sunlight. (Plate X)

Nervous system: Delirium is the commonest severe mental disturbance in acute pellagra. Dementia is more frequently seen in the chronic cases. Milder mental disturbances consisting of irritability, change in disposition, depression, inability to concentrate, poor memory are more common in the mild cases. The symptoms of postero-lateral tract degeneration, ataxia, spasticity and the involvement of the bladder and rectal sphincters are seen in chronic cases.

Methods of assay

The methods available for the assay of niacin may be classified as follows: (*i*) Chemical (*ii*) Microbiological and (*iii*) Biological methods.

Chemical methods

(*a*) *Colorimetric method for niacin:* A chemical method for the assay of niacin in foods, urine and tissues based on Konig's reaction was first reported by Swaminathan (1938). The same author (Swaminathan 1940, 1941) studied the effect of the following modifications on the accuracy of the method:—(*i*) the effect of acid or alkaline hydrolysis (*ii*) the use of different aromatic amines for the development of colour and (*iii*) the effect of pH of the medium on the development and stability of colour. The method consists of the following steps (1) Extraction of niacin and derivatives with dilute acid at 80°C (2) Hydrolysis of niacinamide to niacin (3) Removal of interfering substances and colouring matter using lead acetate or zinc hydroxide and (4) Development of colour by reacting with cyanogen bromide and aniline at neutral pH in aqueous medium. Various modifications of the method have been reported by several workers (American Association of Vitamin Chemists 1966).

(*b*) *Determination of N'-methyl nicotinamide in urine:* The important metabolite of niacin and niacinamide occurring in urine is N'-methyl nicotinamide. A fluorimetric method has been developed by Hochberg, Melnick and Oser (1945). The method involves adsorption of the metabolite from aliquots of urine at pH 4.5 on a column of synthetic zeolite, elution of the metabolite with potassium chloride solution, alkalinization, extraction of the resulting compound with n-butanol and measurement of the fluorescence.

Microbiological method

A method based on the growth of *Lactobacillus arabinosus* (also known as *L-plantarum*) was developed by Elvehjem and coworkers (1943). The method is highly sensitive. The response of the organism to known amounts of standard niacin ranging from 0.15 to 0.75 μg. is found out. The growth of the organism is assessed by titrating the quantity of lactic acid produced with sodium hydroxide. A standard curve is drawn using the data obtained. The response of the bacteria to known amounts of the food extract containing niacin (within the range of 0.15 to 0.75 μg) is also determined. By comparing the response with the test

food with the standard curve, the quantity of niacin present can be calculated.

Biological method

Animal assays for niacin present yield only roughly quantitative results. Puppies and chicks have been used. The chick is more convenient as a larger number can be used. The growth response of niacin deficient chicks to two levels of standard niacin and two or three levels of the unknown food is determined. By comparing the growth response to standard niacin with that obtained for unknown foods, the niacin content of the food can be calculated.

Detection of sub-clinical niacin deficiency

The important metabolites of niacin and niacinamide occurring in urine are N methylnicotinamide and N-methyl-6 pyridone-3 carboxamide. The method for the latter metabolite is considerably more cumbersome than that for N-methylnicotinamide. Hence, only the assay of N-methylnicotinamide in urine is recommended by ICNND (1963) for the detection of niacin deficiency. Values for N-methylnicotinamide excretion of 0.5-1.59 mg/g creatinine in adults are considered low, indicative of dietary inadequacy of niacin. For pregnant women, N-methylnicotinamide values in urine of 0.5-1.59 mg during the 1st trimester, 0.6-1.99 mg during the 2nd trimester and 0.8-2.49 mg during the 3rd trimester, are considered low, indicative of inadequate dietary niacin intake.

Dietary sources

Data regarding the niacin content of foods have been published by several workers (Teply *et al.*, 1942; Swaminathan 1944). These have been summarised in food composition tables published in different countries. The niacin contents of some foods are given in Table 1A in appendix and are summarised in Table 9.17. The richest natural sources of niacin are dried yeast, rice polishings, liver and peanut. Whole cereals, legumes, meat and fish are good sources. Milled cereals, milk, eggs and vegetables are fair sources. The important sources of niacin in the average diets are cereals, pulses, meat, fish, milk, eggs, nuts and oilseeds.

Table 9.17 **Important sources of niacin**

Foodstuffs	Niacin (mg/100g)	Foodstuffs	Niacin (mg/100g)
Rich sources :		*Fair sources :*	
Dried yeast	25-35	Milled cereals	0.5-1.2
Liver	16-20	Maize	0.6-1.2
Rice polishings		Roots and tubers	0.5-1.0
(from raw rice)	16-18	Other vegetables	0.2-0.6
Peanut (groundnut)	14-15	Milk	0.2
Peanut flour	19-20	Egg	0.2
Good sources :			
Whole cereals	3-5		
Legumes	2-3		
Meat	6-7		
Fish	3-4		

Niacin equivalent of tryptophan present in dietary proteins

Tryptophan present in the dietary proteins is converted into niacin in the human and animal body. It has been estimated that 60 mg of tryptophan yield 1 mg. niacin. So, in addition to the preformed niacin present in foodstuffs, the tryptophan present will also provide additional niacin (Table 9.18).

Tabl 9.18 **Niacin equivalent of some foods**

Food	Niacin mg/1000KCal	Tryptophan* mg/1000KCal	Niacin equivalents (Free niacin + niacin* from tryptophan) mg 1000 KCal
Cow's milk	1·2	673	12.4
Human milk	2·5	443	9.7
Beef	24.7	1280	46.0
Egg, whole	0.6	1150	19.8
Wheat flour, white	2.5	297	7.4
Corn grits	1.8	70	3.0
Corn	5.0	106	6.7

* 1 mg niacin derived from 60 mg tryptophan.

Maize and pellagra

Pellagra was endemic in countries where maize was the staple food of the low income groups. Studies of Goldberger and co-workers showed that good quality protein like milk proteins can also cure pellagra. They were at first inclined to believe that the low tryptophan content of maize protein was the cause of pellagra and good quality proteins like milk proteins provided this amino acid. This theory gained support from Wilson (1927) who was working on pellagra in Egypt. Later, they found that yeast extract which is almost free from protein could cure pellagra. They postulated the presence of a pellagra preventive vitamin in yeast. Recent studies have shown that the observations of Goldberger that pellagra was cured by good quality protein as well as by a vitamin are correct. Soon after the discovery that nicotinic acid was identical with the pellagra preventive vitamin and could cure pellagra, studies were carried out by various workers on the nicotinic acid content of maize and other cereals. Aykroyd and Swaminathan (1940) pointed out that poor maize diets which gave rise to pellagra contained more nicotinic acid than poor rice diet consumed in other regions where pellagra was rare. They concluded that there must be some difference between rice and maize other than the nicotinic acid content. Recent studies have shown that tryptophan present in proteins is converted into niacin in the body of man and animals and 60 mg of tryptophan can give rise to 1 mg of niacin. On this basis, one litre of milk, which contains only 1 mg of preformed nicotinic acid, will provide an additional amount of 8 mg of nicotinic acid formed in the body from 480 mg of tryptophan present in 35 g proteins contained in it. This will explain the curative action of milk in pellagra reported in 1926 by Goldberger and co-workers.

High leucine content of maize and sorghum and its relation to pellagra

The extensive studies of Gopalan and co-workers (1960-69) in India have shown that the high leucine content of maize is one of the major factors contributing to the development of pellagra on maize diets. They have shown that leucine interferes in the conversion of tryptophan to niacin and also in the conversion of

niacin to NAD and NADP in the tissues. They found that poor kaffir corn (*Sorghum vulgare*) diet which is rich in leucine like poor maize diet causes pellagra in human beings and black tongue in dogs. They also found that a synthetic diet containing casein with added leucine can produce black tongue in dogs. It may be concluded that the nutritional defects in maize giving rise to pellagra are (*i*) low nicotinic acid content and (*ii*) low tryptophan and high leucine contents of maize proteins. It is also possible that poor maize diets are deficient in riboflavin, folic acid and vitamin B_{12} in addition to niacin which may be responsible for the anaemia, spinal cord changes and scrotal dermatitis observed in subjects suffering from pellagra.

Requirements

Since tryptophan present in dietary proteins is converted into niacin in the tissues, a consideration of niacin requirements should also include the niacin provided by the tryptophan in addition to free niacin present in the diet. Horwitt (1955) introduced the term 'niacin equivalent' of tryptophan. The FAO/WHO Expert Group on vitamin requirements (1967) adopted the value of 60 mg. of tryptophan as equivalent to 1 mg of niacin. The contents of niacin, tryptophan and niacin equivalents (niacin+niacin from tryptophan) of some foods are given in Table 9.18.

Studies carried out with human beings on corn diets have shown that 4.4 niacin equivalents per 1000 KCal approximate to the minimal requirements for the prevention of clinical signs of niacin deficiency. The FAO/WHO Expert Group on vitamin requirements recommended that a niacin equivalent of 6.6 mg per 1000 KCal per day will be adequate for maintaining normal health. The Nutrition Expert Group of ICMR India also adopted the above recommendation. The niacin requirements of humans recommended by the above committee are given in Table 9.19.

Therapeutic uses

Nicotinic acid when taken orally in doses of 100 to 200 mg or intramuscularly in doses of 20 to 30 mg produces a transient vasodilatation of the cutaneous vessels, particularly of the ears, face, neck, upper extremities and trunk in most human beings. The reaction is accompanied by itching, burning, and tingling of the

Table 9.19 **Daily requirements of niacin (ICMR, 1968)**

Group	Age and activity		Free niacin + niacin from tryptophan (mg)
Men	Sedentary work		16
	Moderate work		19
	Heavy work		26
Women	Sedentary work		13
	Moderate work		15
	Heavy work		20
Pregnancy	Second half of pregnancy		+ 2
Lactation			+ 5
Infants :	0-6 months		5
	7-12 months		7
Children	1 year 2 years 3 years		8
	4-6 years		10
	7-9 years		12
	10-12 years		14
Adolescents	13-15 years	Boys	17
		Girls	14
	16-18 years	Boys	21
		Girls	14

affected areas. These reactions last for a few seconds to a minute. In spite of the reaction, blood pressure, pulse rate and body temperature are normal. Nicotinamide does not cause the above reaction. Hence, it is safe to administer niacinamide for the treatment of pellagra. In severe cases of pellagra, nicotinamide can be administered intramuscularly in doses of 50 mg twice daily for 3 to 4 days followed by oral dose of 100 mg thrice daily for about 2 to 3 weeks till the disease is cured. A maintenance dose of 50 mg daily may be continued for a month. The diet should contain large amounts of milk, liver, peanut, eggs, meat and fish in addition to cereals.

Toxic effects

As mentioned above, nicotinic acid should not be administered in large doses to patients as it causes a transient flushing and burning sensation that may alarm the patient. It is safer to administer

nicotinamide. Nicotinamide in oral doses of 100 mg, 4 times daily or by intramuscular injection of 50 mg twice daily is safe for use in the treatment of pellagra.

Prevention of pellagra

In regions, where pellagra is common, nicotinamide tablets (30 mg/tablet) should be distributed to the people. The staple food, usually corn or sorghum flour should be fortified with nicotinic acid. The people should be encouraged to consume peanuts, meat and liver which are rich sources of niacinamide. Improved strains of maize and kaffir corn with higher quantities of tryptophan and niacin and lower amounts of leucine should be introduced in these areas.

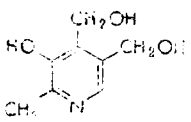
Vitamin B_6 (Pyridoxin and Related Compounds)

History

Pyridoxine is one of the vitamins of the B_2 complex which prevents and cures dermatitis in rats fed on a vitamin B_2 deficient diet. It was isolated in 1938 in a pure form by five different groups of workers (Lepkovsky, 1938; Kerseztesy and Stevens, 1938; Gyorgy, 1938; Kuhn and Wendt, 1938; Itiba and Miti, 1938). It was synthesised in 1939 independently by two groups of workers (Kuhn *et al.*, 1939, Kerseztesy *et al.*, 1939) in Germany and U.S.A. respectively.

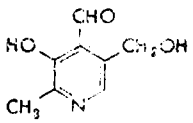
Chemistry and properties

The structures of pyridoxine and two other related compounds —pyridoxal and pyridoxamine are given in Figs. 9.13, 9.14 and 9.15.



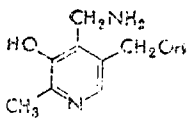
Pyridoxine

Fig. 9.13



Pyridoxal

Fig. 9.14



Pyridoxamine

Fig. 9.15

Pyridoxine contains a pyridine nucleus, two primary alcoholic groups and one phenolic hydroxyl group. Pyridoxal contains an aldehyde group in place of one primary alcoholic group and pyridoxamine contains a primary amine side chain in place of a primary alcoholic group.

Pyridoxine is readily soluble in water. When pyridoxine in neutral or alkaline solution is exposed to light, it is slowly destroyed. When a neutral or alkaline solution of pyridoxine is autoclaved at 120°C for 30 minutes, partial destruction occurs. Since it contains a phenolic hydroxyl group, it reacts with phenol reagent or diazotized sulphanilic acid or 2:6 dichloroquinone chlorimide giving coloured compounds.

Absorption and storage

Pyridoxine is rapidly absorbed from the small intestines. Pyridoxine ingested over the daily needs is not stored in the body but is excreted in urine or metabolised in the tissues.

Physiological functions

Pyridoxine, like other vitamins, functions as a co-enzyme. This co-enzyme is 5-phosphate of pyridoxal. Pyridoxine, pyridoxal and pyridoxamine are converted into 5-phosphate of pyridoxal in the tissues. The most important physiological functions of pyridoxine are as follows:

(*i*) It is essential for growth of infants and several species of animals

(*ii*) Vitamin B_6 deficiency produces degeneration of the nerves, leading to the development of epilyptic form of fits and

(*iii*) Vitamin B_6 deficiency produces microcytic anaemia in animals.

Biochemical functions

Pyridoxal-phosphate acts as a coenzyme for a number of enzyme systems mentioned below:

Transaminase system: The transaminases represent a large number of enzymes. Two important systems are the following:

Glutamic acid + Oxaloacetate → α-Ketoglutarate + Aspartate

Alanine + α-Ketoglutarate → Glutamic acid + Pyruvic acid

Amino acid decarboxylase: These enzymes convert amino acids to the corresponding amines.

Histidine → Histamine + CO_2

Tyrosine → Tyramine + CO_2

Conversion of tryptophan to niacin: The various stages involved in the conversion of tryptophan to niacin are as follows:

Tryptophan → Kynurenine → Hydroxy Kynurenine

↓

Niacin ← 5-Hydroxy anthranalic acid

In pyridoxine deficiency, hydroxy kynurenine is not converted into hydroxy-anthranalic acid and hence niacin is not formed. On the other hand, hydroxy kynurenine is converted into xanthurenic acid and excreted in urine.

Muscle phosphorylase: It contains pyridoxal phosphate.

Dehydrases (Hydrolysases): Dehydrases are important in the catabolism of threonine, serine and homoserine.

Serine + H_2O → Pyruvic acid + NH_3 + H_2O

Racemases: Racemases convert D-amino acids to L-amino acids

D-methionine → L-methionine.

Porphyrin synthesis: Pyridoxal-phosphate is required for the synthesis of δ-aminolevulinic acid, an important intermediate in the synthesis of porphyrin and haem nuclei.

Neurohormones: Pyridoxal-phosphate is essential for the formation of several neurohormones viz., serotonin (from hydroxy-tryptophane), α-amino butyric acid, epinephrine and histamine.

Hypercholesterolemia: Relationship between B_6 deficiency and hypercholesterolemia and atherosclerosis in monkeys has received considerable attention although the exact role of pyridoxine in this condition is not clear.

Oxaluria: Vitamin B_6 deficiency produces oxaluria in experimental animals.

Immune bodies: Immune response is impaired in vitamin B_6 deficiency.

Coenzyme A synthesis: Pyridoxine is involved in the synthesis of coenzyme A from pantothenic acid. In pyridoxine deficiency, coenzyme A level in liver is reduced.

Effects of deficiency

Pyridoxine deficiency has been produced in the rat, dog and monkey and also in man. A brief account of the symptoms is given below.

Rats: The rat loses body weight and develops symmetrical dermatitis in the peripheral areas of the body such as the tail, paws, nose, mouth and ears. The kidneys are damaged. The adrenal glands are enlarged. Anaemia is invariably present. The rat develops nervous disorders which lead to convulsive seizures. These seizures are characterised by hyper-excitability, circular running, and convulsions. All the above symptoms were cured by the administration of pyridoxine.

Dogs: Young puppies fed on a pyridoxine deficient diet developed anaemia, loss of weight, anorexia. When deoxypyridoxine was fed, they developed epileptic form of convulsions.

Pigs: Pigs fed on a pyridoxine deficient diet failed to grow, developed anorexia, anaemia and epileptic form of fits.

Monkeys: Monkeys fed on a pyridoxine deficient diet lost body weight and developed hyperchromic microcytic anaemia. They developed anorexia and thinning of the fur was observed. They did not develop fits. Sclerotic lesions developed in the arteries of the deficient animals.

Adult humans: Pyridoxine deficiency was produced in 8 human subjects by Vilter and co-workers (1950, 1953) by the administration of deoxypyridoxine for 18-55 days. Soborrhoea-like skin lesions developed about the eyes, nose and mouth within 2-3 weeks in 7 out of 8 patients. One patient developed nausea, vomiting, weakness and dizziness. All the symptoms were cured when pyridoxine was administered for a week.

Infants: Several workers have reported that infants receiving proprietary canned (autoclaved) condensed milk food low in pyridoxine, developed nervous irritability and convulsive seizures. A greater part of the pyridoxine present in milk was destroyed during autoclaving. The infants were cured by the administration of pyridoxine.

Methods of assay

Vitamin B_6 occurs in foods in different forms viz., pyridoxine, pyridoxal and pyridoxamine and their phosphorylated forms. Methods available for the assay of vitamin B_6 may be grouped as follows: (*i*) Microbiological (*ii*) Chemical and (*iii*) Biological. The different forms of vitamin B_6 have the same biological activity in the animal. Microbiological methods are used widely as they are very accurate and rapid.

Microbiological methods

The relative activity of various forms of vitamin B_6 to different microorganisms is given below:

Vitamin B_6 forms	Streptococcus fecalis	Lactobacillus casei	Saccharomyces Carlsbergensis	Neurospora Sitophila
Pyridoxine	1.0	1.0	1.0	1.0
Pyridoxamine	6500	8.2	1.1	1.1
Pyridoxal	4500	1170	1.2	1·1

It is evident that *Saccharomyces carlsbergensis* and *Neurospora sitophila* can be used for determining the total vitamin B_6 activity.

(*a*) *Assay of total vitamin B_6 using yeast* (*Saccharomyces carlsbergensis 4228*). This method was evolved by Atkin *et al.*, (1943). The organism responds equally to the three forms of vitamin B_6. Since they may occur in the phosphorylated condition, the material is treated with phosphatase to hydrolyse these esters. Because of the lability of vitamin B_6 to strong light, all the samples should be kept away from strong light. The growth response of the organism to varying levels of pyridoxine ranging from 0-50 mμg. and to known amounts of the extract of the food is determined. The vitamin B_6 potency of the material is calculated by comparison with the standard curve.

(*b*) *Differential assay of pyridoxine, pyridoxal and pyridoxamine:* Using three different organisms, it is possible to estimate the quantity of pyridoxine, pyridoxal and pyridoxamine present. The growth of *lactobacillus casei* is supported only by pyridoxal,

while pyridoxine and pyridoxamine have negligible activity for this organism. For *streptococcus fecalis*, pyridoxal and pyridoxamine are equally active while pyridoxine has negligible activity. With the use of the above two organisms, in conjunction with *Saccharomyces carlsbergensis*, it is possible to estimate them individually.

Chemical methods

Although a number of chemical methods have been developed by different workers (Swaminathan, 1940; Scudi, Koones and Keresztesy, 1940) they are not quite specific for the vitamin.

(*a*) *Diazotized sulphanilic acid test :* A colorimetric method was described by Swaminathan (1940). It was applied to the determination of vitamin B_6 in some foodstuffs, animal tissues and urine. The method yielded reliable data. Since, three members of the vitamin B_6 group give different colours viz., pyridoxine (orange colour), pyridoxamine (orange to pink colour) and pyridoxal (yellow colour), the method could not find general application (Ormsby *et al.*, 1947).

(*b*) *Indophenol test:* A blue colour develops when pyridoxine is reacted with 2:6 dichloroquinonechlorimide. (Scudi *et al.*, 1940; Gird *et al.*, 1942). This was the basis of a colorimetric method. Melnick and co-workers (1945) found that pyridoxamine and pyridoxal gave only about 31 per cent and 15 per cent of the colour given by pyridoxine. Hence, this method could not be used widely.

Biological methods

The three different forms of vitamin B_6 have the same growth promoting activity in rats, chicks and rice moth larvae.

(*a*) *Rat growth test:* Assay procedures based on the growth of rats have been widely used (Edgar *et al.*, 1938). Groups of young rats are fed on a vitamin B_6 deficient diet. Half the number are given known amounts of pure vitamin B_6 while the remaining are given known amounts of the test food. By comparing the growth response obtained on the test food with that obtained on pure vitamin B_6 supplement (0-10μg per rat) the vitamin B_6 potency of the test food can be calculated.

(*b*) *Chick growth test:* Growth test using chicks have been used (Sarma *et al.*, 1948). The test is carried out in the same way as the rat test.

(*c*) *Rice moth larvae growth test:* This method was developed by Sarma (1943). The rice moth larvae is fed on vitamin B_6 deficient diet. The growth rate of the larvae to known amounts of vitamin B_6 and the test food is determined. By comparison of the growth response of the larvae on the test food with that on vitamin B_6 supplement, the vitamin B_6 potency of the test food can be calculated.

Dietary sources

Vitamin B_6 occurs in foods in the form of pyridoxine, pyridoxal and pyridoxamine. Vitamin B_6 contents of foods are given in Table 2A in appendix and are briefly discussed below:

Rich sources: Dried yeast, rice polishings, wheat germ and liver are rich sources.

Good sources: Whole cereals, legumes (pulses), oilseeds and nuts, egg, milk, meat and fish and green leafy vegetables are good sources.

Fair sources: Milled cereals and their products, roots, tubers, other vegetables and fruits are fair sources.

It is evident from the data given in Table 9.20 that whole cereals, legumes, nuts and oilseeds, meat, fish, egg and milk are the important sources of vitamin B_6 in the diet.

Requirements

Human aduits fed on vitamin B_6 deficient diets (with added deoxypyridoxine) developed signs of deficiency in one to three weeks. Repletion studies indicated that approximately 1.25 to 2 mg/day would cure the deficiency (Baker *et al.*, 1964). More recent studies by the same group of workers, have indicated that for a diet containing 100 g. of proteins, the requirement is 1.25 mg. Vitamin B_6 requirements are related to the protein intake and not to calorie intake. In other study, it has been reported that for a diet providing 168 g. proteins an intake of 1.93 mg vitamin B_6 does not prevent the abnormal excretion of xanthurenic acid after a test dose of tryptophan, whereas 2.76 mg. vitamin B_6/day

Table 9.20 **Vitamin B_6 content of foods**

Foodstuffs	mg/100g	Foodstuffs	mg/100g
Rich sources:		*Good sources:*	
Dried yeast	0.7-4.0	Whole cereals	0.3-0.5
Rice polishings	0.6-0.8	Legumes	0.2-0.5
Wheat bran	1.1-1.3	Nuts and Oilseeds	0.3-0.6
Wheat germ	0.8-1.4	Milk powder, whole	0.4-0.7
Liver, (sheep, beef or pig)	0.5-0.7	,, skimmed	0.5-0.8
		Egg, whole	0.5-1.0
		Meat	0.2-0.3
		Milk, fresh	0.06-0.12
		Leafy vegetables	0.2-0.3
		Fair sources:	
		Milled cereals	0.04-0.10
		Fruits	0.02-0.06
		Vegetables	0.02-0.07

prevents. The recommendations of the N.R.C., U.S.A. for the different age groups are given in Table 9.21.

Table 9.21 **Daily requirements of Vitamin B_6 (N.R.C., U.S.A. 1968)**

Group	Particulars		Vitamin B_6 (mg/day)
Men	Sedentary work, Moderate work, Heavy work		2.0
Women	Sedentary work, Moderate work, Heavy work		2.0
Pregnancy	Second half of pregnancy		2.5
Lactation			2.5
Infants	0-6 months		0.2
	7-12 ,,		0.3
Children	1 year		0.4
	2 years		0.5
	3 years		0.6
	4-6 years		0.7
	7-9 years		0.9
	10-12 years		1.2
Adolescents	13-15 years	Boys	2.0
		Girls	2.0
	16-18 years	Boys	2.0
		Girls	2.0

Therapeutic uses and toxicity

Vitamin B_6 has been used in doses of 50-200 mg. in adults by oral or intramuscular route for over two months without any ill-effects (Jolliffe, 1941; Stone, 1944). Prolonged daily administration of pyridoxine to rats (25 mg/kg), dogs (20 mg/kg) and monkeys (10 mg/kg) failed to cause any toxic manifestations or pathological changes in tissues. Rats receiving 2.5 mg/kg were raised through three generations. The rates of growth of the rats were normal. It may be concluded that pyridoxine in daily doses of 50-200 mg. in adults and 2-5 mg in infants is safe for the treatment of vitamin B_6 deficiency states.

Detection of subclinical deficiency

The following methods have been used by some workers for the detection of sub-clinical vitamin B_6 deficiency in human beings (1) Urinary excretion of pyridoxic acid (2) Tryptophan load test and (3) Transaminase activity in RBC.

(1) *Urinary excretion of pyridoxic acid:* The major metabolite of vitamin B_6 excreted in urine is pyridoxic acid. The excretion of pyridoxic acid is related to the intake of vitamin B_6. Recent studies indicate that estimation of pyridoxic acid content in urine may serve as an index of the vitamin B_6 nutrition status of the individual (Reynolds *et al.*, 1958; Woodring *et al.*, 1964). The daily excretion of pyridoxic acid in normal human subjects ranges from 0.5 to 1.3 mg. per 24 hours. Values below 0.5 mg. indicate deficient dietary intake.

(2) *Tryptophan load test:* The discovery by Lepkovsky in 1942 of the excretion of xanthurenic acid, a product of tryptophan metabolism, in the urine of vitamin B_6 deficient dogs and rats led to the development of tryptophan load test for detection of vitamin B_6 deficiency. (Greenberg *et al.*, 1949; Vilter *et al.*, 1953). A test dose of 10 g. DL-tryptophan (or 5 g. L-tryptophan) is administered orally in the case of adults. For infants and children, a dose of 100 mg L-tryptophan per kg body wt. is given orally. The urine is collected for 24 hours. In normal individuals, the quantity of xanthurenic acid excreted is less than 50 mg. while in subjects deficient in vitamin B_6, the excretion may be between 100-500 mg. depending on the degree of deficiency.

(3) *Transaminase activity in RBC:* Recently, it has been shown that sub-clinical deficiency of vitamin B_6 in adults decreases the serum glutamic-oxaloacetic transaminase activity, (Babcock *et al.*, 1960). But this test appears to be less sensitive than the tryptophan load test.

Pantothenic Acid

History

Pantothenic acid is one of the vitamins of the vitamin B_2 complex which can prevent or cure a specific type of dermatitis (chick pellagra) in chicks fed on vitamin B_2 deficient diet. It was isolated in a pure form as its calcium salt, by Williams and co-workers in 1938. Its synthesis was accomplished in 1940 by several groups of workers.

Chemistry and properties

The chemical structure of pantothenic acid is given in Fig. 9.16. Pantothenic acid is a pale viscous oil. It has not been obtained so far in a crystalline form but its sodium, potassium or calcium salts crystalise readily. They are highly soluble in water. Pantothenic acid is stable to autoclaving at 120°C for 30 minutes in neutral solution but is destroyed rapidly in acid or alkaline medium.

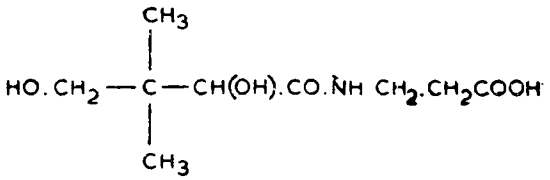


Fig. 9.16 **Pantothenic acid**

Compounds containing pantothenic acid

Coenzyme A: Pantothenic acid exists in tissues in the form of coenzyme A which contains in its molecule pantothenic acid, β-mercaptoethylamine, adenine, ribose and phosphoric acid.

Pantotheine and Pantothine: In 1949, it was reported that a compound containing pantothenic acid stimulated the growth

of *Lactobacillus bulgaricus* to which the name *Lactobacillus bulgaricus* factor (LBF) was given. LBF was found to have the same activity for rats as an equivalent amount of pantothenic acid. LBF has been identified as the compound of pantothenic acid and β-mercaptoethylamine. It exists in two forms i.e., SH form and S.S. form to which the names of pantotheine and pantothine have been given.

CH_2-OH-C $(CH_3)_2$-CHOH-CO-NH-CH_2-CH_2-CO-NH-CH_2-CH_2
Pantotheine

$(CH_2$-OH-C $(CH_3)_2$-CHOH-CO-NH-CH_2-CO-NH-CH_2-$(CH_2)_2$ S
Pantothine

Compounds related to pantothenic acid

D-Pantothenamide: D-Pantothenamide has recently been introduced in the Pharmaceutical industry as a compound with full pantothenic acid activity and having certain advantages when incorporated into dry vitamin preparations, because it is crystalline, stable, free flowing and non-hygroscopic. It has the following structure:

CH_2-OH-C-$(CH_3)_2$ CHOH-CO-NH-CH_2-CH_2-$CONH_2$
D-Pantothenamide

Pantothenyl alcohol (Pantothenol): This was prepared by Pfaltz in 1943. It has the following chemical structure:

CH_2-OH-C$(CH_3)_2$-CHOH-CO-NH-CH_2-CH_2-CH_2-OH
Pantothenyl alcohol

It has the same activity as pantothenic acid for animals and man. It does not, however, promote growth of bacteria.

It was found to be as effective as pantothenic acid in preventing achromotrichia in black rats (Pfaltz, 1943). Pantothenol is converted into pantothenic acid in warm blooded animals (Burley 1944). On prolonged administration of pantothenol, increased amounts of pantothenic acid and coenzyme A were found in tissues and liver (Weiss *et al.*, 1950).

Absorption and storage: Pantothenic acid and its salts are readily absorbed from the small intestines through the portal vein

into the general circulation. When consumed in excess of the requirements, it is not stored in the body, but is excreted in urine or metabolised by the tissues. The pantothenic acid excretion by human beings following a dose of pantothenol was greater than that found with the same amounts of pantothenic acid, the excretion of pantothenic acid in urine being 49-74 per cent of a dose of pantothenol and 23-58 per cent of an equivalent dose of pantothenic acid (Rubin *et al.*, 1950).

Physiological and biochemical functions: The different functions of pantothenic acid are as follows: (*i*) It is essential for the growth of different animals and birds and also for infants and children (*ii*) chicks fed on a diet deficient in this vitamin suffer from a specific dermatitis. (*iii*) Pantothenic acid deficiency interferes with the hatching of eggs in the hen. (*iv*) Pantothenic acid exists in animal and human tissues in the form of Coenzyme A (CoA). This was discovered by Lipmann in 1947. Coenzyme A contains in its molecule pantothenic acid, β-mercaptoethylamine, adenine, ribose and phosphoric acid. CoA participates in a large number of enzyme reactions in the metabolism of carbohydrates, fatty acid synthesis and metabolism, propionate metabolism and the metabolism of branch chain fatty acids and in acetylation reactions. Acetyl CoA (acetyl derivative of coenzyme A) is formed in the oxidation of pyruvic acid and fatty acids. It is involved in many acetylation and condensing and transfer reactions as indicated below:

(*i*) Choline + acetyl CoA ⟶ Acetylcholine + CoA.

(*ii*) Succinyl CoA + acetoacetate ⟶ Succinate + acetoacetyl CoA

(*iii*) Methyl malonyl CoA + pyruvate ⟶ Propionyl CoA + Oxaloacetate

(*iv*) Acetyl CoA + Oxaloacetate ⟶ Citrate + CoA

Effects of deficiency

The effects of pantothenic acid deficiency in certain animals and in human beings are briefly discussed below:

Rats: Rats fed on pantothenic acid deficient diets develop necrotic lesions of the adrenal cortex and other related symptoms. Salt and water balance are affected. A decrease in coenzyme and succinicoxidase activities of liver was observed. The deficient rats were not able to form antibodies in the normal manner.

Chicks: Pantothenic acid deficiency produced keratitis, dermatitis, fatty liver and lesions of the spinal cord. The deficient birds could not reproduce.

Dogs: Pantothenic acid deficiency in dogs produced the following symptoms: retardation of growth, decreased appetite, irritability, sudden prostration or coma, rapid respiratory and heart rate, convulsions and finally death. Pathological examination revealed fatty livers and haemorrhagic degeneration of kidneys.

Pigs: Pantothenic acid deficiency in pigs resulted in loss of weight, dry and rough hair coat and skin and eventually alopecia, diarrhoea, emaciation, loss of appetite, incoordinated movements of the hind legs and spastic gait ('goose-stepping'): Histological examination showed that degenerative changes in the peripheral nerves, posterior root ganglia and the posterior roots and posterior funiculi of the spinal cord. Atrophy of the mucosa cells of small intestines and ulceration of the large intestines were observed.

Monkeys: McCall *et al.*, (1946) reported that pantothenic acid deficiency in rhesus monkeys was characterised by loss of body weight, greying and thinning of the fur, anaemia, diarrhoea and ataxia.

Human beings: Pantothenic acid deficiency has been produced experimentally in man by feeding on a diet deficient in pantothenic acid and by the administration of the antivitamin—Omega-methyl pantothenic acid (Bean *et al.*, 1958, 1960). The visible signs of deficiency included nausea, vomiting, tremor of the outstretched hand and irritability. The biochemical abnormalities include eosinopenic response to adrenocorticotropic hormone and sensitivity to insulin.

Burning feet syndrome: A condition, known as 'burning feet' syndrome was observed in prisoners of war during World War II in Japan and Burma. This syndrome which was also associated with neurological and mental disturbances, did not

respond to treatment with thiamine, riboflavin and niacin. It responded readily to dried brewers' yeast which was a rich source of all B-vitamins (Cruickshank 1946). Gopalan (1946) found that 'burning feet' syndrome observed in Indian subjects responded to treatment with Ca-pantothenate (20-40 mg) administered intramuscularly. He found a mixture of thiamine, riboflavin and niacin was not effective in curing the condition.

Methods of assay

Pantothenic acid occurs in foods and tissues as the free acid and also in the form of coenzyme A, pantotheine and pantothine. In addition to the above, pharmaceutical preparations may contain pantothenol and pantothenamide. The methods available for the assay of pentothenic acid are (1) microbiological (2) biological and (3) physico-chemical. They are briefly discussed below. The methods suitable for the routine assay of foods, tissues, blood and excreta are microbiological methods, while chemical methods can be used for pharmaceutical preparations containing pure pantothenic acid, pantothenol or pantothenamide. The methods of choice are given below:

Materials	Methods
1. Animal and plant foods, blood tissues and excreta	Microbiological 1. *Saccharomyces carlsbergensis* 2. *Lactobacillus plantarum* 3. *Lactobacillus helviticus*
2. Pharmaceutical products	1. Chemical methods 2. Microbiological methods

Microbiological methods: For the assay of pantothenic acid in foods and animal tissues and blood, coenzyme A, pantotheine and pantothine should be hydrolysed to free pantothenic acid by combined treatment with alkaline phosphatase and liver enzymes. The free pantothenic acid is determined using *Saccharomyces carlsbergensis* ATCC 9080. In the presence of asparagine, this organism responds only to pantothenic acid and not to pantotheine.

For the assay of pantotheine, *L-helviticus* is used as the activity of pantotheine is about 100 times that of pantothenic acid, if the organism is maintained in a medium containing pantotheine.

Physico-chemical methods: These methods are very much less sensitive than the microbiological methods and hence can be used only for the assay of pantothenic acid, pantothenol or pantothenamide in pharmaceutical preparations (Gyorgy and Pearson, 1967).

Biological methods: The biological methods are time-consuming and very much less accurate than microbiological methods. Hence, they are not used widely for the assay of pantothenic acid in foodstuffs. The available methods are based on the growth rates of pantothenic acid deficient rats or chicks receiving known amounts of pure pantothenic acid or the test food.

Dietary sources

Pantothenic acid contents of common foods are given in Table 2A in appendix. A summary of the values is given in Table 9.22.

Table 9.22 **Pantothenic acid content of foods**

Foodstuffs	Pantothenic acid (mg/100g)	Foodstuffs	Pantothenic acid (mg/100g)
Rich sources		*Good sources*	
Dried yeast	10-11	Whole cereals	0.6-1.5
Liver (ox, sheep and goat)	7-8	Legumes	0.6-2.2
Rice polishings	3-4	Nuts and Oilseeds	0.6-2.1
Wheat germ	2-3	Egg	1.5-1.6
		Meat	0.3-0.4
		Milk	0.3-0.4
		Fish	0.5-0.6
		Fair sources	
		Milled cereals	0.5-0.7
		Vegetables	0.2-1.0
		Fruits	0.1-0.3

Royal jelly is the richest natural source containing 30 mg/100 g. Rich natural food sources are dried yeast, liver, rice polishings, and wheat germ. Whole cereals, peanut, legumes, milk, meat, eggs and leafy vegetables are good sources. Milled cereals, other vegetables and fruits are fair sources.

Requirements

Data regarding the pantothenic acid requirements of human beings are not available. The following tentative figures are based on the recommendations of the N.R.C., U.S.A.

	Pantothenic acid mg/day/caput
Adults	10
Children	5-8
Adolescents	8-10
Infants	1.5-2.5
Pregnant and lactating women	10-15

Therapeutic uses and toxicity

Pantothenic acid administered in large doses has not produced any ill effects in experimental animals. Rats grew normally when given 200 mg. of calcium pantothenate daily for 190 days. Dogs given 50 mg. pantothenic acid per kg. body weight daily for six months grew normally. Monkeys given 1 g. pantothenic acid for six months showed no ill effects. Intravenous injection of 10 to 50 mg. per kg. of pantothenic acid in cats did not cause any ill effects. Spies *et al.*, (1940) reported that intravenous injection of a solution containing 100 mg. of Ca or Na pantothenate produced no change in the blood pressure, pulse rate, body temperature or respiration of normal human subjects.

FOLIC ACID

History

Folic acid was known under different names from 1933 by its curative effects in different deficiency states in man, monkey, chick, rat, guinea pig and also by its growth promoting effect on microorganisms. Wills (1934) showed that tropical macrocytic anaemia in human beings was cured by a vitamin present in autolysed yeast extract. Day *et al.*, (1935) described leucopenia,

diarrhoea and oral lesions in monkeys as a result of feeding on a vitamin B_2 deficient diet which were cured by a factor present in liver. They called this factor Vitamin M. A new chick growth factor (Factor U) was reported by Stokstad (1938) while Hogan *et al.*, (1939) reported the presence of a factor in liver which cured anaemia in chicks. They called it vitamin B_c. Snell and Peterson (1940) found that two unidentified growth factors present in liver are required by *L-casei*. One of these was adsorbed on norite while the other was not adsorbed. They were accordingly designated norite eluate factor and norite filtrate factor respectively. Mitchell, Snell and Williams (1941) reported the presence of a factor in spinach leaves essential for the growth of *L-casei*. They called this factor-*folic acid* (*folium*-leaf). Day *et al.*, (1943) showed that *L-casei* factor from liver was identical with vitamin 'M' and cured vitamin M deficiency in monkeys. Mitchell, Snell and Williams (1944) purified folic acid to a high state of purity. Stockstad *et al.*, (1946) prepared *L-casei* factor from liver in a crystalline form. Pfiffner *et al.*, (1947) isolated vitamin B_c from liver in a crystalline state. In 1948, Sauberlich and Baumann established the presence of a growth factor for *Leuconostoc citrovorum* in liver and yeast extract.

Keresztesy and Silverman (1950) isolated *citrovorum* factor from liver. The early nomenclature for folic acid is given in Table 9.23.

Table **9.23 Early nomenclature of Folic acid**

Name	Properties and source	Authors
1. 'Wills factor'	Present in autolysed yeast and cures tropical macrocytic anaemia	L. Wills (1933)
2. Vitamin M	Present in liver and cures nutritional cytopenia and diarrhoea in monkeys.	Day *et al* (1935)
3. Factor U	Chick growth factor present in liver	Stockstad (1938)
4. Vitamin Bc	Present in liver and cures anaemia in chicks	Hogan *et al* (1939)
5. Norite eluate factor (*L-casei*)	Present in liver and essential for growth of *L-casei*	Snell and Peterson (1940)
6. Folic acid	Present in spinach and essential for growth of *L-casei*	Mitchell, Snell and Williams (1941)

Chemistry and properties

The chemical structure of folic acid (Fig. 9.17) was established and its synthesis was accomplished by Angier *et al* (1946). The structure of *citrovorum* factor (5-formyl-tetrahydrofolic acid) was established by May *et al* (1951).

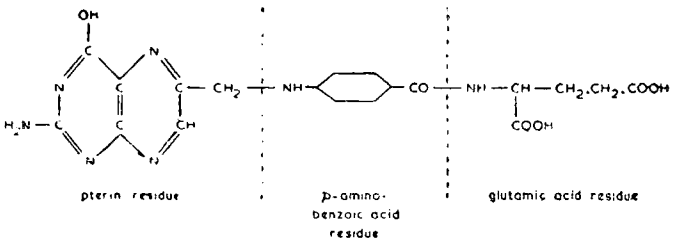


Fig **9.17 Pteroylglutamic acid (Folic acid)**

Folic acid (Pteroylglutamic acid) is an yellow crystalline substance. The molecule consists of a pteridine group linked through p-aminobenzoic acid and L-glutamic acid. The pteridine group linked with p-aminobenzoic acid is called pteroic acid. *Citrovorum* factor is 5-formyl tetrahydrofolic acid. Folic acid is slightly soluble in water (1 mg./100 ml at 0°C and 50 mg/100 ml at 100°C). The disodium salt is soluble to the extent of 1.5 g. per 100 ml. at 20°C. Folic acid is stable to heat at neutral pH. It can be sterilised by heating at 120°C for 30 minutes without loss of activity at neutral pH. But at pH 1.0 a solution of folic acid loses about 70-100 per cent of the activity when autoclaved at 120°C for 30 minutes. When aqueous solution of folic acid is exposed to light, it is rapidly destroyed. Riboflavin accelerates the photo-oxidation of folic acid. *Citrovorum* factor (5-formyl-tetrahydrofolic acid) is also unstable in dilute acid solutions.

Absorption and storage

Folic acid is readily absorbed from the small intestines through the portal vein and passed on to tissues through the general circulation. Folic acid taken in excess of requirements is not stored in the tissues. Liver is the richest source containing about 250 μg/100 g. This value is not increased beyond normal limits when the intake of folic acid is high.

Physiological and biochemical functions:

The different functions are as follows:

(1) It is essential for the maturation of red blood cells. In folic acid deficiency, megaloblastic anaemia develops.

(2) Tetrahydrofolic acid (FAH_4) accepts formyl and hydroxy methyl groups from various sources and acts as an intermediate carrier in the transfer of these groups (single carbon compounds) in biological reactions.

(3) A number of these compounds are known to occur in biological systems. These include 10-formyl FAH_4, 5:10 methenyl FAH_4, 5,10-methylene FAH_4, 5-methyl FAH_4, 5-formyl FAH_4 (*Citrovorum* factor, *Leucovorin*) and 5-formimino FAH_4. The functions of these are as follows:

(*a*) The 2 and 8 carbon atoms of the purine ring are transferred into the ring by 10-formyl FAH_4 and 5, 10 methenyl FAH_4 respectively. The 2nd carbon atom of the imidazole ring and the 2nd carbon atom of the pteridine ring are derived from 10-formyl FAH_4.

(*b*) The CH_2 group of 5, 10-methylene-FAH_4 is utilised in the formation of serine from glycine and in the formation of the methyl group of thymine when thymidylic acid is formed from uracil deoxyribotide.

(*c*) 5-methyl-FAH_4 is formed by hydrogenation of 5, 10-methylene FAH_4 and transfers its methyl group to homocystine to produce methionine.

(*d*) 5-formyl FAH_4 (*Citrovorum* factor, *Leucovorin*) is used in experimental cancer chemotherapy as an antidote to counteract the toxic effects of inadvertent overdosage with folic acid antagonists.

(*e*) 5-formimino-FAH_4 is formed during the degradation of histidine and is deaminated to 5, 10-methenyl-FAH_4 by the enzyme cyclodeaminase.

Effects of deficiency

Various animal species differ markedly in their response to folic acid deficient diets. The chick, the monkey and the guinea pig develop deficiencies on a purified diet low in this vitamin. The

rat, on the other hand, is able to meet its folic acid requirements by intestinal synthesis. Consequently for the production of folic acid deficiency in the rat, bacteriostatic agents like sulphonamides have to be incorporated in the diet to control the growth of intestinal microorganisms.

Rats: Rats fed on a folic acid deficient diet containing sulphaguanadine or sulphasuccidine showed retarded growth rate and haematological changes including leucopenia, agranulocytosis, hypocellularity of the bone marrow and less frequently anaemia. All these symptoms were cured by liver extract or folic acid.

Chicks: A dietary deficiency of folic acid is produced in chicks by feeding on a deficient diet. Reduction in growth rate, macrocytic anaemia, decreased feather growth and depigmentation of feather were the deficiency symptoms observed. Addition of liver extract or folic acid prevented and cured the anaemia and promoted good growth.

Monkeys: The rhesus monkeys fed on a folic acid deficient diet developed anaemia, leucopenia, oral lesions, diarrhoea and ulceration of the colon and increased susceptibility to infections of the intestinal tract. All these symptoms were cured and prevented by liver extract or folic acid.

Folic acid deficiency in man: The most important deficiency syndrome in man due to folic acid deficiency is the development of megaloblastic anaemia. The incidence of this condition in different age groups and their treatment are briefly discussed below. The probable causes leading to the development of megaloblastic anaemia are listed in Table 9.24.

Nutritional megaloblastic anaemia in adults: The classical studies of Wills (1931) in India showed that the megaloblastic anaemia prevalent in pregnant women of the low income groups subsisting on poor vegetarian diets, could be cured by autolysed yeast extract. The haemoglobin content of the blood may range from 6-9 per cent and RBC count from 2-3 million per mm^3 in typical cases. Studies carried out by various workers in India have shown that in a majority of cases, the anaemia could be cured by the administration of 5 mg. of folic acid daily or 5 mg. folic acid and 5 grains ferrous sulphate daily. In a small number of cases, administration of both folic acid 5 mg. orally and vitamin B_{12} (500 micrograms) by intramuscular injection were essential

Table 9.24 **Probable causes leading to the development of macrocytic anaemia**

Megaloblastic anaemia in adults	*Megaloblastic anaemia in children*
1. Deficient intake: Nutritional megaloblastic anaemia	1. Deficient intake:
2. Defective absorption:	(*a*) Nutritional megaloblastic anaemia syndrome of infancy
(*a*) Idiopathic tropical malabsorption, Tropical sprue	(*b*) Nutritional megaloblastic anaemia of Kwashiorkor
(*b*) Secondary malabsorption states	(*c*) Nutritional megaloblastic anaemia.
3. Excessive demands	2. Deficient absorption: Idiopathic tropical malabsorption syndrome, Tropical sprue
(*a*) Pregnancy	3. Excessive demands:
(*b*) Neoplastic condition, haemolytic anaemia, infections	(*a*) Infection
(*c*) Drug therapy	(*b*) Drug therapy

for the cure. It is evident that the primary deficiency in nutritional macrocytic anaemia is folic acid, even though iron deficiency or vitamin B_{12} deficiency may also be associated with the condition.

Megaloblastic anaemia of sprue and other malabsorption syndromes: Megaloblastic anaemia is commonly seen in subjects suffering from malabsorption syndromes. In this condition, folic acid and vitamin B_{12} present in the diet are poorly absorbed. Tropical sprue (malabsorption syndrome) occurs widely among the low income groups in South India. A majority of these patients suffer from megaloblastic anaemia due to deficiency of folic acid and some cases due to deficiencies of folic acid and vitamin B_{12}. The haemoglobin content ranges from 5-8g/100 ml and RBC count between 2.5 to 3 million per mm^3. The treatment consists in the administration of 5-10 mg. folic acid and 5 grains ferrous sulphate daily by the oral route and 500 micrograms of vitamin B_{12} intramuscularly once a week.

Megaloblastic anaemia in infants and children: Megaloblastic anaemia has been reported to occur widely among malnourished children in the developing countries. It has been reported occasionally in U.S.A. in children fed on proprietary infant

foods (Zuelzer and Ogben 1946) and also in Italy among infants fed exclusively on goat's milk. (Wintrobe, 1956). The haemoglobin content is usually low 5-8g/100 ml. blood and RBC count 2.5-3 million per mm^3. The treatment consists in the oral administration of folic acid (1-2 mg daily), iron salts (6 mg iron/kg body weight) and vitamin B_{12} (100 micrograms once a week) intramuscularly.

Methods of assay

Methods available for the assay of folic acid in biological materials and pharmaceutical preparations may be grouped as follows: (1) Physico-chemical (2) Microbiological and (3) Biological.

Physico-chemical methods: These methods are not sensitive enough for application to biological materials. They can be used for the assay of folic acid in pharmaceutical preparations. The minimum quantity of folic acid which can be estimated by these methods is about 20-30 μg. The important methods in this category are (*i*) U.S. Pharmacopea colorimetric method (*ii*) Fluorimetric method of King *et al* (1949) and (*iii*) Paper and thin layer chromatographic methods.

Microbiological methods: The biologically active compounds derived from folic acid and present in biological materials are, folic acid, formyl-tetra-hydrofolic acid (FAH_4) 10-formyl FAH_4 5, 10-methenyl FAH_4, 5, 10-methylene FAH_4, 5-methyl FAH_4 5-formyl FAH_4. All these compounds promote the growth of *L-casei* but not *S-faecalis*. Pteroic acid found in biological materials which has no folic acid activity for the human, does not promote the growth of *L-casei* but promotes the growth of *S-faecalis*. The folic acid conjugate (containing more than 3 glutamic acid) which is biologically inactive for man does not promote the growth of *L-casei*. In view of the above, *L-casei* is the most suitable organism for determining the 'free' folate activity of biological materials.

Biological methods: Biological methods are based on the growth promoting value of folic acid in animals (rats or chicks) fed on a folic acid deficient diet. Two groups of folic acid deficient rats or chicks are given supplements of known amounts of pure folic acid and known amounts of test food respectively. By comparing the growth rates on the test food with that obtained

on pure folic acid supplement, the potency of the test food can be calculated.

Detection of sub-clinical folic acid deficiency

The folic acid content of serum as determined by *L-casei* has been found to be a good index of the folic acid nutrition status of individuals. The serum levels for normal and sub-clinical cases are given below:

Serum L-Casei Folate Levels

Serum Level (mμg/ml)	Interpretation
3	Folate deficiency
3-6	Probable folate deficiency
6-20	Normal
25	Elevated

Dietary sources

The folic acid contents of some common foods are given in Table 9.25. Dried yeast and liver, wheat germ and rice polishings are rich sources. Whole cereals, legumes, green leafy vegetables, cereals, other vegetables, milk and fruits are fair sources.

Requirements

Folic acid occurs in foods in two forms (1) Folic acid, tetrahydrofolic acid and its derivatives and (2) Folic acid conjugate (folic acid polyglutamates). The 'free' folates represent the first group, the 'bound' folate the second group, while the 'total' folates represent both the groups. Among the microorganisms used for the assay of folic acid, *L-casei* responds only to 'free' folates. The conjugates are made available for *L-casei* only after the extract is treated with the enzyme conjugase which hydrolyses the conjugate to 'free' folate. In man, 'free' folate is absorbed almost to the same extent as synthetic folic acid. The 'conjugates' are not well absorbed and utilised in the human system. The Joint FAO/WHO Expert Group on vitamin requirements (1970) and Nutrition Expert Group, ICMR (1968) recommended that folic acid requirements are best expressed in terms of 'free' folate. Their recommendations are given in Table 9.26.

Table 9.25 **Folic acid content of foods***

Foodstuff	Folic acid 'Free' (μg/100g)	Foodstuff	Folic acid 'Free' (μg/100g)
Cereals:		*Other vegetables:*	
Bajra	14.7	Brinjal	5.0
Italian Millet	4.2	Cluster beans	50.0
Jowar	14.0	Cucumber	12.6
Maize, dry	14.0	French beans	15.5
Oatmeal	32.0	Kovai	18.0
Ragi	5.2	Ladies fingers	25.3
Rice, parboiled	8.9	Plantain, green	1.6
Rice, raw, milled	4.1	Pumpkin	3.0
Samai	2.2	Snake gourd	7.5
Varagu	7.4		
Wheat, whole	14.2	*Nuts and Oilseeds:*	
Wheat flour (whole)	12.1	Coconut, dry	15.3
		Coconut, fresh	11.7
Pulses (legumes):		Gingelly seeds	51.0
Bengalgram, whole	34.0	Groundnut	16.0
Bengalgram dhal	32.0		
Bengalgram (roasted)	22.0	*Fruits:*	
Blackgram dhal	24.0	Tomato, ripe	14.0
Cowpea	69.0		
Green gram dhal	24·5	*Fish and other sea foods:*	
Lentil	14.5	Mrigal	9.7
Peas, dry	4.6	Shrimp, fresh	15.7
Red gram	19.0		
Soy bean	8.7	*Other flesh foods:*	
		Buffalo meat	4.6
Leafy vegetables:		Egg, duck	80.0
Amaranth	41.0	Egg, hen	70.3
Cabbage	13.3	Fowl	3.2
Curry leaves	23.5	Goat meat	0.5
Mint	9.7	Liver, goat	61.2
Spinach	51.0	Liver, sheep	65.6
		Mutton	1.0
Roots and Tubers:			
Carrot	5.0	*Milk and Milk products:*	
Colocasia	16.0	Milk, buffalo	3.3
Onion, big	1.5	Milk, cow	5.6
Potato	3.0	Milk, goat	0.7
Yam (ordinary)	0.9	Milk, human	1.3
		Curd, (buffalo milk)	3.3
		Miscellaneous foods:	
		Betel leaves	3.1
		Yeast, dried	150.0

*Nutritive value of Indian Foods—C. Gopalan *et al.* (1971), I.C.M.R. India.

Table **9.26 Recommended daily intakes of 'free' folates**

Age group	FAO/WHO Expert Group (1970)	ICMR Expert Group (1968)
	(μg/day/caput)	
0-6 months	40	25
7-12 ,,	60	50-100
1-12 years	100	50-100
18 years and over	200	100
Pregnancy	400	150-300
Lactation	300	150

Therapeutic uses and toxicity

Folic acid is administered orally in doses of 5-20 mg. daily for the treatment of megaloblastic anaemia in adults. Sometimes it is administered in doses of 2 to 5mg. intramuscularly daily in patients who are very ill. Such doses do not cause any physiological reactions and are well tolerated. The acute intravenous toxicity of folic acid is low. The following values have been obtained for LD 50 in different animals: mice 600; rat, 500; rabbit, 410 and guinea pig, 110 mg. per kg body weight. Since the solubility of folic acid is low, large doses should not be given by the parenteral route as there is danger of crystallisation in the kidney tubules and causing renal injury. Renal injury has been observed in animals receiving large doses (50 mg/kg body wt.) of folic acid intravenously.

VITAMIN B_{12}

History

Minot and Murphy (1926) made the important discovery that liver can cure pernicious anaemia. The active principle was isolated independently by two groups of workers in Great Britain (Smith and Parker, 1948) and in U.S.A. (Rickes *et al.*, 1948) respectively. This was designated vitamin B_{12}. It was found effective in curing pernicious anaemia when administered intramuscularly in small quantities (5-10 μg).

Chemistry and properties

The chemical structure of Vitamin B_{12} is given in Fig. 9.18.

It has a complicated chemical structure. It contains cobalt (4-5 per cent). It has a molecular weight of 1355 and an emperical formula $C_{63}H_{88}N_{14}O_{14}$ PCo. Exposure of an aqueous solution of vitamin B_{12} to sunlight results in the destruction of the vitamin. Vitamin B_{12} exists in liver and other tissues in two forms (1) Cyanocobalamin (vitamin B_{12}) and (2) Hydroxo-cobalamin (Vitamin B_{12a}). Both the forms have the same biological activity in the experimental animals and for the micro-organisms. When cyanocobalamin in solution is autoclaved, it is converted partly to hydroxocobalamin. Since hydroxocobalamin is heat labile, it is destroyed by autoclaving at 120°C for 30 minutes. This will explain the significant deterioration in the potency of cyanocobalamin of a food when autoclaved at 120°C for 30 minutes. Addition of cyanide in small quantities to the food converts the hydroxocobalamin to cyanocobalamin and protects the vitamin from destruction during the preparation of the food extract for assay of the vitamin. If ascorbic acid is added to a solution of vitamin

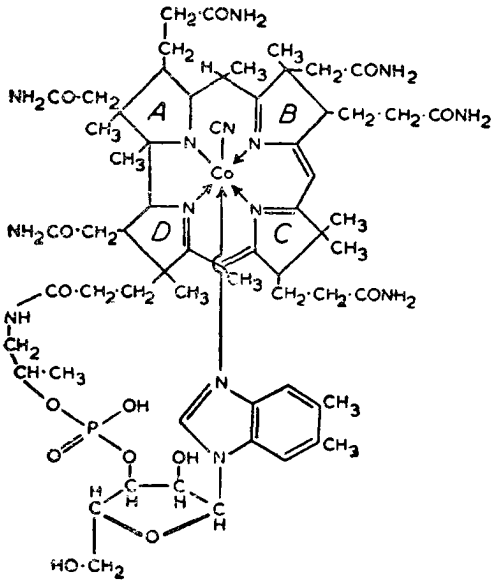


Fig. 9.18 Cyanocobalamin (vitamin B_{12})

B_{12}, the B_{12} activity is slowly destroyed due to the reducing action of ascorbic acid on vitamin B_{12}.

Absorption and storage

Vitamin B_{12} is unique among all essential nutrients in having a highly specialised mechanism for its absorption. Even though the daily requirements are only a few micrograms, its absorption from the intestines requires a factor called 'intrinsic factor (IF) secreted by the stomach. IF binds vitamin B_{12} and the IF-B_{12} complex is absorbed. Many other substances including saliva, lysozyme, nucleic acid, milk and some fractions of the gastric mucosa can bind vitamin B_{12}, but they do not help in the absorption of vitamin B_{12}. Dietary vitamin B_{12} and small oral dose of the vitamin are absorbed by the IF mechanism. The quantity that can be absorbed is limited and is about 1.6 μg from a single dose of 10 μg. Large oral doses are absorbed by diffusion. When 1000 μg of vitamin B_{12} is administered, about 1 per cent is absorbed and serum level rises within 1 hour of administration. Normal subjects have serum levels of 20-90 mμg/100 ml with a total binding capacity of 50-110 mμg/100 ml. so that in normal subjects vitamin B_{12} binding power of serum is 60 per cent saturated. Vitamin B_{12} present in free form in serum is rapidly excreted in urine. It is known that vitamin B_{12} injected in normal human subjects does not appear in urine until the dose exceeds 50 μg. The amount in excess of 50 μg is rapidly excreted in urine. The main storage organ for vitamin B_{12} is liver. Its capacity to store vitamin B_{12} is limited. Vitamin B_{12} given by injection in excess is not stored but is excreted in urine.

Absorption in pernicious anaemia

In normal subjects, a small oral dose of vitamin B_{12} (0.5-1 μg) is almost completely absorbed while in subjects suffering from pernicious anaemia, it is not absorbed unless an intrinsic factor (IF) preparation is given along with it.

Physiological functions

The physiological functions of vitamin B_{12} are as follows:

1. Vitamin B_{12} promotes the maturation of the erythroid cells. Within a few hours of the injection of vitamin B_{12} the bone

marrow begins to change its appearance. The earlier megaloblastic cells which are the dominant cells in pernicious anaemia become transformed into later stages and all the marrow cells change mostly to normoblastic type. As the red cells complete the maturation, reticulocytes begin to appear in the circulation. The newly formed red cells are normal in appearance.

2. Vitamin B_{12} acts on the other marrow elements as shown by the increase in white cell count and blood platelet count.

3. Within a few days of treatment, vitamin B_{12} stimulates the appetite and general health of the subject and

4. It also cures the neurological symptoms of pernicious anaemia.

Biochemical functions

The important functions of vitamin B_{12} are discussed below:

A. SYSTEMS REQUIRING 5-DEOXYADENOSYL CYANOCOBALAMIN

(*i*) A coenzyme containing vitamin B_{12} (5-deoxyadenosyl cyanocobalamin) is required for the isomerisation of glutamic acid to threo-β-methyl aspartate.

(*ii*) *Methylmalonyl COA-mutase:* A coenzyme containing vitamin B_{12} is required for the conversion of methyl malonyl COA-to succinyl COA.

(*iii*) *Dehydrase reaction:* A B_{12} coenzyme is required for the following dehydrase reactions:

Ethylene glycol——→acetaldehyde
1:2 propandiol——→propionaldehyde
Glycerol——→β-hydroxypropionaldehyde

B. SYSTEMS USING CO-METHYL COBALAMIN

(*i*) Homocysteine——→methionine

(*ii*) B_{12} coenzyme is essential for the following reaction:

5-methyl tetrahydrofolic acid + homocysteine——→methionine + tetrahydrofolic acid.

Effects of deficiency

The most important deficiency disease occurring in human beings due to vitamin B_{12} deficiency is pernicious anaemia.

Pernicious anaemia

This disease is caused by the lack of 'intrinsic factor' in the stomach with a consequent failure in the absorption of vitamin B_{12}. The principal signs and symptoms of pernicious anaemia are as follows:

Blood: The RBC count is low-1.5-2.5 million per mm^3 (normal 4.5-5.5 million). The average diameter of the cell is well above normal about 8.2 μ as compared to normal diameter of 7.3 μ. There is evidence that the abnormal circulating red cells are undergoing excessive destruction with consequent increase in the serum bilirubin content. The haemoglobin content is low (8-9 per cent).

Bone marrow: The nucleated red cells of the marrow are greatly increased. If the successive nucleated cell stages in erythropoisis are called stages I, II, III, and IV, then in pernicious anaemia cells of stages I and II constitute 70 per cent and of stages III and IV 30 per cent while in normal persons the reverse is the case. The cells of stage I are peculiar and differ from the normal cells and are called megaloblasts. The overacting bone marrow in pernicious anaemia is described as showing megaloblastic hyperplasia.

Stomach: The cells which secrete acid and enzymes are atrophied. The gastric secretions are devoid of acid, pepsin and intrinsic factor (IF).

Mouth: Soreness and inflammation of the tongue are commonly observed.

Nervous system: In about 80 per cent of the cases, paraesthesia (numbness and tingling) occurs in fingers and toes. Occasionally there are objective signs of involvement of the spinal cord (vitamin B_{12} neuropathy). In advanced cases, demyelination of the white fibres of the spinal cord occurs, affecting first the dorsal column and later, the lateral column (sub-acute combined degeneration of the cord).

Vitamin B_{12} deficiency in Vagans

Persons living exclusively on vegetarian diets (Vagans) have low serum levels of vitamin B_{12}, develop sore tongue, paraesthesia and signs of degeneration of the long tracts of the spinal cord as

a result of low intakes of vitamin B_{12}. Megaloblastic anaemia is not so common, as folic acid intake is adequate (Smith, 1962).

Vitamin B_{12} deficiency in breast fed infants

A few instances of megaloblastic anaemia caused by vitamin B_{12} deficiency has been reported in breast fed babies of mothers who are themselves deficient in vitamin B_{12} (Baker *et al.*, 1962).

Methods of assay

Microbiological methods are widely used for the assay of vitamin B_{12} in foodstuffs. A brief account of the different procedures is given below:

(*i*) *Lactobacillus Lactis assay:* The growth response of the organism to varying amounts (5-100 mμg) of the standard vitamin B_{12} is determined and a standard curve is drawn. The response of the organism to varying amounts of the extract of the test food is also determined. By comparing the response with the test food with that of the standard, the vitamin B_{12} potency can be calculated.

(*ii*) *Lactobacillus leichmannii assay:* The growth response of the organism to varying quantities 0.025 to 0.25 mμg of vitamin B_{12} is determined and a standard curve is drawn. The growth response of the organism to varying amounts of the extract of the test food is determined. By comparing the response with the test food with that of the standard, the vitamin B_{12} potency of the test food can be calculated.

(*iii*) *Protozoan assay:* Two protozoans (*i*) *Euglena gracilis* and (2) *Ochromonas malhamensis* have been used successfully for the assay of vitamin B_{12}. *Ochromonas* has more specific vitamin B_{12} requirements than *Euglena*. It responds only to the clinically active and animal-active forms of vitamin B_{12}—an advantage over *Euglena* and *Lactic acid bacteria*. Both organisms are photosynthetic and heavy growth can be obtained without artificial aeration. They have proved reliable for evaluating the vitamin B_{12} content of serum. The method is highly sensitive (1 $\mu\mu$g/ml).

Dietary sources

Vitamin B_{12} is present only in foods of animal origin. It is not present in foods of vegetable origin. Vitamin B_{12} content

of foods is given in Table 9.27. Liver is the richest natural source of vitamin B_{12}. Meat, fish, kidney, brain and eggs are good sources. Fresh milk, milk powder and cheese are fair sources.

Table **9.27 Important dietary sources of Vitamin B_{12}**

Foodstuff	Vitamin B_{12} μg/100g	Foodstuff	Vitamin B_{12} μg/100g
Rich sources:		*Fair sources:*	
Liver, goat	120	Whole milk powder	2.4
,, ox	118	Skimmed milk powder	3.2
,, pig	59	Cow's milk, fresh	0.5
,, sheep	133	Buffalo milk, fresh	0.4
Good sources:		Goat's milk, fresh	0.1
Meat, goat	11	Human milk	0.02
,, sheep	30		
Fish	23		
Egg, hen's	11		
Egg, duck's	9		

Large scale production and cost

Vitamin B_{12} is manufactured in different countries by microbial synthesis by various organisms. The principal manufacturers are U.S.A., Japan and U.K. The cost of crystalline vitamin B_{12} in U.S.A. in 1968 was $8 (Rs 60) per gram. It is understood Japan is also selling the product at that price. The annual requirement of vitamin B_{12} of an individual is about 1 mg. and this will cost only 0.8 U.S. cents or 6 paise (Indian currency).

Requirements

Estimates of losses of vitamin B_{12} from the body determined recently using whole body counting techniques indicate the loss to be 2.6 μg/day assuming the pool size to be 5 mg (Heinrich, 1964). Studies of vitamin B_{12} absorption by normal individuals, using radioactive vitamin B_{12} indicate that at a dose of 0.5 μg, more than 70 per cent is absorbed and that as the dosage of vitamin B_{12} increases, absorption gradually decreases until at a dose of 5 μg, 30 per cent or less is absorbed. (Kervans *et al.*, 1954);

Vitamin B_{12} from food sources are as well absorbed as the crystalline vitamin (Heyssel *et al* 1966). The recommended daily allowances for vitamin B_{12} by different groups i.e., FAO/WHO Expert Group, NRC, U.S.A. and ICMR Nutrition Expert Group are given in Table 9.28.

Table 9.28 **Recommended daily intakes of Vitamin B_{12}**
(μg/caput/day)

Age group	FAO/WHO	NRC (USA)	ICMR (India)
Infants 0-6 months	0.3	1.0	0.2
7-12 ,,	0.3	1.5	0.2
Children 1-3 years	0.9	2.0	
4-6 ,,	1.5	2.5	0.5
7-9 ,,	1.5	4.0	
10-12 ,,	2.0	5.0	
Boys / Girls 13-19 years	2.0	5.0	1.5
Adult Men and Women	2.0	5.0	1.0
Pregnancy	3.0	8.0	1.5
Lactation	5.2	8.0	1.5

Therapeutic uses and toxicity

For the treatment of pernicious anaemia, the general practice is to give 1000 μg. of vitamin B_{12} by injection twice in the first week, followed by 250 μg every week for about 2 months. Subsequently a dose of 250 μg. every month for a few months will prevent recurrence. Vitamin B_{12} administered in doses of 250-1000 μg. intramuscularly does not have any toxic effects.

Detection of deficiency

For the detection of vitamin B_{12} deficiency the following tests are useful (*i*) Serum vitamin B_{12} assay and (*ii*) Absorption of test dose of vitamin B_{12}.

(*i*) *Serum vitamin B_{12} assay:* The vitamin B_{12} content of serum is determined using the protozoan *Euglena gracilis.* A serum level of below 140 $\mu\mu$g/ml indicates vitamin B_{12} deficiency.

(*ii*) *Absorption of test dose of vitamin* B_{12}

(*a*) *Absorption of radioactive* B_{12}. The first test proposed was the direct determination of faecal radioactivity following oral administration of 0.5 μg labelled vitamin B_{12}. In normal subjects, about 30 per cent of the test dose and in pernicious anaemia about 90 per cent of the test dose are not absorbed.

(*b*) *Shilling's urinary excretion test:* This test is based on the kinetics of vitamin B_{12} absorption. A dose of 0.5 μg labelled vitamin B_{12} is given by mouth and followed in 2 hrs. by an injection of 1000 μg unlabelled vitamin B_{12} which saturates the binding protein in the blood so that any radioactive B_{12} absorbed will be carried in the blood in the free state subject to excretion by the kidney. Normal subjects will excrete 10-30 per cent of the labelled test dose in 24 hours. Patients with pernicious anaemia show a total excretion less than 2.0 per cent of the labelled test dose in 24 hours.

Vitamin B_{12} and folic acid in megaloblastic anaemia

Both vitamin B_{12} and folic acid are effective in curing megaloblastic anaemia. The nervous lesions in pernicious anaemia respond only to vitamin B_{12}. If pernicious anaemia is treated with folic acid, the anaemia generally improves but the nervous lesions are aggravated. Hence, pernicious anaemia and megaloblastic anaemia due to vitamin B_{12} deficiency should never be treated with folic acid.

BIOTIN

History

In 1927, Boas made an important observation that when raw egg white was incorporated as the main source of protein in the diets of rats, they developed deficiency symptoms characterised by dermatitis, loss of hair and muscular inco-ordination. All these symptoms were prevented or cured by yeast, liver and egg yolk. The factor was called 'anti egg white injury factor'. Later Gyorgy (1931) gave the name vitamin H to this factor. Vitamin H was isolated in 1939 by Gyorgy, Kuhn and Lederer. Later Gyorgy *et al.* (1940) found that vitamin H was identical with biotin (a growth factor for yeast) isolated from egg yolk by Kogel and Tonnis in 1936. Vitamin H was also isolated from milk by Melville *et al.*, in 1942. This vitamin was given the name Biotin.

Chemistry and properties

The chemical structure of biotin (Fig. 9.19) was established by du Vigneaud and colleagues (1940-42) and synthesis of biotin was accomplished by Harris and co-workers in U.S.A. (1943-44). Biotin is sparingly soluble in cold water and is freely soluble in hot water. It forms salts with alkali hydroxides. It is sparingly soluble in alcohol but insoluble in fat solvents. It is stable to autoclaving at 120°C for 30 min. in aqueous medium at neutral pH.

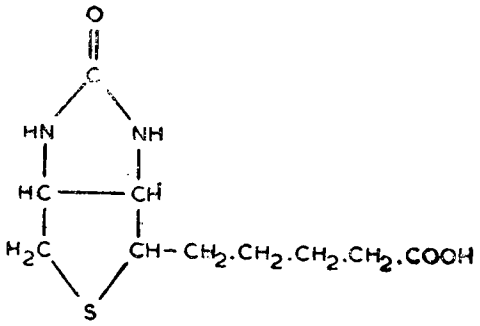


Fig. **9.19 Biotin**

Absorption and storage

Biotin is absorbed readily from the small intestines through the portal vein into the general circulation. Biotin ingested in excess of requirements is not stored but is mostly excreted in urine.

Physiological functions

The important physiological functions of biotin are as follows: (*i*) In biotin deficiency, the skin shows extensive hyperkeratosis, some parakeratosis, acanthosis and oedema. (*ii*) Biotin is essential for normal gestation and for lactation in rats and (*iii*) Biotin deficiency causes paralysis of the hind legs in experimental animals.

Biochemical functions

Biotin takes part as a coenzyme in several metabolic functions. Recently the CO_2 biotin-enzyme complex was isolated and the

active component was shown to be N^1-carboxy biotin. Another compound biocytin (e-N-biotinyl-L-lysine) occurs in biological materials. Its biochemial role is not known.

A. Carboxylation reactions

(*i*) *Propionyl-CoA-carboxylase:* Biotin acts as a coenzyme in the conversion of propionic acid to succinic acid.

(*ii*) *β-methylcrotonyl-CoA-carboxylase:* β-methylcrotonyl-CoA carboxylase is important in the degradation of leucine. The enzyme catalyses the conversion of β-methylcrotonyl CoA to β-methylglutaconyl-CoA in the presence of biotin.

(*iii*) *Acetyl-CoA-carboxylase:* This enzyme catalyses the conversion of acetyl CoA to malonyl CoA in the presence of biotin.

(*iv*) *Pyruvic carboxylase:* This enzyme catalyses the conversion of pyruvic acid to oxaloacetic acid in the presence of biotin.

B. Transcarboxylase reactions

(*i*) *Transcarboxylase:* This enzyme catalyses the following reactions in the presence of biotin:

Pyruvic acid + methylmalonyl CoA → oxaloacetic acid + propionyl CoA

(*ii*) *Oxalosuccinate-Acetyl CoA-transcarboxylase:* This enzyme catalyses the following reaction in the presence of biotin:

Oxalosuccinic acid + Acetyl CoA → α-keto glutaric acid + malonyl CoA

Effects of deficiency

The effects of biotin deficiency have been studied in experimental animals and in man.

Rat: There is retardation of growth and development of a progressive scaly desquamative dermatosis of the seborrheic type. There is loss of hair. Paralysis of the hind legs develops. If untreated the animals die.

Chicks: Dermatitis and perosis are the chief manifestations of biotin deficiency in chicks.

Pigs: The deficiency syndrome in pigs is characterised by alopecia, seborrheic skin changes, spasticity of the hind legs and cracks in the feet.

Human beings: Sydenstricker and co-workers (1942) produced biotin deficiency in human beings by feeding them a diet low in biotin and with 30 per cent of the total calorie intake in the form of fresh egg white. Fine scaly desquamation of the skin without pruritis appeared during the third and fourth weeks; more marked maculosquamous dermatitis became evident around the seventh week on the neck, hands, arms, and legs of one of the subjects. During the 9th and 10th weeks, all patients showed fine brawny desquamation accompanied by mild depression and followed by extreme lassitude, somnolence, muscle pain, and hyperaesthesia. After the tenth week, anorexia with occasional nausea developed. Slight anaemia and increase in serum cholesterol level were noted. The urinary excretion of biotin was reduced after 7-8 weeks to 3.5-7.3 μg per day as compared with 29-52 μg per day on a normal diet. Injection of biotin in doses of 75-300 μg. brought about rapid cure in 3-5 days and the urinary excretion of biotin rose to 55 μg per day.

Biotin deficiency has been reported in an adult who consumed daily 4-12 raw eggs and 1-4 quarts of wine (Williams 1943).

Leiner's disease: Leiner's disease (erthroderma desquamativum or exfoliative dermatitis) in young infants has been reported by several workers (Brown 1948). The disease often occurs in breast fed infants frequently in association with persistent diarrhoea. The low biotin content of human milk, together with the poor absorption of biotin due to diarrhoea, appears to cause biotin deficiency. Administration of biotin has been reported to effect a cure in many of these cases.

Avidin

Egg white injury factor: The active principle present in egg white and responsible for producing 'egg white injury' in experimental animals has been isolated in a pure state (Eakin, Snell and Williams 1941). It is a protein called 'avidin' which has a molecular weight of 53,100. The iso-electric point of avidin is about pH 10-10.5. Avidin present in egg white or in a purified form is denatured and inactivated by prolonged heating (steaming

at 100°C for 60 minutes). Spray drying of egg white or drying it at low temperature in trays 80°C for 1 hour does not destroy avidin completely. Purified avidin when incorporated in the diet produces effects similar to that caused by raw egg white. One molecule of avidin can combine with 3 molecules of biotin. One mg. of avidin has been found to combine with 13.8 μg of biotin. The site of binding of biotin with avidin has been shown to be the tryptophan residue in the avidin molecule.

Methods of assay

In natural foods, biotin is present only in small quantities. For the estimation of biotin, biological and microbiological methods are available. The microbiological methods have the advantage of being technically simple and requiring less time; however, they have certain limitations. Most of the micro-organisms used in the assay of biotin will respond only to free biotin and not to its bound forms. Hence the foods must be digested with enzymes to liberate the bound biotin. Micro-organisms also tend to respond to close homologues, break down products and precursors such as pimelic acid and to apparently unrelated compounds such as oleic acid.

Animal assays

The bio-assay can be made with rats or chicks made biotin deficient by the use of special diets.

Rat assay: Under ordinary dietary conditions, the biotin requirements of rat appear to be met by the biotin synthesised by the intestinal microflora. Hence it is essential to introduce raw egg white or avidin to bind the biotin and to prevent it from being absorbed. The rat assay is based on the growth of young rats fed on diet containing avidin to known quantities of pure biotin 0.1 to 1.0 μg per rat per day or natural food to be tested.

Chick method: The daily biotin requirement per chick is about 0.65-1 μg (7-10 μg per 100 g. of diet). Under normal conditions the intestinal synthesis of biotin in chicks is too small to meet the needs so that biotin deficiency can be produced by feeding chicks a biotin deficient diet. The criteria for the assay is the increase in the growth of biotin deficient chicks to known amounts of pure biotin or the test food.

Microbiologicc l assay: A large number of micro-organisms have been used for the assay of biotin. Among these, the most widely used and successful method is that of Wright and Skeggs which utilises *Lactobacillus plantarum* as the test organism. The growth response to standard biotin is determined in the range 0.02 to 0.2 mμg/ml. medium and to varying quantities of the test food extract. The growth response is measured either by titration of the acid produced after 72 hours incubation at 30-37°C or turbidity after 24 hours.

Dietary sources

Biotin occurs widely both in foods of vegetable and animal origin. The biotin contents of foods are given in Table 2A in Appendix and are briefly discussed below. A summary of biotin content of foods is given in Table 9.29.

Table 9.29 **Biotin content of foods**

Foodstuffs	Biotin (μg/100g)	Foodstuffs	Biotin (μg/100g)
Rich sources:		Eggs	20-22
Dried yeast	100-200	Milk, cow's (fresh)	5-6
Rice polishings	57-60		
Wheat germ	55-66	*Fair sources:*	
Liver (sheep, goat, ox)	100-127	Milled cereals and cereal flours	1.5-3.0
Peanut	35-40	Vegetables	3-5
Soybean	45-55	Fruits	1.7-2.5
Good sources:		Milk, human	0.4-0.5
Whole cereals	6-15		
Legumes (except soybean)	12-18		
Mutton	5-8		

Dried yeast, rice polishings, wheat germ, liver, peanut and soybean are rich sources. Whole cereals, legumes, meat, fish, poultry, milk, eggs, oilseeds and nuts are good sources. Milled cereals, milled cereal flours, vegetables, fruits and human milk are fair sources.

Requirements

The minimum daily requirements for biotin are not known. Since biotin deficiency has not so far been reported in normal

human subjects, it may be presumed that the diets provide adequate amounts of biotin. The biotin content of poor diets consumed in the developing countries is about 50-60 μg while that of diets consumed in the developed countries vary from 150-300 μg. Cow's milk contains approximately 50 μg biotin per litre while human milk contains only 4 μg. per litre. Cases of biotin deficiency occurring in infants fed on breast milk and suffering from diarrhoea have been reported. The daily biotin requirements of adults may be estimated to be between 50-60 μg. of children between 20-40 μg and of infants 10-15 μg.

Therapeutic uses and toxicity

In the experimental biotin deficiency produced in adult human beings, injection of about 300 μg. of biotin per day for 5 days brought about rapid cure. Crystalline biotin has been administered in large quantities (1 g/kg body wt.) in experimental animals without producing any harmful effects. It may be assumed that 200-300 μg of biotin can be safely administered by the parenteral route for the treatment of the deficient subjects.

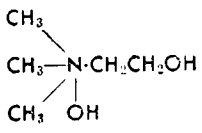
CHOLINE

History

The importance of choline in nutrition was established by Best and Huntsman (1934) who discovered that choline deficiency in the rat produces fatty liver. Choline is an integral part of phospholipids which are present in nervous tissues and are essential for the life processes. In addition, it plays an active role in transmethylation and its acetyl ester (acetyl choline) is involved in the humoral transmission of parasympathetic and other nerve impulses to effector organs. In view of its importance in nutrition it is usually considered as a member of the vitamin B complex.

Chemistry and properties

The chemical structure of choline is given below:



Choline

It is hydroxy ethyl trimethylammonium hydroxide. It is a colourless crystalline compound which is extremely hygroscopic. It is a strong base. It is highly soluble in water and alcohol. Dilute solutions of choline (less than 4 per cent) are stable to heat but when concentrated solutions are boiled, it decomposes, giving trimethylamine.

Absorption and storage

Free choline present in foods is readily absorbed from the small intestines through the portal vein into the general circulation. Choline consumed in excess of requirements is not stored in the tissues. Part of the ingested choline is converted into phospholipids but a greater part is metabolised in the liver into trimethylamine which is excreted in the urine.

Physiological and biochemical functions

Choline has the following important functions.

(*i*) It prevents accumulation of fat in the liver in many experimental animals.

(*ii*) It is essential for the growth of many experimental animals.

(*iii*) Choline deficiency reduces egg production in the hen.

(*iv*) Choline deficiency causes paralysis of hind legs in the nursing rat and slipped tendon (perosis) in chicks and young turkeys.

(*v*) Choline is a constituent of phospholipids which are essential constituents of all cells in the body, including cytoplasmic particles such as mitochondria cell walls and nervous tissues.

(*vi*) Phospholipids are directly concerned in the transport of fatty acids and cholesterol in the blood and from the liver and with their deposition and removal in adipose tissue. In choline deficiency, neutral fat and cholesterol esters accumulate in the liver.

(*vii*) Acetyl choline plays an important part in the humoral transfer of para-sympathetic and other nerve impulses to effector organs and

(*viii*) Choline takes part in transmethylation reactions in the formation of methionine from homocystine. Choline is oxidised to betaine and betaine reacts with homocystine yielding methionine and dimethylglycine in the presence of the appropriate enzymes.

$$\text{Choline} \xrightarrow{\text{oxidation}} \text{Betaine}$$

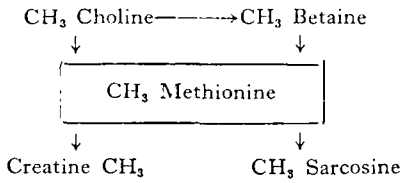
$$\text{Betaine} + \text{homocystine} \longrightarrow \text{methionine} + \text{dimethylglycine}$$

Synthesis of choline in the body

Choline is being synthesised in the animal and human tissues to some extent. The reactions involved are as follows:

$$\text{Serine} \longrightarrow \text{Ethanolamine} \longrightarrow \text{Choline}$$

The transmethylation reactions in the body involving transfer of methyl group from choline to homocystine to form methionine and from methionine to ethanolamine to form choline are shown below:



Effects of deficiency

Rats: Choline is essential in the weanling rat not only for the prevention of fatty liver but also for growth, survival and maintenance of tissue structure in normal condition. Choline deficiency in rats produces fatty liver and haemorrhagic degeneration of the kidney.

Chicks: The primary effects of choline deficiency in chicks are cessation of growth and appearance of perosis or 'slipped tendon' disease.

Pigs: Decreased growth rate, fatty liver and abnormality of gait have been reported in pigs.

Methods of assay

Methods available for the assay of choline in biological materials may be grouped as follows: (1) Chemical and (2) Microbiological.

Chemical methods: The most widely used chemical method for the quantitative determination of choline consists of the following steps (*i*) extraction of choline (*ii*) precipitation of choline as reineckate salt and (*iii*) colorimetric determination of red colour of the reineckate salt after dissolving it in acetone.

Microbiological method: A microbiological method for the determination of choline by the growth of the 'Cholineless mutant' of *Neurospora crassa* was described by Horowitz and Beadle (1943). This method is highly sensitive and widely used for the assay of choline.

Requirements

Choline requirements of human beings are not known. The diets commonly consumed all over the world contain adequate amounts of choline and choline deficiency is not likely to occur in human beings.

Dietary sources

The choline contents of foods are given in Table 2A in Appendix. A summary of the data is given in Table 9.30.

Table 9.30 **Important dietary sources of choline**

Foodstuffs	Choline (mg/100g)	Foodstuffs	Choline (mg/100g)
Rich sources:		*Fair sources:*	
Egg, whole	504	Milled cereals	50-60
Egg, yolk	1490	Vegetables	20-80
Liver (pork, beef, goat)	550-660	Fruits	12-24
Wheat germ	406-450	Milk	15-18
Legumes	210-340		
Good sources:			
Mutton (lamb, beef, pork)	84-96		
Whole cereals	110-146		
Rice polishings	150-180		
Nuts and oilseeds	95-165		

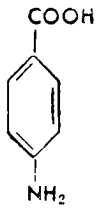
PARA-AMINO BENZOIC ACID

History

In 1941, Ansbacher reported that p-amino benzoic acid (PABA) is essential for normal growth of rats. It was also found necessary for normal lactation in rats and sows and for the prevention of greying of hair in the black rat. It is present in folic acid as a part of its molecule in combination with pteridine and glutamic acid.

Chemistry and properties

The chemical structure of p-amino benzoic acid (PABA) is given below.



p-Aminobenzoic acid

It is a crystalline substance, and is slightly soluble in water (0.5 per cent at 25°C). It is soluble in alcohol. It is stable to heat in neutral and acid solutions.

Absorption and storage

PABA is absorbed from the intestines through the portal vein and passes through general circulation to all tissues. It is partially acetylated in the liver. It is also converted into p-amino hippuric acid which is excreted in urine. PABA consumed in excess of requirements is not stored but is metabolised and excreted in urine.

Physiological and biochemical functions

The important functions are as follows: (1) An excess of p-amino benzoic acid inhibits the bacteriostatic effect of sulphonamides because of structural similarities. (2) Sulphonamide resistant strains synthesise PABA (3) In some animal species PABA has been reported to increase the action of insulin. (4) It has been reported to inhibit the production of thyroid hormones. (5) It inhibits the oxidative destruction of adrenaline in the tissues

and (6) The injection of hydroquinone in mice results in greying of fur which is prevented by PABA.

Effects of deficiency

The deficiency of PABA retards growth in rats and brings about greying of hair. The milk production of lactating rats is reduced in PABA deficiency.

Methods of assay

No satisfactory chemical method is available for the assay of PABA in biological materials. Microbiological methods have been used for this purpose. The commonly used organisms are *Acetobacter suboxydans* and *P-amino benzoicless* mutant of *Neurospora crassa* (Cheldelin and Bennett 1945; Agarwala and Peterson, 1950).

Dietary sources

PABA occurs in plant and animal tissues both in the free and bound form. The PABA contents of foods are given in Table 9.31.

Table **9.31 PABA contents of foodstuffs**

Foodstuffs	Free (μg/100g)	Total (μg/100g)	Foodstuffs	Free (μg/100g)	Total (μg/100g)
Cereals:			*Flesh foods:*		
Corn meal	30	—	Liver (beef, calf)	20-30	180-250
Oats, rolled	33	—	Meat (beef)	30	60-70
Wheat, whole	25	49-63	Pork	30	70-80
Wheat, germ	50-100	170-180			
			Vegetables:		
Oilseeds and nuts:			Cabbage	33	50-70
Peanut	50-80	160-170	Carrot	8	10-22
			Potato	34	40-60
Egg and milk:					
Egg, whole	7	25-40	*Fruits:*		
Milk, cow's	8-15	—	Banana	9-12	41-46
Dried yeast	500-900	4000-5000			

Requirements

The requirements of PABA for human beings are not known. Average diets provide adequate amounts of PABA.

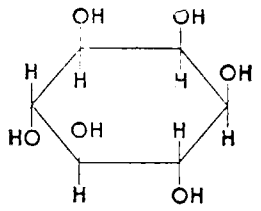
INOSITOL

History

Inositol occurs in both plant and animal tissues. The hexaphosphoric ester of inositol (Phytic acid) has been known for a long time. There are seven optically inactive and two optically active forms of inositol. Of these only one optically inactive form which occurs in nature has some nutritional importance. Woolley *et al* (1940) reported that inositol is essential for mice and prevents the development of alopecia in mice.

Chemistry and properties

Inositol (also called meso-inositol or myo-inositol) is hexahydroxy-cyclohexane (Fig. below). It is a crystalline compound and has a sweet taste. It is highly soluble in water and stable to heat in neutral, acid and alkaline medium.



meso-Inositol ($C_6H_{12}O_6$)

Absorption and storage

Inositol is readily absorbed from the small intestines through the portal vein and passes through the general circulation to the tissues. Inositol consumed in excess of requirements is not stored but is partly excreted in urine or metabolised. Anderson and Bosworth (1916) found that when 0.5 g. of inositol per kg. body weight was given by mouth, only 9 per cent was excreted in urine and none in faeces. On the other hand, studies in animals have shown that absorption is poor and a greater part is excreted in the faeces.

Bound forms of inositol

Although inositol occurs in the free state in muscle and other tissues, a greater part of it exists in bound forms. In animal

tissues, it exists in the form of phospholipids, while in plant tissues it exists mostly in the form of phospholipids, phytic acid and phytin.

Inositol containing phospholipids: Brain and liver tissues contain phospholipids containing inositol. Inositol mono and diphosphates have been isolated by the hydrolysis of the phosphatides. Claude (1946) has reported that mitochondria and microsomes contain large amounts of inositol containing phosphatides.

Phytic acid: Cereals, legumes, oilseeds and nuts contain large amounts of phytic acid and phytin (Ca, Mg salt of phytic acid). Phytic acid is inositol-hexaphosphoric acid. This is broken down by an enzyme phytase present in plants into inositol and phosphoric acid. Phytic acid is not absorbed from the intestines unless it is broken down by the enzyme phytase. Since the phytase activity of the intestinal juices is low, only a small percentage of the phytic acid present in the diet is hydrolysed and absorbed.

Physiological and biochemical functions

The important functions are given below: (1) When glucose C^{-14} is fed to the rat, inositol C^{-14} has been isolated from the tissues, indicating that some inositol is synthesised in the tissues. (2) An enzyme—cyclase catalyses the formation of inositol-1-phosphate from glucose-6-phosphate in the presence of DPN. (3) Inositol is oxidised to glucuronic acid in the liver by an enzyme, oxygenase. (4) Labelled inositol gives rise to labelled glucose in the body (5) Studies in tissue culture have revealed that inositol is required for the growth of fibroblasts (6) Inositol is present in certain phospholipids. In certain conditions, inositol exerts a lipotropic effect. (7) Inositol occurs in large quantities in mammalian heart muscle and shark skeletal muscle. Sacks (1906) found that inositol in concentrations found in heart muscle increased the amplitude and rate of contraction. (8) In animals, it has been shown that inositol increases the peristalsis of the small intestines (9) Inositol causes an increase in nerve chronaxia in the rat and it abolishes the reduction in chronaxia caused by adrenochrome. (10) Young mice fed on inositol deficient diet lose hair (alopecia). (11) Inositol occurs in

large quantities in the secretion of the seminal vesicle of the boar and (12) It stimulates the growth of yeast and other fungi.

Effects of deficiency

Experimental animals: Woolley (1940) showed that in mice fed on inositol deficient diet, growth was retarded and loss of hair over the body (alopecia) occurred. Later, Pavcek and Baum (1941) found that inositol cured a condition known as 'Spectacled eye' (a condition due to loss of hair around the eye) in rats fed on inositol deficient diet. Hegsted *et al.* (1941) reported that inositol supplementation increased slightly the growth rate of chicks fed a diet deficient in inositol. Hogan and Hamilton (1942) reported that addition of inositol increased the growth rate of guinea pigs fed on a deficient diet. Gavin and McHenry (1941) found that addition of biotin to a purified diet caused fatty liver in rats which could be prevented by inositol. The lipotropic action of inositol in rats has been confirmed by other workers.

Human beings: Evidence for the specific need of inositol in human nutrition is not available. However, the wide distribution of inositol in the human body and the need of inositol in the nutrition of animals, make it reasonable to assume that inositol may be essential in human nutrition. Ariel *et al.* (1944) found a high incidence of fatty liver in patients with gastro-intestinal cancer. Administration of inositol in doses of 280 to 1200 mg daily brought about a rapid reduction in the liver fat content.

Methods of assay

Since only meso-inositol is of nutritional importance, only microbiological methods which are specific for meso-inositol can be used for the assay of inositol.

(1) *Assay with saccharomyces carlsbergensis 4228:* This organism responds to concentrations of 0.1 to 1 μg. of inositol and yields reliable results. The growth of the organisms to standard inositol solution (0.1 to 1 μg) and to known amounts of the extract of test food is determined. From the data obtained, the inositol content of the unknown can be calculated.

(2) *Assay with inositol-less mutant of Neurospora crassa:* The growth of the mycelium to known amounts of standard inositol

and test food extract is determined. Inositol content of the test food is calculated from the data obtained.

Dietary sources

The inositol contents of foods are given in Table 2A in Appendix. Inositol is present in the free form in animal tissues and plant tissues and also in the form of phospholipids in nervous tissues and liver and in the form of phytin in cereals, legumes, nuts and oilseeds. The availability of inositol from phytin is poor. The inositol contents of some important foods are given in Table 9.32.

Table **9.32 Important dietary sources of inositol**

Foodstuffs	Inositol mg/100g	Foodstuffs	Inositol mg/100g
Vegetables	27-160	Whole cereals	120-180
Fruits	22-210	Fish	17-44
Liver	300-400	Legumes	180-250
Cow's milk	50	Oilseeds and Nuts	170-260
Human milk	33	Dried yeast	50-270

Requirements

Inositol requirements for human beings are not known. Average diets provide adequate amounts of inositol.

BIOFLAVONOIDS (VITAMIN P)

History

Szent-Gyorgy prepared in 1936 an extract from paprika and lime juice and found it essential in addition to ascorbic acid, in preventing capillary fragility in human beings. He gave the name Vitamin 'P' to this factor. Many investigators have confirmed the value of vitamin P (Bioflavonoids) along with ascorbic acid in preventing capillary fragility. The Joint Committee on nomenclature of the American Society of Biological Chemists and American Institute of Nutrition (1950) recommended that the name vitamin 'P' be dropped. The term 'bioflavonoids' was proposed for these groups of compounds having vitamin 'P' activity.

Chemistry and properties

Citrin and other glycosides (called anthoxanthins) having vitamin 'P' activity have been isolated from paprika, citrus juice and peel. Chemically they are called flavonoids. Citrin from lemon peel is said to be a mixture of two flavanone glucosides-hesperidin and eriodictin. The other important bioflavonoids occurring in nature are rutin and quercetrin.

Absorption

Bioflavonoids taken orally are absorbed from the small intestines through the portal vein and passes through the general circulation to all tissues.

Physiological and biochemical functions

The important functions are as follows: (1) Studies with experimental animals have shown that bioflavonoids control capillary permeability in experimental animals.

(2) Citrin also cures intestinal haemorrhages in scorbutic guinea pigs when administered along with ascorbic acid. and

(3) It has been suggested that the effect of bioflavonoids is indirect, based on their inhibitory effect on the oxidation of adrenaline.

Effects of deficiency

The effects of bioflavonoid deficiency in human beings have been studied in detail by Scarborough (1940). The signs of bioflavonoid deficiency are (i) decreased capillary resistance leading to petechial bleeding, accompanied by pain across the shoulders and in the legs, (ii) lassitude and (iii) fatigue. Administration of bioflavonoids is effective in curing the above symptoms.

Methods of assay

A biological method for assaying the bioflavonoid activity of foodstuffs based on the measurement of capillary resistance has been developed by Bacharach, Coates and Middleton (1942). The method is as follows: A suction cup with a diameter of 12mm

is applied to the greased and shaven area on the back of a guinea pig. The pressure is gradually reduced by 5 mm. stages and maintained at each stage for three to five seconds. The pressure is noted at which petechiae are first seen when the area under suction is viewed through the cup. This is called the critical petechial pressure. If this is plotted against the logarithm of the dose of vitamin P administered to the animals a straight line graph is obtained.

Dietary sources

Bioflavonoids occur mainly in fresh fruits and vegetables. (Table 9.33). One 'Provisional Unit' (P.U.) is defined as the activity of 1 mg. of citrin i.e., the water soluble glycoside complex derived from citrus peel. (Bacharach and Coates, 1943). Recrystallised hesperidin had an activity of only 0.1 P.U. per mg while black-currant concentrate had an activity of 10 P.U. per mg.

Table 9.33 **Bioflavonoid activity (Vitamin P) of some fruits and vegetables (I unit = activity of 1 mg of citrin from lemon peel)**

Food	Vitamin P content (units/100g)	Food	Vitamin P content (units/100g)
Apple	60	Parsnip	40
Beetroot	15	Pea	40
Blackberry	60	Plum	50
Cabbage	60	Potato	25-40
Carrot	40	Rose hips-syrup	240-350
Cauliflower	40	Spinach	130
Cherry	60	Tomato	60-70
Lemon, peel	500	Turnip	20-30
,, juice	450	Walnut	100
Lettuce	80-100	Watercress	70
Orange (juice and peel)	490		

Requirements

The bioflavonoid requirements for human beings are not known. Daily consumption of liberal amounts of fresh fruits and vegetables will meet the requirements.

Therapeutic uses and toxicity

Bioflavonoids are generally given orally in doses of about 200 mg. four times a day. They have been found effective in curing decreased capillary resistance in allergic subjects. Scarborough (1942) reported that two cases of vascular purpura (purpura senilis) was cured by bioflavonoids. Bioflavonoids when administered by intramuscular route may produce some toxic effects.

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CHAPTER 10

MINERALS

The body contains about 24 minerals, all of which must be provided by the diet. These include calcium, phosphorus, potassium, sodium, chlorine, magnesium, iron, manganese, copper, iodine, cobalt, zinc, aluminium, arsenic, bromine, fluorine, nickel, chromium, cadmium, selenium, silicon, vanadium, and molybdenum. These minerals are necessary for the following different functions: (1) As constituents of bones and teeth. e.g., calcium, phosphorus and magnesium, (2) As constituents of body cells of soft tissues such as muscles, liver etc., e.g., phosphorus. (3) As soluble salts which give to the body fluids and cell contents, their composition and stability which are both essential for life, e.g., sodium, potassium, chloride and phosphorus. (4) Some minerals are required in small quantities for specific functions, e.g. (*a*) iron and copper—formation of haemoglobin (*b*) iodine—formation of thyroxine (*c*) zinc—constituent of an enzyme, e.g., carbonic anhydrase and a hormone, e.g., insulin, and (*d*) cobalt—constituent of a vitamin, e.g., vitamin B_{12} and (*e*) some other elements are essential for the activity of various enzymes (Table 10.1).

In poor diets consumed by a large majority in India and other developing countries, deficiencies of calcium and iron commonly occur. Iodine deficiency occurs in people living in certain hilly tracts in India and in some other countries, where the soil and water are deficient in iodine. Addition of extra sodium chloride to the diet is of special importance in tropical countries, as the loss of NaCl in sweat is heavy. The deficiencies of other minerals do not normally occur in average diets.

CALCIUM

Among the different minerals, calcium occurs in the highest amounts in the body. About 99 per cent of the calcium is present in the skeleton and the remaining 1 per cent in soft tissues. The body of the infant at birth contains about 27.5 g. of calcium while the adult body contains about 1,000-1,200 g. All this calcium is deposited in the bone during the growth of the body.

Table **10.1 The catalytic function of mineral elements in enzyme systems of plants and animals**

Enzymes	Metal(s) required
Succinic dehydrogenase	Ca. Al.
Arginine phosphopherase	Ca, Mn. Mg.
Lecithinase	Ca, Mg. Co. Zn, Mn.
Ascorbic acid oxidase	Cu
Tyrosinase	Cu
Glycylglycin dipeptidase	Co, Mn, Zn
Catalase	Fe
Cytochromes	Fe
Peroxidases	Fe
Carboxypeptidase	Mg
Creatine phosphopherase	Mg
Flavokinase	Mg
Glutamic phosphokinase	Mg
Inorganic pyrophosphatase	Mg
Nicotinamide methylkinase	Mg
Acid and alkaline phosphatases	Mg, Mn
DPN phosphopherase	Mg, Mn
Isocitric dehydrogenase	Mg, Mn
Leucine amino-peptidase	Mg, Mn
Pyruvic carboxylase	Mg, Mn
Oxaloacetic decarboxylase	Mg, Co, Zn
Deoxyribonuclease	Mg, Mn, Co, Fe
Oxalosuccinic decarboxylase	Mn
Arginase	Mn, Co, Ni and Fe
Carbonic anhydrase	Zn
Xanthine oxidase	Mo

The important physiological functions of calcium are: (1) it is essential for the formation of bone and teeth (2) it is essential for the clotting of blood (3) it regulates the permeability of capillary walls (4) it is essential for the contraction of the heart and muscle, and (5) it regulates the excitability of nerve fibres and nerve centres.

Calcium content of the body and rate of accretion of calcium in the body

Mitchell and co-workers (1945) calculated the calcium increment in the body based on the following assumptions: (1) the calcium content of the body at birth (0.8 per cent) and that of the adult (1.6 per cent) on whole body basis and (2) the percentage of calcium increasing progressively during growth with the increase in body weight. The second assumption is based on findings with growing rats, chicks and lambs, and it appeared valid to apply the same criterion to humans. The estimates of Mitchell *et al.* (1945) were revised by Leitch and Aitken (1959). The data for the calcium increment in the body for different ages are given in Table 10.2.

Table **10.2 Calcium content and daily calcium increment in the body of boys (I. Leitch and F. A. Aitken, 1959)**

Age (years)	Weight (kg.)	Total Ca (g)	Calcium increment	
			g per annum	mg per day
0	3.5	28.5	—	—
1	9.4	84.3	55.8	153
2	12.4	116.4	32.1	88
3	14.6	141.6	25.2	69
4	16.7	167.0	25.4	70
5	19.0	196.3	29.3	80
6	21.2	225.6	29.3	80
7	23.9	263.4	37.8	104
8	26.5	301.6	38.2	105
9	29.4	346.6	45.0	123
10	32.6	399.0	52.4	144
11	36.2	461.2	62.2	170
12	40.2	534.7	73.5	201
13	45.4	637.0	102.3	280
14	51.0	755.3	118.3	324
15	57.0	892.1	136.8	375
16	62.6	1028.5	136.4	374
17	65.9	1113.7	85.2	233
18	68.2	1175.1	61.4	168
19	69.4	1207.6	32.5	89
20*	70.0	1223.6	16.0	44

* Taken as young adult

Endogenous loss of calcium

When the calcium intake is negligible, there will be depletion of body calcium. This is known as 'endogenous loss' of body calcium. The 'endogenous' calcium is excreted in the urine, faeces and sweat. In practice, the total endogenous loss of calcium is assessed as the loss of body calcium when the intake of dietary calcium is zero and this is obtained by extrapolation from the regression of retention on intake estimated at two or more levels. The losses so estimated vary from 150 to 300 mg calcium/day in adults. The isotopic studies of Bauer *et al* (1957), showed the endogenous losses in an infant to be 109 mg/day. It may be assumed that the endogenous calcium losses in children will be between the values for infants and adults.

Loss of calcium in sweat

In tropical climates, the loss of calcium in sweat will be appreciable. Under severe sweating conditions, the daily loss of calcium in sweat in adults may vary from 42 to 121 mg. This may account for the apparent retention of calcium in adults reported by several workers, as calcium loss in sweat is usually not determined in calcium balance studies.

Calcium balance

Dietary calcium which is not absorbed in the intestines is excreted in the faeces. A small part of the absorbed calcium is excreted in urine. The calcium balance, i.e., the difference between the quantity of calcium ingested and that excreted in urine and the faeces is *positive* during growth, pregnancy, lactation and in normal adults. The excretion of calcium continues on a calcium deficient diet when the body will be in *negative calcium balance*.

Factors affecting calcium absorption

The various factors affecting calcium absorption from the diets are discussed below:

Phosphates and phytic acid: Excess of phosphates lowers calcium absorption. Phytic acid forms insoluble calcium salts and interferes with the absorption of calcium.

Reaction of the intestinal contents: Calcium is well absorbed at the normal pH of the intestines. If the contents become alkaline, calcium absorption is lowered due to the formation of insoluble tricalcium phosphate.

Fats and fatty acids: Faulty absorption of fats leading to the presence of large amounts of fatty acids in the stools interferes with calcium absorption, as insoluble calcium salts of fatty acids are formed and excreted in the faeces.

Protein: Higher levels of proteins in the diet help to increase the absorption of calcium.

Fibre: Presence of excess of fibre in the diet interferes with the absorption of calcium.

Oxalic acid: Several workers have shown that oxalic acid present in certain foods forms insoluble calcium oxalate which is excreted in the faeces, thus lowering calcium absorption.

Citric acid: A number of earlier publications indicated the beneficial effect of citric acid and citrates in rickets. Some of the recent studies have not, however, confirmed the earlier observations.

Lactose: Lactose has been reported to increase the absorption of calcium. The beneficial effect of lactose is due to increased acidity (lactic acid) of the intestinal contents which leads to increased calcium absorption.

Calcium absorption and retention in human subjects

Studies have been carried out by several workers on the absorption and retention of calcium in human subjects. The absorption and retention of calcium will depend on (1) calcium intake (2) presence of interfering substances and (3) presence of vitamin D. On normal intake of calcium, the retention varies widely from 10 to 30 per cent depending on the diet and the age of the subject. A summary of the results is presented in Table 10.3.

Adaptation to low calcium intake

Infants fed on breast milk receive only 103-208 mg. calcium daily and retain about 80 mg. Infants fed on cow's milk receive

Table **10.3 Retention of calcium in human subjects**

Subject	Nature of diet	Calcium intake (mg)	Retention %
Infants (1-12 months)	Human milk	103-208	40-70
,, ,,	Cow's milk	600-900	15-30
Preschool children	Cow's milk	400-1200	18-24
,, ,,	$CaHPO_4$	350-550	18
Early school age	Milk, Cow's	750-1300	21
School children	Rice diet	259-277	4-15
,,	Ragi diet	1151	20
,,	Pearl millet diet	479	19
,,	Sorghum diet	441	17

daily 600-900 mg. calcium and may retain about 120-150 mg. Infants fed on human milk adapt to the low intake and the calcium absorption on human milk is about two or three times as high as on cow's milk. When calcium intake is low, the percentage absorption is high. Nicholls and Nimalasurya (1939) reported that in Ceylonese children with calcium intakes of 183 to 245 mg. daily from their diet, the retention of calcium was high ranging from 63 to 169 mg. The children, however, were stunted in growth. X-ray examination showed that the calcification of the bones was normal.

Calcium and parathyroids

The parathyroid regulates calcium level in blood and calcium metabolism in the bone. It acts directly on the bone releasing calcium from it and thus raising the plasma calcium level. It also increases the excretion of phosphates by the kidney. In hypoparathyroidism, the serum calcium level may fall to a low level of 4-8 mg./100 ml. and tetany may result. The symptoms of tetany can be relieved temporarily by injecting parathyroid hormones. In hyperparathyroidism, the calcium level in serum may increase from the normal level of 9-11 mg. to 16-20 mg. Rarefaction of the bones and spontaneous fracture may occur.

Effect of calcium deficiency

Young animals and children: The effects of deficiency are (1) decreased rate of growth; (2) negative calcium balance;

(3) loss of calcium from bone leading to the development of osteoporosis; (4) hyperplasia (a diffuse overgrowth) of parathyroid glands; and (5) hyperirritability and tetany leading to death.

Adults: Osteoporosis in adults, is a condition in which rarefaction (decalcification) of the bone occurs due to calcium deficiency in the diet. Fractures of the brittle bones occur even after minor accidents. Pain due to fractures of vertebrae may radiate round the trunk, to the buttocks or down the legs. Healing of fractures is not impaired in osteoporosis and pain subsides as soon as the fractures are healed.

The treatment consists in giving a well balanced diet containing about 1 to 1.5 g. calcium along with vitamin D (400-800 I.U.).

Calcium content of blood

The calcium content of blood serum is fairly constant ranging from 9-11 mg. per 100 ml. This level is maintained constant in healthy subjects by the following factors: (1) The calcium absorbed from food through the intestines and (2) The rate of secretion of parathyroid hormone which controls the level of calcium in blood.

Hypocalcemia

The motor nerves become over-susceptible to stimuli. This particularly affects the face, hands and feet, producing the clinical features of tetany. Muscles lose tone and become flabby.

Hypercalcemia

Recently, Lightwood (1952) described a new disease—idiopathic hypercalcemia—in infants. Since then, the disease has been reported to occur in infants by other workers (Mitchell, 1967). The disease usually occurs between the ages of 5 and 8 months. The infants affected suffer from loss of appetite, vomiting, wasting, constipation, flabby muscles and a characteristic facial appearance. The level of ionised calcium in the blood is raised. Increases in the levels of blood urea and plasma cholesterol are also observed. Abnormal calcification may be present in the kidneys. Many of these infants have received

excessive amounts of vitamin D resulting in excessive absorption of calcium and hence hypercalcemia. The treatment consists of giving a low calcium diet devoid of vitamin D.

Calcium: Phosphorus ratio in the diet

Diets having distorted calcium: phosphorus ratios have been used to produce experimental rickets in animals. Observations on several species have indicated that during rapid growth and calcification, the diet should have a calcium: phosphorus ratio 1:1. When calcium is required only for maintenance as in adults, the calcium requirement is lower, both absolutely and relatively tothe phosphorus requirement. Thus, the calcium: phosphorus ratio is highest at the earliest age, (1:1), decreases with the attainment of adult status (1:2) and then, in the case of the female increases again (1:1) in the latter part of pregnancy and during lactation. In the normal adult mammal, the calcium: phosphorus ratio of the whole body is a little under (1:1) and that of the bone is a little over 2:1.

Requirements

Surveys have shown that a large majority of the population in the developing countries consume diets providing 300-500 mg of calcium. Metabolic studies have shown that they maintain positive calcium balance on such diets. The FAO/WHO Expert Group on calcium requirements (1962) considered the question of calcium requirements and suggested 'practical allowances' for calcium which will be adequate to meet the needs of different age groups (Table 10.4). The I.C.M.R. Nutrition Expert Group (1968) adopted the above recommendations with slight modifications. The basis for arriving at the above recommendations is briefly discussed below:

Infants: Calcium requirements of infants birth to 6 months can be computed from the calcium intake from breast milk when they are adequately and solely fed on breast milk. On this basis, the daily calcium intake will range from 250-300 mg. The FAO/WHO and I.C.M.R. Expert Groups have suggested a higher intake of 500-600 mg for the age group birth-12 months, as infants fed on cow's milk will receive 600-900 mg calcium daily.

Table **10.4 Recommended allowances for calcium**

Subject	FAO/WHO (1962) (mg/day)	ICMR (1968) (mg/day)
Infants (0-12 months)	500- 600	500-600
Children (1-9 years)	400- 500	400-500
,, (10-15 years)	600- 700	600-700
Adolescents (16-19 years)	500- 600	500-600
Adults	400- 500	400-500
Pregnancy and Lactation	1000-1200	1000

Children (1-9 years): Calcium requirements during growth can be calculated from the rate of accretion of calcium in the body (Table 10.4). The calcium accretion in the body from 2-9 years ranges from 92 to 140 mg. per day. Assuming that the percentage absorption of dietary calcium is about 25 per cent, dietary intakes of 400-560 mg. calcium would be adequate to meet the needs. The FAO/WHO and the I.C.M.R. Expert Groups suggested allowances of 400-500 mg. daily.

Adolescents: The calcium accretion data have yielded abnormally high values for the amounts of calcium retained in the body between the age-group 12-15 years. This may be due to the fact that the calculations have been based on the increase in body weight. It is likely that the increase in body weight during adolescence consists of a smaller proportion of skeleton and a larger proportion of soft tissues and adipose tissue. Metabolic studies and diet surveys have shown that children between the age-group 10-19 years have maintained positive calcium balance on calcium intake of 500-600 mg. daily. The FAO/WHO and the I.C.M.R. Expert Groups have recommended practical allowances of 600-700 mg. (for 10-15 years) and 500-600 mg. (for 16-19 years).

Adults: Metabolic studies have shown that daily intakes of 400-500 mg will maintain positive calcium balance in adults.

Pregnancy and lactation: Additional calcium in the diet is required during pregnancy to meet the calcium deposited in the foetus and during lactation to meet the calcium excreted in milk.

An allowance of 1,000-1,200 mg. have been recommended by FAO/WHO and I.C.M.R. Expert Groups to meet the above needs.

Important dietary sources of calcium

Data regarding the calcium content of foods are given in Table 1A in Appendix and Table 10.5. The important sources of calcium in the diet are milk and milk products, sesame seeds and green leafy vegetables. Milk is the best natural source and skim milk powder is very rich source (1.37 per cent) of calcium. Ragi is the cheapest natural source of calcium, containing about 0.3 to 0.36 per cent. Though sesame seeds are rich in calcium (1.45 per cent), the calcium is present as calcium oxalate mostly in the skin. The skin is usually removed before consumption. The dehusked sesame seed is only a fair source of calcium (0.15 per cent). Green leafy vegetables are one of the cheapest natural sources of calcium containing about 0.44 to 1.13 per cent. Since green leafy vegetables are also very rich sources of carotene and ascorbic acid, they should be included in the diet as a source of all the above nutrients. Small fish eaten along with bones is an excellent source of calcium.

Table **10.5 Important dietary sources of calcium**

Foodstuffs	Calcium content mg/100g	Foodstuffs	Calcium content mg/100g
Milk and milk products:		*Cereals:*	
Milk, cow's	120	Ragi	330
Milk, buffalo	210		
Milk, goat	170	*Green leafy vegetables :*	
Curds from cow's or buffalo milk	120-210	Sesbania leaves	1130
		Carrot leaves	340
Milk powder whole	950	Amaranth leaves	500
Milk powder, skimmed	1370	Drumstick leaves	460
Cheese	790	Curry leaves	810
Khoa (from whole buffalo milk)	650	*Fish:*	
		Small fish, dried	1800
Oilseeds and nuts:			
Sesame seed	1450		
Sesame seed (without skin)	150		

PHOSPHORUS

An adult human body contains about 400 to 700 g. of phosphorus as phosphates. A greater part of this is present in the bone and teeth and the rest in other tissues. Phosphorus is present in the body as inorganic salts of phosphoric acid or in combination with organic compounds.

Inorganic P: As calcium phosphate in bones and teeth and as phosphates of sodium and potassium in soft tissues and body fluids.

Organic P: The important organic compounds containing phosphorus are the following: (1) Phospholipids e.g., lecithin, cephalin. (2) Nucleoproteins and nucleic acid. (3) Creatine phosphate, A.T.P. and A.D.P. and Coenzymes, I, II and Co-carboxylase, and (4) Hexose phosphates, triosephosphates and glycerophosphates. Data regarding the phosphorus content of foods are given in Table 1A in Appendix and Table 10.6.

Food sources

The important food sources are milk, eggs, meat and fish. Vegetables are fair sources. A greater part of the P present in cereals, pulses, nuts and oilseeds exists in the form of phytic acid or phytin. Phytic acid is a compound of inositol and phosphoric acid and phytin is Ca, Mg salt of phytic acid.

Functions

The important functions are as follows:

1. Phosphorus is necessary for the formation of bone and teeth.
2. It is necessary for the formation of phospholipids—lecithin and cephalin which are integral parts of cell structure and also act as intermediates in fat transport and metabolism.
3. It is essential for carbohydrate metabolism as phosphorylation of glycogen requires inorganic phosphorus and phosphoric esters like adenylic acid, adenylpyrophosphate and creatine phosphate.

Table 10.6 **Important sources of phosphorus**

	Phosphorus (g./100g.)		*Phosphorus (g./100g.)*
Milk and Milk products		Rice, raw, homepounded	0.21
Milk, cow's	0.09	Rice, raw, milled	0.11
Milk, buffalo's	0.13	Rice, parboiled, home-pounded	0.22
Milk, powder, skimmed	1.00	Rice, parboiled, milled	0.17
Milk powder, whole	0.73	Wheat, whole	0.32
Cheese	0.52	Wheat flour (maida)	0.09
Khoa (from whole buffalo milk)	0.42	*Pulses*	
Flesh foods		Bengal gram dhal	0.31
Egg, hen's	0.22	Black gram dhal	0.37
Egg, duck's	0.26	Cow gram	0.49
Fish	0.41	Field bean, dry	0.45
Mutton	0.24	Green gram dhal	0.28
Liver, sheep	0.38	Horsegram	0.39
Cereals		Lentil (Masur dhal)	0.25
Bajra (Cambu)	0.35	Peas, dried	0.30
Barley	0.23	Red gram dhal	0.26
Cholam (Jowar)	0.28	Soybean	0.69
Italian millet	0.29	*Nuts and oilseeds*	
Maize (corn)	0.33	Almond	0.49
Oatmeal	0.38	Cashewnut,	0.45
Ragi	0.27	Coconut, fresh	0.24
Rice, raw, husked	0.23	Gingelly (sesame) seeds	0.57
		Groundnut	0.39

Note: 1. Most of the common foodstuffs are rich in phosphorus.

2. The phosphorus present in milk and animal foods is available to a greater extent than that present in cereals, pulses, nuts, and oilseeds.

4. It is a constituent of certain coenzymes e.g. Coenzyme I, and Co-carboxylase which take part in the enzyme systems concerned in the oxidation of carbohydrates, fats and proteins and

5. It is an essential constituent of nucleic acid and nucleo-proteins which are integral parts of the cell nuclei.

Metabolism

Phosphorus is absorbed in the small intestines as inorganic phosphates. Phosphorus present in organic combination. e.g. phytic acid, should be hydrolysed to inorganic phosphate before absorption. Since the enzyme—phytase—is not present in human digestive juices, phytin phosphorus is absorbed only to a very slight extent in human beings. Phosphorus present in animal foods such as milk, meat and eggs is absorbed to a greater extent than that present in cereals and legumes as the latter exists mostly in the form of phytic acid. The kidney is the major pathway of excretion of the phosphorus absorbed. The retention of phosphorus in children on different diets has been reported to vary widely from 10-40 per cent. The retention of phosphorus will depend on the following factors (*i*) the quantity of P ingested (*ii*) the calcium content of the diet (*iii*) the form in which phosphorus exists in the diet and (*iv*) vitamin D intake.

Phosphorus content of blood serum

The inorganic phosphorus content of blood serum in normal human adults ranges from 2.5 to 4.0 mg/100 ml and in children from 4.0-5.0 mg/100 ml. In rickets, the level of phosphorus is reduced to less than 3 mg/100 ml.

Requirements

Phosphorus requirements depend on the availability of phosphorus present in diets. Phosphorus present in cereals and legumes are available to a lesser extent (as it is present in the form of phytic acid) than that present in milk, meat, eggs and fish. In view of this, phosphorus requirements of persons consuming predominantly cereal based diets, will be greater than those consuming large quantities of milk, meat, eggs and fish. The optimal Ca: P ratio for infants and children is 1:1 and for adults 1:2. The Ca: P ratios of diets consumed in different countries vary widely from 1:1.5 to 1:3. Metabolism studies conducted on these diets have shown that the subjects maintained positive calcium and phosphorus balance. N.R.C. (U.S.A.) has recommended an allowance for phosphorus equal to

that for calcium for all age groups excepting infants. In the case of infants, they have recommended a Ca: P ratio of 1.5:1.

MAGNESIUM

The adult human body contains about 25 g. of magnesium. About half this quantity is present in the bones in combination with phosphate and carbonate and about one-fifth of the total magnesium in the body is present in the soft tissues.

Functions

1. Magnesium is found in certain enzymes e.g., co-carboxylase which decarboxylates pyruvic acid.
2. It acts as an activator of several enzymes e.g. alkaline phosphatase, all phosphorylating enzymes and
3. It is required as a co-factor for oxidative phosphorylation.

Metabolism

The average intake from the diet by adults is about 300-400 mg. A greater part of this (40-50 per cent) is not absorbed and hence excreted in the stools. About one third of the amount ingested is excreted in urine. The magnesium content of normal human serum is about 2-3 mg/100 ml. and of whole blood 1.6 mg. per 100 ml.

Effects of magnesium deficiency

Rats: In weanling rats fed on a Mg deficient diet, vasodilation manifested by erythema and hypermia appeared within 3 to 5 days followed by pallor and cyanosis 12 days later. The animals became hypersensitive to slight noise. Generalised convulsions were observed after 18 days. Many of them died during the first convulsions. Incorporation of 5 mg magnesium per 100 g. of the same diet prevented the deficiency and promoted normal growth.

Adult human beings: Magnesium deficiency was produced in 3 patients on low magnesium diet. The principal clinical features were depression, muscular weakness, vertigo and liability to convulsions. The serum magnesium level was below 1 mg/100 ml. All the clinical symptoms were cured by the parenteral

administration of magnesium salts (100 mg magnesium chloride) within 4 hours. Magnesium deficiency has also been observed in chronic alcoholics as indicated by low serum magnesium and muscular weakness. Valee *et al.* (1960) reported the occurrence of muscular tremors, delirium and tetany in human subjects suffering from magnesium deficiency. The symptoms were cured when magnesium salts were administered parenterally.

Children: It is possible that magnesium deficiency exists in cases of kwashiorkor as the serum Mg. level has been reported to be low. Magnesium deficiency may contribute to some extent to the apathy and weakness found in kwashiorkor.

Requirements

Magnesium requirements for different age groups are given below:

Adults—200-300 mg./day.
Older children—150-200 mg./day.
Infants and preschool children—100-150 mg./day.

Magnesium contents of foods

Data regarding magnesium content of foods are given in Table 3A in Appendix.

SODIUM CHLORIDE

All minerals except NaCl are usually present in sufficient amounts in a well-balanced diet. Sodium chloride is the only mineral which is taken in more or less pure form in addition to the amount present in natural foods.

NaCl intake and excretion

A healthy adult excretes daily in urine about 10 to 15 g. NaCl. All this is derived from the salt taken in food. Sodium chloride is also excreted in sweat. During excessive sweating in hot climates, while doing hard work, the loss of NaCl in sweat may vary from 10 to 20 g. daily. During fasting or on salt-free diets, the excretion of chlorides in urine may be decreased or only a trace of NaCl may be found. Absence of NaCl in urine is an indication for adding more NaCl to the diet. NaCl is stored in the subcutaneous tissue. During NaCl deprivation, the store is used up.

Heat cramps due to NaCl deficiency

Men doing hard work in hot humid climates e.g., as in mines suffer from heat cramps i.e., intense and painful contractions of skeletal muscle. This is due to NaCl deficiency caused by loss of NaCl from the body by excessive sweating. The loss may be about 2 to 5 g. of NaCl per hour of very hard work or 10-20 g. per day. Heat cramps may be prevented by adding NaCl (0.3 to 0.5 per cent) to drinking water. Drinking pure water without replacing the salt loss, aggravates the deficiency.

Excessive intakes of NaCl

Consumption of excessive amounts of NaCl causes oedema in protein deficiency and increases blood pressure in hypertension patients.

Sodium chloride requirements

NaCl requirements depend on the climate and occupation and on the salt content of the diet. Foods of animal origin contain more NaCl than those of vegetable origin. The sodium chloride requirements for persons living in the tropics are given below:

NaCl requirements (g/day) for tropical countries

Adults: light work	10-15
hard work	15-20
very hard work	25-30
Children	5-10
Adolescent boys and girls	10-25
Women, pregnancy: first half	10
second half	5
Lactation	15

SODIUM

The adult human body contains about 100 g. of sodium ion. It is distributed entirely in the extra cellular fluid (plasma, tissue fluid and lymph) of the body. On the average 5-10 g. of sodium corresponding to about 10-20 g. NaCl is ingested per day in an average diet.

Functions of Na ion

Na ion exists in the body in association with the following anions; Cl, bicarbonate, phosphate, lactate and proteinate. The functions of Na ion are: (1) Regulation of acid-base balance of the body (2) Regulation of the osmotic pressure of the plasma or tissue fluids (3) Na ions play a special role in originating and maintaining the heart beat.

Excretion

On an average diet about 3 to 5 g of sodium (corresponding to 8 to 12 g NaCl), is excreted in urine. On a low salt diet and in starvation urinary excretion may fall to very low levels.

Low sodium diets

Low Na diets are prescribed for patients suffering from high blood pressure. Data regarding the sodium content of foods are given in Table 3A in Appendix.

POTASSIUM

The adult human body contains about 250 g. potassium which is present almost entirely in the cells of different tissues, muscle etc. Only small quantities are present in extracellular fluid. While plasma contains only traces of potassium, the red blood cells contain large amounts of potassium ion. Potassium is the major basic ion of the body cells and apparently serves in the cells the same functions as sodium in the extracellular fluids. Potassium occurs in abundance in foods and so there is no danger of potassium deficiency.

Functions of potassium

The important functions of potassium are: (1) Regulation of pH of cell contents, (2) Regulation of the osmotic pressure of cell contents and (3) Potassium ion increases the relaxation of heart muscle which is antogonised by Ca ion.

Potassium deficiency and excess

Potassium deficiency causes weakness and muscular paralysis. In animals, hypertrophy of the heart has been observed.

Consumption of excessive amounts of potassium causes muscular weakness and apathy—symptoms similar to those of potassium deficiency.

Potassium content of foods

Data regarding the potassium content of foods are given in Table 3A in Appendix.

IRON

The total iron content of the normal adult man (70 kg wt) is estimated to be about 4-5 g. A greater part of the iron in the body is present as haemoglobin. Most of the body iron exists in complex forms bound to protein either as porphyrin or heme compounds or as ferritin and transferrin. Free inorganic iron occurs in the body only in very small amounts. The hemo protein and flavo-protein enzymes also contain iron.

Some compounds of biological importance containing iron are given below:

(*i*) *Iron porphyrin (heme) compounds:* Blood haemoglobin, Myoglobin (in muscle).

(*ii*) *Heme enzymes:* Mitochondrial cytochromes, Microsomal cytochrome, Catalase, Peroxidase.

(*iii*) *Flavin-enzymes:* Succinic dehydrogenase, Xanthine oxidase, DPNH-Cytochrome C reductase, Iron chelate enzyme aconitase, and

(*iv*) *Transport and storage of iron:* Transferrin (2 Fe+ globulin), Ferritin [+ (FeOOH)n+globulin], Hemosiderin (Ferric hydroxide+non-nitrogenous compound).

Iron metabolism

In monogastric species, it is known that iron is absorbed principally in the ferrous state in the duodenum. In the rat and dog, ferric salts have been shown to be as well utilised as ferrous salts indicating that gastro-intestinal conditions in these animals are favourable to reduction of ferric iron. In man, on the other hand, ferrous salts have been shown to be more effective for haemoglobin formation than ferric salts. Iron absorption is reduced by the presence of phytate, oxalates and excessive amounts

of inorganic phosphate. The rate of absorption of iron is also affected by the magnitude of iron store in the body and the rate of erythropoiesis. About 2-20 per cent of an oral dose of radio-active iron is absorbed in normal subjects, compared with 20-60 per cent in iron deficiency anaemia, the percentage absorption falling as the intake is increased.

Storage of iron

The storage of iron in the body occurs predominantly in the liver in the form of the two non-hemecompounds, ferritin and hemosiderin. Ferritin is a iron protein compound containing about 20 per cent iron. Iron stored as hemosiderin is not available to the body.

Blood iron

Iron occurs in blood as haemoglobin in the RBC and as transferrin in plasma in the ratio of nearly 1000 to 1. Transferrin (also known as siderophilin) is a globulin which binds 2 atoms of ferric iron per molecule and appears to be the main carrier of iron.

Excretion

The iron present in the faeces consists mainly of unabsorbed food iron. True excretory iron is estimated to be 0.3-0.5 mg/day, by radio active iron technique. This is derived from bile and desquamated mucosal cells. The amount of iron eliminated in the urine of normal human adults may vary from 0.1 to 0.3 mg/day. There is a continuous dermal loss of iron in the sweat, hair, nails etc. 'Cell rich' sweat may contain 1.15 to 1.61 mg Fe/litre while 'cell free' sweat may contain 0.34 to 0.44 mg Fe/litre in healthy men and women. In anaemic women, the 'cell rich' sweat contains 0.44 mg Fe/litre and 'cell-free' sweat contains no iron.

Iron deficiency anaemia

Iron deficiency anaemia is widely prevalent among children, adolescent girls and expectant and nursing mothers in all developing countries.

Signs and symptoms of anaemia: The clinical features are the results of diminished oxygen carrying power of the blood due to low haemoglobin content (5 to 9 g./100 ml. blood).

Women of child bearing age: The clinical features are general fatigue and lassitude, breathlessness on exertion, giddiness and pallor of the skin. In severe cases, there may be some oedema of the ankles. The haemoglobin levels commonly range between 5 and 9 g/100 ml. blood (normal 13-15 g/100 ml) and the RBC count (3-4.5 million per cumm).

Weaned infants and young children: Iron deficiency anaemia is widely prevalent among weaned infants and young children in India and other developing countries. The haemoglobin levels in severe and moderately severe anaemic cases, may range from 5-9 g/100 ml. The children are dull, and inactive and show pallor of the skin. The appetite is poor and growth and development of children are retarded due to low food intake. There is a tendency for children below 3 years to eat mud.

Treatment: Anaemic women should receive ferrous sulphate tablets (0.2 g) 3 times a day. For a child of below 12 months a mixture containing 0.2 g. ferrous ammonium citrate sweetened with glycerine three times a day and for children 1-5 years 0.4 to 0.9 g. of ferrous ammonium citrate in the form of mixture will be effective in curing the anaemia. The treatment should be continued for a few months till the haemoglobin is restored to normal level.

Disorders of iron metabolism

Three types of disorders of iron metabolism are known (1) Siderosis (2) Nutritional siderosis and (3) Haemochromatosis.

Siderosis: This term is used to describe the presence of excess of iron in the body as demonstrated by the presence of haemosiderin in the tissues. Iron stored in this form is not available to the body.

Nutritional siderosis: This disorder has been observed among Bantus in South Africa. Bantus cook their food in large iron pots and the daily iron intake may be as high as 100 mg. or more. The absorption of iron also appears to be high leading to the development of nutritional siderosis. Examination of the

livers of Bantus who died of accidents revealed the presence of large amounts of iron (more than 100 mg/100 g. fresh liver). Iron is also present in parenchymal cells, Kupffer cells and portal tracts.

Haemochromatosis: This is a rare disease in which excessive amounts of iron are absorbed from the intestinal tract. The absorbed iron is deposited chiefly in the liver, spleen, pancreas, skin and several other organs, giving rise to various disorders including cirrhosis of the liver, diabetes mellitus and grey discolouration of the skin.

Important dietary sources of iron

Data regarding the iron content of foods are given in Table 1A in Appendix and Table 10.7.

Table **10.7 Important sources of Iron**

Foodstuffs	Iron (mg/100g)	Foodstuffs	Iron (mg/100g)
Cereals	2.0-8.8	*Green leafy vegetables:*	3.9-21.4
Bajra	8.8	Amaranthus, tender	21.4
Barley	3.7	Coriander	10 0
Cholam	6.2	Drumstick	7.0
Maize, Yellow	2.1	Mint	15.6
Oat meal	3.8	Radish leaves	4.8
Ragi	5.4	Spinach	5.0
Rice, parboiled milled	3.7		
Rice, raw milled	2.8	*Meat and fish and eggs:*	2.1-6.3
		Egg	2.1
Legumes:	3.8-11.3	Fish	2.3
Bengalgram dhal	8.9	Liver, goat	6.3
Blackgram dhal	9.8	Mutton, goat	2.5
Cow gram	3.8		
Field bean, dry	5.0	*Nuts and oilseeds:*	1.7-10.5
Green gram dhal	8.4	Cashew nut	5.0
Redgram dhal	8.8	Groundnut	1.7
Soybean	11.3	Sesame seeds	10.5
		Miscellaneous foods:	
		Jaggery	11.4

Cereals are the most important sources of iron in the diets of a large majority of the population in India and other developing countries. Other important sources are legumes, green leafy

vegetables and jaggery. Meat, fish and eggs are important sources of iron in all advanced countries. Milk is a poor source of iron.

Requirements

Iron requirements are influenced by the availability of iron present in foods. Iron present in cereals, legumes and green leafy vegetables is available to a lesser extent (due to the presence of phytates and oxalates) than that present in eggs, meat and fish. In view of this, iron requirements of persons consuming a predominantly cereal based diet, will be greater than those consuming large quantities of meat and eggs. Iron requirements suggested by different Expert Groups are given in Table 10.8.

Table **10.8 Recommended allowances for Iron (mg/day/caput)**

Age groups	I.C.M.R. Expert Group (1968)	N.R.C. (U.S.A.) (1968)	Expert Group (U.K.) (1969)
Birth to 1 year	1 mg/kg	6-15	6
1-5 years	15-20	15	7-8
6-12 years	15-20	10	10-13
13-18 years: Boys	25	18	15
Girls	35	18	15
Man	20	10	10
Woman	30	18	20
,, Pregnancy	40	18	15
,, Lactation	30	18	15

TRACE ELEMENTS

Many elements occur in tissues and foodstuffs in small amounts i.e., in traces. Hence these elements have been termed 'Trace Elements'. The important trace elements are copper, iodine, zinc, cobalt, manganese, molybdenum, fluorine, selenium, nickel, chromium, cadmium, silicon, vanadium and strontium.

COPPER

The first conclusive evidence, that copper is an essential element for the formation of haemoglobin in rats suffering

from iron deficiency anaemia emerged from the studies of Hart and co-workers in 1928. Later studies have indicated that copper is a constituent of several enzymes and is found as a complex with some proteins in blood.

Distribution of copper in the body

The healthy human adult body contains about 100-150 mg. of copper. Copper present in the blood exists in the form of copper protein complex-*haemocuprin* in red blood cells and *ceruloplasmin* in plasma.

Copper metabolism

Numerous studies with stable and radio-active copper emphasize the poor absorption of ingested copper in all species investigated. Only about 5-10 per cent of the copper in ordinary diets appear to be absorbed and retained by the body. The absorbed copper is transported in the blood plasma loosely bound to a plasma protein, probably serum albumin from which it is transported to different tissues. Copper is stored in the liver and other tissues and released for the formation of copper containing enzymes, and such compounds as haemocuprin and ceruloplasmin. Copper is excreted in the bile into the intestinal tract. Copper administered by injection is better retained in the body than orally administered copper. The biliary system is the major pathway of copper excretion in man and several animals.

Blood copper levels

The copper content of normal human whole blood ranges from 0.5 to 1.5 mg/litre and of plasma 0.7 to 1.6 mg/litre. Plasma levels are not changed as a result of ingestion of copper or during fasting. The plasma copper level increases in pregnancy. Low plasma copper levels are found in man in Wilson's disease in which increased urinary excretion of copper has been observed. In Wilson's disease, the ceruloplasmin content of plasma is also reduced (ceruloplasmin content in Wilson's disease, 9 mg/100 ml and in normal subjects 34 mg/100 ml).

Wilson's disease: This disease is associated with cirrhosis of the liver. The clinical symptoms include tremors as in paralysis, muscular weakness, difficulty in swallowing and speaking

and alteration of emotional balance. The cause of the disease is not known. Copper metabolism is affected in this disease. Only traces of copper are excreted in normal human urine (14 to 22 μg/day) while in Wilson's disease the excretion may be as high as 1500 μg/day. Negligible amounts of copper are lost in the sweat. Some amount of copper is excreted in human milk (0.4 mg/day).

Effects of copper deficiency

Animals: A wide variety of clinical disorders have been associated with a dietary deficiency of copper in animals. These include anaemia, depressed growth, bone disorders, depigmentation of hair or wool, abnormal wool growth, neonatal ataxia and impaired reproductive performance.

Human beings: In human beings the only condition observed, is anaemia due to copper deficiency.

Copper in human nutrition

Anaemia produced in infants fed exclusively on milk can be cured only by giving copper salts along with iron. The estimates of average daily intakes in adult diets range from 2-3 mg. in U.S. and U.K. diets as compared with 4.5 to 5.8 mg. on Indian diets. The high intake in Indian diets may be due to contamination of copper from brass vessels used in cooking. Copper deficiency is likely to occur only in infants fed exclusively on milk diet.

Copper content of foods

Data regarding the copper content of foods are given in Table 3A in Appendix.

Requirements

The copper requirements are given below:

	mg/day/caput
Adults	2
Pregnancy	3
Lactating	3
Infants (1-12 months)	0.5-1.0
Children	2
Adolescents	3

Plate XI: A case of goitre

IODINE

Iodine is a constituent of thyroxine, the active principle of the thyroid gland. The thyroid gland, weighing about 25 g. in a normal adult, contains only about 10 mg. of iodine. The adult body as a whole contains about 50 mg. of iodine. The thyroid gland plays an important part in energy metabolism and in the growth of the body.

Food sources of iodine

Iodine is present only in small amounts in common foods, the quantity of iodine present depending on the iodine content of the soil. The soil of mountainous regions usually contains less iodine than the soil of the plains near the sea. Crude common salt prepared from sea water and sea fish are good sources of iodine.

Iodine requirements

Iodine requirements for adults are about 0.15 to 0.2 mg. daily. This is normally supplied by an ordinary well-balanced diets and by drinking water except in mountainous regions where the food and water are deficient in iodine. The iodine requirements of different age groups are given in Table 16.2 in Chapter 16.

Iodine deficiency in human beings

If sufficient iodine is not taken in the diet, enlargement of the thyroid takes place, resulting in the disease called *goitre*. The thyroid gland of the adult which normally weighs about 25 g. may weigh as much as 200 to 500 g. or even more in goitre. Histological examination shows diffuse overgrowth of the glandular tissue, known as general hyperplasia of the glands. The vesicles contain little or no colloid. (Plate XI). If treatment with iodine is started very early the thyroid may become normal. But if treatment is delayed, the enlargement of the gland persists. In children, severe iodine deficiency may result in serious retardation of growth. This condition is known as *cretinism*.

Incidence and prevention of goitre

A survey of the incidence of goitre in different parts of the world shows that the disease is related to deficiency of iodine

in the water and food. Goitre commonly occurs among people living in mountainous regions. In India, goitre occurs in the hilly districts along the foot of the Himalayas e.g., in Kashmir, Kangra Valley etc., Goitre can be prevented by the regular use of iodised salt (1 g. of sodium iodate being added to 100,000 g. of common salt). By the use of iodised salt, goitre has been prevented in countries such as Switzerland, U.S.A. India etc. (Chapter 2. Vol, II).

Goitrogenic substances in foods

There is evidence indicating many foods such as cabbage, cauliflower and radish contain substances which react with the iodine present in the food and make it unavailable. Consumption of large quantities of these foods leads to the development of goitre by making the iodine present in the food not available to the body. These substances are known as 'goitrogenic' substances. The chemical nature of these substances is discussed in Chapter 3 (Volume II).

ZINC

The importance of zinc for the growth and well-being of the albino rat was demonstrated by Todd *et al.*, in 1934. This was confirmed by other workers for mice and birds. In 1939, Keilin and Mann showed that zinc is a constituent of the enzyme carbonic anhydrase. Since that time, zinc has also been found in some other enzymes. It is also found in insulin.

Occurrence in the body

The whole body of a normal man weighing 70 kg. may contain 1.4 to 2.3 g. of zinc. Zinc is present in small amounts in all tissues. Bones, teeth and pancreas contain slightly higher amounts than other tissues. Whole blood contains about 0.7 mg./100 ml. and blood serum or plasma 0.1 mg/100 ml.

Metabolism

Studies in rats using radio-active isotope of zinc showed that only 3-10 per cent of dietary zinc was absorbed. Phytic acid interferes in the absorption of zinc. Zinc, whether taken by oral route or by injection, leaves the body largely through the

faeces. Faecal zinc consists largely of unabsorbed dietary zinc with a small proportion of zinc absorbed and secreted in the intestines through pancreatic juice and bile. Very little zinc is found in the urine.

Functions

The important functions are as follows: (1) Zinc is a constituent of the enzyme, carbonic anhydrase present in R.B.C. and stomach walls. (2) It is a constituent of insulin—the hormone present in the Islets of Langerhans of pancreas and (3) It is a constituent of several other enzymes viz. carboxypeptidase, alkaline phosphatase etc.

Deficiency

Zinc deficiency has been experimentally produced in rats. The signs and symptoms include (1) retardation or failure of growth (2) alopecia (loss of hair) accompanied by gross epithelial, especially cutaneous lesions and (3) dermatitis, scaling and cracking of the paws. Histological studies revealed a condition of parakeratosis i.e. hyperkeratinisation of the epithelial cells of the skin. In chicks, in addition to the above symptoms, shortening and thickening of long bones are observed. Zinc deficiency in the diet has been reported to be the cause of anaemia, growth retardation and delayed genital maturation ('Dwarfism') in children in Middle East countries. Zinc deficiency does not occur in other countries.

Human requirements

Normal well balanced diets contain about 10-15 mg. zinc. Human requirements are not known.

MANGANESE

Nutritional importance of manganese was discovered in 1936-37, when Norris and co-workers and Lyons and Insko reported the development of 'slipped tendon' and nutritional chondrodystrophy in poultry fed on manganese free diet. Recent studies of Hurley and Everson and their associates, (1961) have thrown more light on the relation of manganese to growth, skeletal development, reproduction and the functioning of central nervous system.

Occurrence in the body

The body of a normal man weighing 70 kg. contains about 12-20 mg. manganese. It is distributed throughout the body tissues and fluids. The following tissues viz., bone, kidney, liver, pancreas and pituitary contain more manganese than other tissues. The manganese content of human blood is very low-2 to 3 microgram/100ml.

Manganese metabolism

Only 3-4 per cent of the Mn present in the diet is absorbed, the remaining being excreted in the faeces. The urine contains only traces of manganese.

Manganese deficiency in animals

Manganese deficiency has been produced experimentally by feeding on diets low in Mn The most important effects observed are, impaired growth, skeletal abnormalities, depressed reproductive function, and ataxia of the new born.

Manganese and bone growth: Skeletal abnormalities ranging from gross and crippling deformities to mild and generalised rarefaction of bone, occur in all species, depending on the degree and duration of manganese deficiency.

Manganese and reproductive function: In manganese deficiency, the animals give birth to young ones which develop ataxia. In a more severe stage of deficiency, sterility results. In poultry, lowered egg production and decreased hatchability are observed even in mild manganese deficiency.

Lipotropic action of manganese: The livers on manganese deficient rats contain excessive amounts of fat. This fat accumulation is prevented either by manganese or choline.

Manganese and enzyme activity: Liver arginase activity and alkaline phosphatase activity of blood and bone are significantly reduced in manganese deficiency.

Manganese in human nutrition

Clinical signs and symptoms due to manganese deficiency has not so far been reported in man. Diets normally consumed in different countries appear to contain enough manganese.

COBALT

The first conclusive evidence that cobalt is a dietary essential came in 1935 from work done in Australia on certain debilitating diseases of sheep and cattle known locally as 'coast disease' and 'wasting disease'. Addition of small amounts of cobalt to the diet prevented the diseases. In 1948, it was shown that vitamin B_{12} contained cobalt. Later it was found that cobalt deficiency in ruminants can be cured by injecting vitamin B_{12}.

Distribution in the body

Cobalt occurs in small amounts in all tissues, highest concentrations occurring in liver and kidneys. Most of the cobalt is present in vitamin B_{12}.

Cobalt metabolism

Cobalt present in the diet is absorbed to the extent of 70-80 per cent and about half the absorbed cobalt is excreted in the urine. The excretion in the faeces is 20-30 per cent of the intake.

Cobalt in ruminant nutrition

Sheep and cattle fed on fodder grown in some regions in Australia and New Zealand developed severe anaemia and wasting of muscles and died. The diseases are known as 'pining' 'salt-sick', 'bush-sickness', 'wasting disease' or 'coast disease'. Supplementation of the feed with small amounts of cobalt cures and prevents the diseases. The deficiency can also be cured by injection of vitamin B_{12}.

Cobalt requirements

Daily supplements of 0.1-0.2 mg. cobalt for sheep and 0.3 to 1.0 mg. cobalt for cattle prevent the deficiency and keep the animals in good health.

Cobalt and vitamin B_{12} in ruminants

The mode of action of cobalt in preventing the deficiency disease in ruminants is through the production of vitamin B_{12} by the rumen microorganisms. In the absence of cobalt, vitamin

B_{12} is not synthesised. The fact that cobalt deficiency can be cured by the injection of vitamin B_{12} indicates, cobalt acts by being incorporated in vitamin B_{12}. Dietary cobalt, incorporated in vitamin B_{12} synthesised by rumen organisms is available to the ruminants while that synthesised in the cecum and large intestines cannot be absorbed. Ruminants synthesise their requirements of vitamin B_{12}, while man and other animals cannot synthesise vitamin B_{12} and need a dietary source of this vitamin.

Cobalt requirements of man

Cobalt deficiency has not been observed in human beings. Cobalt requirements, if any, appear to be met by traces of cobalt found in foods. Human beings require a dietary source of vitamin B_{12} and cannot synthesise it in the body.

Cobalt toxicity

Cobalt administered in large amounts to animals or man is toxic. It causes a condition known as polycythemia i.e., increased number of R.B.C. in blood.

MOLYBDENUM

Attention was first directed to the importance of molybdenum in 1938 in England, when it was found that severe diarrhoea-*'teart'* in cattle was the result of intake of excessive amounts of molybdenum from the herbage. Interest in the biological significance of molybdenum received a further stimulus from the discovery that the flavoprotein enzyme, xanthine oxidase contained molybdenum.

Occurrence in animal and human tissues

Small amounts of molybdenum are present in all tissues. Liver and kidney contain larger amounts than other tissues.

Absorption and excretion

About 50 to 70 per cent of the intake is absorbed and half the absorbed molybdenum is excreted in urine.

Molybdenum and xanthine oxidase of tissues

The need for dietary molybdenum for maintaining the xanthine oxidase levels in the tissues of rats was established by some workers.

Molybdenum toxicity

Consumption of pasture rich in molybdenum in certain parts of U.K., California and New Zealand has been reported to cause severe diarrhoea and ill health in cattle known as 'teart' or 'peat scours'. This is cured by the administration of fairly large doses of copper salts. High molybdenum intakes by rats are accompanied by the loss of body weight often with marked anorexia. Changes in the connective tissues occur.

Molybdenum-copper relationship

The diarrhoea caused in cattle by the consumption of forage rich in molybdenum can be cured by the administration of copper salts. It has been reported that molybdenum decreases the retention of copper in cattle.

Dietary sources

Pulses, cereal grains and some green leafy vegetables are good sources. Other vegetables and fruits are poor sources. Liver and kidney are good sources.

Human requirements

The average diets provide adequate amounts of molybdenum. The exact requirements are, however, not known.

FLUORINE

Interest in the importance of fluorine in nutrition was created by the discovery of high incidence of chronic endemic fluorosis in man and farm animals in 1931 in certain parts of India. (Shortt *et al.* 1937). An association between fluorine deficiency and dental caries in rats was reported in 1925 by McCollum and co-workers.

Fluorine and dental caries

Studies by several workers have shown that the incidence of dental caries in animals is high on a fluorine deficient diet and incorporation of fluorine at a level of 1-2 ppm. in the diet, prevented the disease. Surveys carried out on the incidence of dental caries in human beings have yielded the following results (1) The incidence of dental caries in children is high in areas where the drinking water contained less than 0.5 ppm. fluorine and was low in areas where the water contained 1-2 ppm. fluorine and (2) Addition of fluorine to drinking water at a level of 1 ppm. brought about a significant reduction in the incidence of dental caries.

Toxic effects of excess of Fluorine

Fluorosis in man: Dental fluorosis (Mottled enamel)

In many parts of the world where the drinking water contains excessive amounts of fluorine (3-5 ppm.) signs and symptoms of dental fluorosis have been observed. The enamel of the teeth loses its lustre and becomes rough. Chalky white patches with a secondary infiltration of yellow or brown staining are found irregularly over the surface of the teeth. The enamel is structurally weak and in severe cases there is marked loss of enamel accompanied by 'pitting' which gives the tooth surface a corroded appearance.

Skeletal fluorosis

Chronic fluorine intoxication through drinking water containing excessive amounts of fluorine (over 10 ppm.) or among workers handling fluoride containing minerals, results in pathological changes in bones. There is (sclerosis) increased density and hypercalcification of the bone of the spine, pelvis and limbs. In addition, the ligaments of the spine become calcified, producing a 'poker back'. It is possible that collagen in the bone is calcified. There may also be ossification of the tendinous insertions of muscles. Neurological disturbances secondary to the changes in the vertebral column are common. Such persons are

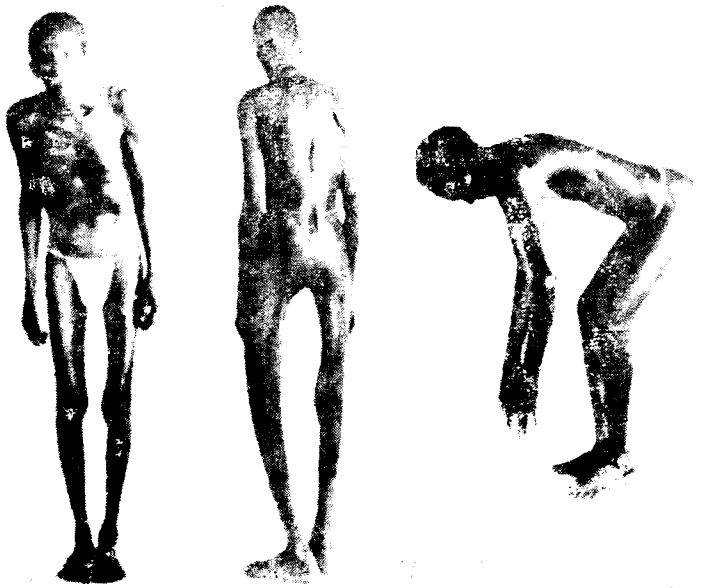

Plate XII: An advanced case of fluorosis showing spinal deformity and rigidity

crippled and cannot perform simple daily tasks, such as bending, squatting etc., as the joints become stiff (Plate XII).

Prevention of toxic fluorosis

Toxic fluorosis can be prevented only by removing fluorine from the water supplies by treatment with activated carbon or by some other suitable adsorbents.

Requirements

Fluorine is found in small amounts in normal bones and teeth. Since water containing 1-2 ppm. prevents dental caries and does not do any harm, the fluorine requirements of the body are met by the quantity normally present in drinking water (1-2 ppm.) in most of the regions.

SELENIUM

Selenium was shown to be toxic to animals as early as 1842, but nearly a century passed before two naturally occurring diseases of livestock, known as '*alkali disease*' and '*blind staggers*' were identified as manifestations of chronic and acute selenium poisoning respectively. Schwartz and Foltz in 1957 discovered that necrosis of liver in rats produced by feeding on diets based on torula yeast can be prevented by minute quantities of selenium salts. They also discovered that selenium was present in organic combination termed by them as 'Factor 3' in brewer's yeast and liver which can prevent necrosis of liver. Later, some workers demonstrated that this element is necessary in small amounts for promoting growth and maintaining good health in chicks.

Effects of selenium deficiency in animals

The following effects of selenium deficiency have been observed in animals.

Necrosis of liver: Feeding rats on synthetic diets based on torula yeast and low in cystine and vitamin E produces necrosis of the liver which has been prevented by supplements of minute amounts of selenium.

Muscular dystrophy: Muscular dystrophy is a degenerative disease of the muscles which occurs in animals. It has been

reported to occur in calves and lambs in many countries. Studies carried out in Scotland have shown that the disease can be prevented by injection of 15 mg. of selenium at birth or 5 mg. selenium in every 4 weeks.

Exudative diathesis in chicks. Chicks fed selenium deficient diets fail to grow and develop a diseased condition known as exudative diathesis.

Selenium and vitamin E

Selenium and vitamin E are both essential for curing certain diseased states in experimental animals mentioned below (1) liver necrosis in rats and pigs (2) exudative diathesis in birds and (3) muscular dystrophy in cattle.

Selenium deficiency in the presence of vitamin E: When animals are provided with adequate amounts of vitamin E, selenium deficiency causes the following signs and symptoms: (1) rats—retardation of growth and muscular wasting and (2) chicks: retardation of growth and fertility. Selenium is thus an essential trace element which has close metabolic relationship with vitamin E.

Toxic effects of excessive amounts of selenium: Selenium poisoning in cattle due to consumption of fodder containing excessive amounts of selenium has been reported in different countries.

Chronic poisoning: The disease known as 'alkali disease' results from chronic selenium poisoning. The signs and symptoms of 'alkali disease' are dullness, lack of vitality, emaciation, roughness of coat, loss of hair from the body and tail, soreness and sloughing of the hoofs, stiffness and lameness, cirrhosis of the liver and anaemia.

Acute poisoning: In the final stages of acute selenium poisoning ('blind staggers') the affected animals suffer from salivation, grating of teeth, paralysis and blindness. Death often results from respiratory failure.

Requirements

Requirements are not known. Average diets contain adequate amounts of selenium.

NICKEL

Nickel occurs in traces in human and animal tissues. The essential nature of nickel has been demonstrated for the chick. (Nielsen, 1970).

Nickel content of tissues and blood

Nickel is not accumulated in the tissues with age. The mean nickel contents of tissues are as follows: small intestines, 2; bladder, 0.5; lung, muscle and heart, 0.4 and liver 0.12 ppm. on wet tissue basis. The nickel content in human plasma is 0.06 ppm.

Metabolism

Studies carried out in U.S.A. have shown that adults excrete about 30 μg Ni/litre in urine on normal diets. Studies with radioactive nickel (63_{Ni}) in mice showed that 61 per cent of a single injection of the salt was excreted in urine in 3 days and 60 per cent in faeces. The radio activity completely disappeared from blood after 48 hrs. Only kidney contained significant amounts of 63_{Ni}.

Biochemical role

Nickel activates several enzyme systems, including arginase, carboxylase, trypsin and acetyl coenzyme A synthetase. It is also found in traces in RNA.

Deficiency in animals

Albino rats fed on a nickel deficient diet, do not show any growth retardation or any other deficiency signs. Chicks maintained on a nickel deficient diet developed within 3 weeks the following signs of deficiency:—Bright orange colour of leg instead of pale yellow brown, slightly enlarged hocks, legs slightly thickened and edematous type of dermatitis of the skin. None of these abnormalities developed in the control group receiving the same diet supplemented with nickel at 4 ppm.

Toxicity

The toxicity of nickel varies with the animal species. Albino rats grew at a normal rate on diets containing 250-1000 ppm,

nickel. Adult monkeys remained in good health on similar diets. Chicks, however showed toxic symptoms on diets containing 750 ppm nickel, such as growth depression, reduction in food intake etc.

Requirements

Human requirements are not known.

CHROMIUM

Chromium occurs in traces in human and animal tissues. Recent studies have shown that chromium may be a co-factor for insulin.

Occurrence in tissues and blood

The chromium content of an adult human body is estimated to be 6 mg. Most adult tissues contain 0.02 to 0.4 ppm. of chromium on dry basis. The blood contains about 0.009 to 0.055 ppm.

Metabolism

Absorbed or injected chromium is excreted mainly in the urine, with small amounts being lost in bile and faeces. Tissue uptake is quite rapid and plasma is cleared of a dose of ^{51}Cr within 3 or 4 days. The bones, spleen, testis, and epididymis retained more ^{51}Cr after 4 days than the heart, lungs, pancreas or brain. Chromium is mobilised from the tissues in response to glucose administration.

Deficiency

Chromium deficiency is characterised by impaired growth and disturbances in glucose, lipid and protein metabolism. Rats fed on diets low in protein and in chromium (less than 0.1 ppm Cr) developed corneal opacity. Chromium supplementation prevented the opacity.

Biochemical functions

Chromium plays an important role in carbohydrate, lipid and protein metabolism. These are briefly discussed below:

Carbohydrate metabolism: In 1959, Schwarz and Mertz showed that Cr (111) (trivalent chromium) cured the impaired glucose tolerance observed in rats on chromium deficient diet. Later, Schroeder induced severe chromium deficiency in rats and the animals developed a syndrome resembling diabetes mellitus. It was rapidly cured when 5 ppm of Cr (111) was supplied in drinking water. Cr (111) has also been reported to be effective in improving glucose tolerance in some patients suffering from diabetes mellitus while it was not effective in few others.

Lipid metabolism: The serum cholesterol level was raised in rats fed on chromium deficient diet. Addition of 5 ppm of Cr (111) in drinking water brought the cholesterol level to normal.

Protein synthesis: In rats fed diets deficient in chromium and protein, the capacity for the incorporation of certain amino acids in heart muscle was reduced.

Chromium toxicity

Oral administration of excessive amounts (50 ppm) of Cr (111) has been reported to produce growth depression and liver and kidney damages in some experimental animals.

Requirements

The exact requirements are not known. Average diets appear to meet the chromium requirements.

CADMIUM

Cadmium occurs in small quantities in animal and human tissues and foodstuffs. The importance of cadmium as a trace element is due to recent observations on the possible relation of cadmium excess to hypertension in human beings and its interactions with zinc and other trace elements.

Cadmium content of tissues and blood

The cadmium content of an adult human body is about 30 mg of which 10 mg occurs in the kidney and 4 mg in the liver. Cadmium is virtually absent from the kidney of the new born. It accumulates in this organ as age advances. Adult human

blood contains about 0.7 ppm on an average. The mean excretion in the urine of adults is about 13 μg/litre. The mean cadmium content of cow's milk is about 15 μg/litre.

Metabolism

On a daily intake of 200 μg cadmium by an adult man, about 40 μg appears in the urine, 2 to 4 μg are retained and the rest is excreted in the faeces. Excess of zinc and copper interferes with absorption of cadmium and *vice versa*.

Toxicity

Cadmium when fed at 100 ppm level reduced weight gain in rats. Rabbits fed cadmium developed hyperplastic bone marrow and hypochromic microcytic anaemia similar to that produced by iron deficiency.

Cadmium and hypertension

When 5 ppm cadmium was included in drinking water, a progressively increasing incidence of systolic hypertension developed in rats after 1 year. The hypertension can be reversed by injecting a chelating agent (trans-cyclohexane—1,2 diamine-tetraacetic acid). It depleted renal and hepatic cadmium and repleted renal and hepatic zinc. The pathological changes associated with cadmium hypertension in rats, included renal arterial and arterioralar lesions and glomerular changes.

Cadmium and reproduction

Cadmium in excess causes injurious effects on the testes of male rats. Massive hemorrhagic necrosis occurred in the non-ovulating ovaries of young rats. Injection of cadmium salts into pregnant rats resulted in complete destruction of the *pars fetalis* of the placenta.

Requirements

The requirements of cadmium for human beings are not known. Available data would indicate that most of the diets contain liberal amounts of cadmium and cadmium deficiency is not likely to occur.

VANADIUM

Vanadium occurs in traces in human and animal tissues. Recent studies have indicated of a possible relationship between vanadium and dental caries and vanadium and cholesterol synthesis.

Vanadium content of animal and human tissues

The body of an adult man has been estimated to contain about 10-25 mg of vanadium most of which is present in the bones, teeth and body fat.

Metabolism

Only a small percentage (less than 1 per cent) of the dietary vanadium is absorbed. About 60 per cent of the absorbed vanadium was excreted in the urine, the remaining being retained in bone, teeth, liver and body fat.

Vanadium and dental caries

Vanadium occurs in enamel and dentin. Experiments with animals have, however, yielded conflicting results regarding the value of vanadium salts in preventing dental caries.

Vanadium and cholesterol synthesis

Vanadium inhibits synthesis of cholesterol from acetate as well as from mevalonic acid but not from squalene.

Vanadium toxicity

Vanadium fed as sodium vanadate at 25 ppm showed toxic effects such as depression in growth etc. At intakes of 50 ppm, the animals developed diarrhoea and mortality increased.

Requirements

The human requirements of vanadium are not known.

SILICON

Silicon occurs in small amounts in animal and human tissues. Recent studies have indicated the possible role of silicon in bone calcification and in the development of urolithiasis.

Silicon content of animal tissues

The silicon content of human foetal tissues ranges from 40-400 ppm as Sio_2 (on dry basis) while in adult human tissue, the values range from 50-1000 ppm. The silicon content of blood in man and animals ranges from 2-5 μg/ml. as Sio_2.

Metabolism

Silicon enters the alimentary tract from the food as monosilicic acid and as solid silica. Only a small part i.e. less than 1 per cent is absorbed. A greater part of absorbed silica is excreted in urine. Small amounts are stored in various tissues.

Silicon and bone calcification

There is evidence that silicon is associated with calcium in the mineralisation process of bones.

Silicon and Urolithiasis

Normally the silicon present in urine is in soluble form. Under some circumstances, a part of the silicon is deposited in the kidney, bladder, or urethra to form calculi or uroliths. When the calculi is large, they block the urine passage. Silica urolithiasis is a serious problem among some animals in Australia, Canada and U.S.A.

STRONTIUM

Strontium is present in small amounts in foodstuffs and animal and human tissues and bones. In view of the fact that ^{90}Sr is an abundant and potentially dangerous radio-active element of nuclear fission, there is a possibility of ^{90}Sr being absorbed and deposited in the bone.

Strontium content of tissues

The following mean values have been reported for the strontium content of adult human organs: muscle, 0.07; liver 0.10; brain 0.12; heart 0.15; kidney 0.35; lungs, 0.5 ppm of dry tissue. The bone ash of human adults contains from 0.016 to 0.024 per cent Sr.

Metabolism

Strontium is poorly absorbed and retained from the diet. About 80 per cent of the ingested Sr is lost in the faeces and about 16-18 per cent of the ingested Sr is excreted in urine. Only 2 to 3 per cent of the ingested Sr is retained in the body.

Functions of strontium

In rats and guinea pigs fed on a strontium deficient diet, growth depression and impairment of the calcification of bone and teeth and higher incidence of carious teeth have been reported.

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CHAPTER 11

THE COMPOSITION OF BLOOD AND CLASSIFICATION OF ANAEMIAS

Blood consists of a suspension of different types of cells (red blood cells, white blood corpuscles and platelets) in a fluid called plasma. It is primarily a medium for the carriage of oxygen, nutrient materials, hormones and anti-infective agents (e.g. antibodies) to the tissues and for the removal of carbon dioxide and other waste products from the tissues and their elimination from the body. The normal values for nutrients and other constituents of blood are given in Table 5A in the Appendix.

The different constituents of blood are given below:

I. Cellular fraction (Volume: 45 per cent.)
Constituent cells:
1. Erythrocytes (5,000,000 per cumm)
2. Leukocytes (6,000 per cumm)
3. Platelets (250,000 per cumm)

II. Plasma fraction (Volume: 55 per cent.)

A. Non-diffusible constituents
1. Albumin
2. Globulins
3. Fibrinogen
4. Enzymes, lipids, etc.

B. Diffusible constituents.
1. Hormones, vitamins etc.
2. Anabolic constituents: glucose, amino acids, creatine, etc.
3. Electrolytes: Na^+, K^+, Ca^+, Mg^+, Cl^- HCO_3^-, HPO_4^-, $H_2PO_4^-$ etc.
4. Catabolic products: urea, creatinine, uric acid etc.

NORMAL BLOOD STANDARDS

Blood volume: The total volume of blood present in the adult is about 7 per cent of the body weight. An adult body weighing about 70 kg. will contain about 5 litres of blood.

Plasma volume: The plasma volume is about 4.0 per cent of the body weight and is about 2.83 litres in an adult of 70 kg. It can be determined by the dye (Evan's blue T 1824) method. This dye is bound to serum albumin and escapes only slowly from the capillaries. The degree of dilution of a known volume of the dye is measured.

Packed red cell volume and haematocrit ratio: The relative volume of plasma and packed red blood corpuscles is determined by centrifuging heparinised blood in a graduated tube (haematocrit) and measuring the volume of packed red blood cells (which sediment below the plasma). The plasma volume is usually 55 ml. and red blood cell volume is 45 ml per 100 ml. blood and the haematocrit ratio is 45/55=0.82 in normal human beings.

Red blood cells (RBC): The RBC is a circular, non-nucleated byconcave disc. It is highly elastic and can undergo deformation while passing through minute capillaries. The average diameter of RBC in normal healthy subjects is 7.3 μ (Range 5.5 to 8.8 μ). The average red cell count in adult male is about 5.5 million and in the female is 4.8 million per mm^3.

Haemoglobin: Haemoglobin consists of a protein globin united with the pigment haem. The mean haemoglobin content of blood in normal males is 15.8 g. and in normal females, 13.7 g. per 100 ml. blood. Haem contains iron in the ferrous form. Iron content of haemoglobin is 0.340 per cent. The molecular weight of haemoglobin is about 67,000. Under optimal conditions, 1 g of haemoglobin can carry 1.34 ml of oxygen. The functions of haemoglobin are as follows: (*i*) It is essential for carrying oxygen (*ii*) It is essential for the transport of CO_2 formed in the tissues, and (*iii*) It plays an important part in the regulation of the reaction of blood. Haemoglobin readily combines with carbon monoxide forming carboxy haemoglobin. When oxidised, it is converted into methemoglobin which contains iron in the ferric state.

Mean corpuscular volume: This is calculated as follows:

$$\text{MCV} = \frac{\text{Volume of packed cell (ml) per litre of blood}}{\text{RBC in millions per } mm^3}$$

The value is expressed in cubic microns (μ^3). For example, if the packed cell volume is 450 ml. per litre and the red cell

count 5 million per mm^3, the mean corpuscular volume (the mean volume of RBC) is 450/5 million=90μ^3. The normal range is 78-94 μ^3. Cells of normal volume are called *normocytes*. Cells having larger volumes are called *macrocytes*, and cells having smaller volumes are called *microcytes*.

Average corpuscular haemoglobin content: This is calculated as follows:

$$\frac{\text{Haemoglobin (g) per litre of blood}}{\text{RBC in millions per } mm^3}$$

The average value is 29.5±2.5 pg

(1 pg=picogram or micro micro-gram or 10^{-12}g.)

Mean corpuscular haemoglobin concentration per cent: This is the quantity of haemoglobin expressed as a percentage of the packed cell volume.

$$\frac{\text{Haemoglobin (g) per 100 ml. blood}}{\text{Packed cell volume (ml) per 100 ml. blood}}$$

For example, if the haemoglobin is 15 g. per 100 ml. and packed cell volume is 45 ml. then the mean corpuscular haemoglobin concentration per cent will be

$$\frac{15}{45}\times 100=33.3 \text{ g/100 ml cells.}$$

The average value is 35±3 g/100 ml. red blood cells. If the above value is in the normal range then the cell is *normochromic*. If it is below the normal range, then the cell is *hypochromic* (contains less haemoglobin than normal cells). Low values indicate that the individual is suffering from iron deficiency anaemia.

DEVELOPMENT OF RED BLOOD CELLS

The development of red blood cells after birth takes place in the red marrow. At birth all the bones are filled with red marrow. In the adult, red marrow is present only in vertebrae, sternum, ribs and bones of the skull and pelvis. All other bones contain only yellow marrow. The functions of the red marrow are as follows (*i*) Formation of red blood cells (*ii*) Formation of granulocytes and to a lesser extent of monocytes and lymphocytes (*iii*) Formation of blood platelets and (*iv*) Destruction of red blood

cells by macrophages which constitute part of the lining of the blood sinuses.

The stages of development of red blood cells (erythrocytes) are shown in Table 11.1.

Table **11.1 Stages of Erythropoiesis**

Cell stage	British terminology	American terminology	Cell diameter
I	Proerythroblast	Megaloblast	15-20μ
II	Early normoblast	Early erythroblast	12-16μ
III	Intermediate normoblast	Late erythroblast	10-14μ
IV	Late normoblast	Normoblast	7-10μ
	Reticulocyte	Reticulocyte	—
	Erythrocyte	Erythrocyte	5.5-8.8μ

Reticulocytes: In the new born, 2-6 per cent of the red cells in the circulation are reticulocytes. This level falls to about 1 per cent during the first week and remains at this level throughout life. The reticulocyte count increases wherever red cells are formed rapidly as during hemolysis of blood due to drugs or during the treatment of pernicious anaemia with vitamin B_{12}.

Life span of RBC: Studies carried out with red cells tagged with radio active sodium chromate have shown that the full life span of red blood cells is 120 days. This means that destruction and formation of red blood cells are going on continuously.

Regulation of Erythropoiesis: The factors regulating erythropoiesis are briefly discussed below.

Erythropoietin: There is evidence indicating that RBC formation is regulated by a humoral agent known as erythropoietin which has been isolated from plasma. Erythropoietin (also called haemopoietin or erythrocyte stimulating factor ESF) is a glycoprotein of low molecular weight produced by renal tissue.

Maturation factors: The maturation of nucleated red cells (erythroblasts) require vitamin B_{12} and folic acid.

Factors affecting haemoglobin formation: Iron, copper and protein are required for the formation of haemoglobin.

Vitamins: In addition to folic acid and vitamin B_{12}, ascorbic acid and pyridoxin deficiencies have been reported to cause anaemia in animals.

ANAEMIAS

Anaemia refers to the condition in which there is disturbance in the formation of haemoglobin or maturation of red blood cells or depression or destruction of the haemopoietic cells in the red marrow.)

Anaemia may be broadly classified under three heads (*i*) due to nutritional deficiency i.e., deficiency of iron, copper, folic acid and vitamin B_{12} (*ii*) due to loss of blood or destruction of RBC (haemorrhage and haemolysis) and (*iii*) due to depression of the RBC forming tissues of red marrow due to toxins or exposure to X-rays etc. Anaemia may be further classified according to the size of the RBC as normocytic, macrocytic and microcytic and according to haemoglobin content of the cells e.g., orthochromic and hypochromic. In practice, a combination of both exists in many cases. A comprehensive morphological classification of anaemia based on the above is given below:

(*i*) *Macrocytic orthochromic:* The cells are large in size but they contain enough haemoglobin, e.g., (*a*) pernicious anaemia due to vitamin B_{12} deficiency, (*b*) nutritional macrocytic anaemia due to folic acid deficiency (*c*) anaemia of pregnancy and (*d*) anaemia of sprue.

(*ii*) *Macrocytic hypochromic or dimorphic:* This represents deficiency of iron and folic acid or vitamin B_{12} e.g., Anaemia of pregnancy and malnutrition.

(*iii*) *Normocytic orthochromic:* Anaemia of malignancy and aplasia of bone marrow, anaemia of malaria, kala-azar and of acute haemolysis.

(*iv*) *Normocytic hypochromic:* Anaemia of scurvy or thyroid deficiency and

(*v*) *Microcytic hypochromic:* This represents all types of iron deficiency anaemias.

I. ANAEMIAS DUE TO NUTRITIONAL DEFICIENCIES

The anaemias due to nutritional deficiencies are (*i*) Iron deficiency anaemia (*ii*) Folic acid deficiency anaemia (Nutritional macrocytic anaemia) and (*iii*) Pernicious anaemia due to vitamin B_{12} deficiency.

IRON DEFICIENCY ANAEMIA

Iron deficiency anaemia is by far the most common anaemia in all countries of the world. It occurs most commonly among pregnant and nursing women, infants, preschool children and adolescent girls.

Clinical features

The general clinical features are fatigue, lassitude, breathlessness on exertion, giddiness, dimness of vision, pallor of the skin, palpitation, anorexia and dyspepsia.

Blood picture: The haemoglobin content of the blood is low-generally below 10 g/100 ml. blood and red cell count between 3 and 4.5 million per mm^3.

Treatment

Women and adolescent girls: The treatment consists in the administration of ferrous sulphate tablets (200 mg. per tablet providing 60 mg. iron) three times a day for a period of 30 days till the haemoglobin returns to normal level followed by 1 tablet daily for 3 months.

Infants and preschool children: Mixtures providing iron salts should be administered three times a day so that the child receives daily 50 to 60 mg. of iron, till the haemoglobin level in blood increases to normal levels.

NUTRITIONAL MACROCYTIC ANAEMIA

This is due to the deficiency of folic acid in the diet. It occurs commonly among preschool children suffering from protein-calorie malnutrition and pregnant women.

Blood picture

The red cell count is usually low-2-3 million per mm^3 and haemoglobin between 6-9 per cent in typical cases. The cells have larger diameter. Examination of bone marrow aspirate reveals the presence of characteristic megaloblasts, indicating that the maturation of red blood cells are affected in folic acid deficiency.

Treatment

Adults: Folic acid 5 mg. orally twice daily and ferrous sulphate tablets (200 mg) thrice daily should be administered for a month or longer till the blood picture returns to normal.

Preschool children: Folic acid (1-2 mg) orally daily, ferrous sulphate mixture to provide 6 mg. iron per kg body weight daily should be given for a period of one month or longer till the blood picture returns to normal.

Pernicious anaemia

Pernicious anaemia is caused by the lack of intrinsic factor in the stomach with the consequent failure in the absorption of vitamin B_{12} from the intestines.

Diagnosis

The diagnosis can be made by the examination of blood, bone marrow aspirate and stomach acidity.

Blood: The RBC count is low-1.5-2.5 million per mm^3. The average cell diameter is above normal indicating the macrocytic nature of the cells. The haemoglobin content is also low (8-9 per cent).

Bone marrow: Microscopic examination of bone marrow aspirates indicates the presence of megaloblasts in large numbers.

Stomach: The gastric secretion is devoid of acid, pepsin and intrinsic factor.

Treatment

It is general practice to give 1000 μg of vitamin B_{12} intramuscularly twice a week in the first week followed by 250 μg. every week for about 2 months. Subsequently a dose of 250 μg. once in every month for a few months will prevent recurrence.

II. ANAEMIAS DUE TO LOSS OF BLOOD AND DESTRUCTION OF RED CELLS

A. *Haemolytic anaemias:* The causes of haemolytic anaemias may be grouped as follows: (*i*) inherent hereditary defect in the red cells leading to rapid haemolysis e.g., familial haemolytic

jaundice, sickle cell anaemia, Mediterranean anaemia (Cooley's anaemia) (*ii*) due to action of haemolytic agents liberated by microbial infections e.g., malaria, kala-azar and haemolytic streptococcal infection or chemical poisons e.g., arsenic, sulphonamide etc., or snake venom.

B. *Loss of blood:* Anaemias due to loss of blood occur due to hookworm infection or as a result of haemorrhage.

C. *Aplastic anaemia:* (Anaemia due to depression of bone marrow): This is caused by the failure of the regeneration of red cells in the red marrow caused by some toxins or toxic drugs or exposure to X-rays.

The White Blood Corpuscles

The white blood corpuscles (leucocytes) are divided into three groups. The total normal W.B.C. count ranges from 4000-11,000 per mm^3.

(*i*) *Granulocytes:* These cells have diameter ranging from 10-14 μ. They have granules in the cytoplasm. They form about 70 per cent of the total count. They consist of three types of cells (neutrophils 50-70 per cent; eosinophils 1-4 per cent; basophils 0-1 per cent).

(*ii*) *Lymphocytes:* The small lymphocytes have diameter of 7-10 μ and the large 10-14 μ. They form about 20-40 per cent of the total white cell count.

(*iii*) *Monocytes:* The diameter ranges from 10-18 μ. They form about 2-8 per cent of the total white cell count.

Eosinophils and basophils contain histamine. The basophil leucocytes also contain heparin. Lymphocytes contain γ-globulin which is also present in the blood serum.

Changes in WBC in disease

An increase in the total number is called *leucocytosis* and decrease below 4000 is called *leucopenia*.

Neutrophil leucocytosis: This occurs in many inflammatory conditions and in certain types of fevers.

Eosinophilic leucocytosis: This occurs in infections of parasitic worms, in allergic conditions (hay fever, asthma etc.), and many skin diseases.

Lymphocytosis: This is a rare condition. An increase in lymphocyte count occurs in infections with tubercular bacillus.

Leukemias: Leukemias are a group of diseases in which one or more of the types of WBC undergo malignant changes.

PLASMA

The plasma contains about 6.4-8.3 g proteins per 100 ml. The proteins are broadly grouped under two heads: albumin and globulins. The globulin fraction consists of α_1, α_2, β and γ-globulins and fibrinogen. Plasma contains in addition nucleoproteins and conjugated proteins such as seromucoid and lipoproteins. If the fibrinogen is removed from plasma by converting it into fibrin the resulting fluid is called serum. Serum can be obtained directly by allowing blood to clot. The quantities of different proteins present in plasma are given in Table 11.2.

Table **11.2 Distribution of proteins in citrated plasma as determined by Electrophoretic analysis**

Constituents		Grams/100 ml plasma	As per cent of total proteins
Total Protein	...	6.03	100
Albumin	...	3.32	55
Alpha-globulin	...	0.84	14
Beta-globulin	...	0.78	13
Gama-globulin	...	0.66	11
Fibrinogen	...	0.43	7

Functions of plasma proteins

The plasma proteins have several physico-chemical and biochemical functions.

Physico-chemical functions

The physico-chemical functions of plasma proteins include (1) maintenance of normal water content of the tissues by virtue of their osmotic pressure and (2) maintenance of normal acid base balance.

Exchange of fluid between blood and tissues: The blood pressure of capillaries at the arterial end is 32 mm Hg and at the venous

end is 12 mm Hg. The osmotic pressure of plasma proteins is 25 mm Hg. At the arterial end of the capillaries, the blood pressure is greater than the osmotic pressure of plasma. Hence fluid enters the blood from the tissue space. At the venous end, the osmotic pressure of plasma is greater than the capillary blood pressure and hence fluid passes from blood to tissue space.

Formation of oedema in protein deficiency: In protein deficiency the albumin content of plasma may fall to about 1-1.5 per cent and hence the osmotic pressure of plasma proteins also falls to about 10-12 mm. Hg. Under these conditions, fluid from blood is driven into tissue space at the arterial end of the capillaries resulting in the formation of oedema. When protein deficiency is cured by feeding on a high protein diet, plasma albumin level increases to the normal level, resulting in an increase of plasma protein osmotic pressure to 25 mm Hg. The oedema also disappears.

Maintenance of acid-base balance of blood: A second physicochemical function of the plasma proteins is in the maintenance of normal acid base balance of blood. This aspect is discussed in Chapter 12.

Biochemical functions

The important biochemical functions of plasma proteins are as follows:

(*i*) *Transport of lipids:* Many of the plasma lipid fractions are bound to plasma proteins. Such fractions are called lipoproteins. These complexes permit the water-insoluble lipids to be transported through the vascular system without coalescing or forming emboli. The major lipids carried in this way are cholesterol esters, and phospholipids. In addition, plasma proteins also transport fat soluble vitamins.

(*ii*) The antibodies found in blood are mainly associated with γ-globulins. The functions of important proteins present in plasma are given in Table 11.3.

Platelets

Platelets appear as round or oval bodies 2.5 μ in diameter. The normal platelet count ranges from 250,000-500,000 per mm^3.

Table **11.3 Functions of important proteins present in plasma**

Protein component	Functions
Albumin	Osmotic pressure regulation and exchange of fluid and transport of some nutrients
α-Lipoproteins	Lipid transport
Ceruloplasmin	Copper transport
Prothrombin	Blood coagulation
β-Lipoproteins	Lipid transport
Transferrin	Iron transport
Fibrinogen	Blood clotting
γ-Globulins	Associated with antibodies

The important functions of platelets are as follows: (1) When blood is shed, the platelets disintegrate and liberate a thromboplastin which is concerned with the activation of prothrombin to thrombin in the clotting of blood and (2) Platelets on disintegration liberate 5-hydroxy tryptamine (serotonin) which causes vasoconstriction and brings about local haemostasis.

Coagulation of blood

The essential reaction in the coagulation of blood is the conversion of the soluble protein fibrinogen into the insoluble protein, fibrin by means of an enzyme, thrombin. Fibrinogen exists in the circulating blood while thrombin does not exist as such. Thrombin is formed from the inactive circulating precursor—prothrombin, when the blood is shed. The conversion of prothrombin to thrombin depends on the presence of a Ca ion and of 'thromboplastins' which are derived from factors present in damaged tissues, disintegrating platelets and from plasma. The process of coagulation is shown below and in Fig. 11.1.

$$\text{Prothrombin} + \text{Ca} + \text{Thromboplastins} \longrightarrow \text{Thrombin.}$$
$$\text{Thrombin} + \text{Fibrinogen} \longrightarrow \text{Fibrin clot.}$$

The different factors concerned in the coagulation of blood are given below:

Blood coagulation factors

Factor	I.	Fibrinogen
Factor	II.	Prothrombin
Factor	III.	Thromboplastin
Factor	IV.	Calcium
Factor	V.	Labile factor, Proaccelerin
Factor	VII.	Proconvertin
Factor	VIII.	Antihaemophilic Factor A.
Factor	IX.	Antihaemophilic Factor B (Christmas factor)
Factor	X.	Stuart-Prower Factor
Factor	XI.	Plasma thromboplastin antecedent
Factor	XII.	Hageman Factor
Factor	XIII.	Fibrin stabilizing Factor (FSF)

The formation of thromboplastin from damaged tissues or blood platelets requires several factors as shown in Fig. 11.1.

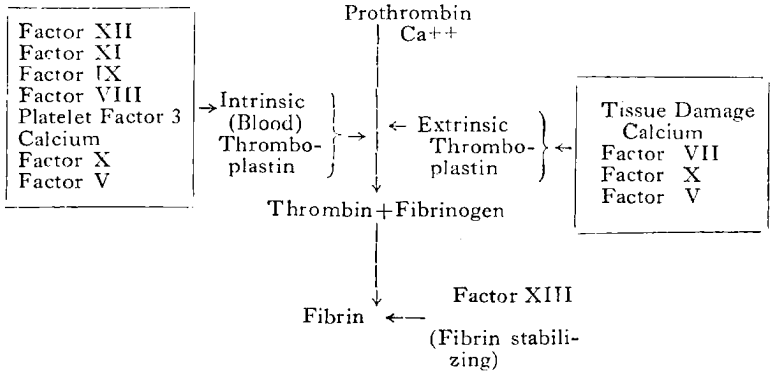

Fig. **11.1 The Factors involved in the Process of Coagulation of Blood**

ABNORMAL HAEMOGLOBINS

The present knowledge of abnormal haemoglobins is incomplete and some of the associated conditions are relatively rare. A large number of abnormal haemoglobins have been reported. In addition, haemoglobin F (Foetal haemoglobin) when occurring in high concentration must be considered abnormal.

Recommended nomenclature designates the capital letters A for normal adult, haemoglobin F for foetal haemoglobin and S for sickle-cell haemoglobin and consecutive letters of the alphabet

for other abnormal types in order of their identification. Case reports have established the importance of types, C, D and E, which occur in association with well-defined haematologic entities. The *racial incidence* of haemoglobins is exemplified by the predominance of the abnormal haemoglobins—S and C in Negroes and E in Thais and Burmese.

Important biochemical differences exist between adult haemoglobin and the form elaborated by the human foetus during prenatal life. While the haem component of the two haemoglobin molecules is similar, the differences relate to the structure and properties of globin and possibly of the haemoglobin linkages. The property by which foetal haemoglobin is best known and most readily identified is its increased resistance to denaturation in alkaline solutions.

Haemoglobin P-(primitive haemoglobin)-is different from haemoglobin F and is found in early foetal life. This haemoglobin prevails during the first half of embryonic life and is later replaced by foetal haemoglobin. The primitive haemoglobin is associated with severe hypoproteinaemia and agammaglobulinaemia, but its biological significance is not yet clear.

Two simple procedures serve to identify the various types of haemoglobins: the detection of foetal haemoglobin by the method of alkali denaturation and the separation of the abnormal haemoglobins by the technique of *paper electrophoresis*.

Foetal haemoglobin: It comprises 44 to 89 per cent of the haemoglobin in the newborn infant, decreases rapidly during the first year of life and is rarely demonstrable after the age of 30 months. Following this period and in the child and adult, 2 per cent represents the upper limit of normal. In certain of the chronic hereditary haemolytic disorders foetal haemoglobin reappears in substantial amounts. Large quantities of foetal haemoglobin are synthesized in patients with *sickle-cell anaemia* (5 to 15 per cent) and severe *Mediterranean anaemia* and to a lesser extent in *spherocytic anaemia* (usually less than 10 per cent).

Thalassaemia (Mediterranean Anaemia, Cooley's Anaemia): This combines the features of a refractory haemolytic anaemia with characteristic roentgenographic, clinical and blood changes. The anaemia presents a wide spectrum of severity. The various grades are usually designated as 'minima', 'minor', 'intermedia'

and 'major'. The disease is most common in individuals of *Mediterranean ancestry*, but occurs also occasionally in India, Ceylon and Thailand. The fundamental defect in this disease consists of the inability to synthesize adult haemoglobin in normal amounts. Except for increased amounts of haemoglobin F, which, as mentioned, occurs in smaller amounts in many conditions, no other haemoglobin abnormality has, so far, been found in thalassaemia.

Target cells: These are particularly prominent in the blood smear of patients with abnormal haemoglobins and often provide a clue to diagnosis. Present in both mild and severe Mediterranean anaemia, they are further increased in sickle-cell anaemia and in haemoglobin E disease. Few or none of these cells are noted in reported cases of sickle-cell haemoglobin D disease, or with G, H, I and J haemoglobins.

Haemoglobin D appears to be more common in Indians than in any other people, both in India and among Indians in Africa. It is, however, apparently without notable pathological significance.

Haemoglobin E was originally discovered in Thailand, where it was found in 13 per cent of the Thais, but it is just as common in Burma and is not rare in Indonesia. Since the first case described was in an American child of partial Indian ancestry it may perhaps also occur in India. The haemoglobin E trait is found in Ceylon.

Haemoglobin H has been found in Thais and in Nepalese. It is the only known abnormal haemoglobin which in the heterozygous state produces clinical anaemia. In supravital stained smears intra-erythrocytic inclusion bodies were found.

Haemoglobin S is especially common in Africa but has been found also in India. Current thought attributes the sickle-cell abnormality to a mutant gene which is responsible for the synthesis of a different type of haemoglobin.

Blood Groups

Three main groups of 'Factors' are present in human blood cells which enable the cells of individuals to be differentiated; these factors have great clinical, medico-legal and genetic interest. The chief groups are:

(1) A, B, AB and O (2) Rh (Rhesus) factor and (3) M and N.

In the serum of the four classical groups, the agglutinins are found to be distributed as follows: Group A contains β; Group B contains α; Group AB contains no agglutinin; Group O contains α and β. The full description of the four groups taking into account both agglutinogens and agglutinins would be, therefore: Aβ; Bα; AB; O$\alpha\beta$.

It is usual to carry out grouping tests by the use of tested sera belonging to different Groups as given below:

Blood:	of Group AB	is agglutinated by	serum of	A and B (containing β and α)
,,	Group A	,,	,,	B (containing α)
,,	Group B	,,	,,	A (containing β)
,,	Group O	,,	,,	neither

The corpuscles of Group O are not agglutinated by the plasma of any group and Group O are accordingly called 'Universal Donors', since their blood is safe for transfusion into any corpuscles so that people belonging to this group could receive blood from any healthy person.

The classification of blood groups is made still more precise, especially in examination of medico-legal cases, by the discovery that, quite independently of the system mentioned above, another classifiation can be made, based on different agglutinogens called M and N. These are identified by special immune rabbit sera, and provide three independent types, M, N, MN. As these occur independently of the AB groups, the combination yields twelve possible blood types, and in fact, yet other subsidiary groups are known.

An important factor, called the Rh factor was discovered in 1940. It is an antigen of the Rhesus monkey and is present in the blood of 85 per cent of white people and may be transmitted from father to child. If the mother is Rh negative, she forms antibodies to it and these pass through the placenta to the foetus and causing extensive blood destruction in the new-born child.

REFERENCE

C. H. Best, and N. B. Taylor (1961), The Physiological basis of Medical Practice, Balliere, London.

CHAPTER 12

WATER, ELECTROLYTES AND ACID BASE BALANCE

Water is the largest constituent of the body, about 60-70 per cent of the total body weight consisting of water. The water content of soft tissues ranges from 70-80 per cent while that of bone about 20 per cent. Body water is distributed as follows: (*i*) Inside the cells of tissue—intracellular water (50 per cent); and (*ii*) Outside the tissue cells—extracellular water (20 per cent). The extracellular water is further subdivided into (*i*) Water in blood plasma (about 4 per cent) (*ii*) Interstitial water—water in tissue space (9 per cent) and (*iii*) Lymph in the lymphatic vessels (7 per cent).

Additional minor divisions of extracellular water are cerebrospinal fluid and aqueous humour (in the anterior chamber of the eye.)

Approximate quantitative data for an adult man weighing 70 kg. are given in Table 12.1.

Table 12.1 **Distribution of water in the body**

		As % of body wt.
Total body weight	70 kg.	100
Total water	50 kg	70
(i) Intracellular	35 kg	50
(ii) Extracellular (thiosulphate space)	12 kg	17
(a) Plasma	3L	4
(b) Lymph	5L	7
(c) Intertitial water (tissue fluid)	4L	6

Water intake and loss

Water is lost continuously from the body in the following ways (*i*) via kidney as urine (*ii*) via the skin in the form of insensible perspiration and as sweat (*iii*) via the lungs in the expired air, (*iv*) to a small extent via the large intestines in the faeces, and (*v*) in lactating women in the milk. Water is taken in food and also

as drinking water. In addition, water is formed in the tissues by the oxidation of H_2 present in fats, carbohydrates and proteins. Approximate quantitative data per day for an adult weighing 70 kg. are given in Table 12.2.

Table **12.2 Water intake and loss from the body**

	Temperate climate (ml)	Tropical climate (ml)
Water intake:		
Drinking water	1500	2000—5000
In food	1000	1000—2000
By oxidation of carbohydrates, fats and proteins in tissues	300	300
Total	2800	3300—7300
Water loss		
In urine	1500	1000—1500
Via skin	800	1800—5200
Via lungs	400	400 400
In faeces	100	100— 200
Total	2800	3300—7300

Water intake: This is taken as drinking water, about 1500 ml; in food 1000 ml and from oxidation of carbohydrates, fats and proteins in tissues, 300 ml.

Water loss: This occurs in urine, about 1500 ml; via skin 800 ml, via lungs 400 ml and in faeces, 100 ml. In a normal individual, the water intake is approximately equal to water lost from the body and the water content of the body is maintained fairly constant.

Exchange of water in the body

Water is absorbed rapidly from the small intestines through the portal vein to the general circulation. It rapidly passes to the tissue space as tissue fluid. A greater part of the ingested water is excreted by the kidneys within an hour. The mechanism of exchange of water between blood and tissues is briefly discussed below:

Composition of body fluids

Data regarding the composition of plasma and lymph are given in Table 12.3 and briefly discussed below:

Table 12.3 **Approximate composition of plasma and thoracic lymph**
(Values per 100 ml)

	Plasma	Thoracic Lymph
pH	7.35—7.45	—
Sodium (Na^+) (mg)	300—355	271—304
,, (mEq)	131—154	118—132
Potassium (K^+) (mg)	14—22	15—22
,, (mEq)	3.6—5.6	3.8—5.6
Chloride (Cl^-) (mg)	352—382	305—361
,, (mEq)	98—108	87—103
Bicarbonate (HCO_3^-) (mg)	55.2—71.3	—
,, (mEq)	24—31	—
Total Protein (g)	6—8	2.9—7.3
Albumin (g)	4.6—6.7	1.5—2.7
Globulin (g)	1.2—2.3	1.5—4.8
Creatinine (mg)	0.1—1.2	—
Urea (mg)	28—40	—
Urea N (mg)	13—26	—
Cholesterol (mg)	110—390	34—106
Fatty acids (mg)	149—483	—

Plasma: Plasma contains proteins (about 7-8 per cent) and small quantities of organic substances like glucose, amino acids, urea etc., and inorganic salts like sodium bicarbonate and sodium chloride.

Lymph: Lymph is the fluid found in the lymphatic vessels. The composition of lymph varies widely. The protein content varies from 2-7 per cent. The electrolyte content of lymph is similar to that of plasma.

Interstitial fluid: There is no direct information regarding the composition of interstitial fluid. i.e., fluid bathing the tissue cells. Since the blood capillaries do not permit proteins to pass out, it is presumed that interstitial fluid has very little proteins and it has a crystalloid composition similar to that of plasma.

Intracellular fluid: The intracellular fluid (ICF) cannot be determined directly. The difference between the total body water and the total extracellular fluid (ECF) is taken as the ICF. The cell contents have large amounts of proteins (17-18 per cent). The concentration of sodium in the ICF has been estimated to be 20 mEq. per litre and that of potassium 110 mEq.

Permeability of cell membranes: The cell membrane is freely permeable to water, glucose, lactate, amino acids and aceto-acetate. The intracellular fluid is hypertonic to extracellular fluid. The cell has active transport mechanism which prevents water from entering the cell freely from the extracellular fluid.

Water exchange between plasma and interstitial fluid

This is controlled by the protein osmotic pressure in the plasma which is 25 mm. Hg and the arterial capillary pressure (32 mm. Hg.) and the capillary pressure at the venous end (12 mm. Hg.). At the arterial end, the capillary blood pressure is greater than the protein osmotic pressure of plasma by 7 mm. Hg. Hence, fluid passes from capillaries to tissue space. At the venous end, the capillary blood pressure is less than the protein osmotic pressure by 13 mm. Hg. Therefore, fluid passes from tissue space into the capillaries. This plasma-interstitial fluid exchange takes place on a large scale i.e., at about 3 litres per minute.

Effect of excess water intake on water balance in the body

If 2 litres of water are taken, the water is distributed rapidly throughout the body. The kidney responds to the increased water intake after about 15-30 minutes. The flow of urine rises from the normal value of 50 ml per hour to its peak of 1500 ml. per hour. The extra water is excreted by the kidney within 3 hours. The excess urine output may (in temperate climate) be almost equal to the water ingested. In tropical climate, a part of the ingested water is lost in the sweat.

Effect of excess of sodium chloride on water balance

When NaCl is taken in excess in the form of solution, the plasma crystalloid osmotic pressure rises. Water moves from the interstitial spaces into the plasma, initially increasing the plasma volume and hence the blood volume; but at the same time NaCl diffuses

into the interstitial fluid. The outflow of salt causes also flow of water from plasma to interstitial fluid. The net result is an uniform increase in the salt concentration of the extracellular fluid. The raised osmotic pressure of ECF leads to a flow of water from the cells into the ECF. This results in an increase in the volume of ECF and decrease in the volume of ICF. In an experiment in which 28 g. NaCl (484 mEq. per litre) were ingested, the urine flow increased from 30 to 120 ml. per hour and the rate of chloride excretion rose from 0.2 to 1.2 g. per hour. The maximum concentration of NaCl in urine occurred between the 3rd and 12th hour when it was 3.4 per cent NaCl as compared with normal value of 1 per cent. It is evident that the rate of excretion of salt and water taken with salt is thus comparatively slow and water is retained in the body along with salt upto 12 hours or longer.

Effects of NaCl depletion on water balance

When a subject is fed on salt free diet, the following changes take place. A decrease in the concentration of Na and Cl ions in plasma and the interstitial fluid occurs. Owing to a fall in the crystalloid osmotic pressure of ECF, water moves from the interstitial fluid into the cells. This results in a decrease in the volume of ECF and an increase in the volume of ICF. The loss of NaCl in urine rapidly decreases and only traces of NaCl will be found in urine after 3 or 4 days on salt free diet. The body weight falls due to water loss. The plasma volume and blood volume decrease which will be accompanied by a decrease in cardiac output. Water drinking does not cause the usual striking diuresis.

Effects of water deprivation on water balance

Water is being constantly lost from the body in urine, sweat, expired air and faeces. If corresponding amounts of water are not ingested, water depletion occurs in the body leading to changes in body fluids. A reduction in volume of the extracellular fluid (ECF) and intracellular fluid (ICF) takes place. The urine output is reduced. There is a rapid decrease in body weight and a state of dehydration of the cells occurs. After a few days, a decrease in plasma volume (and also in blood volume) occurs

which will reduce cardiac output and lead to circulatory failure. An adult who has lost 5-10 litres of water from the body will be seriously ill and death will occur when the water loss from the body is about 15 litres.

ELECTROLYTE BALANCE

Electrolytes are present in intracellular and extracellular fluids. The concentration of electrolytes is affected by water balance, sodium chloride intake and intake of other minerals present in the diet. The body has several mechanisms by which it can keep the electrolyte balance in the intracellular and extracellular fluids at a constant level. These are briefly discussed below.

Electrolyte concentration in intracellular fluid

Tissue cells and red blood cells contain large amounts of potassium phosphates, bicarbonates and small amounts of sodium and chloride ions. The concentration of K is about 110 mEq/litre while that of sodium is about 20 mEq.

Electrolyte concentration in extracellular fluid

Extracellular fluid includes, plasma, lymph and interstitial fluid. They contain large amounts of sodium, chloride and bicarbonate ions and small amounts of potassium and phosphate ions. Concentration of Na in the above fluids is about 143 mEq/litre while that of potassium is 5 mEq.

Permeability of cell membranes: Cell membranes are permeable to water and to nutrients like glucose, lactate, acetoacetate, amino acids and inorganic phosphate ions.

Active transport across cell membranes

Control of free movement: Eventhough intracellular fluid (ICF) contains large quantities of K ions and extracellular fluid (ECF) contains large quantities of Na ions, the movement of Na ion into ICF or K ion into ECF is controlled by active transport mechanism. This 'active transport mechanism' prevents the passage of Na ion into ICF and K ion into ECF by the continuous expenditure of chemical energy.

Movement against the gradient: Potassium ion can be taken by tissue cells or red cells even when the intracellular concentration of K ion far exceeds the extracellular concentration. The cells can maintain, at equilibrium, a higher concentration of K ion inside its membrane boundary than the concentration in the ECF.

Some examples of active transport are given below:

(1) Prevention of movement of K ions from cells to ECF and Na ions from ECF into cells (2) Movement of Na, K, and Ca ions in nerve; (3) Secretion of HCl by gastric oxyntic cells; (4) Alkaline secretion from pancreas; (5) Absorption and secretion by kidney tubules and (6) Secretion of saliva by salivary glands, pancreatic juice by pancreas and hormones by ductless glands.

Excretion of electrolytes by the kidney: In an adult, the kidney excretes normally 5 g. of Na ion, 9 g. Cl ion, 2.2 of K ion, 1.2 g. of phosphate ion, and 0.2 g. of Ca ion daily in urine. The quantity of these ions excreted will depend on the intake of these ions from the diet.

ACID-BASE BALANCE

The acid-base balance of body fluids is maintained within normal range by buffer system present in them. The pH of blood is about 7.3-7.4. The pH of other body fluids viz., lymph, cerebrospinal fluid are about the same as that of blood. The pH of intracellular and interstitial fluids are believed to be of same order as those of blood and lymph. The methods available for maintaining the acid-base balance in the body are (1) buffer systems which can prevent change in pH within reasonable limits (2) breathing and (3) kidneys. The important buffer systems present in body fluids are (1) bicarbonate—HCO_3 (2) Proteins and (3) Phosphates of Na or K.

Buffer systems

Bicarbonate—HCO_3 system: When non-volatile acids like lactic, phosphoric or sulphuric acids are produced in the cells, they react with the bicarbonate present with the liberation of carbonic acid. The excess carbonic acid is eliminated as CO_2 by the lungs. The term'alkali reserve' is used to indicate the bicarbonate present in plasma.

Buffering action of proteins: Proteins can react with K or Na ions forming proteinates. They exist in this form in body fluids. H_2CO_3 can react with alkaline proteinates forming bicarbonates.

Phosphate buffer system: Both inorganic and organic phosphates exist in body fluids as alkaline phosphates. These act as buffers in neutralising acids formed in the metabolism of carbohydrates, fats and proteins in the tissues.

Breathing

The role of lungs in eliminating CO_2 and thereby reducing the concentation of HCO_3 ion in the body fluids is important in maintaining the acid-base balance of the body fluids. When H concentration of blood tends to increase, the pulmonary ventilation is increased, more CO_2 is eliminated. This also helps to lower the amounts of HCO_3 ion in the blood and other tissue fluids. Such a response occurs in the following conditions (1) During secretion of alkaline digestive juices (pancreatic juice, bile and intestinal juice) during digestion of food (2) In diabetes mellitus and starvation due to excessive production of β-hydroxybutyric acid and acetoacetic acid in the body and (3) By ingestion of $NaH_2(PO_4)_2$ or ammonium chloride. On the other hand, when the H-ion concentration of blood tends to fall, the pulmonary ventilation is diminished, CO_2 is retained, alveolar CO_2 rises and the arterial CO_2 and HCO_3 ions rise. This increase in HCO_3 implies an increase in bicarbonate i.e., alkali reserve and tendency to alkalaemia. This may occur (1) during secretion of gastric juice in the stomach and (2) by ingestion of disodium or dipotassium phosphates.

Role of kidney

Two renal mechanisms are available for regulating the acid-base balance of body fluids (1) Excretion of B H_2PO_4, B_2HPO_4 and $BHCO_3$ in varying proportions when their concentration increases beyond normal limits in the body fluids and (2) the formation of NH_3 by renal tubules from amino acids. Under normal conditions, the pH of urine is about 6.0, representing excretion of 70mEq. of acid per day.

(*a*) *Excretion of phosphates and bicarbonates:* The glomerular filtrate contains BH_2PO_4, B_2HPO_4, and $BHCO_3$ in the same proportions as in plasma. In extreme acidemia, practically all the $BHCO_3$ is reabsorbed in the renal tubules and all the phosphates are excreted as BH_2PO_4. In extreme alkalaemia, large amounts of $BHCO_3$ are excreted and nearly all the phosphates are excreted as the alkaline salt B_2HPO_4.

(*b*) *Phosphate conversion mechanism:* Conversion of acid phosphate to alkaline phosphate and *vice-versa* takes place in the tubules in acidemia and alkalaemia respectively. The mechanism of conversion is as follows: In acidemia, Na_2HPO_4 is converted in the lumen of tubule into NaH_2PO_4 by losing one Na ion and gaining one H ion from H_2CO_3. The newly formed acid phosphate NaH_2PO_4 is excreted in urine, additional H ion thus being eliminated. The released Na is reabsorbed into the blood presumably as Na HCO_3, thus conserving the reserves of base in the body fluids. In alkalaemia, the NaH_2PO_4 in the lumen of the tubules is transformed into Na_2HPO_4 by gaining Na ion from other tubular constituents and H ion released from NaH_2PO_4 is reabsorbed into the blood. Additional fixed base is thus eliminated and the H ion is conserved.

(*c*) *Bicarbonate mechanism:* The bicarbonate mechanism is really effective only when compensating for states of alkalaemia. The tubules excrete bicarbonate and thus help to decrease the base present in the body.

(*d*) *Ammonia mechanism:* The epithelia of renal tubules produce ammonia by the oxidation of amino acids. The NH_3 reacts with acidic substances excreted forming ammonium salts. Ammonium salts react with alkaline salts and free certain amounts of Na or K ion which are reabsorbed. For example, sodium lactate is converted into ammonium lactate and excreted while sodium ion is reabsorbed as bicarbonate. The ammonia mechanism operates when acidemia develops.

REFERENCE

C. H. Best, and N. B. Taylor (1961), The Physiological basis of Medica Practice, Balliere, London.

CHAPTER 13

THE NUTRITIVE VALUE OF FOODS

Foods differ widely in their contents of various nutrients. Data regarding the chemical composition of foods consumed in many countries are available. A list of important food composition tables is given at the end of this chapter. Most of the analysis of vegetables and fruits relate to the 'edible portion'. Foods are broadly divided into various groups depending on their nutritive value. The classification generally adopted is as follows: (1) Cereals, and millets (2) Legumes (Pulses), (3) Oilseeds and nuts, (4) Vegetables (*a*) Green leafy, (*b*) Roots and tubers and (*c*) Other vegetables, (5) Fruits, (6) Fats and oils, (7) Foods of animal origin, (8) Milk and milk products (9) Starchy and sugar foods and (10) Spices and condiments. The nutritive value of different categories of foods is discussed in this chapter.

1.0 CEREALS

Cereals and millets form the staple food of the human race. These include wheat, rice, maize (corn), barley, oats, rye and the various millets. They are rich sources of starch and fair to good sources of proteins, certain minerals and B-group of vitamins. The chemical composition and the amino acid contents of different cereals and millets are given in Tables 1A and 4A in the Appendix.

1.1 WHEAT

Wheat is grown in large quantities in temperate climates in most parts of the world. It is extensively grown in the U.S.A., Canada, Argentina, Australia, Europe, Russia, Egypt, Pakistan, China and India. The wheats of commercial importance belong to the following botanical species—(1) *Triticum vulgare* (common hard wheat), (2) *Triticum durum* (Durum wheat) and (3) *Triticum compactum* (soft white wheat).

The wheat grain consists of three main parts: (1) the germ or embryo which is rich in protein and oil, (2) the endosperm which is rich in starch and is fair source of proteins and (3) the

various outer converings such as pericarp, testa or seed coat, hyaline layer and aleurone cell layer.

The relative per cent by weight of different constituents of wheat grain is given in Table 13.1.

Table **13.1 Relative per cent by weight of constituents of wheat grain**

Constituent	*% of whole grain by weight*
Germ	2-3
Total bran	13
Aleurone layer	5-6
Testa and hyaline	2-3
Pericarp	3-4
Endosperm	83-85

The different products obtained in the milling of wheat are (1) flour,(2) bran and (3) germ. The chemical composition of commercial samples of flour, bran and germ is given in Table 13.2.

Table **13.2 The Chemical composition of flour, bran and germ from wheat**
(Values per 100 g)

Nutrient	Flour			Germ	Bran
	Whole meal	85% extraction	70% extraction		
Moisture (g)	13.0	13.0	13.0	11.7	13.2
Protein (g)	13.6	13.4	12.8	28.5	14.4
Fat (g)	2.5	1.7	1.2	10.4	4.7
Ash (g)	1.7	0.8	0.4	4.5	6.3
Total P (mg)	350	188	97	1118	1276
Phytate P (mg)	242	96	30	716	920
Carbohydrates (by diff.) (g)	69.2	71.1	72.6	44.9	61.4
Cellulose (g)	2.2	0.3	0.1	7.5	21.4
Hemicelluloses (g)	3.0	1.2	0.6	6.8	26.2
Sugar (g)	0.8	0.5	0.2	16.2	4.6
Thiamine (μg)	400	360	80	2010	720
Riboflavin (μg)	160	81	53	680	350
Niacin (mg)	5.0	2.0	1.2	4.2	21.0

Wheat flour: The degree of milling of wheat is known as extraction rate. Whole meal flour is called 100 per cent extraction. National wheat flour prepared in U.K. during the last war was of 85 per cent extraction (15 per cent of bran and other parts of the wheat removed), while white flour of commerce is of 70 per cent extraction (30 per cent of bran and other parts of wheat removed). The chemical composition of whole wheat, 85 per cent and 70 per cent extraction flours is given in Table 13.2. It is evident that 70 per cent extraction flour is a poor source of B-vitamins. Low extraction flour (e.g. 70 per cent extraction) has the following advantages over high extraction flour (100 per cent or 85 per cent extraction flour). (1) It is whiter and hence white bread is more acceptable than brown bread, (2) It has a lower fat content and so its keeping quality is better than 85 per cent extraction or 100 per cent extraction flours, and (3) It contains less phytate phosphorus and hence absorption of calcium and iron from this flour is greater than those observed with high extraction flours. As against the above advantages, low extraction flours have less quantities of B-vitamins, calcium, iron and protein than high extraction flours.

High protein flour: High protein wheat flour has been prepared by subjecting wheat flour to a process of air classification. The protein content of high protein flour may range from 20-25 per cent.

Nutritive value of wheat proteins: The protein content of wheat may vary widely from 9 to 16 per cent depending on the variety. The chief proteins are glutelin and gliadin, together known as gluten. Wheat also contains small amounts of globulin, albumin and proteose. The essential amino acid contents of wheat proteins are given in Table 4A in Appendix. The limiting amino acids are lysine and threonine. The protein efficiency ratio of wheat proteins is about 1.6-1.8. This is markedly increased to about 2.2-2.5 when supplemented with lysine and to about 2.8-2.9 when supplemented with a mixture of lysine and threonine.

Effect of improvers on the nutritive value of flour: Several types of chemicals are used for improving the 'strength' of the flour for baking. One such improver is nitrogen trichloride. Sir Edward Mellanby (1946) reported that wheat flour treated

with nitrogen trichloride when fed to puppies, produced characteristic running fits in them (canine hysteria). He found that nitrogen trichloride reacted with methionine present in the proteins and produced a toxic principle known as methionine sulphoxime. At present, the use of nitrogen trichloride as a flour improver has been banned in all countries, and chlorine dioxide is used as an improver. Flour treated with chlorine dioxide does not contain any toxic factor and is wholesome.

Effects of feeding diets containing wheat flour of varying degrees of extraction on growth and N retention in children

McCance and Widdowson (1954) carried out studies on children living in orphanages in Germany to assess the effects of variation in the extraction rate of flour on the growth and N retention in children. The children were divided into 3 groups receiving bread made from one of the following flours (1) whole wheat flour (2) 85 per cent extraction flour and (3) 70 per cent extraction flour. The children received about 75 per cent of the calories from wheat flour. Each child received in addition, about 82-113g. fresh milk, 17-29 g. of meat and fish, 5-7 g. of cheese and 72-80 g. of margarine, and fair amounts of vegetables and fruits. All the flours were fortified with calcium. All children received vitamin tablets providing daily 2000 I.U. of vitamin A, 1000 I.U. of vitamin D and 25 mg. of ascorbic acid. The daily protein intake was about 62 g. of which 9 g. were animal proteins. The children were under observation for one year at the end of which no significant difference was observed in the mean increases in height and weight of children in the different groups. The results showed that addition of small quantities of milk, cheese, meat and fish made up the deficiencies of B-vitamins in 70 per cent extraction flour and made the diet adequate for children.

Processed wheat products

Bread: The nutritive value of bread is similar to that of the flour used in its preparation, if allowance is made for the higher water content of bread (about 33 per cent). The nutrient content of bread (on fresh basis) is about two-thirds that of the flour.

Biscuits: Biscuits are baked from a mixture of wheat flour, sugar and fat or wheat flour, fat and salt. During baking, a part of thiamine present is destroyed. The protein contents of biscuits (5-8 per cent) are lower than that (9-12 per cent) of the flour from which they are prepared. The available lysine content decreases, causing a lowering in the PER of the proteins.

Toast: Toast is prepared by applying dry heat to bread slices. During the process of toasting, a part of the thiamine present is destroyed. The available lysine content and the PER decrease.

Breakfast cereals: Wheat flakes, shredded wheat and grape-nuts are the important products made from wheat. As toasting is involved in the preparation of these foods, a part of the thiamine is lost and the quality of the proteins is adversely affected due to decrease in available lysine contents.

Semolina: Semolina consists of the endosperm of wheat and has nutritive value similar to that of 70 per cent extraction flour.

Wheat germ: Wheat germ is separately extracted in certain flour mills. About half the amount actually present in the grain is usually obtained, the remaining being mixed with flour. Wheat germ is a rich source of protein and fat. It is also a good source of B-vitamins. Wheat germ oil is a rich source of vitamin E.

Nutritive value of poor wheat diets

Poor diets based mainly on wheat and containing small quantities (5 per cent) of legumes, oil and vegetables similar to those consumed in India promote moderate growth in albino rats (9-12 g/week). They are deficient in protein, calcium, vitamin A and riboflavin. Supplementation of poor wheat diet with processed protein foods (based on oil seed meals and legumes and fortified with calcium salts and vitamins) at 10 per cent level so as to provide about 2 per cent extra proteins makes up the deficiencies in the diets and promotes excellent growth in albino rats. Children fed on a poor wheat diet maintain positive N, Ca and P balance. (Swaminathan 1967, 1969).

1.2 RICE

Rice is grown in large quantities in all Asian countries. It is the cereal of choice for most damp tropical climates. The

rice grain consists of the following parts (1) the outer bran layer beneath which is the aleurone layer (2) the endosperm and (3) the germ.

B-vitamins in rice: Hinton (1948) separated the different fractions by micro sections and studied their thiamine contents. The results are given in Table 13.3.

Table **13.3 Thiamine content of fractions of the rice grain**

Part of grain	Proportion of grain (%)	Thiamine content (μg/g)	Proportion of the total thiamine of the grain (%)
Pericarp and aleurone	5.95	31	35.2
Covering to germ	0.20	12	
Epiblast	0.27	78	3.9
Coleorhiza	0.20	94	3.5
Plumule	0.31	46	2.7
Radicle	0.17	62	2.0
Scutellum	1.25	189	43.9
Outer endosperm	18.80	1.3	8.8
Inner endosperm	73.10	0.3	

It is evident that the scutellum contains about 44 per cent and the aleurone layer about 35 per cent of the thiamine present in rice.

Parboiled rice: The process of parboiling of paddy consists in soaking paddy in water, steaming the soaked paddy for a short period, drying and milling. During the process of parboiling, scutellum gets fixed to the grain and a greater part of the thiamine present in the aleurone layer passes into the endosperm and hence not removed during milling. The starch gets gelatinised and the cooking quality of rice improves..

Effect of milling on the vitamin contents of raw and parboiled rice

The effect of milling on the thiamine and niacin contents of rice has been studied by Aykroyd *et al.*, (1940) and Swaminathan (1942). The results are given in Table 13.4.

Table 13.4 **Effect of milling on the thiamine and niacin contents of raw and parboiled rice**

Degree of milling	Thiamine (μg/100g)		Niacin (mg/100g)	
	Raw rice	Parboiled rice	Raw rice	Parboiled rice
Husked	360	450	3.8	4.6
5% Polishings removed	290	420	3.0	4.2
10% ,,	180	380	1.9	3.9
15% ,,	100	290	1.1	3.2
20% ,,	40	200	0.6	2.8

It is evident that raw milled rice is a poor source of thiamine. This will explain the wide prevalence of beriberi among raw rice eaters. Parboiled milled rice, on the other hand, is a good source of thiamine and niacin.

Nutritive value of rice proteins

The protein content of rice varies from 6-9 per cent depending on the variety. The chief protein is glutelin. Small quantities of albumin, globulins and prolamins are also present. The amino acid content of rice proteins is given in Table 4A in Appendix. The proteins are limiting in lysine, and threonine and to a lesser extent in methionine. The protein efficiency ratio of rice proteins ranges from 1.8-2.2 and is increased to about 2.4-2.6, when supplemented with a mixture of lysine and threonine and to 2.8-3.0 when supplemented with lysine, threonine and methionine.

Processed rice products: The important processed rice products are rice flakes or beaten rice, puffed rice (from paddy) and puffed rice (from rice). The chemical composition of these products is given in Table 1A in Appendix. Since puffed rice is a precooked product, it is consumed as a snack. Beaten rice is a partially cooked product and can be reconstituted readily by soaking in warm water for 15-20 minutes.

Nutritive value of poor rice diets

Poor rice diets based mainly on rice and containing small quantities of legumes similar to those consumed by low income

groups in India (5.0 per cent), oil and vegetables promote low growth in albino rats (4-7 g/week). The diets are deficient in calcium, proteins, riboflavin and vitamin A. Supplementation of the rice diets with processed protein foods (based on oilseed meals and legumes and fortified with vitamins and minerals) at 10 per cent level so as to provide about 2 per cent extra proteins makes up the deficiencies in the diet and promotes excellent growth in rats and children. (Swaminathan, 1967, 1969).

1.3 MAIZE

Maize (Corn) is cultivated in large quantities in U.S.A., Africa, Latin American countries and India. In Africa and Latin America, where maize is consumed as a staple food even by weaned infants and preschool children, the incidence of protein-calorie malnutrition has been found to be high. Before the discovery of niacin (the pellagra preventive vitamin), pellagra was widely prevalent among maize eaters in many countries of the world.

Proteins: The chief proteins of maize are glutelin and (zein) prolamins. Zein is completely lacking in tryptophan and lysine. Maize proteins contain excess of leucine and recent studies indicate that excess of leucine interferes in the conversion of tryptophan to niacin and hence aggravates the pellagragenic action of maize (Gopalan *et al.*, 1969). More recently, new strains of maize (e.g. opaque-2) containing more tryptophan and lysine and less isoleucine have been developed. The quality of proteins in opaque-2 is superior to that of the common strains of maize. Opaque-2 promotes good growth in experimental animals and does not produce black tongue in dogs. The PER of the proteins of maize ranges from 0.9 to 1.4 depending on the variety.

B-vitamins: Whole maize is a good source of thiamine, pyridoxine, pantothenic acid, fair source of riboflavin but poor source of niacin. Milled maize products e.g., degerminated maize meal is a poor source of all B-vitamins.

Maize products

The different maize products of commerce are whole maize meal, degerminated maize meal, hominy grits and corn flakes.

Whole maize meal: This is prepared by powdering the whole maize kernel.

Degermed maize meal: The degermed maize is powdered and sieved. The flour is devoid of germ and hence has a low B-vitamin content.

Hominy grits: Whole maize grain is steamed and the pericarp and germ removed. Hominy grits consist of the endosperm of maize and have low contents of B-vitamins.

Corn flakes: Corn flakes are made from hominy grits and hence have low contents of B-vitamins. The proteins undergo damage due to heat processing and have very low protein efficiency ratio.

1.4 RYE

Rye was cultivated widely in Europe about 50 years ago. It is still grown in that continent to an appreciable extent.

Chemical composition

The chemical composition of rye is given in Table 1A in the Appendix. The protein content may vary from 7-14 per cent. The grain is a good source of thiamine and a fair source of riboflavin and niacin. It is a good source of phosphorus and a fair source of calcium and iron.

Proteins: The proteins consist of a mixture of prolamin, glutelin, globulin and albumin. The properties of rye gluten are different from those of wheat gluten, as it is not possible to prepare gluten from rye by washing the dough in water. The amino acid composition of rye proteins is given in Table 4 A in the Appendix.

Milling of rye: Rye milling is an important industry in Europe. Rye flour is a commercial product. It is used for the preparation of bread.

Rye bread: Rye bread is not made, as a rule, by fermentation with yeast but is usually made after fermentation with a sour dough.

1.5 OATS

Oats are cultivated in limited amounts in Europe, U.S.A. Canada and Australia and are used mainly as a breakfast cereal. The important product of commerce is rolled oats.

B-vitamins: Oats is a good source of thiamine, pyridoxine and pantothenic acid and a fair source of riboflavin and niacin.

Proteins: The protein content of rolled oats varies from 12 to 18 per cent. (Table 1A in the Appendix). Oats proteins are fair sources of all essential amino acids and possess protein efficiency ratio values ranging from 2.2-2.4.

1.6 BARLEY

Barley is one of the oldest grain crops. It is cultivated extensively in all countries of the world. It is consumed as a staple food by small groups of the population in some countries. The greatest use of barley, however, is for the preparation of malt, which is used for brewing and in the preparation of malt extract for incorporation in infant and invalid foods.

Proteins: The protein content of barley ranges from 9-12%. Barley proteins consist of a mixture of albumin, globulins, prolamins (hordein) and glutelins and are fair to good sources of all essential amino acids (Table 4A in Appendix). The limiting amino acids in barley are lysine and threonine. The protein efficiency ratio values of barley proteins range from 1.9 to 2.2.

B-vitamins: Barley is a good source of thiamine, niacin, pantothenic acid, and pyridoxine and a fair source of riboflavin.

Pearl barley: Pearl barley prepared by the removal of the outer husk by abration, is consumed as an invalid food. It has low content of B-vitamins.

1.7 MILLETS

The millets consumed in large quantities in different countries of the world are kaffir corn, pearl millet, and ragi. Some other millets e.g., Italian millet, little millet, common millet are cultivated in small quantities and consumed by the people in some countries. The principal millet producing countries are India, China, Africa and U.S.A.

1.7.1 KAFFIR CORN (*Sorghum Vulgare*)

Kaffir corn is also known as Milo or Sorghum. It is grown in large quantities in India, U.S.A. and Africa.

Chemical composition

The chemical composition of kaffir corn is given in Table 1A in the Appendix. The protein content ranges from 9-14 per cent depending on the variety. It is a good source of thiamine and pyridoxine and a fair source of riboflavin and niacin. It is a fair source of calcium and iron and is a good source of phosphorus.

Proteins: The principal proteins of kaffir corn are prolamin and glutelin. The proteins are fair to good sources of the essential amino acids, the limiting essential amino acids being lysine and threonine. Kaffir corn proteins contain excess of leucine. Recent studies in India have shown that excess of leucine interferes in the conversion of tryptophan to niacin and causes pellagra in human beings consuming poor diets based mostly on kaffir corn (Gopalan *et al.*, 1969). The PER values of kaffir corn proteins range from 1.1-1.5 depending on the variety. The PER increases markedly when the proteins are supplemented with lysine.

Nutritive value of poor kaffir corn diet

A poor diet consisting predominantly of kaffir corn and containing small amounts of legumes (5 per cent), oil and vegetables promotes only moderate growth in albino rats (6-9 g/week). The diet is deficient in calcium, proteins, vitamin A and riboflavin. Supplementation of the diet with processed protein foods (based on blends of oilseed meals and legumes and fortified with vitamins and minerals) at 10 per cent level makes up the deficiencies in the diet and promotes good growth in albino rats. Children fed on poor kaffir corn diet maintain positive N, Ca and P balance (Swaminathan, 1967, 1969).

1.7.2 RAGI (*Eleusine Coracana*)

Ragi is cultivated in appreciable amounts in some regions in India, Ceylon and Africa and consumed as a staple food by the local population. Unlike other common millets, the grain has a hard seed coat which is difficult to be separated by mechanical means. The whole grain is powdered, passed through a sieve and consumed as a dumpling.

Chemical composition

The chemical composition of ragi is given in Table 1A in the Appendix. The protein content of the grain varies from 6-9 per cent depending on the variety. Ragi is a rich source of calcium in which other common cereals are deficient. It is a good source of iron and phosphorus. The grain is a good source of thiamine and a fair source of niacin and riboflavin.

Proteins: Ragi proteins consist of a mixture of globulins, prolamin and glutelin. About 30 per cent of the nitrogen present in the grain (mostly in the husk) is not extracted even by dilute sodium hydroxide. The protein is a fair to good source of all essential amino acids, the limiting amino acids, being lysine and threonine. The PER values of ragi proteins range from 1.2 to 1.4. When supplemented with lysine, the PER increases to 2.2.

Nutritive value of poor ragi diets

Poor ragi diets containing small quantities of legumes (5 per cent), vegetables and fat promote moderate growth in rats (7-10 g/ week). The diets are limiting in proteins. When supplemented with processed protein foods at 10 per cent level or legumes at 15 per cent level (to provide 2.5 per cent extra proteins) a marked improvement in the growth rate of rats (16-20 g/week) has been observed. Children fed on a poor ragi diet maintain positive N, Ca and P balance. (Swaminathan, 1967, 1969).

1.7.3 PEARL MILLET

Pearl millet (*Pennisetum typhoideum*) is widely cultivated and consumed by the local population in many countries of Asia and Africa. It is usually powdered in a flour mill and consumed in the form of porridge, dumpling or unleavened bread. Dehusked pearl millet can be cooked in the same way as rice and consumed.

Chemical composition

The chemical composition of pearl millet is given in Table 1A in the Appendix. The protein content varies from 10-14 per cent. It is a good source of thiamine and fair source of niacin and riboflavin. It is a fair source of calcium and iron and a good source of phosphorus.

Proteins: The proteins consist of a mixture of albumin, globulins, prolamin and glutelins. They are fair to good sources of essential amino acids, the limiting amino acids being lysine and threonine. The PER of the proteins ranges from 1.9-2.0 and is improved to a marked extent to about 2.5-2.7 by supplementation with lysine.

Nutritive value of poor pearl millet diet

Poor pearl millet diet based mainly on pearl millet and containing small quantities of pulses (5 per cent), oil and vegetables promotes moderately good growth in albino rats (10-12 g week). It is deficient in calcium, vitamin A, riboflavin and proteins. Supplementation of the diet with processed protein foods (based on oilseed meals and legumes and fortified with essential vitamins and minerals) makes up the deficiencies in the diets and promotes good growth in albino rats. Children fed on poor pearl millet diet maintain positive N, Ca and P balance. (Swaminathan, 1967, 1969).

1.7.4 MINOR MILLETS

The following millets e.g., Italian millet (*Setaria Italica*) Little millet (*Panicum miliare*), Common millet (*Panicum miliaceum*) and Kodu millet (*Paspalum scrobiculatum*) are cultivated and consumed in some regions in India and other countries.

Chemical composition

The chemical composition of these millets is given in Table 1A in the Appendix. The protein contents of these millets range from about 7 to 13 per cent. They are good sources of thiamine and fair sources of riboflavin and niacin. They are fair sources of calcium and iron and good sources of phosphorus.

Proteins: The essential amino acid composition of these millets is given in Table 4A in the Appendix. They are fair to good sources of the different essential amino acids, the limiting amino acids being lysine and threonine. The protein efficiency ratio values range from 0.7 to 0.9.

Nutritive value of poor diets

Poor diets based on these millets promote low growth in albino rats (5-8 g/week).

2.0 LEGUMES (Pulses)

The edible leguminous seeds are important sources of proteins in the dietaries of millions of people in Asian, African and Latin American countries. The common legumes may be broadly grouped under three heads (*i*) grams (*ii*) peas and (*iii*) beans. The term pulses are used for leguminous seeds in India.

Methods of preparation of legumes

Legumes are prepared for consumption in several ways such as whole legumes, dehusked split legumes known as dhal in India and legume flour.

Whole legumes: They are soaked in water overnight and cooked in excess boiling water for 20-30 minutes. Excess of water is discarded. The cooked legume is seasoned with salt and condiments and consumed.

Dhal (dehusked split legumes): Legumes are consumed mainly in this form in India. The husk is removed in a mill and the dehusked legume is split. The dhal thus obtained is cooked in excess water for 20 to 30 minutes to a soft consistency. Salt, is added to taste.

Legume flour: Legume flour is prepared by grinding the dhal (dehusked split legume) in a flour mill. The flour thus obtained is used along with cereal flours in many common Indian preparations.

Chemical composition of legumes

The chemical composition of legumes is given in Table 1A in the Appendix. The protein content ranges from 18-25 per cent. They are good sources of thiamine and riboflavin and fair sources of niacin. Legumes are fair sources of calcium and iron and good sources of phosphorus. They contain fair amounts of unavailable carbohydrates.

Proteins: The essential amino acid composition of the proteins of legumes is given in Table 4A in the Appendix. It will be observed that legume proteins are, in general, good sources of lysine and threonine in which cereal proteins are deficient. They are, however, poor sources of sulphur amino acids and

tryptophan of which cereals are good sources. Hence, legume proteins supplement effectively cereal proteins.

Toxic factors in legumes and their elimination: Many of the legumes contain toxic factors which must be eliminated during cooking before they are consumed. The various toxic factors present in legumes may be broadly grouped under three heads (*i*) trypsin inhibitors (*ii*) haemagglutinins and growth inhibitors and (*iii*) other toxic factors.

Trypsin inhibitors, haemagglutinins and growth inhibitors are destroyed by autoclaving the legumes at 15 lbs pressure for 30 minutes. Other toxic factors present in certain groups of legumes are goitrogenic factors, cyanogetic glucosides, and saponins. These can be leached out by cooking the legumes in excess of water and discarding it. In addition, legumes of lathyrus family contain toxic factors which cause the disease lathyrism. These toxic factors can be eliminated by cooking the legume for 10 minutes in excess of water and discarding the water.

Effect of heat processing on the nutritive value of the proteins

Heat processing, in general, improves the nutritive value of legume proteins, by inactivating trypsin and growth inhibitors and haemagglutinins. The PER values of heat processed legume proteins range from 0.8 to 2.2.

Supplementary value of legume proteins to cereal proteins: Since legume proteins are good sources of lysine and threonine in which cereals are deficient, they supplement effectively cereal proteins.

Supplementary value of legumes to poor cereal diets: Legumes, when incorporated in poor cereal diets at 15 per cent level along with vitamins and minerals bring about a marked improvement in the overall nutritive value of the diets as judged by the growth of albino rats. Hence, increased production and consumption of legumes will help to overcome protein deficiency in the diets of low income groups of the population in the developing countries. (Swaminathan, 1967, 1969).

3.0 NUTS AND OILSEEDS

Nuts and oilseeds are cultivated in different countries and used mainly as a source of oil. They are, however, rich potential

sources of proteins for use as supplements in human diets. The most important of these are peanut (groundnut), soybean, cottonseed, sesame seeds, copra, sunflower seeds, and safflower seeds.

3.1 PEANUT (*Groundnut*)

Peanut is cultivated in large quantities in many Asian and African countries and in U.S.A. It is used mainly for the production of oil. Edible peanut flour and peanut protein isolate (obtained from the meal) are being used in India in the production of processed protein foods, weaning foods and toned milk. Peanut butter is also produced on a large scale in U.S.A. Peanut harvested under moist condition has been found to be infected by *Aspergillus flavus* which produces aflatoxin. This can be avoided by drying the peanut immediately after harvest in a drier to a low moisture content.

Chemical composition

Peanut contains about 22-28 per cent proteins and 42-50 per cent oil. It is a good source of thiamine, niacin, pantothenic acid and a fair source of riboflavin. Peanut is a good source of phosphorus and fair source of iron and calcium.

Proteins: The chief proteins in peanuts are globulins known as *arachin* and *conarachin*. The limiting amino acids in peanut proteins are lysine, threonine and methionine. The PER of peanut proteins ranges from 1.6-1.8.

Peanut products

The important products from peanuts are (1) roasted decuticled kernels (2) peanut candies (3) peanut butter (4) peanut milk and curds and (5) peanut flour.

Roasted peanut kernels: Roasted peanut kernels are commonly consumed in India as a snack. Mild roasting of the kernels does not cause any appreciable destruction of thiamine present. Roasted kernels are also added to several cereal food preparations.

Peanut candies: Candies based on peanut kernels and sugar or jaggery are rich sources of proteins and calories. The traditional candy preparation based on peanut and jaggery (crude

cane sugar) are widely consumed as a snack by the low income groups in India.

Peanut milk and curds: Peanut milk is prepared by grinding decuticled peanut kernel into a fine paste and mixing with 8 times the weight of water. Lime water (calcium hydroxide solution) is added (100 ml per litre of peanut milk) to stabilise the milk and to increase its calcium content. The milk is strained through a muslin cloth and heated to boiling. Sugar is added to taste. The milk can be converted into curds by seeding with lactic curd obtained from cow's milk. Both the milk and curds have a pleasant nutty flavour and are acceptable to a large majority of the consumers.

Peanut butter: Peanut butter is manufactured on a large scale in U.S.A. and used as a supplement in human diets. The method of preparation is as follows (1) roasting, decuticling and sorting (2) grinding to a smooth paste (3) removal of a part of peanut oil and addition of hydrogenated fat and (4) fortification with vitamins and minerals.

Peanut flour: Edible low fat peanut flour is prepared from lightly roasted decuticled kernels as follows: The peanut kernels are lightly roasted and the red skin is removed by rubbing. The spoilt seeds (fungal affected) are removed by handpicking. The cleaned kernels are pressed in an expeller to remove oil. The resulting white cake is powdered in a flour mill. The flour thus obtained has an attractive creamy colour and acceptable taste. The protein content of the flour is about 48 to 50 per cent. It can be consumed in the form of porridge sweetened with sugar. The flour can also be added to cereal flours at 10 per cent level in common food preparations. Peanut flour has been used in the preparation of processed protein foods such as Indian multipurpose food, Bal-ahar and Balanced malt food. (Swaminathan 1967, 1969).

Supplementary value of fortified peanut flour and peanut curds to diets of children

Feeding experiments carried out with school children have shown that a daily supplement of 50 g. peanut flour (fortified with calcium salts, vitamins A and D and riboflavin) or 12 ounces of peanut curds (fortified with vitamins and calcium) over a period

of 3-6 months brings about highly significant improvements in their growth and nutritional status. (Swaminathan, 1967, 1969).

3.2 SOYBEAN

Soybean is cultivated extensively in some Asian countries and in U.S.A. It is a rich source of protein. During recent years a good deal of work has been carried out in different countries on the development of processed products based on soybean for use as supplements in human diets. These include (1) milk substitutes and infant foods (2) soyflour and processed foods based on soy flour and (3) other processed soya products.

Chemical composition

The chemical composition of soybean is given in Table 1A in the Appendix. The protein content ranges from 35-45 per cent and fat content from 17-22 per cent. It is a good source of thiamine, and niacin and a fair source of riboflavin. Soybean contains trypsin and growth inhibitors and haemagglutinins. These can be inactivated by autoclaving soybean at 14 lb pressure for 30 minutes.

Proteins: The chief protein of soybean is a globulin known as glycinin; other proteins present in small amounts are phaseolin and legumelin. Soybean proteins are good sources of all essential amino acids except methionine which is the limiting amino acid (Table 4A in the Appendix). Since the proteins are good sources of lysine and threonine, they supplement effectively cereal proteins. The PER of heat processed soybean proteins ranges from 1.9-2.2 and is increased markedly to 2.8-3.0 by supplementation with methionine.

Processed soybean products

Milk substitutes and Infant foods: Processes for the production of milk substitutes based on soybean have been developed by several workers. The process consists in grinding decuticled soybean in hot water, straining the milk through a fine sieve, heating the milk at 14 lb pressure for 30 minutes to destroy trypsin and growth inhibitors and haemagglutinins and fortifying with sugars, vitamins and minerals. The milk substitute thus

obtained can be consumed as fluid milk or dried in a spray drier. Both the fluid milk and the dried milk substitutes can be used for feeding infants and as a supplement to the diets of weaned infants and preschool children.

Soybean flour and processed protein foods based on soybean flour: Three types of edible soybean flour viz., full fat, low fat and defatted soybean flours, are being manufactured in U.S.A. They can be used in the preparation of processed protein foods and for enriching bakery products, breakfast cereals and paste goods.

Multipurpose food: This consists of low fat or defatted soybean flour fortified with vitamins and minerals.

Soup powder: A soup powder formulation based on a blend of soybean, peanut and pea flours, skim milk powder, condiments and spices and fortified with vitamins and minerals has been described.

Protein foods: Protein foods based on blends of soybean, peanut and coconut flours and fortified with vitamins and minerals have been developed at C.F.T.R.I., Mysore (Swaminathan, 1966). The foods can be used for the treatment and prevention of protein malnutrition in preschool children and expectant and nursing mothers in developing countries.

Tofu (Soybean protein curd): Tofu is manufactured in large quantities in Japan and used as a supplement to the daily diet. Tofu consists of soybean protein curd precipitated from soybean milk by the addition of calcium or magnesium salts. The curd is pressed into the consistency of cheese. Fresh tofu can be preserved in a refrigerator for a few days. Dried or smoked tofu, however, keeps well for long periods and can be readily transported in cans and other suitable packings.

Natto: Natto is a product resembling cheese. The method of preparation of Natto is as follows: Soybeans are cooked in boiling water for 5 hours, and the cooked bean is inoculated with *Bacillus Natto* culture at 60°C, tied in paddy straw and allowed to ferment for 24 hours. The fermented product is consumed in the fresh state in Japan.

Tempeh: This product is widely consumed in Indonesia. The method of preparation is as follows: Cooked soybeans are inoculated with *A. oryzae* and the mycelium is allowed to grow

for about 48 hours after which the material is fried in oil or sliced and cooked in soup.

Miso: Miso is one of the most popular foods in Japan and is mainly consumed along with soup and vegetables. A mixture of soybean flour and rice flour is made into a paste using water and inoculated with fermented rice (*rice Koji*) and fermented for 3 to 4 days. The principal organism in fermentation is *A-oryzae*.

Soya sauce: Soya sauce is consumed widely in Japan. A mixture of cooked soybean and wheat flour is inoculated with *A-oryzae* and incubated at 40°C for 72 hours. Salt solution is added and the fermentation is allowed to proceed for periods of 3 months or longer. The soya sauce is pressed out, filtered, sterilised by heat and bottled.

Supplementary value of soybean flour to poor cereal diets

Studies carried out with albino rats have shown that incorporation of heat processed soybean flour fortified with vitamins and minerals at 7 per cent level in poor cereal diets based on common cereals and millets makes up the deficiencies in the diets and promotes excellent growth comparing well with that obtained when Bengalgram was incorporated in the diet at 15 per cent level. (Swaminathan, 1967, 1969).

3.3 SESAME

Sesame is cultivated in many tropical and sub-tropical regions. The seed is used mainly for the production of sesame oil. Small quantities are consumed directly in various traditional food preparations. Two varieties i.e., black and white are commonly grown.

Chemical composition

The protein content of the seed varies from 16-20 per cent and oil content 55-60 per cent. The whole seed is a good source of thiamine and a fair source of riboflavin and niacin. The seed is rich in calcium. Most of the calcium is present as oxalate in the seed coat. The dehusked seed has low calcium and oxalic acid contents.

Oil: Sesame oil is a rich source of essential fatty acids.

Proteins: Sesame proteins consist of a mixture of globulins. The proteins are fair to good sources of all essential amino acids. It is a rich source of methionine and tryptophan but is deficient in lysine. The PER of sesame proteins ranges from 1.7-1.8 and is increased markedly to 2.7-2.9 when supplemented with lysine.

Sesame products

Edible sesame flour: Edible sesame flour is prepared as follows: Sesame seeds are moistened with water and the seed coat is removed by rubbing. The decuticled seed is crushed in an expeller for removing the oil. The white cake thus obtained is powdered in a flour mill. The flour thus obtained has about 35-40 per cent proteins and 10-12 per cent oil.

Processed protein foods containing sesame flour: Sesame flour has been used along with peanut and soybean flours for the preparation of protein foods suitable for supplementing the diets of children (Swaminathan 1969). The protein foods have been found to be effective supplements to human diets.

Use of sesame seed in food preparations: Sesame seed is used in Mexico and Venizuela along with corn as an ingredient of bread. Sesame seed candy prepared from decuticled seed and jaggery (crude cane sugar) is used widely in India as a snack. Crushed sesame seed is also incorporated in several cereal food preparations in India and middle East Countries.

3.4 COCONUT

Coconut is cultivated in large quantities in Phillipines, Ceylon and Kerala State of India. Coconut is used mainly for the production of oil. The cake prepared carefully for edible purposes, is a good source of proteins and can be used as a supplement to human diets. Coconut is used as such to some extent in the daily diet. Tender coconut water is also consumed to a limited extent during the summer season in India.

Chemical composition: The chemical composition of coconut and its products is given in Table 13.5.

Table : **13.5 Chemical composition of coconut and its products**

Sl. No.		Moisture (%)	Protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Carbohydrate (%)	Calorific value KCal/100g
1.	Coconut kernel (tender)	90.8	0.9	1.4	...	0.6	6.3	41
2.	Coconut kernel from mature nuts	46.3	4.1	37.3	3.4	1.0	7.9	484
3.	Copra	4.8	7.1	64.4	5.9	1.7	16.1	672
4.	Desiccated coconut	3.5	6.3	57.4	7.5	1.3	24.0	618
5.	Coconut water (from *tender coconut*)	95.0	0.14	0.13	...	0.63	4.1	19
6.	Coconut milk (from mature nuts)	89.8	0.8	7.2	...	0.2	2.0	76
7.	Coconut meal	11.2	20.9	13.3	10.5	4.9	39.2	360

Coconut kernel: Coconut kernel from mature nuts contains only 4.1 per cent protein but is a rich source of fat (37.3 per cent). Coconut kernel from tender nuts is a poor source of protein.

Copra: Copra contains only 7.1 per cent protein but is a rich source of fat (64.4 per cent).

Desiccated coconut: It is prepared from mature coconut kernel as follows: The brown testa is removed, the kernel is washed in water, shredded and dried in a hot air drier at 50-55°C. Desiccated coconut is used in cakes, pastries and chocolates.

Coconut meal: Edible coconut meal prepared from good quality copra by expressing the oil in an expeller (screw press) is a good source of protein (20-22 per cent). Since it contains too much of fibre, it can be consumed in small quantities (30-40 g per day) mixed with the cereal flours or pulse flours.

Coconut water: Coconut water from tender coconut is a poor source of protein and calories.

Coconut milk: Coconut milk is obtained from grating mature coconut, blending it with 6 times the weight of water containing small quantities of $NaHCO_3$ (0.1 per cent) and pressing the milk

through a cloth. It is a poor source of protein (0.8 per cent) but a good source of fat (7.2 per cent). If coconut milk is mixed with equal quantities of soybean milk (protein 4.2 per cent and fat 2.2 per cent), the protein content of the mixture will be 2.5 per cent and fat content 4.7 per cent. A blend of equal volumes of coconut and soybean milk sweetened with sugar (at 4.0 per cent of milk) will form a good supplement to the diets of weaned infants and preschool children of the low income groups in the developing countries.

Nutritive value of coconut proteins: Data regarding the essential amino acid composition of coconut proteins given in Table 4A in the Appendix indicate that coconut protein is a moderately good source of all essential amino acids. The protein efficiency ratio is fairly high ranging from 2.1-2.4 at 10 per cent protein level.

Supplementary value of coconut meal to poor cereal diets: Coconut meal fortified with calcium salts, vitamins A and D and riboflavin when incorporated at 10-20 per cent levels in poor cereal diets makes up the deficiencies in the diets and promotes good growth in albino rats. (Swaminathan 1969).

3.5 COTTONSEED

Cottonseed contains on an average 44.5 per cent hulls (including linters) and 55.5 per cent kernels (meat). Delintered cottonseed contains about 36 per cent hulls and 64 per cent kernel.

Chemical composition: Data regarding the chemical composition of cottonseed and kernels (meat) are given in Table 13.6.

Table 13.6 **Chemical composition of whole cottonseed and cottonseed kernels**

	Moisture (%)	Crude Protein (%)	Fat (%)
Cottonseed (whole)	10.0	19.5	18.8
Cottonseed kernels (meat)	7.0	30.5	30.8

Gossypol: The kernel contains a toxic principle known as gossypol. Gossypol reacts with the e-amino group of lysine moiety in proteins during the process of expelling cottonseed oil from the kernel.

Cottonseed proteins: Data regarding the essential amino acid content of cottonseed proteins given in Table 4A in the Appendix indicate that the proteins are fair sources of all essential amino acids. The PER of the proteins ranges from 1.9-2.2. The limiting amino acids are lysine, methionine and threonine.

Cottonseed oil: Crude cottonseed oil obtained by the expeller process contains gossypol. This can be removed by refining and bleaching. The refined and bleached cottonseed oil is a good source of the essential fatty acid-linoleic acid and also vitamin E.

Edible cottonseed flour: Edible cottonseed flour contains about 50 per cent proteins and has been used as an ingredient of weaning foods and processed protein foods suitable for the treatment and prevention of protein-calorie malnutrition in children. *Incaparina* (Formula 9) developed by the INCAP, Guatemala, contains about 38 parts of cottonseed flour, 29 parts of corn, 29 parts of sorghum, 3 parts of torula yeast, 1 part of calcium carbonate and 4500 I.U. of vitamin A. It has been widely used for the treatment and prevention of protein-calorie malnutrition among preschool children in Guatemala. (Swaminathan 1969).

3.6 SUNFLOWER SEED

Sunflower seed is being cultivated extensively in U.S.S.R. and Argentina. The cultivation of sunflower seed on a large scale is being encouraged in India.

Chemical composition: Data regarding the chemical composition of sunflower seed are given in Table 13.7. The seed consists of hull and kernel. The hull forms about 48 per cent and the kernel about 52 per cent by weight of the whole seed. The kernel contains about 28.0 per cent proteins and 42.0 per cent fat.

Table **13.7 The composition of sunflower seed and kernel**

	Moisture %	Crude protein (N×6.25) %	Fat %	Hulls %
Sunflower seed (whole)	4.5	12.5	24.2	48.2
Sunflower seed (kernel)	4.4	24.0	47.3	3.8

Nutritive value of proteins: Data regarding the essential amino acid content of the proteins of sunflower seeds given in Table 4A in the Appendix, indicate that the proteins are good sources of all essential amino acids except lysine. The PER of sunflower seed proteins is fairly high ranging from 2.1-2.4.

Use of sunflower seed meal in feeding infants: Sunflower seed meal has been successfully used along with rice flour, malt extract and sugar in feeding infants, allergic to cow's milk. There is scope for the incorporation of sunflower seed meal in weaning food and protein food formulations.

Sunflower seed oil: Sunflower seed oil is a rich source of the essential fatty acid-linoleic acid, containing about 58 per cent.

4.0 MILK AND MILK PRODUCTS

Cow's milk is being used throughout the world for feeding infants and as a supplement to the diets of children and adults. Buffalo milk is also used in some countries as a supplement to human diets. Milks of other mammals e.g., goat, sheep and camel are used to a very small extent in some countries.

Chemical composition: Data regarding the chemical composition of milk of some mammals are given in Table 13.8. It will be noted that there are wide variations in the contents of proteins, fat and minerals between the milks of different mammals. The discussion which follows relates to the nutritive value of cow's and buffalo milk.

Nutritive value of proteins: The proteins of milk consist of casein (a phospho protein), albumin and small amounts of globulins. The protein contents of cow's and buffalo milks are about 3.5 and 4.1 per cent respectively. Milk proteins are rich sources of all essential amino acids. They are slightly deficient in sulphur amino acids as compared to egg proteins. The PER of cow and buffalo milk proteins ranges from 2.9 to 3.2 and the NPU from 70 to 75. Since milk proteins are good sources of lysine and threonine, they supplement effectively cereal proteins.

Fat: The fat content of cow's milk is about 3.5 per cent while that of buffalo milk is about 7.0 per cent. The fat of cow's or buffalo milk is a poor source of essential fatty acids. The

Table **13.8 Composition of milk from different mammals**
(Values per 100 g)

Species	Fat g	Protein (N×6.38) g	Lactose anhydrous g	Energy value KCal
Human	4.62	1.23	6.94	73
Cow: Friesian	3.50	3.25	4.60	62
Guernsey	4.65	3.65	4.70	75
Buffalo	7.45	3.78	4.90	100
Goat	4.50	3.30	4.40	71
Ewe	7.50	5.60	4.40	105
Mare	1.60	2.20	6.00	47
Ass	1.50	2.10	6.20	46
Camel	4.20	3.70	4.10	70
Yak	7.00	5.20	4.60	100
Llama	3.20	3.90	5.30	65
Reindeer	22.50	10.30	2.40	250
Elephant	9.3	5.1	3.7	121
Pig	8.5	5.8	4.8	122
Rabbit	16.7	10.4	2.0	206

vitamin A content of milk fat (butter) varies from 700 to 1200 I.U. per 100 g.

Lactose: The main sugar present in milk is lactose. The lactose contents of cow and buffalo milks vary from 4.5 to 4.9 per cent.

Vitamins: The vitamin A contents of cow's and buffalo milks range from about 150-200 I.U. per 100 g. The contents of other vitamins per 100 g. are as follows: thiamine 40-50 μg; riboflavin 150-200 μg; Niacin 70-80 μg; Vitamin B_{12} 0.4-0.5 μg; folic acid 3.3-5.6 μg. and ascorbic acid 2-2.5 mg.

Calcium: Milk is a good source of calcium. The calcium contents of cow's and buffalo milks are about 0.12 and 0.19 per cent respectively.

Other minerals: Cow's and buffalo milks are good sources of phosphorus, magnesium and potassium but poor sources of iron (0.2 mg/100 g).

OTHER TYPES OF LIQUID MILK

Skimmed milk (Separated milk). It is prepared by removing the fat from whole milk by using a cream separator. The quantity of fat left is about 0.05-0.1 per cent. Since it does not contain fat and vitamin A, it should not be fed to infants. It can, however, be used as a supplement to the diets of older children and adults. Its nutritive value is given in Table 1A in the Appendix.

Toned milk: Toning can be defined as the addition of reconstituted skim milk to whole buffalo milk so as to reduce the fat content to 3.0 per cent and solids-not-fat (S.N.F.) to 8.5 per cent. Its nutritive value is almost similar to that of fresh cow's milk.

Double toned milk: Double toned milk is prepared by mixing cow's or buffalo milk with fresh skim milk or skim milk reconstituted from skim milk powder so that the fat content is not less than 1.5 per cent and S.N.F. 9.0 per cent. Its nutritive value is similar to that of toned milk except for lower fat and vitamin A contents.

Recombined milk: Recombined milk means the hemogenized product prepared from milk fat, skim milk powder and water. Its fat content should not be less than 3.0 per cent and S.N.F. 8.5 per cent. Its nutritive value is almost similar to that of toned milk.

Filled milk: Filled milk is prepared from refined vegetable oil and skim milk powder and water. It is also fortified with vitamin A. Its fat content should not be less than 3.0 per cent and S.N.F. 8.5 per cent. Its nutritive value is similar to that of toned milk.

Standardised milk: Standardised milk is prepared from buffalo milk or a mixture of buffalo and cow's milk by mixing with skim milk so that the fat content of the mixture is reduced to 4.5 per cent and S.N.F. 8.5 per cent. Its nutritive value is almost similar to that of cow's milk.

Sterilized milk: During the process of sterilization, some portion of the vitamins present is destroyed. The percentage destruction of different vitamins is approximately as follows: thiamine, 30 per cent; ascorbic acid, 50 per cent; vitamin B_{12},

90 per cent; vitamin A, 17 per cent, riboflavin, 15 per cent and pyridoxine, 50 per cent.

Evaporated milk: In the preparation of evaporated milk, the milk is subjected to sterilization at 240-245°F for 15 minutes. Hence, there is appreciable loss of several vitamins. The percentage losses of vitamins are of the same order as that occurring in sterilised milk. It is diluted with an equal amount of boiled water and used in Europe and America for feeding infants.

Sweetened condensed milk: Sweetened condensed milk contains about 40 per cent sucrose but the concentration of milk solids is nearly the same as in evaporated milk. Because of its high sucrose content, it is not suitable for feeding infants. It can, however, be given in small amounts as a supplement to the diets of older children.

Dried milk (milk powder): Two methods are commonly used for the preparation of dried milk (1) roller or drum drying and (2) spray drying. The powder obtained by the roller process has a solubility of only about 85 per cent as compared with a solubility of 98 per cent for spray dried powder.

Whole milk powder: Whole milk powder obtained from standardised milk contains about 26 per cent proteins and 26 per cent fat. It is about 8 times as much concentrated as fresh cow's milk. Milk reconstituted from whole milk powder is used for feeding infants.

Skim milk powder: Skim milk powder contains about 35 per cent proteins and only traces of fat (0.5-1.0 per cent). It is a good source of calcium and B-vitamins. Since it is devoid of fat and vitamin A, it should not be fed to infants. Skim milk powder can be used as a supplement to the diets of children and adults.

Instant skim milk powder: Recently, methods have been developed for the production of instant skim milk powder from spray dried skim milk powder. The product disperses immediately and completely in water. The nutritive value of instant milk powder is similar to that of spray dried skim milk powder.

Malted milk powder: Malted milk powder is prepared from whole milk and malt extract. It contains about 15 per cent proteins and 7 per cent fat. It can be used as a food for invalids and convalescents and as a supplement to the diets of children and

adults. It is not suitable for feeding normal infants as it has a low fat content.

FERMENTED MILK

Yogurt: Yogurt and yogurt-like products are the traditional forms of sour milk consumed in Bulgaria and adjacent countries. The fermentation is brought about by lactic acid bacilli and yeasts. At present, yogurt is being manufactured on a large scale in Western Europe and North American countries. The product is made from whole milk or whole milk concentrated to two-thirds its volume. The souring is brought about by a mixture of lactic acid producing *streptococci* and *lactobacilli acidophilus*. The nutritive value of yogurt is similar to that of the milk from which it has been prepared. During lactic fermentation, a part of the lactose is fermented to lactic acid and galactose is left behind.

Sour curds: This product is prepared on a home scale in India and consumed extensively. The fermentation of milk is brought about by mixed lactic organisms. A part of the lactose is converted into lactic acid and galactose is left behind. The nutritive value of sour curds is similar to that of milk.

Therapeutic value: Fermented milks are generally considered to possess some therapeutic value in intestinal disorders. The development of lactic acid and the presence of lactic organisms inhibit the growth of many pathogenic organisms. Continuous consumption of lactic fermented milk may help to change the intestinal flora. Fermented milk is more readily digested than the unfermented milk and hence is used in the treatment of intestinal disorders.

Cheese: The composition of cheese is given in Table 13.9. Cheese prepared from full fat milk is an excellent source of proteins, fat, vitamin A, calcium and a fair source of riboflavin. It can be used as a supplement to the diets of children and expectant and nursing mothers.

Indigenous Indian milk products

The important indigenous milk products of India are (*i*) khoa (*ii*) Surati cheese (panir) and (*iii*) channa. Khoa from full fat milk

Table **13.9 Chemical composition of different types of cheese (values per 100g)**

Cheese type	Moisture (g)	Calories (KCal)	Protein (g)	Fat (g)	Carbo-hydrates (Total) (g)	Ash (g)	Calcium (mg)	Phos-phorous (mg)	Iron (mg)	Vitamin A (I.U.)	Thia-mine (mg)	Ribo-flavin (mg)
Hard:												
Cheddar	37.0	398	25.0	32.2	2.1	3.7	750	478	1.0	1310	0.03	0.46
Swiss	39.0	370	27.5	28.0	1.7	3.8	925	563	0.9	1140	0.01	0.40
Semi-hard:												
Brick	41.0	370	22.2	30.5	1.9	4.4	730	455	0.9	1240	...	0.45
Roquefort	40.0	368	21.5	30.5	2.0	6.0	315	339	0.5	1240	0.03	0.61
Soft:												
Cottage (creamed)	78.3	106	13.6	4.2	2.9	1.0	94	152	0.3	170	0.03	0.25
Cottage (uncreamed)	79.0	86	17.0	0.3	2.7	1.0	90	175	0.4	10	0.03	0.28
Cream	51.0	374	8.0	37.7	2.1	1.2	62	95	0.2	1540	0.02	0.24
Camenbert	52.2	299	17.5	24.7	1.8	3.8	105	184	0.5	1010	0.04	0.75
Limburger	45.0	345	21.2	28.0	2.2	3.6	590	393	0.6	1140	0.08	0.50

is rich in proteins, fat, calcium and vitamins. Surati cheese and channa are rich in proteins and fat.

Casein products: Soluble casein products such as calcium caseinate or sodium caseinate are being manufactured and marketed for use in the treatment of protein deficiency states. They contain about 80-85 per cent proteins.

Butter and ghee (butter oil): Butter contains about 82 per cent fat and 3500 I.U. of vitamin A per 100 g. Ghee is 100 per cent fat and contains about 2000 I.U. of vitamin A per 100 g. Butter and ghee serve mainly as sources of fat and vitamin A.

Ice cream: Ice cream contains on the average the following: Milk fat, 12 per cent; non-fat-milk solids, 11 per cent; sugar, 15 per cent. It is mainly a source of energy and contains only 4 per cent proteins.

5.0 EGG AND EGG PRODUCTS AND POULTRY

Data regarding the nutritive value of egg and egg products are given in Table 13.10. Whole egg contains about 13 per cent proteins and 13 per cent fat and is a good source of vitamin A, riboflavin and vitamin B_{12}. Its cholesterol content is 498 mg. per 100 g. Egg is a good source of iron and phosphorus but a poor source of calcium.

Egg white: Egg white contains about 11 per cent proteins. The chief protein is egg albumin which accounts for 70 per cent of the proteins, the remaining 30 per cent being accounted by four other proteins viz., ovoglobulin, ovomucin, conalbumin and ovomucoid present in small amounts. Egg white is a good source of riboflavin and contains a heat labile factor called *avidin* which reacts with biotin and makes it unavailable.

Egg yolk: Egg yolk contains about 16 per cent proteins and 31 per cent fat. It is a rich source of vitamin A and riboflavin. It contains large amounts of cholesterol (1330 mg/100 g.) and is a good source of iron and phosphorus.

Egg powder: Egg powder contains about 49 per cent proteins and 43 per cent fat, and is a good source of vitamin A and riboflavin. It is also rich in cholesterol (3900 mg/100 g) and is a good source of iron and phosphorus.

Table **13.10 Chemical composition of whole egg, egg yolk, egg white and egg powder (values per 100g)**

Type	Moisture (g)	Protein (g)	Fat (g)	Carbo-hydrates (g)	Calories (kCal)	Ash (g)	Calcium (mg)	Phospho-rous (mg)	Iron (mg)	Vitamin A (I.U.)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)
Egg, Hen,													
Whole (without shell)	73.7	12.9	11.5	0.9	163	1.0	54	205	2.3	1180	0.11	0.30	0.1
Yolk	51.1	16.0	30.6	0.6	348	1.7	141	569	5.5	3400	0.22	0.44	0.1
White	87.6	10.9	trace	0.8	51	0.7	9	15	0.1	0	trace	0.27	0.1
Duck egg,													
Whole (without shell)	70.4	13.3	14.5	0.7	196	1.1	56	195	2.8	1230	0.18	0.30	0.1
Goose egg,													
Whole (without shell)	70.4	13.9	13.3	1.3	185	1.1	...	...	...	...	...	...	...
Turkey egg,													
Whole (without shell)	72.6	13.1	11.8	1.7	170	0.8	...	...	...	...	...	...	...
Egg, Hen,													
Whole, dried	2.0	48.9	42.9	2.5	609	3.7	194	832	9.0	4460	0.34	1.25	0.7
Egg white (powder)	8.8	80.2	0.2	5.7	372	5.1	66	110	1.0	0	0.04	1.99	0.2

Nutritive value of egg proteins: Data regarding the essential amino acid content of egg proteins are given in Table 4A in the Appendix. Egg proteins are good sources of all essential amino acids and possess the highest nutritive value among dietary proteins. The PER of egg proteins is 4.5 as compared with a value of 3.0 for milk proteins and the NPU 98, as compared with 70 for milk proteins. Egg proteins being rich in S-amino acids and lysine supplement effectively the proteins of cow's milk and of cereals respectively.

Poultry meat: Poultry meat is a good source of proteins. The protein content ranges from 18-22 per cent. It is a good source of certain B-vitamins. The PER of the proteins varies from 2.8-3.3 and the NPU, 65-70. The proteins are good sources of all essential amino acids.

6.0 MEAT AND FISH

Meat: Meat is a good source of proteins. The protein contents of meat from cattle, calf, sheep and goat vary from 17-20 per cent while that from swine 11-15 per cent. Meat is a good source of several B-vitamins. It is a good source of iron and phosphorus but a poor source of calcium. The proteins of meat are good sources of all essential amino acids. The PER of the proteins of meat ranges from 2.7-3.3 and the NPU from 65-70. Meat proteins, being rich in lysine supplement effectively cereal proteins. Data regarding the chemical composition of meat is given in Table 1A in the Appendix.

Liver: Liver is a good source of proteins (17-20 per cent), vitamin A, riboflavin, folic acid and vitamin B_{12}. It is the richest natural source of vitamin B_{12}. Liver is a good source of iron and phosphorus but a poor source of calcium.

Fish: Fish is a good source of proteins containing from 17-20 per cent. It is a fair source of certain B-vitamins. Fish proteins are good sources of all essential amino acids. The PER of the proteins ranges from 2.7-3.3 and NPU, from 65-70. Fish proteins being rich in lysine and threonine, supplement effectively cereal proteins. Large fish are good sources of iron and phosphorus but poor sources of calcium. Small fish eaten along with bones is a rich source of calcium. Fish protein concentrate (fish

flour) is a very rich source of proteins (containing about 70-80 per cent) and of calcium.

7.0 VEGETABLES

Vegetables can be broadly classified under the following three heads: (*i*) green leafy vegetables (*ii*) roots and tubers and (*iii*) other vegetables. Data regarding the chemical composition of different vegetables are given in Table 1A in the Appendix. The range of values is given in Table 13.11.

Table **13.11 The nutritive value of vegetables and fruits (Range of values per 100g)**

Nutrient	Green leafy vegetables	Roots and tubers	Other vegetables	Fruits
Moisture (g)	79—92	59—94	72—96	75—90
Calories (KCal)	32—96	20—160	14—109	10—80
Carbohydrates (g)	4—14	4—38	4—20	2—20
Protein (g)	1.9—6.7	0.7—3.0	0.4—7.0	0.2—2.0
Fat (g)	0.1—1.7	0.1—0.3	0.1—0.4	0—1.0
Calcium (mg)	30—500	10—50	10—130	5—40
Iron (mg)	0.8—16.0	0.4—2.1	0.5—5.8	0.1—1.0
Carotene (μg)	1200—7500	30—3000	5—200	5—500
Ascorbic acid (mg)	43—220	3—24	2—66	2—300
Thiamine (mg)	0.05—0.06	0.05—0.10	0.04—0.25	0.05—0.2
Riboflavin (mg)	0.11—0.14	0.01—0.07	0.01—0.08	0.02—0.1
Nicotinic acid (mg)	0.4—0.8	0.3—1.2	0.2—0.9	0.2—1.0
Folic acid (μg)	10—30	3—6	5—10	6—9
Potassium (mg)	250—570	200—370	200—400	86—250
Sodium (mg)	4—91	18—60	10—48	5—15

Green leafy vegetables: Green leafy vegetables are fair sources of proteins containing about 2-7 per cent. They are rich sources of provitamin A (carotenes) and ascorbic acid and good sources of folic acid and calcium. Some of the leafy vegetables may contain oxalic acid which may interfere in the absorption of calcium present in the diet. Green leafy vegetables form excellent supplements to poor cereal diets.

Roots and tubers: Roots and tubers are, in general, good sources of starch. They are poor to fair sources of proteins,

B-vitamins and ascorbic acid. The most important roots and tubers are potato, sweet potato, carrots, tapioca (cassava) and colocasia.

Potato: Potato is a fair source of proteins, ascorbic acid and B-vitamins. It can be used as a complete or partial substitute for cereals in the diet.

Sweet potato: Sweet potato is a poor source of proteins but a fair source of ascorbic acid and B-vitamins. The yellow flesh variety is a rich source of provitamin (carotene). Sweet potato can be used as a partial substitute for cereals to the extent of about 25 per cent.

Carrots: Carrots are rich sources of provitamin A (carotene) and carbohydrates. They are poor sources of proteins and B-vitamins.

Tapioca (Cassava): Tapioca is a poor source of proteins. In Africa where tapioca is grown and consumed extensively, protein-caloric malnutrition is widely prevalent among preschool children.

Taro (Colocasia) (Colocasia esculentum): This is widely cultivated in the Pacific islands and in parts of Africa and Asia. It is a poor source of proteins. It contains large amounts of non-available carbohydrates.

Yams: Yams are poor sources of proteins and contain large amounts of indigestible carbohydrates.

Other vegetables

The other vegetables may be broadly grouped under the following heads: (*i*) green peas and beans (*ii*) gourds and pumpkin and (*iii*) ladies finger, brinjal etc.

Green peas and beans are good sources of proteins and B-vitamins. Gourds and pumpkins, ladies finger and brinjal are fair sources of ascorbic acid and minerals. Green peas and yellow pumpkin are fair sources of carotene.

8.0 FRUITS

Fruits contain different sugars, organic acids and are fair to good sources of ascorbic acid. A few of them are fair sources of carotene. Most of the fruits are poor to fair sources of B-vitamins.

Fruits are good sources of potassium and pectin. In view of the pleasant taste and flavour, fruit juices help to stimulate the appetite. Data regarding the chemical composition of fruits are given in Table 1A in the Appendix and summarised in Table 13.11 and briefly discussed below:

Soluble carbohydrates: Fruits contain glucose, fructose and sucrose. The carbohydrate content varies from 2-20 g/100 g. The calorific values of fruits also vary widely from about 10-80 KCal/100 g.

Ascorbic acid: The ascorbic acid content varies widely from 2.0-300 mg/100g. Citrus fruits are good sources (60-80 mg/100 g) while guava and Indian gooseberry are very rich sources (300 mg. and 700 mg/100 g. respectively). Apple and banana are poor sources.

B-vitamins: Fruits are poor sources of most of the B-vitamins. They are fair sources of folic acid (6-9 μg/100 g.)

Carotene: Fruits, in general, are poor sources of carotene, the exceptions being mango (3500 μg), Papaya (1500 μg), Orange (250 μg), Tomato (220 μg) and Jack fruit (450 μg) per 100 g.

Pectin: Most of the fruits contain small amounts of pectin. Some e.g., guava, apple, contain appreciable amounts of pectin.

Potassium: Fruits are good sources of potassium. (250-570 mg/100 g.).

9.0 FATS AND PROCESSED FAT PRODUCTS

Fats and fat products serve mainly as a source of energy. They are also important sources of essential fatty acids. Some are also good sources of vitamin A and vitamin E. The essential fatty acid contents are given in Table 3.5 and 3.6 in Chapter 3. The nutritive value of some groups of fats is summarised in Table 13.12 and briefly discussed below:

Fats of animal origin: They are poor sources of vitamin E and essential fatty acids. They also contain cholesterol.

Fats of vegetable origin: Vegetable fats (with the exception of coconut oil) are good sources of essential fatty acids. Sunflower, safflower, sesame, soybean and cottonseed oils are rich sources of essential fatty acids. They are also good sources of vitamin E.

Table **13.12 Nutritive value of fats**

Name of fat	Moisture %	Fat %	Calorific value KCal/100g	Vitamin A value (I.U./ 100g)	Vitamin E (mg/ 100g)
Fats of animal origin					
Butter	14	86	774	700	2.4
Ghee (clarified butter fat)	...	100	900	600	2.0
Animal body fats (lard, suet and beef dripping)	...	100	900	...	1.0—2.0
Fats of vegetable origin:					
Vegetable oils and fats	...	100	900	...	8—140
Red palm oil	...	100	900	4000—10,000	10—15
Processed fats:					
Margarine	14	86	774	700	10—50
Shortenings	...	100	900	...	10—50
Vanaspathi	...	100	900	700	10—30

10.0 STARCHY FOODS, SUGARS AND SYRUPS

Food products based on starch and sugar are widely consumed. They contain mainly carbohydrates and serve only as a source of energy. The carbohydrate content and calorific value of some starchy foods and syrups are given in Table 13.13.

Table **13.13 Carbohydrate content and calorific value of products based on starch and sugar**

	Moisture %	Carbohydrates %	Calories KCal/100g
Starchy foods :			
Custard powder, arrowroot flour and sago	12—14	86—88	344—352
Syrups	30—35	65—70	260—280
Honey	20—25	75—80	300—320

Starchy foods

The common starchy food preparations are custard powder, arrow root flour and sago.

Custard powder: This consists usually of corn starch containing edible colour and flavour.

Arrow root flour: This consists only of arrow root starch. It is used as an invalid food along with milk.

Sago: Sago globules are prepared from cassava starch or sago palm starch. It has the same nutritive value as starch.

Syrups: Syrups contain sugars, edible flavour and colour.

Honey: Honey contains sugars and traces of minerals and vitamins.

11.0 BEVERAGES

A large variety of beverages are consumed by human beings all over the world. These may be broadly grouped under the following heads: (1) Coffee, tea and cocoa, (2) Fruit juices and squashes, (3) Miscellaneous beverages such as coconut water, *Neera*, sweetened carbonated drinks, etc. and (4) Alcoholic drinks. A brief account of the different beverages is given below:

Coffee, Tea and Cocoa

Coffee, tea and cocoa are widely used as beverages. They are invigorating drinks and remove a sense of exhaustion, due to the stimulating action of substances like *caffeine* or *theobromine* present in them.

Coffee

The stimulating effect of coffee is due mostly to its content of *caffeine*. Coffee also contains some essential oils which are pleasing to the taste. *Caffeine* stimulates gastric secretion and hence may aid digestion to a certain extent. It has a delaying action on the emptying time of the stomach and hence it allays a sense of hunger.

Nutritive value: The chemical composition of a cup of coffee containing 6 ozs. of decoction, 2 ozs. of milk and 15 grams of sugar is given in Table 13.14.

Effects of excessive consumption: Excessive consumption of coffee is likely to be harmful, due to high intake of *caffeine*. *Caffeine* in excess produces insomnia and causes irritability and

rapid heart action. It also causes increased excitability of the nervous system, particularly in the very young and the old.

Tea

The stimulating effect of tea also is due to its *caffeine* content.

Nutritive value: Since very little milk goes into the making of a cup of tea, its nutritive value is far less than that of coffee. The nutritive value of one cup of tea containing 7 ozs. of decoction, 1 oz. of milk and 15 grams of sugar is given in Table 13.14.

Table **13.14 The chemical composition of coffee, tea and cocoa**
(Values per cup)

	Coffee	Tea	Cocoa
Protein (g)	1.8	0.9	7.2
Fat (g)	2.2	1.1	8.8
Carbohydrates (g)	17.8	16.4	26.2
Calcium (g)	0.068	0.034	0.27
Phosphorus (g)	0.050	0.025	0.20
Vitamin A (I.U.)	102	51	408
Calories (KCal)	98	79	213

Excessive consumption of tea has the same effects as excessive consumption of coffee. Since, it is a rich source of oxalic acid, excessive consumption of tea may lead to the development of renal calculi.

Cocoa

Cocoa contains a substance called *theobromine* which has stimulating properties like *caffeine*. Cocoa by itself has a higher protein content than coffee or tea (without milk). Cocoa gives a pleasant flavour to milk. Since more milk goes into the making of a cup of cocoa than of coffee or tea, a cup of cocoa has a higher nutritive value than a cup of coffee or tea. The chemical composition of one cup of cocoa containing 8 ozs. of milk, 6 g. of cocoa and 15 g. of sugar is given in Table 13.14.

Taken in moderation, these beverages act as mild stimulants and add to the nutritive value of the diet, because of the milk and sugar taken along with them.

Fruit Beverages

The beverages based on fruits include fruit juices, squashes and cordials.

Fruit juices: The fresh juices extracted from the various fruits contain mainly sugars and small quantities of vitamins and minerals. The acid taste is due to the presence of organic acids. The chemical composition of some fruit juices is given in Table 13.15.

Fruit juice squashes: Squashes are prepared by the addition of sugar, organic acids and preservatives to freshly expressed fruit juices. Squashes are usually diluted with water before consumption (Table 13.15).

Fruit juice cordials: Fruit juice cordials differ from the fruit juice squashes in that the suspended fruit pulp has been removed. They are usually diluted with water before consumption.

Miscellaneous Beverages

Coconut water: The water present in tender coconut is a popular drink all over India. It contains about 6 per cent sugars and small quantities of vitamins and minerals (Table 13.15).

Sweet toddy (Neera): The freshly drawn up sap of different palms usually preserved by the addition of calcium hydroxide is a popular drink in some parts of India. It contains about 14 per cent sugars and small quantities of vitamins and minerals (Table 13.15).

Sugarcane juice: Freshly expressed sugarcane juice with added lemon juice is commonly consumed in some parts of India. It contains about 9 per cent sugar and small amounts of vitamins and minerals (Table 13.15).

Coconut milk: Coconut milk is a milk-like emulsion expressed from freshly grated mature coconut kernel with the addition of 6 times the amount of water. It is rich in fat (7.2 per cent) and contains small amounts of proteins (0.8 per cent), vitamins and minerals and fair amounts of carbohydrates (2 per cent) (Table 13.15).

Sweetened carbonated beverages: The sweetened carbonated beverages contain about 10-15 per cent sugar.

Table **13.15 The nutritive value of some beverages** (values per 100g.)

Name of beverage	Water (g)	Fat (g)	Total carbo-hydrates (g)	Calcium (mg)	Iron (mg)	Carotene (Pro-vitamin A) (μg)	Thiamine (mg)	Riboflavin (mg)	Vitamin C (mg)	Calorific value (KCal)
Fruit juice										
Apple juice ...	85.9	...	13.8	6	0.5	4	0.02	0.02	1	55
Grape juice ...	85.3	...	13.7	8	0.3	20	0.02	0.02	3	55
Mango juice ...	84.2	...	14.6	12	0.5	1500	0.03	0.02	49	44
Orange juice ...	87.5	...	11.0	19	0.2	190	0.03	0.02	49	44
Pine apple juice ...	86.2	...	13.0	15	0.5	80	0.03	0.02	19	52
Tomato juice ...	93.5	...	4.3	7	0.4	1050	0.03	0.02	16	17
Dilute squash										
Orange squash ...	83.2	...	15.2	5	0.2	20	0.01	0.01	6	61
Lemon squash ...	83.4	...	15.0	7	0.2	...	0.01	0.01	5	60
Pine apple squash ...	82.9	...	15.2	6	0.2	50	0.01	0.01	5	61
Mango squash ...	82.9	...	15.6	8	0.2	500	0.01	0.01	6	62
Miscellaneous beverages										
Coconut water ...	93.9	...	5.9	10	0.1	...	0.01	0.01	2	24
Coconut milk ...	89.8	7.2	2.0	20	0.2	...	0.02	0.01	1	76
Neera (sweet toddy) ...	84.7	...	14.3	40	1.0	...	0.02	0.02	13	57
Soft drinks, carbonated (cold and other sweet drinks) ...	88.0	...	12.0	...	...	...	...	...	...	48
Sugarcane juice ...	90.2	...	9.1	10	1.1	...	0.02	0.02	5	36

Flavoured syrups (Sharbat): These consist of sugar syrup flavoured with artificial flavouring agents. They are usually diluted with water before consumption.

Alcoholic Beverages

A large variety of alcoholic beverages are consumed by human beings in many countries. They contain varying amounts of alcohol. Alcohol in moderate doses stimulates gastric secretion and is absorbed and serves as a source of energy. Alcohol is a depressent of the nervous system and excessive amounts of alcohol causes severe brain depression and incoordination of movements and loss of consciousness. The alcohol content of some beverages is given below:

Toddy and beer contain appreciable amounts of B-vitamins.

Alcohol content of alcoholic beverages

	Alcohol (%)
Beers	3.5-5.0
Toddy	3.5-7.0
Wines	10-16
Wines fortified with spirit	17-23
Spirits	
(Brandy, gin, rum and whisky)	35-45

12.0 SPICES AND CONDIMENTS

Spices and condiments are used extensively in many countries as flavouring agents in food preparations. They contain essential oils and other active principles. Data regarding the chemical composition of different spices and condiments are given in Table 1A in the Appendix. The condiments, in general, contribute very little food value to the diet as they are consumed in small amounts. Their importance lies in the fact that they improve the flavour and acceptability of cooked food preparations and make them more appetising. The value of condiments may be broadly considered under the following heads: (1) Improvement of taste and flavour (2) Effects on appetite and digestion (3) Effect on intestinal flora and (4) Harmful effects of excessive amounts of spices.

Improvement of taste and flavour: Condiments and spices such as chillies, pepper, turmeric, coriander seeds etc., are used in

curry powders. Tamarind soups containing curry powders are used for flavouring cooked rice before consumption in Asian countries. Spices are also used to modify the flavour of vegetable, meat and fish preparations.

Effect on appetite and digestion: Spices and condiments improve the flavour and acceptability of different food preparations. The active principles present in spices and condiments act on the mucous membranes of mouth, stomach and intestines and stimulate the secretion of digestive juices.

Effect on intestinal flora: Studies carried out at C.F.T.R.I., Mysore have shown that incorporation of certain spices e.g., garlic and asafoetida in the diet helps to reduce the total count of intestinal flora and prevent flatulence.

Harmful effects of excessive amounts of spices: The active principles of some spices e.g., chillies, black pepper, asafoetida possess strong pungency. Use of excessive amounts of these spices causes irritation of the mucous membranes and may be one of the contributory factors in the causation of peptic and duodenal ulcers among people.

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CHAPTER 14

EFFECT OF COOK NG AND HEAT PROCESSING ON THE NUTRITIVE VALUE OF FOODS

The object of processing and cooking is threefold (*i*) to improve their digestibility and appearance (*ii*) to develop new flavours and (*iii*) to destroy harmful microorganisms present. The important processing and cooking methods are the following: (*i*) Boiling in water (*ii*) Steaming (*iii*) Baking (*iv*) Roasting (*v*) Frying (*vi*) Canning and (*vii*) Dehydration.

Effect of cooking on various nutrients

Carbohydrates: Cooking is essential for the proper digestion of starch which is the main source of calories in the diets of a large majority of the population in the developing countries. Cooking also helps the breaking of cell walls in vegetables and thus facilitate digestion of protein present in them. When heat is applied to moist starch, the starch granules swell and burst and the starch gets gelatinised. Cooked starch is digested more readily than raw starch.

Fat: Cooking, under ordinary household conditions, has very little effect on fats; but during prolonged heating as in frying for long periods, a part of the essential fatty acids present is destroyed and toxic polymerised products are formed. Fats heated for long periods depress the growth of rats, thus indicating the presence of deleterious substances in them.

Proteins: Application of moderate moist heat to proteins causes coagulation and shrinking. Moderately cooked proteins are more easily digested than raw proteins. But severe heat processing such as roasting, baking, frying has been reported to effect adversely the nutritive value of the proteins of certain cereals, oilseeds and animal foods.

The adverse effect is due to the reaction of the end amino group of the essential amino acid-lysine-in the intact protein with reducing sugars present in them. This reaction is known as 'Maillard reaction'. In addition to lysine, certain other amino

acids e.g., arginine, tryptophan and histidine may also react with reducing sugars when the heat processing is drastic. The availability of lysine and other amino acids from the heat processed proteins are less than the unprocessed proteins due to the 'Maillard reaction'. On the other hand, the nutritive value of the proteins of legumes is improved to a considerable extent as a result of heat processing. Legumes contain trypsin inhibitors, haemagglutinins and growth inhibitors which affect adversely the utilisation of proteins and hence the growth of experimental animals. Most of the inhibitors are inactivated by optimal heat processing.

Vitamins

Vitamin A and Carotene: As these are insoluble in water, no loss occurs by discarding cooking water. There is slight destruction of vitamin A and carotene during cooking in water due to oxidation by air. Frying or roasting of vegetables causes considerable losses of vitamin A and carotene.

Thiamine: Loss of thiamine during cooking occurs in two ways (*i*) its destruction by heat during cooking and (*ii*) its dissolution in the cooking water. Hence, the quantity of thiamine loss during cooking varies depending on the method of cooking. Discarding the cooking water accounts for a loss of nearly 20-50 per cent depending on the quantity of water used in cooking. If sodium bicarbonate is added to dhal during cooking, most of the thiamine is destroyed. Hence, cooking soda should not be added to dhals for improving their cooking quality. The loss of thiamine during cooking under pressure is greater than that occurring during steaming.

Riboflavin: Loss of riboflavin during cooking occurs in four ways. (*i*) exposure of the food during cooking to strong light (*ii*) loss of riboflavin due to heat (*iii*) loss of riboflavin due to leaching by discarding excess of cooking water and (*iv*) loss of riboflavin due to addition of cooking soda during cooking of dhal and vegetables. Bottled milk exposed to strong sunlight loses a part of riboflavin present.

Nicotinic acid: Loss of nicotinic acid occurs by leaching of the vitamin when excess cooking water is discarded.

Pantothenic acid: Loss of pantothenic acid during cooking occurs in two ways (*i*) by leaching of the vitamin when excess cooking water is discarded and (*ii*) by heat during toasting and frying.

Pyridoxine: Loss of pyridoxine during cooking occurs in two ways: (*i*) by destruction due to excessive heat as in cooking under pressure (14 lbs) and (*ii*) by leaching of the vitamin when excess cooking water is discarded.

Folic acid and vitamin B_{12}: Losses of folic acid and vitamin B_{12} during cooking occur in two ways (*i*) destruction by heat as in pressure cooking, toasting or frying and (*ii*) by leaching when excess cooking water is discarded.

Ascorbic acid: Loss of ascorbic acid during cooking occurs in two ways (*i*) by oxidation due to exposure to air during cooking and (*ii*) by leaching in water when excess cooking water is discarded. Contamination with copper also accelerates the rate of destruction of the vitamin. The quantity of ascorbic acid lost during cooking of vegetables may vary widely from 10 to 60 per cent depending on the vegetable cooked and the method of cooking.

MINERALS

Calcium and phosphorus: Losses of calcium and phosphorus during cooking occur when excess of cooking water is discarded. When rice and vegetables are cooked in hard water, appreciable amounts of calcium present in the water are incorporated in them.

Iron: Loss of iron during cooking can occur if excess of cooking water is discarded. When vegetables are cut with iron knives, appreciable amounts of iron are incorporated in the vegetables. When foods are roasted in cast iron pans, appreciable amounts of iron are incorporated in them.

Sodium, potassium and magnesium: Losses of sodium, potassium and magnesium occur by leaching, when excess of water used in cooking is discarded. Sodium chloride used in cooking increases the sodium content of cooked foods.

Trace elements: Losses of trace elements occur by leaching when excess of water used in cooking is discarded.

CEREALS

Cereals are cooked or heat processed before consumption. The effects of cooking on the nutritive value of rice and of baking on the nutritive value of wheat are discussed below. Cereals are also processed into breakfast cereals and consumed widely. The effect of processing on the nutritive value of breakfast cereals is also discussed briefly.

Rice

Rice is generally washed and then cooked in excess of water. The gruel present in cooked rice is usually drained off. These procedures cause marked losses of vitamin B_1, nicotinic acid and other B-vitamins in the case of home-pounded and milled raw rice. The losses of B-vitamins are very much less in the case of home-pounded and milled parboiled rice. The results are given in Table 14.1.

Table **14.1 Effect of washing and cooking on vitamin B_1 and nicotinic acid contents of raw and parboiled rice**
(Values per 100 g. original rice)

Treatment	Parboiled home-pounded rice		Parboiled milled rice		Raw home-pounded rice		Raw milled rice	
	Vit. B_1 (mg)	Nicotinic acid (mg)	Vit. B_1 (mg)	Nicotinic acid (mg)	Vit. B_1 (mg)	Nicotinic acid (mg)	Vit. B_1 (mg)	Nicotinic acid (mg)
Original rice	0.32	3.5	0.24	2.4	0.22	2.2	0.11	1.0
Washed rice	0.29	3.2	0.22	2.1	0.10	1.1	0.07	0.6
Washed and cooked* rice	0.16	1.9	0.13	1.5	0.06	0.5	0.04	0.3

*Gruel removed

It is evident that washed and cooked raw milled rice is a very poor source of vitamin B_1 and nicotinic acid while washed and cooked parboiled rice is a good source of the two vitamins.

Wheat

The effect of baking on the nutritive value of wheat is briefly discussed below:

Bread: Pale, medium and dark baked bread retained 82.1, 80.4 and 73.8 per cent of thiamine respectively. Similar data on dinner rolls were 89.5, 84.5 and 77.5 per cent and on pan rolls were 92.6, 91.5 and 87.8 per cent respectively.

Biscuits: If biscuits made from self-rising flour are baked under optimum conditions, about 15 per cent of the added thiamine is destroyed, if the pH of the baked product is 7.0 or lower. Addition of 0.25 per cent calcium acid phosphate to enriched self-rising flour will help to maintain the pH value at 7.0. If the pH is greater than 7.0, then the loss of thiamine is high.

Breakfast cereals: Breakfast cereals are prepared on a large scale in many countries from different cereals such as corn, wheat, rice, oats etc. They are extensively consumed as they do not require any cooking. The heat processing involved in their manufacture is, however, somewhat drastic and many of the B-vitamins are likely to be destroyed to a great extent. For this reason, these foods are commonly fortified with these vitamins. The heat processing also is likely to affect adversely the quality of the proteins. Data presented in Table 14.2 indicate that the proteins in some foods are damaged severely and do not promote any growth in albino rats.

Table **14.2 The protein efficiency ratio of some breakfast cereals***

Breakfast cereals	PER
Corn flakes	negative
Corn soya flakes	0.88
Wheat flakes	negative
Puffed wheat	negative
Grape nuts	negative
Quick oats	1.67

* B. Sure (1951) *Food Research*, 16, 163.

LEGUMES

Legumes are good sources of proteins and B-vitamins. They form excellent supplements to cereal diets. Many legumes suffer from the disadvantage in containing trypsin and growth inhibitors which affect adversely the nutritive value of the proteins. Fortunately, these inhibitors can be inactivated by suitable heat treatment. The results of studies on the different modes of processing on the nutritive value of legumes are given below:

Soaking and cooking: Soaking in water overnight is the first step in cooking whole legumes (with the seed coat intact). The soaked legumes are then cooked for 20-30 minutes by boiling in water before consumption. The losses of nutrients in the water will be small if the seed coat is intact.

The decorticated legumes are usually split and sold as dhals. The method of cooking dhals is similar to that of whole legumes. The losses of B-vitamins are appreciable if the dhal is cooked in excess of water and the cooked water is rejected.

Steaming or cooking under pressure: When legumes are soaked in minimum amount of water and cooked in steam under pressure, the growth and trypsin inhibitors present in them are destroyed and the nutritive value of the proteins is enhanced. The losses of some B-vitamins are appreciable when cooked in steam under pressure.

Germination: The fact that germinated or sprouted legumes will prevent and cure scurvy has been known since 18th century. On germination, the vitamin C content of legumes has been found to increase. Germinated legumes are fair sources of this vitamin. (Table 14.3).

Table **14.3 Vitamin C content of germinated legumes**

Period of germination	Vitamin C (mg/100 g).
Nil (Dry legumes)	Trace
After 24 hours germination	7—12
After 48 hours germination	11—18
After 72 hours germination	13—20

There is also some increase in the content of riboflavin and niacin, while there is no appreciable change in the thiamine content.

Toasting and puffing: Toasting improves the taste and acceptability of legumes. As a result of toasting, the trypsin and growth inhibitors present are destroyed, thus bringing about an improvement in the nutritive value of the proteins. Toasting at high temperatures for prolonged periods may affect adversely the nutritive value of the proteins as the available lysine content is likely to be reduced due to 'Maillard reaction'. Puffing does not affect appreciably the nutritive value, as the heat treatment is given only for a short period of 1-2 minutes.

OILSEEDS, NUTS AND THEIR MEALS

The effect of heat treatment on the nutritive value of oilseeds and nuts and of screw pressing on the nutritive value of the oilseed meals, is discussed below.

Peanut (Groundnut)

Roasted peanut: Roasting of peanut causes destruction of appreciable amounts of thiamine. The trypsin inhibitor present in it is destroyed, resulting in an improvement in the nutritive value of proteins.

Peanut butter: Since the peanut is roasted to a high degree for the preparation of peanut butter, the loss of thiamine and pantothenic acid may amount to 30-50 per cent.

Peanut meal: Peanut meal is a good source of B-vitamins and proteins. During screw pressing, very little vitamin present in it is destroyed. The quality of the proteins is not affected if the temperature of expelling is kept at the optimum level. The PER of the proteins of the meal ranges from 1.7-1.9. Autoclaving at 15 lbs pressure for 30 minutes brings about a significant reduction in the PER.

Cottonseed meal: Cottonseed contains a toxic factor known as gossypol. Optimal heat processing and screw pressing of the cottonseed kernel yields an edible meal containing proteins of medium nutritive value and low free gossypol content. The PER of the proteins of the meal ranges from 1.7-2.0. Autoclaving at 15 lbs pressure brings about a reduction in the protein efficiency ratio.

Sesame meal: Sesame is a good source of proteins. The seed coat imparts dark colour and bitter taste to the meal. Further, it contains large amounts of oxalates. In view of this, it is essential to remove the seed coat for the preparation of edible sesame meal. Sesame meal obtained from the dehulled seed by screw pressing contains proteins of moderate nutritive value (PER 1.7-1.8). Sesame proteins are deficient in lysine but are rich sources of sulphur amino acids. Steaming and autoclaving bring about a reduction in the nutritive value of the proteins.

Coconut meal: Coconut meal is an important additional source of proteins in many Asian countries, viz., Philippines, Indonesia, Malaysia, Ceylon and Kerala State in India. The edible meal obtained by screw pressing contains about 20 per cent proteins of fairly high nutritive value (PER 1.7-2.2) and fair amounts of B-vitamins. Autoclaving at 15 lbs pressure for 30 minutes brings about a lowering in the nutritive value of the proteins.

Soybean meal: Soybean is a rich source of proteins (35-40 per cent). The proteins of raw soybean possess a low PER (0.5-0.8) due to the presence of trypsin and growth inhibitors. Optimal heat treatment inactivates the inhibitors and brings about marked improvement in the PER of the proteins (1.9-2.2). The soybean meal is prepared by powdering the cake obtained by screw pressing. The optimally heat processed soybean meal is a rich source of proteins (48-50 per cent) of high nutritive value.

FATS AND OILS

Effect of heat and rancidity development

Application of heat as in frying, brings about destruction of a part of the vitamins A and E and essential fatty acids present in them. Thermally induced polymers are formed. In rancid fats, peroxides are formed. These will destroy a part of the vitamins A and E present in them.

Effect of heat: Numerous studies with oils and fats left after frying foods in household have shown that incorporation of these fats and oils in the diet at 10-20 per cent level promotes normal growth of rats and the tissues are normal. There are several studies to indicate that feeding of fats and oils used

repeatedly for frying of foods over long periods brings about a depression in growth and produces morphological changes in liver, intestines, etc., and may produce malignant lesions in the stomach and intestines.

Effect of rancid fats: Rancid fats may exert an adverse effect on the experimental animals in a number of ways (1) they may bring destruction of vitamins A and E and essential fatty acids, (2) they may decrease the food intake of animals and (3) the peroxides present may affect the intestinal flora and may have an irritating effect on the intestines.

MILK AND MILK PRODUCTS

Milk is processed in several ways for human consumption. The effects of various methods of processing on the nutritive value of milk and milk products are outlined below.

Pasteurization: During pasteurization, about 20-30 per cent of vitamin C is destroyed. The other vitamins are not affected to an appreciable extent.

Effect of exposure of bottled milk to sunlight: When bottled milk is exposed to sunlight or artificial light, there is considerable amount of destruction of riboflavin, vitamins A and C.

Sterilised milk: The relatively prolonged and drastic heat treatment brings about appreciable losses of several vitamins—thiamine, 30-40 per cent, vitamin C, 50-60 per cent, vitamin B_6, 50-60 per cent and vitamin B_{12}, 80-90 per cent. Both vitamin A and riboflavin are stable to heat, but they are slowly destroyed when the sterilised milk in bottles is kept exposed to sunlight or artificial light.

Evaporated milk: There is destruction of vitamin C, vitamin B_{12}, thiamine and vitamin B_6 to the extent of 50-80 per cent.

Sweetened condensed milk: The losses of vitamins in sweetened condensed milk are similar to those of evaporated milk.

Milk powder: During the preparation of milk powder, vitamin C is destroyed to the extent of 70-90 per cent. The losses in other vitamins are small (10-20 per cent). During storage of milk powder, however, there is progressive destruction of vitamin A.

EGG POWDER

Spray drying of eggs under normal commercial practices does not cause any appreciable loss of vitamin A, vitamin D, thiamine, riboflavin or pantothenic acid. Only vitamin A is slowly destroyed during storage, the loss of vitamin A at the end of 6 month's storage at 15°F, 70°F and 98°F being 40, 50 and 70 per cent respectively. The losses of thiamine were 0, 10 and 30 per cent respectively under the above conditions.

FISH PRODUCTS

The loss of vitamin A in canned fish is negligible. The losses of B-vitamins may range from 50-70 per cent. The loss of vitamin A in dried fish is appreciable (20-30 per cent). The losses of B-vitamins during dehydration are small (10-20 per cent).

MEAT PRODUCTS

The losses of B-vitamins during the curing of ham amount to about 20-40 per cent. The loss of thiamine is quite high (70-80 per cent) during canning of meat. The losses of other B-vitamins may range from 50-70 per cent. The losses of B-vitamins are small during dehydration of meat.

POULTRY

The losses of thiamine in canned poultry meat may range from 60-70 per cent and of other B-vitamins 40-50 per cent. In contrast to high sterilisation temperatures required for canned poultry, pre-cooked frozen poultry can be prepared under relatively mild processing conditions. The loss of thiamine is of the order of 20-40 per cent, and of other B-vitamins, 10-20 per cent.

VEGETABLES

Effects of different methods of cooking: Vegetables are cooked either in steam or by boiling in water. During the above processes a certain amount of vitamins present in them is lost. The available data indicate that when vegetables are cooked in boiling water (without rejecting the water), the losses of thiamine amount to 15-32 per cent, of riboflavin 9-20 per cent and of ascorbic acid, 23-45 per cent. When vegetables are cooked in steam, the

losses of thiamine range from 16-24 per cent, of riboflavin 9-14 per cent and of ascorbic acid 20-30 per cent. The results are given in Table 14.4.

Table **14.4 The losses of vitamins in some vegetables during cooking**

Name of vegetable	Method of cooking	(Losses %)		
		Thiamine	Riboflavin	Ascorbic acid
Beans, snap	ng in water	29	20	42
Cabbage	,,	32	18	45
Carrots	,,	20	10	30
Peas	,,	28	17	40
Potato, whole	,,	25	9	23
Spinach	,,	15	10	28
Sweet potato	,,	20	13	24
Beans, Lima	Steaming	22	14	32
Cabbage	,,	18	9	38
Carrots	,,	15	10	25
Peas	,,	24	11	30
Potato	,,	20	12	22
Spinach	,,	16	12	25
Sweet potato	,,	18	12	20

Dehydration: During dehydration, a greater part of the ascorbic acid present is destroyed. The losses of B-vitamins and carotene are low. The retention of carotenes in dehydrated vegetables range from 70-95 per cent. Thiamine is destroyed to a greater extent than riboflavin, niacin and pantothenic acid. The retention of ascorbic acid varies widely from 20-59 per cent.

The available data are given in Table 14.5.

FRUITS

Dehydrated fruits: The losses of vitamins during dehydration will depend on the method of drying and on the stability of the vitamin to heat and air. Carotene losses are quite high (50-70 per cent) during sun-drying of fruits. As in the case of vegetables,

Table **14.5 Average percentage retention of ascorbic acid thiamine, riboflavin, niacin and pantothenic acid in certain vegetables during dehydration**

Vegetable	Ascorbic acid	Thiamine	Riboflavin	Niacin	Pantothenic acid
		(Retention %)			
Snap beans	42	88	89	—	—
Cabbage	45	82	86	—	—
Carrots	38	59	92	93	94
Peas, green	52	90	87	—	—
Potato	42	77	95	94	95

sulphuring causes losses of thiamine to the extent of 50-60 per cent. Riboflavin losses are low. The effects of different methods of drying on the ascorbic acid content of peaches are given in Table 14.6.

Table **14.6 Loss of ascorbic acid during dehydration of peaches**

Treatment	(Ascorbic acid mg/100 g) Fresh fruit *(on dry basis)*	Dried fruit	Loss
Sulphured and sun dried	52	34	33
Blanched, sulphured and sun dried	68	39	42
Sulphured and dehydrated	64	52	13
Blanched, sulphured and dehydrated	60	52	13

Canned fruit juice: The loss of ascorbic acid during canning of fruit juices may range from 20-40 per cent. The losses of B-vitamins are low. Data for tomato juice are given in Table 14.7.

Canned fruits: Canned fruit products lose considerable quantities of ascorbic acid and thiamine as a result of processing and storage. Higher storage temperatures accelerate these losses. It has been reported that canned orange juice stored at 40°F for 18 months retained 93.2 per cent of ascorbic acid while at 76°F it retained only 59.8 per cent. Thiamine retention was 80 per cent at 70°F and only 30 per cent at 100°F. Ascorbic acid

Table **14.7 Retention of vitamins during canning of tomato juice**

Vitamins	Retention of vitamins (%)
Ascorbic acid	67
Thiamine	89
Riboflavin	97
Niacin	98
Carotene	67

retention to the extent of 80 to 92 per cent during the canning of grape fruits has been reported. Ascorbic acid lósses in amla (Indian gooseberry) juice during preparation and after storage for one year at room temperature (26-30°C) ranged from 40 to 50 per cent. It has been reported that canned cashew apple juice (containing 200 mg. ascorbic acid per 100 g) retained nearly 70 per cent ascorbic acid on storage for 8 months at room temperature. Numerous references are available in the literature giving the extent of losses of nutrients of important canned fruits and fruit pulps like mango, papaya, guava, etc. All these indicate that ascorbic acid is the most affected and carotene the least. Thiamine is only partially destroyed and is retained better in canned fruits. Losses of vitamins during canning are less than 10 per cent while during storage at higher temperatures, they become considerable. Ascorbic acid retentions after 12 months' storage at room temperature (30-37°C) have been reported to be 25 per cent, 16 per cent, 55 per cent and 76 per cent in canned mangoes, papaya, guava and orange respectively. Under similar storage conditions, the carotene retentions have been reported to be 85.6 and 100 per cent in mango and papaya respectively. Retention of other vitamins under the same storage conditions has been reported to be thiamine 21.0 and 27.8 per cent; riboflavin 99.0 and 100 per cent; niacin, 61.1 and 65.8 per cent in mango and papaya respectively.

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CHAPTER 15

LATHYRISM

Lathyrism is a crippling disease characterised by paralysis of the leg muscles occurring mostly in adults consuming large quantities of the seeds of *L-sativus* or other lathyrus species over long periods. In India, the disease occurs in Madhya Pradesh, Bihar, and Uttar Pradesh. The disease has also been reported to occur in Spain, Algeria and occasionally in certain parts of France and Italy where *lathyrus* peas are consumed by the people.

Signs and symptoms

Acton (1922) has described four stages of the development of the disease in human beings (Plate XIII.) The first stage is characterised by weakness of the lower limbs with spasticity of leg muscles so that movement at the ankle and knee joints are restricted and painful. In the second stage, flexion of the knee is more marked and there is a certain amount of inversion of foot with a tendency to walk on toes. In the third stage, the symptoms described above become more marked and the subject can walk only with the help of crutches or sticks. In the fourth stage, the knee becomes completely flexed and erect posture and walking becomes impossible. There is atrophy of the thigh and leg muscles.

Animal experiments with lathyrus species

Lathyrus sativus: Feeding experiments with *L-sativus* seeds with different animals have shown that the seeds produce toxic symptoms only in some species. A syndrome resembling human lathyrism has been produced in monkeys by feeding the mature seeds of *L-sativus* or an aqueous extract of the seeds. Fowls and pigeons have been reported to consume the seeds without any ill effect while ducks and guinea pigs, on the other hand, have been reported to develop toxic symptoms as a result of consuming the seed. Acton and Chopra (1922) isolated a

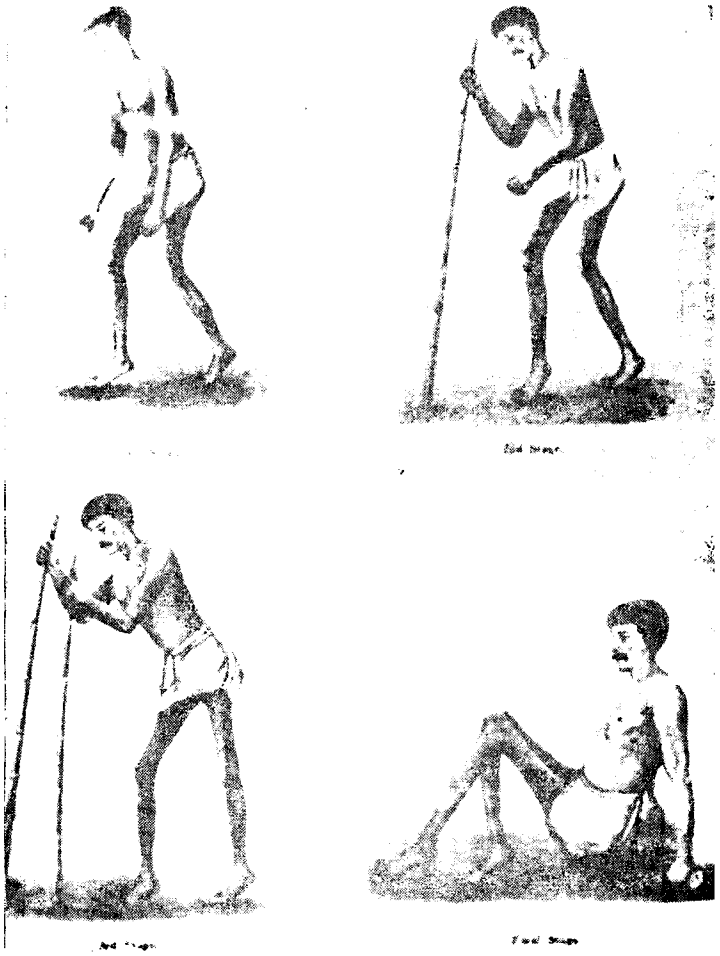


Plate XIII: Four stages of lathyrism

crystalline compound from *L-sativus* seeds which produced a transient spastic paralysis in guinea pigs. Horses have been reported to develop toxic symptoms when fed on *L-sativus* while albino rats are not susceptible to the disease. Acton (1922) made the important observation that feeding ducks on *L-sativus* caused paralysis and death in ducks; but if the pulse has been steeped in water for 24 hours and the soaked pulse fed to the birds, they thrive well on it. The above work indicated that the toxic factor was water soluble and could be removed by soaking in water.

Lathyrus odoratus: Geiger *et al* (1933) fed *L-odoratus* (the common flowering sweet pea) to rats. The animals developed toxic symptoms different from human lathyrism. The animals showed an extreme abnormality of the skeleton, including kyphosis, increased shaft diameter, exostoses of the long bone and generalised osteoporosis. This condition has been called *odoratism* or *osteolathyrism.* In addition, some animals developed hernia. *L-odoratus*, when fed to pregnant rats, caused the death of the foetus.

Lathyrus pusillus (Singletary pea): Lee *et al* (1956) showed that the ingestion of a diet containing 70 per cent singletary pea seed (*L. Pusillus*) led to the development of osteolathyrism in rats.

Lewis *et al* (1948) found that *L-hirsutus* and *L-tingitanus* also produced toxic symptoms in rats similar to those of *L-odoratus.*

L-Sylvestris Wagneri produced neurotoxic symptoms in rats. The toxic substance present in it appears to be different from that present in *L-odoratus.*

Identification of toxic factors in the lathyrus and the vicia species

It is evident from the above account that two kinds of lathyrism occur in animals and human beings viz. osteolathyrism (odoratism) where pathological changes occur in the bones resulting in skeletal deformities and neurolathyrism in which the muscles of the legs are paralysed sometimes resulting in convulsions and death in severe cases. Different species of *Lathyrus* cause one or the other type of disease though sometimes both types may be caused by the same species. The toxic principles isolated so far from some species of *lathyrus* or *vicia* are listed in Table 15.1.

Table 15.1 **Toxic factors in lathyrus and vicia species**

Lathyrus species		Chemical nature of toxic factors
L-odoratus	...	γ-glutamyl-β-amino propionitrile
L-latifolius and *L-sylvestris*	...	α,γ-diamino butyric acid
Lathyrus sativus	...	β-oxalyl amino alanine
Vicia sativa	...	β-cyanoalanine
V-angustifolia and *L-sylvestris*	...	γ-glutamyl-β-cyanoalanine

The osteolathyrogenic factor of the seeds of *L-odoratus*, grown in America and the Mediterranean countries was identified as γ-glutamyl-β-aminopropionitrile (I). It has since been found in other species of *Lathyrus* also. The neurolathyrogenic compounds of the seeds of the American species *L-latifolius* and *L-sylvestris* is α,γ-diaminobutyric acid (II). This basic amino acid was earlier known as a constituent of some antibiotics, e.g., circulin, comirin and the polymyxins; but its neurotoxic properties have not been noticed till recently. It is present in the free state in the seeds of other *Lathyrus* species and many other plants. The wide occurrence of this toxic amino acid emphasizes the need for a careful survey of food plants for the presence of this compound.

Lathyrus sativus seeds have been implicated for a long time as responsible for lathyrism in some parts of India. This disease is mainly of the neurological type. Ganapathy *et al* (1958) reported the presence of an unknown compound (R_f value 0.37 in n-butyl alcohol-acetic acid-water) which on hydrolysis with hydrochloric acid yielded another ninhydrin positive compound (R_f value 0.34 in n-buty lalcohol-acetic acid-water). It is likely this compound is identical with the toxic factor, β-oxalylamino-alanine isolated by later workers. The main neurotoxic substance was recently obtained in crystalline form and its structure was established as β-oxalylamino alanine (BOAA) (III). This compound causes nervous derangement in young chicks but rats and mice are not appreciably affected. This might be the reason for many of the earlier contradictory reports regarding the toxicity of the seeds of *L-sativus* when different test animals were employed. There is evidence to the presence of two neurotoxic factors in addition to BOAA. Kumagai *et al*, (1952) have re-

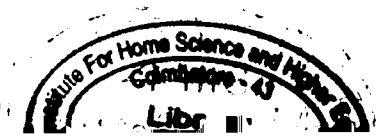
ported the isolation of a blood sugar lowering compound from the leaves and stems of *Lathyrus palustris var. macranthus*. They consider it to be a 'natural' form of oxalic acid. The seeds of *Vicia sativa* and *V. angustifolia* are closely similar to those of the *Lathyrus* species and previous reports attributed the poisonous effects of the latter to contamination with *Vicia* seeds. Ressler (1962) identified the neurotoxic compound in two species of *Vicia* seeds as β-cyanoalanine (IV); Recently γ-glutamyl-β-cyanoalanine (V) has been found to be present in *Vicia sativa* and *L-sylvestris*. Data regarding the content of toxic principles in certain varieties of *lathyrus* and *vicia* species are given in Table 15.2.

Table: **15.2 Classification and lathyrogenic contents of seeds**

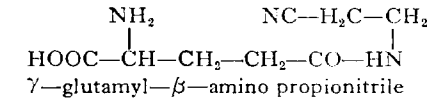
Seed	Content (%)	Seed	Content (%)
I. *Osteolathyrogens*		*α-γ-Diaminobutyric acid*	
β-Aminopropionitrile		L. aurantius	1
L. odoratus	0.05-0.16	L. cirrhosus	1
L. pusillus	0.062	L. gorgoni	1
L. hirsturus	0.021	L. grandflora	1
L. roseus	0.02	L. heterophyllus	1
		L. laevigatus	1
II. *Neurolathyrogens*		L. lut.us	1
β-Cyano-L-alanine		L. multiflora	1
L. sativa	0.15	L. rotundifolius	1
V. angustifolia	0.09-0.12	L. tuberosus	1
α-γ-Diaminobutyric acids		*β-N-Oxalyl-α-β-diaminopropionic acid*	
L. latifolius	0.51-0.67	L. sativus	0.1-2.5
L. sylvestris W.	1.4		

Mechanism of action of the toxic factors

The occurrence of neuro and osteotoxic substances in these legumes has stimulated interest in the mechanism of their action. The chief osteolathyrogenic principle is β-aminopropionitrile (BAPN) which has bone-deforming properties and causes connective tissue damage. Other amino acid nitriles also have similar action. The free amino group of BAPN is essential for physiological activity and its acyl derivatives are inactive unless they

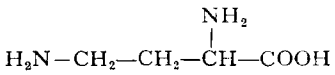


could be hydrolysed enzymatically *in vivo*. Though the exact mechanism of its action is uncertain, it appears that BAPN functions by interfering with collagen formation. Ganapathy *et al.* (1959) found that incorporation of β-amino propionitrile in the diet brought about a decrease in the bone ash content and in butyric acid and choline dehydrogenase activities of liver in albino rats. It has been suggested that the toxic nature of α,γ-diamino-butyric acid may arise from its capacity to act as an antimetabolite to its higher homologues, ornithine and lysine. Since β-aminoalanine also is a lower homologue of these amino acids, it could function as an antagonist to them. This compound has been reported to inhibit the growth of *Corynebacterium diphtheriae*. However, only β-oxalylamino alanine is neurotoxic to chicks and β-aminoalanine and oxalic acid are not. Recent studies using α,γ-diamino butyric acid 2-C^{14} in rats have shown that oxidation takes place at the α or the γ carbon atom with the formation of β-alanine, aspartic acid and carbon dioxide. This



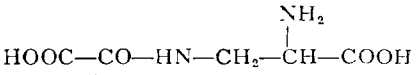
γ—glutamyl—β—amino propionitrile

I



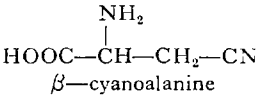
α,γ—di-amino butyric acid

II



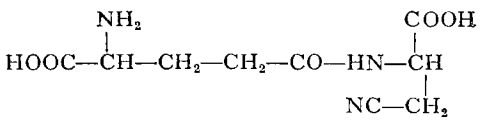
β—oxalyl amino alanine

III



β—cyanoalanine

IV



γ—glutamyl—β-cyanoalanine

V

may indicate a possible method of detoxification. No information is yet available about the mode of action of the neurolathyrogenic compounds present in the *Lathyrus* and the *Vicia* species.

However, it is important to note that, while BAPN and its γ-glutamyl derivatives are osteolathyrogenic, their carboxy derivatives, viz., β-cyanoalanine and γ-glutamyl-β-cyanoalanine have neurotoxic properties. It thus appears that the carboxyl group has an important role in determining the nature of the toxicity and one type may change into another by decarboxylation or carboxylation in plants. This may explain how both types of toxicity may occur together in some plants.

Prevention

There is now conclusive evidence that excessive consumption of *L-sativus* seeds is the cause of the high incidence of lathyrism in certain parts of India. Although the harmful effects of *L-sativus* have been realised for some years, effective steps for the prevention of the disease have not so far been taken by the government. There are three possible approaches for the eradication of the disease. (1) Treatment of the seed by leaching out the toxic factors in hot water both on home scale and factory scale (2) Education of the public on the dangers of consuming untreated *L-sativus* and (3) Cultivation of other pulses in place of *L-sativus*.

Removal of toxic factors by leaching in water: Acton (1922) was the first worker to point out that *L-sativus* seeds soaked in three changes of water are non-toxic. Later workers have confirmed the above observation that the toxic factors present in *L-sativus* could be leached out in water. On the basis of the results obtained by Nagarajan *et al.*, (1965) it is evident that the toxic factors can be removed by (1) cooking the seed or decuticled seed in excess of water and draining the water and (2) steeping the whole seed or decuticled seed in hot water (60-70°C) for 4-5 hours and rejecting the soak water. The latter process can be carried out on industrial scale and the resulting detoxified dhal could be sold to the people through ration shops and co-operative stores.

Education of the public: There is urgent need for educating the public on the dangers of consuming *L-sativus* seeds. This

should be done through leaflets distributed in schools, hospitals and M.C.H. centres pointing out the dangers of consuming the untreated *L-sativus* seeds and the need for the removal of the toxic factors from the seed and dhal by steeping in hot water and rejecting the soak water.

Cultivation of other pulses in place of L-sativus: The governments of different states where lathyrism is prevalent should, if necessary, by enactment of law, enforce the cultivation of other pulses commonly grown in the region in place of *L-sativus.* This will be the only effective and permanent solution to the problem of lathyrism.

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CHAPTER 16

RECOMMENDED DIETARY ALLOWANCES

For maintaining good health and physical efficiency, the diet should provide adequate amounts of all nutrients. For designing balanced diets, it is essential to know the daily requirements of different nutrients. Allowances for different nutrients have been recommended by various National Committees and some International Committees. The allowances recommended by the following Committees (*i*) Nutrition Expert Group, I.C.M.R. India (1968) (*ii*) Food and Nutrition Board, National Research Council, USA (1968) (*iii*) Panel on recommended allowances of nutrients, Department of Health and Social Services, U.K. (1969) and (*iv*) Recommendations of FAO/WHO Expert Groups on vitamins (1967), (1970) are given in Tables 16.1, 16.2, 16.3, 16.4 and 16.5 respectively.

Calories: In estimating the caloric requirements the following factors will have to be taken into account (1) Physical activity (2) Body size and composition (3) Age and sex (4) Physiological state and (5) Climate and environment. In view of the lower body size and warm climate prevalent in the tropical countries, the calorie requirements for the corresponding age groups are lower for persons living in India and other tropical countries as compared with those of persons living in temperate climates. This is evident from the recommended allowances given in Tables 16.1, 16.2 and 16.3.

Proteins: Proteins are required for maintenance, growth, pregnancy and lactation. Periodic infections and infestations widely prevalent among people living in the developing countries, decrease protein absorption and hence increase the protein requirements. Physiological stresses (e.g. pregnancy and lactation) also increase the protein requirements. Since the nutritive value of dietary proteins vary widely it is better to express the requirements in terms of reference protein which is assumed to possess an NPU of 100 and is fully digested and utilised. The reference protein requirements can be converted into proteins of customary

Table 16.1 **Daily Allowances of Nutrients for Indians (Recommended by the Nutrition Expert Group in 1968)**

Group Particulars	Net calories (Kcal)	Proteins (g)	Calcium (g)	Iron (mg)	Vitamin A: Retinol (μg) OR Carotene (μg)	Thiamine (mg)	Riboflavin (mg)	Nicotinic acid (mg)	Ascorbic acid (mg)	Folic acid (μg)	Vit. B_{12} (μg)	Vit. D (I.U.)
Man												
Sedentary work	2400	55[a]	0.4–0.5	20	750 3000	1.2	1.3	16	50	100	1	
Moderate work	2800					1.4	1.5	19				
Heavy work	3900[a]					2.0	2.2	26				
Woman												
Sedentary work	1900					1.0	1.0	13				
Moderate work	2200	45[a]	0.4–0.5	30[a]	750 3000	1.1	1.2	15	50	100	1	
Heavy work	3000					1.5	1.7	20				
Pregnancy (second half of pregnancy)	+300	+10[a]	1.0	40[b]	750 3000	+0.2	+0.2	+2	50	150–300[a]	1.5	
Lactation (upto 1 year)	+700	+ 20[a]		30[a]	1150 4600	+0.4	+0.4	+5	80	150		
Infants 0–6 months	120/kg	2.3–1.8/kg	0.5–06.[a]	1.0mg./kg	400 1600							200
7–12 ,,	100/kg	1.8–1.5/kg			300 1200				30	25	0.2	
Children												
1 year	1200	17	0.4–0.5	15–20	250 1000	0.6	0.7	8	30–50	55–100	0.5–1.0	
2 years		18										
3 years		20										
4– 6 years	1500	22			300 1200	0.8	0.8	10				
7– 9 years	1800	33			400 1600	0.9	1.0	10				
10–12 years	2100	41			600 2400	1.0	1.2	14				
Adolescents												
13–15 years Boys	2500	55	0.6–0.7	25	750 3000	1.3	1.4	17				
Girls	2200	50		35		1.1	1.2	14				
16–18 years Boys	3000	60	0.5–0.6	25	750 3000	1.5	1.7	21				
Girls	2200	50		35		1.1	1.2	14				

FOOTNOTES TO THE TABLE 16.1—DAILY ALLOWANCES OF NUTRIENTS FOR INDIANS

1. Calories

(*a*) Calorie allowance for heavy work does not include work under special conditions like high altitude.

2. Proteins

(*a*) Adult allowance corresponds to 1g/kg. of dietary protein of NPU 65.

(*b*) Infant allowance during 0-6 months is in terms of milk proteins. During 7-12 months, part of protein intake will be in the form of milk and supplementary feeding will be derived from vegetable proteins. Total daily protein allowance is calculated from the ideal weight. Protein allowance during infancy will be—

0-3 months	2.3 g/kg.
3-6 months	1.8 ,,
6-9 months	1.8 ,,
9-12 months	1.5 ,,

(*c*) Allowances for children and adolescents have been computed using body weights as obtained in well-nourished group and assuming NPU of 50-60 for the dietary proteins.

3. Calcium

In the absence of precise information on calcium requirement of different groups, a range of allowance has been suggested.

(*a*) Calcium allowance for infants 0-6 months will be for artificially-fed infants. Calcium intake from breast milk will however, satisfy the needs of breast-fed infants upto 6 months.

4. Iron

(*a*) This allowance of 30 mg. iron is for adult woman during her premenopausal period. For the post menopausal woman iron allowance is the same as for man.

(*b*) This allowance for pregnant woman will be throughout pregnancy.

(*c*) This allowance is for lactating woman who is not menstruating. If a woman is lactating and also menstruating, her iron allowance will be 35 mg/day.

5. Vitamin A

Dietary allowance for vitamin A is given in terms of retinol (vitamin A alcohol) and β-carotene. Either of these is used depending upon the dietary source of vitamin A. The factor to be used to convert β-carotene to retinol is: 1 μg β-carotene=0.25 μg of retinol (vitamin A).

If the diet contains both vitamin A and β-carotene, its content can be expressed as retinol using the following formulae:

(i) Retinol content μg=μg retinol+μg β-carotene$\times$0.25, if the retinol and β-carotene contents of foods are given as μg in food composition tables.

(ii) Retinol content (μg) = Vit. A (IU)×0.3+β-carotene (IU)×0.15 if vitamin A and carotene values are given in terms of I.U.

6, 7, 8. Thiamine, Riboflavin and Nicotinic acid

Daily allowances of these three vitamins are related to caloric intake, the basic allowances per 1000 calories are Thiamine—0.5 mg. Riboflavin—0.55 mg. and Niacin—6.6 mg. (60 mg. tryptophan being equal to 1 mg. of niacin.)

Niacin equivalents in a diet are computed as follows:

$$\text{Niacin equivalents (mg)} = \text{Niacin content (mg)} + \frac{\text{Tryptophan content (mg)}}{60}.$$

9. Folic acid

Dietary allowance of folic acid will be in terms of free folic acid (*L. Casei* activity) present in foods.

(i) Folic acid requirement appears to be considerably increased during pregnancy. Since the exact requirement is not known, a range rather than a single figure has been suggested for the daily allowance of folic acid during pregnancy.

10. Vitamin B_{12}

Vitamin B_{12} is derived entirely from foods of animal origin.

11. Vitamin D

Since the exact requirement of vitamin D is not known, an arbitrary allowance of 200 I.U./day is made. This allowance is in addition to some amounts of vitamin D that might be derived from exposure to sunlight.

12. Fat

Since human requirement of fat is not known, no specific allowance is recommended. A desirable range for fat in the diet is, however indicated. Diet should contain at least 15g. fat derived from vegetable oils like sesame, safflower or groundnut. It is also desirable that calories derived from fat in the daily diet should not exceed 30 per cent of total calories.

diets according to the following formula. Protein requirements as dietary proteins are calculated as follows:

$$\frac{\text{Reference protein requirements}}{\text{NPU of dietary proteins}} \times 100$$

Since the NPU values of proteins of Indian diets (45-55) are lower than those of American and British diets (60-70), protein requirements in terms of proteins of Indian diets should be greater than those in terms of proteins of American and British diets. The factors used for calculating the protein requirements by various Committees by the factorial method have varied.

(Chapter 4). The recommended allowances for proteins of various Committees are given in Tables 16.1, 16.2 and 16.3.

Fat: Fat provides the essential fatty acids such as linoleic, linolenic and arachidonic acids. Further, fats are essential for the absorption of fat-soluble vitamins like vitamin A, provitamin A (Carotene), etc. Phrynoderma, a nutritional deficiency disorder of the skin is attributed partly to the dietary deficiency of the essential fatty acids. Excess of fat consumption amounting to over 40 per cent of the dietary calories has been found to be associated with hypercholesterolemia and atherosclerosis. Present knowledge does not permit fixing with certainty the minimum fat requirements and the safe upper level of fat in the diet. The tentative recommendations of the ICMR Nutrition Expert Group (1968), are as follows: (*i*) Minimum daily fat requirements of 15g fat providing 5-10g essential fatty acids and (*ii*) upper safe limit of fat consumption equivalent to 30 per cent of total daily calorie requirements. The approximate minimum fat requirements for different age groups are given below:

Adults: 10 per cent of total calories in the diet from fat.

Children (1-11 years): 15 per cent of total calories in the diet from fat.

Adolescents: 15 per cent of total calories in the diet from fat.

Infants (birth to 1 yr): 30 per cent of total calories in the feed from fat.

Calcium: There is good agreement between the recommended allowances of calcium by FAO/WHO Expert Group (1962), the ICMR Nutrition Expert Group (1968) and the U.K. Expert Panel (1969). The recommendations of the Food and Nutrition Board, NRC, USA are higher than those of the U.K. Expert Panel.

Phosphorus: The requirements of phosphorus are closely related to those of calcium. It is generally agreed that phosphorus requirements are about 20 per cent higher than calcium requirements. A large part of the phosphorus present in cereals, pulses and nuts is in the form of phytin. Only a small part of phytin phosphorus is available to the human body. Further, phytin phosphorus interferes with the utilisation of dietary calcium and iron.

Table **16.2 Food and Nutrition Board, National Academy of Sciences —Allowances,**
Designed for the maintenance of good nutrition

Age year from upto	Weight		Height				Fat-Soluble Vitamins		
	(kg.)	(lbs)	(cm.)	(in)	Calories (Kcal)	Protein (gm)	Vitamin A activity (IU)	Vitamin D (IU)	Vitamin E activity (IU)
Infants									
0–1/6	4	9	55	22	kg×120	kg×2.2	1,500	400	5
1/6-1/2	7	15	63	25	kg×110	kg×2.0	1,500	400	5
1/2–1	9	20	72	28	kg×100	kg×1.8	1,500	400	5
Children									
1–2	12	26	81	32	1,100	25	2,000	400	10
2–3	14	31	91	36	1,250	25	2,000	400	10
3–4	16	35	100	39	1,400	30	2,500	400	10
4–6	19	42	110	43	1,600	30	2,500	400	10
6–8	23	51	121	48	2,000	35	3,500	400	15
8–10	28	62	131	52	2,200	40	3,500	400	15
Males									
10–12	35	77	140	55	2,500	45	4,500	400	20
12–14	43	95	151	59	2,700	50	5,000	400	20
14–18	59	130	170	67	3,000	60	5,000	400	25
18–22	67	147	175	69	2,800	60	5,000	400	30
22–35	70	154	175	69	2,800	65	5,000	—	30
35–55	70	154	173	68	2,600	65	5,000	—	30
55–75+	70	154	171	67	2,400	65	5,000	—	30
Females									
10–12	35	77	142	56	2,250	50	4,500	400	20
12–14	44	97	154	61	2,300	50	5,000	400	20
14–16	52	114	157	62	2,400	55	5,000	400	25
16–18	54	119	160	63	2,300	55	5,000	400	25
18–22	58	128	163	64	2,000	55	5,000	400	25
22–35	58	128	163	64	2,000	55	5,000	—	25
35–55	58	128	160	63	1,850	55	5,000	—	25
55–75+	58	128	157	62	1,700	55	5,000	—	25
Pregnancy					+200	65	6,000	400	30
Lactation					+1,000	75	8,000	400	30

National Research Council—Recommended Daily Dietary Revised 1968.
of practically all healthy people in U.S.A.

Water-Soluble Vitamins							Minerals				
Ascorbic acid (mg)	Folacin (mg)	Niacin (mg equiv)	Riboflavin (mg)	Thiamine (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (μg)	Calcium (g)	Phosphorus (g)	Iodine (μg)	Iron (mg)	Magnesium (mg)
35	0.05	5	0.4	0.2	0.2	1.0	0.4	0.2	25	6	40
35	0.05	7	0.5	0.4	0.3	1.5	0.5	0.4	40	10	60
35	0.1	8	0.6	0.5	0.4	2.0	0.6	0.5	45	15	70
40	0.1	8	0.6	0.6	0.5	2.0	0.7	0.7	55	15	100
40	0.2	8	0.7	0.6	0.6	2.5	0.8	0.8	60	15	150
40	0.2	9	0.8	0.7	0.7	3	0.8	0.8	70	10	200
40	0.2	11	0.9	0.8	0.9	4	0.8	0.8	80	10	200
40	0.2	13	1.1	1.0	1.0	4	0.9	0.9	100	10	250
40	0.3	15	1.2	1.1	1.2	5	1.0	1.0	100	10	250
40	0.4	17	1.3	1.3	1.4	5	1.2	1.2	125	10	300
45	0.4	18	1.4	1.4	1.6	5	1.4	1.4	135	18	350
55	0.4	20	1.5	1.5	1.8	5	1.4	1.4	150	18	400
60	0.4	18	1.6	1.4	2.0	5	0.8	0.8	140	10	400
60	0.4	18	1.7	1.4	2.0	5	0.8	0.8	140	10	350
60	0.4	17	1.7	1.3	2.0	5	0.8	0.8	125	10	350
60	0.4	14	1.7	1.2	2.0	6	0.8	0.8	110	10	350
40	0.4	15	1.3	1.1	1.4	5	1.2	1.2	110	18	300
45	0.4	15	1.4	1.2	1.6	5	1.3	1.3	115	18	350
50	0.4	16	1.4	1.2	1.8	5	1.3	1.3	120	18	350
50	0.4	15	1.5	1.2	2.0	5	1.3	1.3	115	18	350
55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	100	18	350
55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	100	18	300
55	0.4	13	1.5	1.0	2.0	5	0.8	0.8	90	18	300
55	0.4	13	1.5	1.0	2.0	6	0.8	0.8	80	10	300
60	0.8	15	1.8	+0.1	2.5	8	+0.4	+0.4	125	18	450
60	0.5	20	2.0	+0.5	2.5	6	+0.5	+0.5	150	18	450

Table **16.3 Recommended Daily Intakes of**

Age range	Occupational category	Body weight (kg)	Energy (Kcal)	MJ	Protein (g)	Thiamine (mg)
Children						
0 to 1 year		7.3	800	3.3	20	0.3
1 to 2 years		11.4	1200	5.0	30	0.5
2 to 3 years		13.5	1400	5.9	35	0.6
3 to 5 years		16.5	1600	6.7	40	0.6
5 to 7 years		20.5	1800	7.5	45	0.7
7 to 9 years		25.1	2100	8.8	53	0.8
Boys						
9 to 12 years		31.9	2500	10.5	63	1.0
12 to 15 years		45.5	2800	11.7	70	1.1
15 to 18 years		61.0	3000	12.6	75	1.2
Girls						
9 to 12 years		33.0	2300	9.6	58	0.9
12 to 15 years		48.6	2300	9.6	58	0.9
15 to 18 years		56.1	2300	9.6	58	0.9
Men						
18 to 35 years	Sedentary	65	2700	11.3	68	1.1
	Moderately active		3000	12.6	75	1.2
	Very active		3600	15.1	90	1.4
35 to 65 years	Sedentary	65	2600	10.9	65	1.0
	Moderately active		2900	12.1	73	1.2
	Very active		3600	15.1	90	1.4
65 to 75 years	Assuming a	63	2350	9.8	59	0.9
75 and over	sedentary life	63	2100	8.8	53	0.8
Women						
18 to 55 years	Most occupations	55	2200	9.2	55	0.9
	Very active		2500	10.5	63	1.0
55 to 75 years	Assuming a	53	2050	8.6	51	0.8
75 and over	sedentary life	53	1900	8.0	48	0.7
Pregnancy 2nd & 3rd trimesters			2400	10.0	60	1.0
Lactation			2700	11.3	68	1.1

[1] Report of the Panel on Recommended Allowances of Nutrients, *Reports on* London, 1970

Energy and Nutrients for the United Kingdom[1]

Riboflavin (mg)	Nicotinic acid (mg equivalents)	Ascorbic acid (mg)	Vitamin A (μg retinol equivalents)	Vitamin D (μg calciferol)	Calcium (mg)	Iron (mg)
0.4	5	15	450	10	600	6
0.6	7	20	300	10	500	7
0.7	8	20	300	10	500	7
0.8	9	20	300	10	500	8
0.9	10	20	300	2.5	500	8
1.0	11	20	400	2.5	500	10
1.2	14	25	575	2.5	700	13
1.4	16	25	725	2.5	700	14
1.7	19	30	750	2.5	600	15
1.2	13	25	575	2.5	700	13
1.4	16	25	725	2.5	700	14
1.4	16	30	750	2.5	600	15
1.7	18	30	750	2.5	500	10
1.7	18	30	750	2.5	500	10
1.7	18	30	750	2.5	500	10
1.7	18	30	750	2.5	500	10
1.7	18	30	750	2.5	500	10
1.7	18	30	750	2.5	500	10
1.7	18	30	750	2.5	500	10
1.7	18	30	750	2.5	500	10
1.3	15	30	750	2.5	500	12
1.3	15	30	750	2.5	500	12
1.3	15	30	750	2.5	500	10
1.3	15	30	750	2.5	500	10
1.6	18	60	750	10	1200	15
1.8	21	60	1200	10	1200	15

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Table **16.4 Intakes of Thiamine, Riboflavin, Niacin and Vitamin A recommended by the FAO/WHO Expert Group (1967)**

Age	Thiamine (mg.)	Riboflavin (mg.)	Niacin[1] equivalents (mg)	Vitamin A (retinol) (μg)
Infants				
0– 3 months[2]	—	—	—	—
3– 6 months[2]	—	—	—	—
7–12 months	0.4	0.6	6.6	300
Children				
1 year	0.5	0.6	7.6	250
2 years	0.5	0.7	8.6	250
3 years	0.6	0.8	9.6	250
4– 6 years	0.7	0.9	11.2	300
7– 9 years	0.8	1.2	13.9	400
10–12 years	1.0	1.4	16.5	575
Adolescents				
13–15 years: Boys	1.2	1.7	20.4	725
Girls	1.0	1.4	17.2	
19–16 years: Boys	1.4	2.0	23.8	750
Girls	1.0	1.3	15.8	
Adults				
Man	1.3	1.8	21.1	750
Woman	0.9	1.3	15.2	
Pregnancy	0.9	1.3	15.2	750
Lactation	1.3	1.9	21.8	1200

[1] A niacin equivalent is 1 mg niacin or 60mg L-tryptophan

[2] For children 0 to 6 months it is accepted that breast feeding by a well-nourished mother is the best way to satisfy the nutritional requirements for thiamine, riboflavin and niacin.

Iron: Iron requirements are computed mainly by the factorial method. Iron lost in urine, sweat and in menstruation is estimated. Iron requirements for growth are calculated from the iron increase in the body during growth. An average absorption of 10 per cent of food iron is assumed in computing iron requirements.

Table **16.5 Recommended daily intakes of Ascorbic acid, Vitamin D Vitamin B_{12} and Folate (FAO/WHO Expert Group 1970)**

	Ascorbic acid (mg)	Vitamin D[b] (μg)[c]	Vitamin B_{12} (μg)	Folate (μg)
Infants:				
0– 6 months[a]	20	10	0.3	40
7–12 ,,	20	10	0.3	60
Children:				
1– 3 years	20	10	0.9	100
4– 6 ,,	20	10	1.5	
7– 9 ,,	20	2.5	1.5	
10–12 ,,	20	2.5	2.0	
Boys / Girls 13–19 years	30	2.5	2.0	200
Adults:				
Men / Women	30	2.5	2.0	200
Pregnancy	50[d]	10[d]	3.0	400
Lactation	50	10	2.5	300

[a] It is accepted that for infants aged 0-6 months breast-feeding by a well-nourished mother is the best way to satisfy the requirements of ascorbic acid, vitamin B_{12} and folate, but not of vitamin D.

[b] Adequate exposure to sunlight may partially or totally replace dietary vitamin D.

[c] 2.5 μg of cholecalciferol are equivalent to 100 IU of vitamin D.

[d] For 2nd and 3rd trimesters.

Vitamin A: Vitamin A requirements are expressed in terms of retinol (vitamin A alcohol) or provitamin A (carotene). The ICMR Nutrition Expert Group (1968) recommended that 1 μg retinol equals 4 μg β-carotene while the U.K. Expert Panel (1969) and FAO/WHO Expert Group recommended that 1μg. retinol=6μg of β-carotene as the average absorption from diet was assumed to be 33 per cent. The recommended allowances of vitamin A and β-carotene of the different Committees are given in Tables 16.1 to 16.4.

Thiamine: Thiamine requirements are directly related to calorie requirements. The FAO/WHO Expert Group (1967)

Table 16.6 **Energy and Protein requirements (Joint FAO/WHO *Ad. hoc* Expert Committee 1973)**

Age	Body wt (kg)		Protein [1] (g/kg/day)		Energy requirements			
					Males		Females	
	M	F			(Kcal/kg/day)	(Kcal/day)	(Kcal/kg/day)	(Kcal/day)
Birth-3 months	—	—	2.40		120	—	120	—
3-5 ,,	—	—	1.85		115	—	115	—
6-8 ,,	—	—	1.62		110	—	110	—
9-11 ,,	—	—	1.44		105	—	105	—
1 year	11.4	11.1	1.27		103	1180	106	1180
2 years	13.6	13.4	1.19		100	1360	100	1350
3 ,,	15.6	15.4	1.12		100	1560	99	1520
4 ,,	17.4	17.5	1.06		99	1720	96	1670
5 ,,	20.7	20.0	1.01		91	1870	90	1790
6 ,,	23.2	22.4	0.98		87	2010	85	1900
7 ,,	23.9	25.0	0.92		83	2140	80	2010
8 ,,	28.6	27.6	0.87		79	2260	76	2110
9 ,,	31.3	30.4	0.85		76	2380	73	2210
			M	F				
10 ,,	33.9	33.8	0.82	0.81	74	2500	68	2300
11 ,,	36.7	37.7	0.81	0.76	71	2600	62	2350
12 ,,	40.2	42.4	0.78	0.74	67	2700	57	2400
13 ,,	45.5	47.0	0.77	0.68	61	2800	52	2450
14 ,,	51.7	50.3	0.72	0.62	56	2900	50	2500
15 ,,	56.6	52.3	0.67	0.59	53	3000	48	2500
16 ,,	60.3	53.6	0.64	0.58	51	3050	45	2420
17 ,,	62.4	54.2	0.61	0.57	50	3100	43	2340
18 ,,	63.7	54.6	—	—	49	3100	42	2270
19 ,,	65.0	55.0	—	—	47	3020	40	2200
Adult man	65.0	—	0.57	—	46	3000	—	—
Adult woman	—	55.0	—	0.52	—	—	40	2200
Pregnancy I quarter	—	—	—	+ 1[a]	—	—	—	+ 150
,, II ,,	—	—	—	+ 4[a]	—	—	—	+ 150
,, III ,,	—	—	—	+ 8[a]	—	—	—	+ 350
,, IV ,,	—	—	—	+ 9[a]	—	—	—	+ 350
Lactation (1-6 months)	—	—	—	+17[a]	—	—	—	+ 550

(a)= g/day (1) as egg or milk proteins

recommended an allowance of 0.4mg/1000Kcal while the ICMR Expert Group and Food and Nutrition Board, NRC, U.S.A. (1968) recommended 0.5 mg/1000 Kcal. The thiamine allowances recommended by various Committees are given in Tables 16.1 to 16.4.

Riboflavin: Riboflavin requirements are related directly to the calorie requirements. The FAO/WHO Expert Group (1967) recommended an allowance of 0.55mg/1000Kcal. The same figure has been adopted by ICMR Nutrition Expert Group (1968) and UK Expert Panel (1969). The recommended allowances of different Panels are given in Tables 16.1 to 16.4.

Nicotinic acid: Nicotinic acid requirements are related to the calorie requirements. The FAO/WHO Expert Group (1967) recommended an allowance of 6.6 mg of nicotinic acid equivalents per 1000 Kcal. This allowance has been accepted by the ICMR Nutrition Expert Group and UK Expert Panel. In calculating nicotinic acid equivalent 60mg of tryptophan present in proteins are assumed to be converted into 1 mg of niacin in the body.

$$\text{Niacin equivalents (mg)} = \text{Niacin content mg} + \frac{\text{Tryptophan in dietary protein (mg)}}{60}$$

The recommended allowances of different panels are given in Tables 16.1 to 16.4.

Folic acid: Folic acid exists in foods in two forms viz. (*i*) Free form as folic acid and tetrahydrofolic acid and its derivatives and (*ii*) Bound folic acid and folic acid conjugate (folic acid polyglutamate). The 'free' folic acid is readily absorbed while the folic acid conjugate must be degraded by enzymes to monoglutamate to permit absorption. Folic acid conjugate by itself is not absorbed. Hence, folic acid requirements are best expressed in terms of 'Free' folic acid. The recommended allowances of ICMR Expert Group, Food and Nutrition Board, N.R.C. U.S.A. and FAO/WHO Expert Group (1970) are given in Tables 16.1, 16.2 and 16.5 respectively.

Vitamin B_{12}: Vitamin B_{12} occurs only in foods of animal origin while foods of plant origin do not contain this vitamin. For the absorption of this vitamin, an intrinsic factor present in the gastric juice is essential. The recommended allowances for vitamin B_{12} of various Expert Panels are given in Tables 16.1, 16.2 and 16.5.

Vitamin B_6: Vitamin B_6 activity is possessed by three closely related compounds occurring in foods namely pyridoxine, pyridoxamine and pyridoxal. There is no information on the relative 'biological activity' of the three compounds in man but in rats they possess nearly the same biological activity. The recommended allowances of Food and Nutrition Board, NRC, U.S.A. (1968) are given in Table 16.2.

Ascorbic acid: There are two views on ascorbic acid requirements. One group (U. K. Expert Panel, 1969) maintains that the quantity of this vitamin required to prevent signs of deficiency, with a safety margin for individual variations and for stresses of everyday life, can be recommended as dietary requirements. Another group (Food and Nutrition Board, NRC, U.S.A. 1968) advocates that the ascorbic acid intake should be able to saturate the tissues. The recommended allowances of various Expert Groups are given in Tables 16.1, 16.3 and 16.5.

Vitamin E: Vitamin E is essential in human nutrition. Increased consumption of polyunsaturated fatty acids causes an increase in vitamin E requirements. The recommended allowances for vitamin E of NRC, U.S.A. are given in Table 16.2. One International Unit (I.U.) of vitamin E activity is possessed by 1 mg synthetic dl-α-tocopherol.

Vitamin D: Vitamin D is essential for the absorption of calcium and phosphorus and for the formation of bone and teeth. Body needs of vitamin D are met partly from the vitamin D formed in the body by exposure to sunlight in the tropical countries. The U.K. Expert Panel and Food and Nutrition Board, NRC, U.S.A. have recommended allowances of 400 I.U. per day while the ICMR Expert Group recommended an allowance of 200 I.U.

Other B-vitamins

An average well-balanced diet will provide adequate amounts of pantothenic acid, biotin, choline and inositol.

Other elements

Iodine: Data regarding the iodine requirements for various age groups recommended by Food and Nutrition Board, NRC, U.S.A. are given in Table 16.2.

Copper: Data regarding the exact copper requirements are not available. The Food and Nutrition Board, NRC, U.S.A. has estimated copper requirements as 1-2 mg per day depending on age.

Fluorine: Fluoride is present in traces in water supplies. Fluoride is an essential component in the structure of teeth and is necessary for the prevention of dental caries. Addition of fluoride to water supplies to raise the concentration to 1 ppm has been found to be effective in preventing the incidence of caries.

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CHAPTER 17

NUTRITION AND FEEDING OF NORMAL INFANTS

Milk is a product of evolution designed specifically for the nutrition of infant mammals. Milks of different mammals differ from each other in the proportions of different nutrients present. Human milk is universally regarded as the ideal food for the nutrition of human infants during the early months. Yet, owing to various causes an infant may not derive adequate supplies of human milk and feeding with animal milks has to be resorted to. Cow's milk is widely used in different countries for the feeding of infants. In India and certain other countries buffalo, goat and sheep milks are also being used to some extent for this purpose. During recent years, milk substitutes prepared from soybean have also been used successfully for the feeding of infants. For the satisfactory feeding of infants with animal milks or vegetable milk substitutes, a knowledge of the nutritional requirements and the digestive capacity of the infants is necessary. The use of processed milk foods in the feeding of infants is also on the increase in different parts of the world. This Chapter gives an outline of the present knowledge regarding the nutritional requirements of infants and the use of various types of milk and processed milk products and milk substitutes in the feeding of infants.

Nutritional requirements of infants

The nutritional requirements of artificially fed infants up to one year of age suggested by the Food and Nutrition Board, National Research Council (FNB. NRC) U.S.A. (1968) are given in Table 17.1. For infants, fed entirely on mother's milk, the requirements of all nutrients will be less than those indicated in Table 17.1. The recommended allowances of I.C.M.R. Expert Group (1968) and U.K. Expert Panel (1969) are given in Chapter 16. The requirements of the different nutrients are briefly discussed below:

Proteins: As the healthy new born infant will double its weight in 6 months and treble it in 12 months, the importance of

Table **17.1 Recommended daily dietary allowances for infants***

Nutrients	Birth to 2 months	3 months to 6 months	7 months to 12 months
Calories (Kcal)	120/kg	110/kg	100/kg
Proteins (g)	2.2/kg	2.0/kg	1.8/kg
Vitamin A (I.U.)	1500	1500	1500
Vitamin D (I.U.)	400	400	400
Vitamin E (I.U.)	5	5	5
Ascorbic acid (mg)	35	35	35
Folic acid (μg)	50	50	100
Niacin (mg)	5	7	8
Riboflavin (mg)	0.4	0.5	0.6
Thiamine (mg)	0.3	0.4	0.5
Vitamin B_6 (mg)	0.2	0.3	0.4
Vitamin B_{12} (μg)	1.0	1.5	2.0
Calcium (g)	0.4	0.5	0.6
Iron (mg)	6	10	15
Iodine (μg)	25	40	45

* Food and Nutrition Board, National Academy of Sciences, National Research Council, Washington D.C., U.S.A.

Table **17.2 Recommended daily dietary allowances for infants**

Nutrients	ICMR Nutrition Expert Group (1968)		U.K. Expert Panel (1969)
	Birth to 6 months	7 months to 12 months	Birth to 12 months
Calories (Kcal)	120/kg	100/kg	800
Proteins (g)	2.3–1.8/kg	1.8–1.5/kg	20
Vitamin A (μg) as Retinol	400	300	450
Vitamin D (I.U.)	200	200	400
Ascorbic acid (mg)	30	30	15
Folic acid (μg)	25	25	—
Niacin (mg)	—	—	5
Riboflavin (mg)	—	—	0.4
Thiamine (mg)	—	—	0.3
Vitamin B_{12} (μg)	0.2	0.2	—
Calcium (g)	0.5–0.6	0.5–0.6	0.6
Iron (mg)	1/kg	1/kg	6

adequate protein intake for promoting optimal growth is evident. The breast-fed infant normally receives from 1.5 to 2.0 g of proteins per kilogram body weight per day. This is slightly less than the recommended allowances given in Table 17.1 for infants fed on cow's milk. The limited evidence available would indicate that the proteins from cow's milk are utilised less efficiently than the proteins of human milk when compared on the same levels of protein intake and that feeding higher levels of proteins as those given in Table 17.1 will cause a higher retention of nitrogen than that observed with infants fed human milk. Adequate data, however, are not available on the performance of artificially fed infants receiving protein levels comparable to those obtained from breast milk. The average minimum essential amino acid requirements of infants, as determined by different workers, are given in Table 17.3. The daily amino acid requirements per kilogram body weight are provided by 1.6g of human milk proteins or 2.0g of cow's milk proteins.

Table **17.3 Average minimal daily essential amino acid requirements of infants and the quantities of milk protein required to provide the same**

Amino acid	Infant[1] (mg/kg)
Histidine	32
Isoleucine	90
Leucine	150
Lysine	105
Methionine	85,65
Phenylalanine	90
Threonine	60
Tryptophan	22
Valine	93
Protein required to meet amino acid needs	g/kg
Human milk	1.6
Cow's milk	2.0

[1] Data taken from the report "Evaluation of Protein Nutrition" *Pub.* 711-*Food and Nutrition Board,* National Research Council, National Academy of Sciences, U.S.A.

Fat: Fat provides in addition to energy, the essential fatty acids needed by the infants. It also serves as a vehicle for fat soluble vitamins. Weise *et al*, (1958) reported that for the normal nutrition of infants, 4 per cent of the calories have to be supplied by linoleic acid when fat provides about 30-40 per cent of the calories in the diet. Hansen *et al*, (1963) observed the development of a clinical syndrome of a skin rash (similar to eczema) in infants who received no essential fatty acids for several months. The same group have also investigated the effects of essential fatty acids on the growth of infants. They found that the growth and general health of the infants were satisfactory when linoleic acid supplied 1.3 to 7.3 per cent of the calorie intake. Linoleic acid comprises 4-5 per cent of the calories in human milk and only 1.5 to 2 per cent in cow's, buffalo and goat's milks. It may, therefore, be desirable to add vegetable fats rich in linoleic acid to infant foods based on animal milks in order to raise the linoleic acid content to the same level as in human milk fat.

Carbohydrates: The chief carbohydrate in milk is lactose. The physiological functions of lactose have been reviewed by Duncan (1955). Lactose helps in the absorption of calcium and phosphorus and in maintaining a normal intestinal microflora. In view of this, a fair part of the carbohydrates in the infant's diet should consist of lactose. Other carbohydrates commonly added to cow's milk in infant feeding are sucrose, malt-dextrin mixture, invert sugar syrups and dextrose. In the choice of carbohydrates the following points should be taken into consideration (1) the ease of digestion and absorption (2) the degree to which the carbohydrate is fermented in the intestinal tract (3) relative sweetness and (4) relative cost. Schults *et al*, (1938) reported that the increase in the fasting blood sugar after the ingestion of different carbohydrates was in the following decreasing order; glucose, dextri-maltose, honey, sucrose, fructose and lactose. Sucrose, because of its ready availability and low cost, is commonly added to milk for feeding infants. It has the advantage of being digested and absorbed more rapidly than lactose but less rapidly than glucose and maltose. Since it is too sweet, a mixture of dextri-maltose and sucrose may be more suitable for general use in infant feeding formulae.

Calories: The caloric requirements of infants from birth to one year of age have been calculated by Clements (1949) from data available for basal metabolism and the calorie requirements for physical activity and growth. The daily calorie requirements ranged from about 100Kcal/kg body weight in the first week to about 90Kcal/kg body weight at the 50th week. The F.N.B., N.R.C., U.S.A. recommended an allowance of 120Kcal/kg for infants of 1-3 months to 100Kcal/kg for infants of 7-12 months of age. Adequate caloric intake is essential for promoting optimal growth of infants.

Minerals. The infant needs different minerals and these must be provided in the milk for proper growth of the infant.

Data regarding the calcium and iron requirements of infants are given in Table 17.1. Exact figures for the requirements of other minerals are not available. Human and cow's milks appear to provide adequate amounts of all minerals except iron, since infants fed exclusively on human milk or modified cow's milk formulae develop only iron deficiency anaemia and do not show deficiency of any other minerals. For this reason, infant foods based on animal milks will have to be fortified with iron salts or the infants will have to be provided with supplementary sources of iron. Infant foods based on soybean and peanut will have to be fortified with iodides also, besides other minerals as these materials have been reported to contain goitrogenic principles.

Vitamins. The recommended allowances of certain vitamins suggested by the FNB, NRC, U.S.A., are given in Table 17.1. A statement issued by the Council on Food and Nutrition of the American Medical Association in 1959 stressed the need for avoiding the use of excessive intakes of certain vitamins as they may produce toxic effects. A normal infant requires 1500 I.U. vitamin A, 400 I.U. vitamin D, 35 mg vitamin C, 0.3 to 0.5 mg thiamine, 0.4 to 0.6 mg riboflavin and 6 to 9 mg niacin. Data regarding the exact requirements of other vitamins are not available. Infants fed on unfortified cow's milk formulae will need daily the following additional amounts of vitamins: vitamin A (800 I.U.), vitamin D (300 to 400 I.U.) vitamin C (20 to 30 mg) and niacin (2 to 3 mg). There is evidence that vitamin B_6 present in cow's milk is destroyed to a considerable extent (40 to 70 per cent) during heat processing and infants fed on processed milk

foods may need supplements of vitamin B_6. Gyorgy *et al*, (1952) have shown that the greater rate of haemolysis of red blood cells observed in some new born infants could be prevented by the administration of vitamin E. Since vitamin E content of animal milk is very low, infants fed on these milks may need a supplement of vitamin E (5 mg/day).

Digestion of food

The digestion of food by infants differs in several respects from that of older children. The most important differences in infants are (1) low gastric acidity and (2) absence or low activity of amylase in the pancreatic and intestinal juices of infants.

Gastric digestion. Miller studied the composition of fasting gastric juice of infants and found that the gastric acidity was highly variable. Wolman (1946) studied the pH of the gastric contents over a period of 1½ hours after the ingestion of cow's milk in the young infant (1-2 months old) as compared with infants aged 6 to 11 months and young children. The results showed that (*i*) the pH of the gastric contents of infants aged 1-2 months was about 6 and almost constant and (*ii*) the pH of the gastric contents of infants aged 6-11 months fell from 6 to 4.8 while the pH of the gastric contents of young children fell steadily from 5.6 to 2.5. These results support the observations of other workers that the secretion of hydrochloric acid is very low in young infants (1-2 months) and the amount of acid secreted increases as the infant grows. The enzymes present in the gastric juice of normal infants are pepsin, cathepsin and lipase. Pepsin can digest proteins at a pH of 2.5 to 4. Pepsin is also responsible for the formation of curd from milk, the optimum pH for the conversion of casein to paracasein by pepsin being 5.3. The presence of another proteolytic enzyme-cathepsin-in the gastric juice of infants was discovered by Bucks (1940) and Freudenberg (1943). The enzyme has a maximum activity at pH 4.7. They found that the proteolytic activity of cathepsin in the gastric juice of 54 infants was approximately 50 per cent of that of pepsin. In view of the low acidity of the gastric contents of young infants (1-2 months), it is evident that much of the proteolytic activity of gastric juice on milk is due to cathepsin. Some digestion of the fat of milk may take place in the stomach

by the action of gastric lipase. This is, however, usually negligible. The changes in the pH of the stomach contents of infants after the ingestion of cow's or human milk have been studied by some workers. The maximum acidity was found to be attained at 1½ hours after the ingestion of milk. The change in the pH of stomach contents was from 3.0 to 6.4 at 1½ hours after the ingestion of cow's milk and 3.0 to 4.9 after the ingestion of human milk. This difference is evidently due to the high buffering capacity of cow's milk. It has been reported that feeding cow's milk diluted with water or cow's milk acidified with lactic acid or citric acid, brings about a lowering in the pH of the stomach contents similar to that observed after the ingestion of human milk. Wolman (1946) found that the pH of the stomach contents exerted a strong influence on the physical state of curd formed from the ingested milk.

Pancreatic digestion: Klumpp and Neale (1930) studied the enzymes in duodenal contents of infants. They found that the proteolytic activity was quite high, the lipolytic activity fairly high, while the amylolytic activity very low in infants 1 to 3 months old. The amylolytic activity was found to increase steadily after the 5th month. Due to lack of adequate amylase activity, young infants cannot digest starchy foods to an appreciable extent.

Intestinal microflora: The digestive tract of the new born infant is usually sterile. Within a day or two after birth, bacteria appear in considerable numbers in the intestinal tract. According to Kendall (1952) the predominating organism in the intestines of infants fed on human milk is *lactobacillus bifidus*. In addition, *bacillus lactic aerogenus* and *B. coli* are also found in appreciable numbers. On the other hand, the intestinal flora of infants fed on cow's milk is characterised by a preponderance of *B. coli*. Gyorgy (1953) has made extensive studies of the factors responsible for the predominantly *lactobacillus bifidus flora* in human milk-fed infants and contrasted these with the mixed flora of cow's milk-fed infants. He found a new strain of *lactobacillus bifidus L, var Penn.* in human milk-fed infants, the growth of which was stimulated by some growth factors viz. nitrogen containing polysaccharides present in human milk. These growth factors are found in much greater quantity in human milk than in cow's milk. In contrast to the acid reaction of the faeces of

normal breast-fed infants, the pH of the faeces of infants given the usual cow's milk formula is neutral or alkaline. This is evidently due to the difference in the intestinal microflora of infants fed on the two types of milks.

The chemical composition of human milk and certain animal milks

The composition of human milk as compared with those of cow, buffalo and goat is given in Table 17.4. The differences in the composition of the different milks are briefly discussed below:

Table **17.4 The composition of human milk as compared with that of cow's, buffalo's and goat's milks (values per 100ml)**

Constituents	Human	Cow	Buffalo	Goat
Protein (g)	1.2	3.3	3.8	3.3
Casein (g)	0.4	2.8	3.0	2.5
Lactalbumin (g)	0.3	0.4	0.4	0.4
Lactoglobulin (g)	0.2	0.2	0.2	0.3
Fat (g)	3.8	3.7	7.5	4.1
Lactose (g)	7.0	4.8	4.4	4.7
Calories (Kcal)	71	69	100	76
Mineral matter (g)	0.21	0.72	0.8	0.77
Calcium (mg)	33	125	210	130
Phosphorus (mg)	15	96	130	106
Chlorine (mg)	43	103	112	159
Magnesium (mg)	4	12	15	16
Potassium (mg)	55	138	142	181
Sodium (mg)	15	58	65	41
Iron (mg)	0.15	0.10	0.2	0.05
Copper (mg)	0.04	0.03	0.02	0.04
Iodine (mg)	0.007	0.021	0.004	—
Manganese (mg)	0.7	2	—	8
Zinc (mg)	0.53	0.38	—	—
Vitamins				
Vitamin A (I.U.)	160	158	200	120
Vitamin D (I.U.)	1.4	2.0	—	2.3
Thiamine (mg)	0.017	0.04	0.05	0.05
Riboflavin (mg)	0.04	0.18	0.10	0.12
Nicotinic acid (mg)	0.17	0.08	0.28	0.20
Pantothenic acid (mg)	0.20	0.35	—	—
Vitamin B_6 (mg)	0.001	0.035	—	—
Folic acid (μg)	1.3	5.6	3.3	0.7
Biotin (μg)	0.4	2.0	—	1.5
Vitamin B_{12} (μg)	0.03	0.50	0.30	0.10
Vitamin C (mg)	4.0	2.0	2.5	2.0

Proteins: Cow's, buffalo's and goat's milks contain about 3 times as much proteins as human milk. The ratio of casein to albumin in human milk is nearly 1:1, and about 7:1 in the animal milks. The high casein content has been the important cause in the formation of hard curd from these milks in the stomachs of infants. Data regarding the essential amino acid composition of the proteins of the different milks are given in Table 17.5. Human milk proteins contain greater amounts of tryptophan and sulphur amino acids especially cystine than the animal milk proteins. The NPU of the human milk proteins (87.0) has been reported to be higher than that of the proteins of cow's milk. Barness *et al*, (1957) have reported that in normal infants fed on human milk, nitrogen retention was greater than that observed in infants fed on cow's milk when the protein intakes from the two milks were the same. In view of this, infants require greater amounts of proteins from animal milks than from human milk.

Table **17.5 Essential amino acid composition of milk proteins** (*g/16gN*)

Amino acid	Human	Cow	Buffalo	Goat
Arginine	4.7	3.7	3.3	2.2
Histidine	2.5	2.7	2.1	2.0
Isoleucine	5.5	6.5	5.0	5.0
Leucine	9.1	10.0	10.2	8.7
Lysine	6.6	7.9	8.1	6.3
Cystine	2.0	0.9	1.4	0.7
Methionine	2.4	2.5	2.7	2.2
Phenylalanine	4.4	4.9	4.3	4.4
Tyrosine	5.2	4.2	—	—
Threonine	4.5	4.7	5.2	4.3
Tryptophan	1.7	1.4	1.4	1.3
Valine	6.3	7.0	6.2	5.8

Fat: The fat contents of cow's and goat's milks are nearly of the same order as that of human milk but buffalo milk contains nearly twice as much fat as human, cow's and goat's milks. The main differences (Table 17.6) between human milk fat and animal milk fats are (1) the absence of butyric acid and certain other lower fatty acids in human milk fat and (2) the presence of higher percentage of oleic acid and essential fatty acids (e.g. linoleic acid) in the former as compared with the latter.

Table **17.6 Fatty acid composition of fats of human milk as compared with certain animal milks**

Fatty acid	Human	Cow	Buffalo	Goat
Saturated:				
Butyric acid	0.4	3.1	4.1	2.6
Caproic acid	0.1	1.0	1.4	2.3
Caprylic acid	0.3	1.2	0.9	2.7
Capric acid	1.7	2.6	1.7	8.4
Lauric acid	5.8	2.2	2.8	4.5
Myristic acid	8.6	10.5	10.1	11.1
Palmitic acid	22.6	26.3	31.1	28.9
Stearic acid	7.7	13.2	11.2	7.8
Arachidic acid	1.0	1.2	0.9	0.4
Unsaturated				
Oleic acid	36.4	32.2	33.2	27.0
Linoleic acid	8.3	1.6	2.6	2.6
Linolenic acid	0.4	—	—	
C^{20-22} acids	4.2	1.0	—	0.4
Arachidonic acid	0.8	1.0	—	1.5
Melting point°C	31	35.3	36.5	—
Iodine number	61.6	38.4	33.0	28.8

Minerals: The mineral contents of animal milks are about 2 to 5 times those of human milk except in the case of certain trace elements such as iron, copper etc. The buffering capacity of animal milks is very much greater than that of human milk. The relation of buffering capacity to peptic digestion has been referred to in an earlier section. Further, the extra amounts of minerals present in animal milks may increase water requirements and impose strain on the kidneys of young infants.

Vitamins: The vitamin A content of human milk is nearly equal to that of cow's, buffalo's and goat's milks. All the milks are poor sources of vitamins E and D, niacin, folic acid and vitamin C. The animal milks contain about 2 to 4 times as much thiamine, riboflavin and vitamin B_{12} as those present in human milk. Both human and animal milks are fair sources of vitamin B_6. A fair part of vitamin B_6 activity is, however, destroyed during the heat processing of milk as mentioned earlier.

Modification of animal milks for infant feeding

It is a matter of common experience that most young infants have difficulties in digesting untreated cow's or buffalo's milk. This is mainly due to the higher protein and buffer salts content of most animal milks. The large amounts of casein present in animal milks form a dense curd in the stomachs of infants when acted upon by pepsin and the pepsin digestion is retarded by the neutralisation of the hydrochloric acid present in the gastric juice by buffer salts. Further, buffalo milk contains excessive amounts of fat. Buffalo milk can be partially skimmed or 'toned' by the addition of an equal volume of reconstituted skim milk. The animal milks can be modified in several ways for infant feeding. These include (*i*) Boiling (*ii*) Dilution with water (*iii*) Acidifying with organic acids and (*iv*) Addition of buffer salts. All these treatments bring about changes in the curd tension and also in the composition of the milk.

Boiling: Boiling of fresh milk is an essential step to destroy harmful bacteria. This step also brings about changes in the physico-chemical properties of milk and helps to lower the curd tension to a considerable extent.

Dilution with water: Dilution of animal milk with water in proportion of 1:1, 2:1 or 3:2 is adopted for feeding infants of varying ages. Milks diluted with water will have to be fortified with vitamin A, besides other nutrients such as vitamin D, iron and ascorbic acid as mentioned earlier.

Acidifying with organic acids: In this method, sufficient quantities of lactic or citric acid are added so as to neutralise the buffering action and to cause a finely divided suspension of proteins. Milk treated in this way has a low curd tension. To each litre of milk, 60-80 drops of 40 per cent lactic acid or one teaspoon of 25 per cent citric acid or one ounce of fresh lemon juice or 2 ounces of fresh orange juice are added. Milk obtained in this way has a low buffering capacity and is readily digested by infants.

Addition of buffer salts: The addition of sodium citrate and sodium phosphate results in lowering of curd tension. This procedure, however, has the *disadvantage* of increasing the buffering capacity and hence is likely to prevent the digestive

action of pepsin so that the milk may leave the stomach without being digested.

Homogenisation: In this process, milk is subjected to a temperature of 165°F and to a pressure of 2000 lbs/sq.inch. The fat globules are broken down and the protein is redispersed over their surface, resulting in a lowering of the curd tension of milk.

Feeding schedule for infants

The nutritional requirements of normal infants may be met by the use of simple mixtures of whole boiled cow's milk or partially skimmed or toned buffalo's milk, sugar and water, together with food supplements containing vitamin D, ascorbic acid and iron.

The chemical composition of human, cow's and buffalo's milks is given in Table 17.4. Whole buffalo's milk is rich in fat and hence is not suitable as such for feeding infants. It should be partially skimmed or toned. Toned buffalo's milk can be readily prepared in the home by mixing equal quantities of pure buffalo's milk and milk reconstituted from skimmed milk powder. Infants vary much in their ability to digest undiluted boiled milk. Dilution of the milk serves to make it more readily digestible and, at the same time, permits a more generous fluid intake. Dilution is required only for the young infant. Undiluted boiled milk usually can be digested without difficulty at seven or eight months of age. The quantities of milk, water and sugar required for infants of age varying from 1 to 12 months are given in Table 17.7. In addition to this, the infant up to the age of 6 months requires a supplement of fruit juice (preferably orange or tomato juice) as a source of vitamin C and shark liver oil as a source of vitamins A and D. For infants older than 6 months, small amounts of cooked and mashed green leafy vegetables, egg yolk, cereals and fruit pulp should be introduced in gradually increasing amounts as sources of iron and other nutrients.

Feeding experiments with infants using human milk and cow's milk

Only a few reports are available in the literature on the comparative performance of infants fed on human milk and cow's milk. Sterns (1939) reported that infants fed from birth to six

Table **17.7 Feeding of infants with fresh cow's or toned buffalo milk**

	Age (months)							
	1	2	3	4-5	6-7	8-9	10-11	12
No. of feeds per day	7	6	6	6	6	5	5	5
Cow's milk or toned buffalo milk (ml)	400	500	600	700-750	800-850	900-950	900-950	1000
Water	300	300	300	300	200	100	100	—
Cane sugar (g)	20	30	40	50	60	60	60	60
Vitamin D (I.U.)	400	400	400	400	400	400	400	400
Egg yolk	—	—	—	—	½	½	1	1
Fruit juice (ml)	—	—	10	15	20	25	30	35
Cereal food (g)	—	—	—	10	15	25	30	40
Mashed vegetables and fruits (Teaspoons)	—	—	—	—	4	4	6	8

months of age on modified cow's milk showed a steady increase in the per cent of body nitrogen in contrast to breast-fed infants whose body nitrogen per cent remained at birth level or showed a slight decrease. The greater nitrogen in the body of artificially fed infants must necessarily represent larger amounts of tissue proteins, since nitrogen is not stored in any other form. A large part of the increase in tissue proteins was represented by an increase in muscle mass which was approximately 25 per cent greater than that observed in breast-fed infants. Similarly, the calcium retention in the body of infants fed on cow's milk has been found to be higher than that observed in breast-fed infants. The significance of the above findings on the health and physiological functions of infants is, however, not clear. Simpson (1953) reported a greater gain (9-10 ounces) in the average weight of infants fed on cow's milk formula than that observed with breast-fed infants. In an extensive experiment carried out in Malaysia, the weight gains of infants fed breast milk was compared with those of infants on cow's milk. The results indicated that breast-fed infants belonging to the low income groups showed greater gain in weight in the first six months than those receiving

cow's milk as the latter did not receive adequate amounts of milk. There was no difference in the gain in weight of the two groups of infants belonging to the well-to-do families. A most extensive comparison of human and cow's milk has been made by Mellander *et al*, (1959). In a group of 402 infants, they found no difference at seven months between the breast-fed and early weaned infants with regard to deficiency diseases, haemoglobin values, antibody response or contageous diseases. They found that the gain in weight, height, and gamma globulin in early weaned infants was greater than that of the breast-fed infants at 7½ months of age. On the other hand, breast-fed infants had significantly fewer upper respiratory and gastro-intestinal infections and had higher serum calcium at 7½ months than the early-weaned infants.

Processed infant foods based on animal milks and vegetable milk substitutes

During recent years the use of processed infant foods based on milk and milk substitutes have been on the increase. The different types of infant foods may be discussed under the following heads. (1) Foods based on animal milks (2) Foods containing non-fat milk solids and vegetable fat and (3) Foods based on vegetable milk substitutes.

Foods based on animal milks

Evaporated milk: Evaporated milk is being widely used in U.S.A. for feeding infants as it can be readily reconstituted and fed to infants. In the unopened can it will keep for months without refrigeration. The standard can contains 14.5 ounces (by weight) and is diluted with an equal amount of water and carbohydrate is added before being fed to infants. It contains about 7.9 to 9 per cent of proteins and of fat, with a total solids content of 26-31 per cent and is commonly fortified with vitamin D. In the process of manufacture, milk is concentrated to about half its original volume and the evaporated milk thus obtained is homogenised, filled in cans and sterilised at 240°F. As a result of heat treatment, the casein is altered so that the curd formed in the stomach is softer and smaller than that of boiled milk. Homogenisation of the milk also makes the fat more readily

digestible and helps to lower the curd tension. The lactalbumin present in the milk is also altered in its properties and is less allergenic than that present in the boiled milk. There are reports indicating that a greater part of vitamin B_6 present in the milk is destroyed in the process. Evaporated milk will, therefore, have to be fortified with vitamin B_6 and other vitamins which are destroyed during heat processing.

Dried milk foods: Dried milk foods suitable for feeding infants are usually prepared by drying the concentrated milk (with or without various additions) using spray or roller driers. The advantages of dried milk foods over milk are (1) the small bulk and ease of transportation (2) the long keeping quality and (3) the hygienic quality of the product. The infant milk foods available on the market may be broadly grouped under three heads (1) Full cream milk foods (2) Half cream milk foods and (3) Humanised milk foods. Most of these products are made from cow's milk. Recently, processes have been described for the preparation of infant foods from buffalo milk (Swaminathan and Parpia 1968). These foods have been found to be readily digested and promote good growth in infants. The nutritive value of milk foods based on buffalo milk and containing varying levels of proteins (10-28 per cent) and of fat (8-26 per cent) has been studied by growth experiments in albino rats. The results showed that milk foods containing 15 per cent proteins and 15 per cent or 26 per cent fat promoted nearly the same growth rate as full-fat milk powder (26 per cent proteins and 26 per cent fat), while a milk food containing 10 per cent proteins and 26 per cent fat (similar to that of dried human milk) promoted significantly less growth. Similar studies with infants are needed to find out the optimal protein and fat levels for promoting good growth in infants. Dried and evaporated milk foods based on goat's milk are also being used in U.S.A. for feeding infants who develop allergic symptoms when fed on cow's milk.

Foods based on non-fat milk solids and vegetable fats

Infant foods based on non-fat milk solids and vegetable fats are being manufactured on a large scale in U.S.A. and used extensively for feeding infants. Such foods will have higher

contents of essential fatty acids and vitamin E than milk foods containing butter fat. It has been reported that the feeding of infant foods containing soybean or corn oil results in a lower blood cholesterol level than that observed with infants receiving milk foods containing butter fat.

Foods based on vegetable proteins or blends of vegetable proteins and non-fat milk solids

Recent investigations indicate that infant foods based on soybean can meet the nutritional requirements and promote good growth of infants. In U.S.A., infant foods based on soybean are being used for feeding infants who develop allergic symptoms when fed on cow's milk; but in India and other developing countries such foods can be used for the feeding of normal infants as there is an acute shortage of milk in these countries. Care should be taken, however, to ensure that these foods are adequately fortified with all essential vitamins and minerals and limiting essential amino acids. It may be desirable to incorporate some quantity of lactose in foods meant for infants allergic to cow's milk, as this sugar appears to be an essential nutrient for infants. If, however, the foods are used for the feeding of normal infants in developing countries, it will be desirable to incorporate about 20 per cent of non-fat milk solids in the foods in place of lactose. Investigations carried out at the C.F.T.R.I., Mysore have shown that infant foods prepared from blends of groundnut and soybean or groundnut protein isolate and non-fat milk solids, possess high nutritive value. There is considerable scope for the production of these infant foods in India and other developing countries (Swaminathan and Parpia 1968).

Supplementary foods

Milk is the basic food of the infant in that it supplies a greater part of the required nutritional essentials. Other foods are commonly added to the diet (some from the beginning) in order to provide dietary essentials lacking in milk and also to accustom the baby to varieties in flavour and consistency in food so that good feeding habits are developed. These foods include a source of vitamin D, fruit juices, strained vegetables, fruits and meat, and pre-cooked cereals.

Vitamin D: A source of vitamin D is essential for infants receiving unfortified milk and milk foods. Great care should be taken to ensure that excess of vitamin D is not administered.

Fruit juices: Fruit juices commonly given to infants are orange or tomato juices. These provide the required amounts of vitamin C. The organic acids present in fruit juices help to lower the curd tension of milk and thereby aid in the digestion of milk proteins and in the absorption of calcium.

Egg yolk: Milk is a poor source of iron and a fair source of thiamine. Egg yolk is a fair source of both these nutrients and it has become customary in Western countries to add egg yolk to the diets of infants from 4th to 6th month onwards.

Strained vegetables and fruits: Strained fruits and vegetables are added to the diets of infants from the 4th month onwards. They provide iron, vitamin C and small amounts of indigestible carbohydrates. The latter may help to prevent constipation. Several types of strained baby foods are being manufactured on a commercial scale and widely used as a supplement to milk in the feeding of babies in Western countries.

Strained baby foods based on meat: Strained baby foods containing meat, liver and egg yolk are being manufactured on a large scale and used as a supplement to milk in the feeding of infants from the 6th month onwards in Western countries.

Precooked enriched cereal foods: The feeding of cooked cereals as the baby's first solid food has been a custom widely prevalent in different countries. There is increasing evidence that cereals add something of value to the diet and that babies who receive it thrive better than those who do not. Precooked roller dried cereal foods are widely used for this purpose. These are fortified with iron so that they can meet the iron requirements of babies.

Nutrition and feeding of infants in developing countries

In view of the acute shortage in the production of milk and other animal foods in developing countries, a large majority of infants have to depend on breast milk for their nutrition. Infants over the age of 6 months do not receive adequate quantity of mother's milk. Hence, malnutrition and undernutrition are widely prevalent among them. During recent years, considerable

amount of work has been carried out on the utilisation of oilseeds and legumes in the supplementary feeding of infants. Vegetable protein foods used in infant feeding may be broadly grouped under the following heads: (1) Milk substitutes based on oilseeds and nuts (2) Precooked cereal-based protein supplements and (3) Protein foods based on oilseed meals, legumes and fortified with vitamins and minerals.

Milk substitutes based on oilseeds and nuts: As already mentioned in an earlier section, investigations carried out by several workers have shown that milk substitutes based on soybean promote good growth in infants. Milk substitutes based on blends of soybean, groundnut and sesame can be used as supplements to breast milk in the feeding of infants. Such infants may need an extra supplement of calcium salts, vitamins A and D, if these have not been incorporated in the milk substitutes.

Pre-cooked cereal-based, protein supplements: Studies carride out by various workers have shown that pre-cooked roller dried foods based on blends of cereals, legumes and oilseed meals and skim milk powder fortified with vitamins and minerals, could be used as supplementary foods for feeding infants, over 6 months of age.

Protein foods based on oilseed meals and legumes and fortified with vitamins and minerals: Studies carried out by various workers have shown that processed protein foods based on blends of cereals, legumes and oilseed meals could be used in the form of porridge or pudding as supplements to the diets of infants over 6 months of age.

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CHAPTER 18

NUTRITION OF PRE-SCHOOL CHILDREN

The infant and the pre-school child are extremely vulnerable. They succumb readily where the diet is poor in quality and quantity and infectious diseases and infestations are widespread. The rate of growth and development of infants and pre-school children depend to a large measure on the adequacy of the diet consumed by them. Frequent attacks of infectious diseases further increase the requirements of various nutrients.

Growth of normal infants and pre-school children

Data regarding the growth rate of normal infants and pre-school children are given in Tables 18.1 and 18.2.

Table **18.1 Increase in height of normal well-fed infants and pre-school children**[1]

Age (months)	Height (male infants) (cm)	Increase in height (cm/yr)	Height (female infants) (cm)	Increase in height (cm/yr)
At birth	50.6		50.2	
3	60.4		59.5	
6	66.4		65.2	
9	71.2		70.1	
12	75.2	24.6	74.2	24.0
24	87.5	12.3	86.6	12.4
36	96.2	8.7	95.7	9.1
48	103.4	7.2	103.2	7.5
60	108.7	5.3	109.1	5.9

[1] Figures for 50th percentile from data obtained in studies of Child Health and Development, Department of Maternal and Child Health, Harvard School of Public Health.

Increase in body length: The average infant grows about 25 cm. in body length during the first year distributed approximately as follows: 10, 7, 5 and 3 cm. in the first, second, third and fourth

Table 18.2 **Increase in weight of normal well-fed infants and pre-school children**[1]

Age (months)	Body wt. (male infants) (kg.)	Increase in body wt. (kg/yr)	Body wt. (female infants) (kg.)	Increase in body wt. (kg/yr)
At birth	3.40		3.36	
3	5.72		5.62	
6	7.58		7.26	
9	9.07		8.71	
12	10.07	6.67	9.75	6.39
24	12.56	2.49	12.29	2.54
36	14.61	2.05	14.42	2.13
48	16.51	1.90	16.42	2.00
60	18.37	1.86	18.37	1.95

[1] Figures for 50th percentile from data obtained in studies of Child Health and Development, Department of Maternal and Child Health, Harvard School of Public Health.

quarters respectively. The yearly increases in height in pre-school children are as follows: 1-2 years, 12.4; 2-3 years, 8.9; 3-4 years, 7.3 and 4-5 years, 5.6 cm.

Increase in weight: The increase in body weight in the 1st year is 6.5 kg. as compared with 2.5 kg. during 1-2 years, 2.1 kg. during 2-3 years; 2.0 kg. during 3-4 years and 1.9 kg. during 4-5 years.

Growth rate of infants and pre-school children in developing countries

There is some evidence that infants in the developing countries grow well during the first six months of life when they are solely breast-fed. The growth declines during the second half i.e., 6 months-12 months, as they receive cooked cereals and roots and tubers as supplements and hence do not get adequate nutrition. Studies carried out by several workers have shown that a large majority of pre-school children in the developing countries grow at a subnormal rate as the diets consumed by them are inadequate both in quantity and quality. Data regarding the growth of pre-school children in India as compared with American standards are given in Table 18.3. It is of interest to note that the

mean weight of pre-school children (1-5 years) in India is about 20-25 per cent less than that of American pre-school children for the corresponding age group. On the other hand, the mean height of pre-school children in India is only 6-8 per cent less than that of American pre-school children.

Table **18.3 Mean weight and height of pre-school children in India as compared with American Standards**

Age (years)	Height (cm)				Weight (kg)			
	Indian[1]		American[2]		Indian[1]		American[2]	
	Male	Female	Male	Female	Male	Female	Male	Female
1	73.9	72.5	75.2	74.2	8.4	7.8	10.1	9.8
2	81.6	80.1	87.5	86.6	10.1	9.6	12.6	12.3
3	88.8	87.2	96.2	95.7	11.8	11.2	14.6	14.4
4	96.0	94.5	103.4	103.2	13.5	12.9	16.5	16.4
5	102.1	101.4	108.7	109.1	14.8	14.5	18.5	18.4

[1] Nutrition Atlas of India, I.C.M.R. New Delhi, 2nd Edition (1971).

[2] Figures for 50th percentile from data obtained in studies of Child Health and Development, Department of Maternal and Child Health, Harvard School of Public Health.

Nutrient requirements of pre-school children

Data regarding the mean daily nutritional requirements of various Expert Committees are given in Tables 16.1-16.5 in Chapter 16 and summarised in Table 18.4. They are briefly discussed below:

Calories: The mean calorie requirements range from 100 Kcal/kg. at one year to 80 Kcal/kg at 5 years. This will work out to about 1000 Kcal at 1 year to 1450 Kcal at 5 years. The calorie allowances of various Expert Groups range from 1050 Kcal at one year to 1600 Kcal at 6 years.

Proteins: The protein allowances recommended by various Expert Groups are given in Tables 16.1-16.3 (Chapter 16). The allowances recommended by the ICMR Nutrition Expert Group (1968) range from 17-22 g as compared with 25-30 g. recommended by Food and Nutrition Board, N.R.C. U.S.A. (1968) and 30-40 g. recommended by U.K. Expert Panel (1969). It is evident that the recommendations of ICMR Expert Group are low and need to be increased to 25-30 g.

Table **18.4 Recommended daily nutrient allowances for pre-school children (1-5 years)**

Nutrient	I.C.M.R. India (1968)	N.R.C. U.S.A, (1968)	U.K.Expert Panel (1969)
Calories (Kcal)	1000-1500	1100-1600	1200-1600
Proteins (g)	17-22	25-30	30-40
Calcium (g)	0.4-0.5	0.7-0.8	0.5
Iron (mg.)	15-20	10-15	7-8
Vitamin A (μg).	250-300	2000-2500 (I.U.)	300
Ascorbic acid (mg)	30-50	40	20
Thiamine (mg)	0.6-0.8	0.6-0.8	0.5-0.6
Riboflavin (mg)	0.7-0.8	0.6-0.9	0.6-0.8
Nicotinic acid (mg)	8-10	8-11	7-9
Folic acid (μg)	50-100	100-200	—
Vitamin B_{12} (μg)	0.5-1.0	2-3	—
Vitamin D (I.U.)	200	400	400
Vitamin E (I.U.)	—	10	—

Calcium: The recommended allowances for calcium by various Expert Groups are given in Tables 16.1-16.3 (Chapter 16). The recommendations of ICMR Expert Group range from 0.4-0.5g, while those of Food and Nutrition Board, USA (1968) range from 0.7-0.8g and that of U.K. Expert Panel is 0.5g. The ICMR Expert Group recommendations are adequate to meet the calcium needs of pre-school children.

Iron: Data regarding the allowances for iron recommended by various Expert Groups are given in Tables 16.1-16.3 (Chapter 16). The recommendations of ICMR Expert Group range from 15-20mg, while those of Food and Nutrition Board, USA range from 10-15mg and those of U.K. Expert Panel from 7-8mg.

Vitamin A: The recommended allowances of vitamin A by various Expert Groups are given in Tables 16.1-16.3 (Chapter 16). The recommendations of ICMR Expert Group vary from 250-300μg, as compared with 2000-2500 I.U. recommended by Food and Nutrition Board, USA and 300μg recommended by U.K. Expert Panel.

Thiamine: The thiamine requirements recommended by ICMR Expert Group range from 0.6-0.8mg, those of Food and Nutrition Board, USA from 0.6-0.8mg and those of U.K. Expert Panel from 0.5-0.6mg.

Riboflavin: The riboflavin requirements recommended by various Expert Committees are as follows: ICMR Expert Group, 0.7-1.0mg, Food and Nutrition Board, U.S.A., 0.6-0.9mg and U.K. Expert Panel, 0.6-0.8mg.

Nicotinic acid: The recommended allowances for nicotinic acid by various Expert Groups are as follows: ICMR Expert Group, 8-10 mg, Food and Nutrition Board, U.S.A., 8-11 mg and U.K. Expert Panel, 7-9mg.

Ascorbic acid: Ascorbic acid allowances recommended by various Expert Groups are as follows: ICMR Expert Group, 30-50mg; Food and Nutrition Board, U.S.A., 40mg and U.K. Expert Panel, 20mg.

Vitamin D: The recommended allowances for vitamin D by various Expert Committees are as follows: ICMR Expert Group, 200 I.U. Food and Nutrition Board, U.S.A., 400 I.U. and U.K. Expert Panel, 400 I.U. Since children in the tropics derive some vitamin D from exposure to sunlight, the recommendations of ICMR Expert Group will be adequate for children in the tropics.

Folic acid: The recommendations of various Expert Groups for 'free' folic acid requirements are as follows: ICMR Expert Group, 50-100μg; Food and Nutrition Board, U.S.A., 100-200μg, and FAO/WHO Expert Group 100μg.

Vitamin B_{12}: The recommendations of various Expert Groups for vitamin B_{12} requirements are as follows: ICMR Expert Group 0.5-1.0μg; Food and Nutrition Board, U.S.A., 2-4μg and FAO/WHO Expert Group 0.9-1.5μg.

Deficiencies in the diets of pre-school children in developing countries

Surveys carried out by a large number of workers in developing countries have shown that the diets consumed by a large majority of pre-school children are deficient in calories, proteins, and several essential vitamins and minerals particularly vitamin A, riboflavin, folic acid and iron. Signs and symptoms due to deficiencies of the above nutrients are widely prevalent among them. Incidence of protein-calorie malnutrition and vitamin A deficiency diseases is particularly high among this age group (Jelliffe, 1968).

Balanced diets for pre-school children

Balanced diets at high, moderate and low costs for pre-school children are given in Tables 18.5-18.7.

Table 18.5 **Balanced diets for pre-school children (High cost)**

Foodstuffs	1-3 years V (g)	1-3 years NV (g)	4-6 years V (g)	4-6 years NV (g)
Cereals	100	100	140	140
Pulses	30	20	40	30
Green leafy vegetables	50	50	75	75
Other vegetables, roots and tubers	30	30	30	30
Fruits	100	100	100	100
Milk	1000	700	1000	700
Egg	—	30	—	30
Meat and fish	—	20	—	30
Fats and oils	20	20	25	25
Sugar and jaggery	30	30	40	40

Table 18.6 **Balanced diets for pre-school children (Moderate cost)**

Foodstuffs	1-3 years V (g)	1-3 years NV (g)	4-6 years V (g)	4-6 years NV (g)
Cereals	120	120	170	170
Pulses	50	40	60	50
Green leafy vegetables	50	50	75	75
Other vegetables, roots and tubers	30	30	50	50
Fruits (tomato or papaya or guava)	100	100	100	100
Milk	600	400	600	400
Fats and oils	20	20	25	25
Meat, fish and eggs	—	40	—	50
Sugar and jaggery	30	30	40	40

Table 18.7 **Balanced diets for pre-school children (Low cost)**
(I.C.M.R. Nutrition Expert Group)

Foodstuffs	1-3 years		4-6 years	
	V (g)	NV (g)	V (g)	NV (g)
Cereals	150	150	200	200
Pulses	50	40	60	50
Green leafy vegetables	50	50	75	75
Other vegetables, roots and tubers	30	30	50	50
Fruits	50	50	50	50
Milk	300	200	250	200
Fats and oils	20	20	25	25
Meat, fish and eggs	—	30	—	30
Sugar and jaggery	30	30	40	40

V—Vegetarian NV—Non-Vegetarian

Balanced diets at high costs: These diets contain large amounts of milk, fruits, eggs, meat and fish which are costly (Table 18.5).

Balanced diets at moderate costs: These diets contain moderate amounts of milk, fruits, eggs, meat and fish (Table 18.6).

Balanced diets at low costs: These diets contain small amounts of milk and fair amounts of legumes and green leafy vegetables (Table 18.7).

Supplementary foods for pre-school children in developing countries

Since a large majority of pre-school children in developing countries consume inadequate diets and suffer from malnutrition, there is an urgent need to institute supplementary feeding programmes in the developing countries. Such supplements should be based on locally available foods such as legumes, oilseeds and nuts. Studies carried out by several workers have shown that a daily supplement of about 30 g of processed legumes or roasted groundnut or processed protein foods based on blends of cereals, oilseed meals and legumes will help to overcome malnutrition among pre-school children. Further details on the above aspects are given in Chapters 21 and 22 in Vol. II.

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CHAPTER 19

NUTRITION OF SCHOOL CHILDREN AND ADOLESCENTS

A large number of diet and nutrition surveys have been carried out by different workers on the nutritional status of school children and adolescents in the developing countries. The results have shown that a majority of the school children and adolescents consume inadequate diets and are malnourished. The main contributory causes are (1) Inadequate food production (2) Poverty and (3) Lack of nutrition education.

Growth rate of normal school children and adolescents

Data regarding the growth rate of normal American school children are given in Table 19.1.

Increase in height: The yearly increase in height varies from 4.0 to 7.7 cm. in boys (5-14 years old) and 4.9 to 7.2 cm in girls (5-12 years old). The increase in height slowly declines after the 14th year in boys and 12th year in girls.

Increase in weight: The yearly increase in weight varies from 2.5 to 6.6 kg. in boys between 5-14 years and from 2.3 to 5.2 kg in girls between 5-13 years. The yearly weight increase steadily declines after the age of 14 in boys and 13 in girls.

Growth rate of school children in India: Data regarding the height and weight of children in India as compared with American standards are given in Table 19.1. It is of interest to note that the mean weights of school children and adolescents in India are about 20-25 per cent less than those of American children for the corresponding age group. On the other hand, the mean heights of school children and adolescents in India are only 6-8 per cent less than those of American counterparts. The mean height and weight of Indian children belonging to the higher income groups are significantly greater than those reported for children of all income groups in India. This is evidently due to the more nutritious diets consumed by the high income groups.

Table **19.1 Mean weight (kg) and height (cm.) of Indian children (6-18 years) compared with American children**

Age (years)	American Children				Indian Children				Indian Children			
	Weight (kg)[1]		Height (cm)[1]		Weight (kg)[2]		Height (cm)[2]		Weight (kg)[3]		Height (cm)[3]	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
6	21.91	21.09	117.5	115.9	22.1	21.4	118.9	117.3	16.3	16.0	108.5	107.4
7	24.54	23.68	124.1	122.3	24.5	24.8	123.3	122.7	18.0	17.6	113.9	112.8
8	27.26	26.35	130.0	128.0	26.4	26.1	127.9	126.8	19.7	19.4	119.3	118.2
9	29.94	28.94	135.5	132.9	30.0	29.7	133.6	132.3	21.5	21.3	123.7	122.9
10	32.61	31.89	140.3	138.6	32.4	33.5	138.5	138.5	23.5	23.6	128.4	128.4
11	35.20	35.74	144.2	144.7	35.3	36.5	143.4	144.1	25.9	26.4	133.4	133.6
12	38.28	39.74	149.6	151.9	38.8	42.6	148.9	150.3	28.5	29.8	138.3	139.2
13	42.18	44.95	155.0	157.1	42.9	44.4	154.9	153.0	32.1	33.3	144.6	143.9
14	48.81	49.17	162.7	159.6	48.3	46.7	161.7	155.1	35.7	36.8	150.1	147.5
15	54.48	51.48	167.8	161.1	52.2	48.2	165.3	155.3	39.6	36.8	155.5	149.6
16	58.83	53.07	171.6	162.2	55.4	49.8	168.4	155.4	43.2	41.1	159.5	151.0
17	61.78	54.02	173.7	162.5	59.0	49.9	168.9	156.4	45.7	42.4	161.4	151.5
18	63.05	54.39	174.5	162.5	—	—	—	—	47.4	42.4	163.1	151.7

[1] Figures for 50th percentile from data obtained in studies of Child Health and Development, Department of Maternal and Child Health, Harvard School of Public Health.

[2] Data for children belonging to high income group
[3] Data for children belonging to all income groups
} Nutrition Atlas of India 2nd Ed. I.C.M.R., 1971.

Table 19.2 **Balanced diets (g) at high cost for school children and adolescents**

Foodstuffs	Children				Adolescents					
					Boys				Girls	
	7–9 yrs.		10–12 yrs.		13–15 yrs		16–18 yrs.		13–18 yrs	
	V	NV	V	NV	V	NV	V	NV	V	NV
Cereals	200	200	260	260	370	370	390	390	290	290
Pulses	40	30	40	30	50	30	50	30	50	30
Green leafy vegetables	75	75	100	100	100	100	100	100	150	150
Other vegetables (roots and tubers)	50	50	75	75	75	75	100	100	75	75
Fruits	100	100	100	100	100	100	100	100	100	100
Milk	1000	700	1000	700	1000	700	1000	700	1000	700
Fats and oils	30	30	35	35	35	40	45	50	35	40
Meat, fish and eggs	—	90	—	90	—	120	—	120	—	120
Sugar and jaggery	50	50	50	50	30	30	40	40	30	30
Peanut (roasted)	—	—	—	—	—	—	50	50	—	—

V: Vegetarian NV: Non-vegetarian

Table 19.3 **Balanced diets (g) at moderate cost for school children and adolescents**

Foodstuffs	Children				Adolescents					
					Boys				Girls	
	7–9 yrs		10–12 yrs		13–15 yrs		16–18 yrs		13–18 yrs.	
	V	NV	V	NV	V	NV	V	NV	V	NV
Cereals	220	220	290	290	400	400	420	420	320	320
Pulses	70	60	70	60	70	50	70	50	70	50
Green leafy vegetables	75	75	100	100	100	100	100	100	150	150
Other vegetables (roots and tubers)	50	50	75	75	150	150	175	175	150	150
Fruits	100	100	100	100	100	100	100	100	100	100
Milk	600	400	600	400	600	400	600	400	600	400
Fats and oils	30	30	30	30	30	30	40	40	30	30
Meat, fish and eggs	—	60	—	60	—	80	—	80	—	80
Sugar and jaggery	30	30	30	30	30	30	30	30	30	30
Peanut	—	—	—	—	—	—	50	50	—	—

V: Vegetarian NV: Non-vegetarian

Table 19.4 **Balanced diets (g) at low cost for children and adolescents (I.C.M.R. Expert Group, 1968)**

Foodstuffs	Children						Adolescents			
							Boys		Girls	
	7-9 yrs		10-12 yrs		13-15 yrs		16-18yrs		13-18 yrs	
	V	NV	V	NV	V	NV	V	NV	V	NV
Cereals	250	250	320	320	430	430	450	450	350	350
Pulses	70	60	70	60	70	50	70	50	70	50
Green leafy vegetables	75	75	100	100	100	100	100	100	150	150
Other vegetables (roots and tubers)	50	50	75	75	150	150	175	175	150	150
Fruits	50	50	50	50	30	30	30	30	30	30
Milk	250	200	250	200	250	150	250	150	250	150
Fats and oils	30	30	35	35	35	40	45	50	35	40
Meat, fish and eggs	—	30	—	30	—	30	—	30	—	30
Sugar and jaggery	50	50	50	50	30	30	40	40	30	30
Peanut (roasted)	—	—	—	—	—	—	50*	50*	—	—

V: Vegetarian NV: Non-vegetarian

* an additional 30 g. fats and oils can be included in the diet in the place of peanut.

SCHOOL LUNCH AND ITS ROLE IN IMPROVING THE HEALTH OF CHILDREN

School lunch is a measure for improving the health and nutrition of children. During recent years a great majority of schools in all Western countries and Japan have been providing nutritious lunches for children. In India, only a beginning in this direction has been made recently. It is hoped that in due course all children in schools will receive nutritious lunches based on regional food materials.

School lunch patterns in different countries

Experience has shown that school lunch programmes can be undertaken effectively both in food surplus and in food deficit countries. In the latter case, they help to ensure that the best use is made of available foodstuffs. The composition of school lunches in U.S.A. is given in Table 19.5.

Table **19.5 Composition of school lunches in U.S.A.**

Constituent	Type A*	Type B†	Type C‡
Milk (whole)	½ pint	½ pint	½ pint
Fresh or processed meat, poultry, cheese or fish	2 ounces	1 ounce	...
Beans, cooked	½ cup	½ cup	...
Egg	One	Half	...
Vegetables and fruits (raw, cooked or canned)	6 ounces	4 ounces	...
Bread	1 portion	1 portion	...
Butter or margarine	2 teaspoons	1 teaspoon	...

*Complete lunch, hot or cold, providing one-third to one-half of the day's nutritional requirement.

†A partially complete lunch, hot or cold, which is less adequate nutritionally.

‡One-half pint of whole milk as a beverage.

A regional Nutrition Committee for South East Asia convened by FAO in 1943 at Philippines suggested a general pattern for school lunch suitable for the region (Table 19.6).

Table **19.6 Pattern of school lunch (FAO)**

Foodstuffs	(Ounces/day/caput)
Cereals	2.25
Pulses	0.25
Fish (rich in Ca)	0.25
Leafy vegetables	1.00
Oil	0.25
Salt	0.16

A lunch of the type given in Table 19.6 provides about 400 calories and about one-third the daily requirements of several nutrients.

The School Health Committee set up by the Government of India (1960) suggested a lunch, the composition of which is given in Table 19.7.

Table **19.7 Pattern of school lunch (India)**

Foodstuffs	g/day/child
Cereals and millets	75
Pulses	30
Oils and fats	8
Leafy vegetables	30
Non-leafy vegetables	30

The Committee recommended that it is desirable to include a protein-rich supplement such as fish (15gms) or skim milk reconstituted (120gms) or Indian Multi-purpose food (15gm) in place of 15gm of pulses.

Processed protein foods as supplements to the diets of school children

In view of the shortage in the supply of milk and other protein rich foods of animal origin in the developing countries, successful attempts have been made by several workers on the development of processed protein foods based on locally available oilseed meals and legumes and fortified with essential vitamins and minerals and their use as supplements to the diets of school children. FAO and UNICEF have been helping various developing countries in the large scale production of supplementary foods (Swaminathan, 1964). Further details are given in Chapters 21 and 22, in Vol. II.

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CHAPTER 20

NUTRITION OF EXPECTANT AND NURSING MOTHERS

Maternal mortality records collected in different countries over a period of several years have shown that for women of all races reproduction involves considerable physiological stress, as pregnancy, parturition and lactation make great nutritional demands on their bodies and are accompanied by additional health hazards. Reports on maternal and infant mortality rates indicate that these are very high in India and other developing countries (Tables 20.1 and 20.2) where the mothers are poor and malnourished and live under insanitary conditions. There is no doubt that with improvements in the income, diet and sanitary conditions, the maternal and infant mortality rates will be reduced and the nutrition and health of the mothers and infants will improve in the developing countries.

Table 20.1 **Infant and child mortality in countries (A) where protein-calorie malnutrition is unknown or extremely rare and (B) where it has been frequently encountered**

Country	Infant mortality per 1,000 live births
A	
Canada	23.6
Czechoslovakia	23.7
England	19.6
France	21.7
Japan	18.5
U.S.A.	23.4
B	
U.A.R. (Egypt)	130.5
Chile	107.1
Costa Rica	75.1
Mexico	60.7
Ceylon	57.5
China (Taiwan)	33.3
India	69.0
Philippines	109.2

Table 20.2 **Maternal mortality rates/100,000 live births in some selected countries**

U.S.A.	...	33.1
Denmark	...	15.6
Finland	...	42.3
Netherlands	...	33.1
Sweden	...	19.6
England and Wales	...	25.9
Northern Ireland	...	17.5
Scotland	...	23.0
Australia	...	32.7
New Zealand	...	32.2
India	...	253.0

PREGNANCY

Foetal development is accompanied by extensive changes in maternal body composition and metabolism. There is considerable increase in the nutritional needs of mother as pregnancy advances.

Physiological adjustments during pregnancy

The various physiological adjustments in pregnancy are briefly discussed below:

Energy metabolism: Basal metabolism increases during pregnancy. Hytten and Leitch (1964) concluded that the increased energy cost due to the development of the foetus and other physiological changes averages about 150 Kcal per day during the later half of pregnancy. There is some evidence indicating that the anabolic activity may be increased.

Alimentary function: There is some evidence of reduced gastric tone, motility and secretion. Nausea and constipation are commonly observed. The efficiency of absorption of calcium, iron, vitamin B_{12} and certain other nutrients is increased. More research is needed to establish definitely the increased efficiency of absorption of different nutrients during pregnancy particularly at lower levels of nutrient intake and with diets containing various supplementary foods.

Renal function: The glomerular filtration rate is considerably increased in pregnancy as are the clearances of several substances such as creatinine, urea, uric acid etc. The rate of excretion of water is very high in mid-pregnancy and very low in advanced pregnancy. Mild glycosuria is common. Free amino acids are excreted in increased amounts in the urine. There is increased loss of iodine in urine while the excretion of calcium and certain B-vitamins in urine has been found to decrease.

Blood composition: The plasma volume increases on an average by about 50 per cent and the red cell mass by about 20 per cent. The concentrations of haemoglobin and the packed cell volume usually fall despite the absolute increase in total haemoglobin. The total serum protein also falls but not all fractions change to the same extent. There is a decrease in the concentration of vitamin A, ascorbic acid and increase in the concentration of carotene, tocopherols, cholesterol and N-methyl nicotinamide.

Water balance: The total body water may increase by as much as seven litres and in late pregnancy the kidney may have some difficulty in disposing of the surplus water ingested. Hytten and Leitch (1964) estimated the total body water in their patients (gaining 12.5 kg. in body weight) to be 7000 g. They could account for about 5800 g. on theoretical grounds and postulated that the difference of 1200 g. which appeared only in the last 10 weeks of pregnancy probably existed as extracellular fluid.

The nature of weight gain in pregnancy: The weight gain in pregnancy, its nature and significance should be considered in both well-fed and poorly fed populations. Most average weight gains in well-nourished pregnant women reported from U.S.A. and Europe range between 10 and 12 kg. A limited amount of data reported from India and West Africa indicate that the weight gains of poorly fed pregnant women from rural communities are between 5 and 7 kg. These values are very much lower than those reported for well-fed pregnant women in Western countries.

Average healthy well-fed Scottish women were estimated to gain 12.5 kg. during pregnancy as calculated by Hytten and Leitch (1964). The weight gains of foetus, placenta, liquor amnii, uterus, mammary glands and increase in maternal blood volume, accounted for about 7300 g. out of a total gain of 12,500 g. About

5200 g. of the weight gain could not be accounted. By determining the total body water at intervals, Hytten and Leitch (1964) concluded that 1 kg. of body weight not accounted for at term, may represent extracellular fluid. The rest (4 kg.) has been considered to consist of body fat. This could be true in well-fed communities, as serial skinfold measurements have indicated such an increase in body fat.

Factors affecting the course and outcome of pregnancy

The clinical course of pregnancy and child birth depends on a number of factors such as genetic, dietary and environmental factors. The socio-economic factors influence profoundly the quality of diet consumed and exposure to infection and infestation as a result of living in insanitary surroundings. The standard of medical care available also influences the outcome of pregnancy.

Diet: Diet as a single factor has probably a profound influence on the health of the mother. Consumption of ill-balanced and inadequate diets leads to deterioration in the physical and mental strength, development of anaemia and general weakness.

Foetal and neonatal mortality: Stillbirth and neonatal death rates usually are high among pregnant women belonging to low socio-economic status. The rates are, in general, low in the advanced countries and high in the developing countries. There are a few exceptions. For example, the rates among poor class Chinese women in Hong Kong have been reported to be low. The low standard of living, consumption of poor quality diets and living in insanitary surroundings being constantly exposed to infection and infestation, appear to be the most important contributory causes for the high mortality rates in the developing countries.

Adult body size in relation to birth weight of infants: It would be expected that tall women will have babies with greater body length and weight than those born to short women. This has been, in general, found to be true. The results reported by various workers indicate that (1) babies born to short women are about 8 per cent lighter than babies born to tall women; (2) babies born to underweight women are about eight per cent lighter than babies born to over-weight women and (3) babies born to tall

and heavy women are about 14 per cent heavier than those born to short and light women. (Hytten and Leitch, 1964).

Birth weight and prematurity: There is a large amount of literature on birth weight of infants born to mothers of different socio-economic status. Despite the large variation in the socio-economic status of the mothers in the different countries, the results are remarkably constant. In most European and American countries, the mean birth weight is of the order of 3300 g. In many of the developing countries, the mean birth weights among the well-to-do sectors of the people range from 2850 to 3250 g. while among low income groups the mean weights range from 2580 to 2880 g. There is definite evidence that low socio-economic status of the mother is associated with low birth weight of infants.

Premature infants have low birth weights. The cause for low birth weight in premature children may be due to differences in the foetal growth and duration of gestation. Babies of low birth weights have relatively high mortality rates (de Silva *et al.*, 1962).

Pre-eclampsia and eclampsia: The toxaemias of pregnancy are difficult to define with sufficient precision as the causative factors are not fully known. The United Kingdom Obstetric Medical Research Unit has adopted the working definition of pre-eclampsia suggested by Nelson (1955) which is as follows: A rise in diastolic blood pressure to 90 mm. of mercury or higher after the 24th week of pregnancy, without proteinuria (mild pre-eclampsia) or with definite proteinuria not attributable to infection (severe pre-eclampsia). Oedema is neglected in this definition. Thomson (1959) found that women who developed pre-eclampsia consumed on the average, diets of higher calorie value and nutrient content than those who maintained normal health. It is possible that high intake of salt and low intake of proteins and certain B vitamins may influence the onset of the condition. There is evidence that lack of antenatal care is an important contributory cause for the development of pre-eclampsia and eclampsia.

Anaemia: The concentration of haemoglobin in the blood tends to be lower during pregnancy than in normal women, because the plasma volume increases by about 50 per cent on the

average and the volume of circulating red cells by about 20 per cent. The resulting fall in haemoglobin concentration may be about 2 g./100 ml. of blood. The physiological change resembles the manifestations of iron deficiency anaemia and the haemoglobin concentration can be increased by giving iron salts in therapeutic quantities. The WHO study group (1959) on 'Iron deficiency anaemia' has suggested that a haemoglobin concentration of less than 10 g./100 ml. is an indication of anaemic condition. The most important causes of iron deficiency anaemia are (*i*) increased blood loss due to systemic or intestinal parasitism (*ii*) increased iron requirements for foetal growth or impaired iron absorption or both (*iii*) low iron intake and low physiological availability of dietary iron and (*iv*) too frequent pregnancies with consequent inability of the body to make up the blood losses in short periods. In addition to iron deficiency anaemia, megaloblastic anaemias due to deficiency of folic acid and less often due to deficiency of vitamin B_{12}, also occur in a fair proportion of pregnant women in the developing countries (WHO 1959).

Vomiting in pregnancy: In the early pregnancy, most women suffer from nausea and vomiting. The resulting metabolic changes may be mild, moderate or severe. The pregnant woman must be under the care of the physician who will give appropriate treatment. It is obvious that undernutrition and malnutrition will result due to lack of food intake. Consequently, greater amounts of nutrients will have to be given after the stoppage of vomiting in order to make up for the dietary deficiency in the early months of pregnancy caused by vomiting.

Nutritional requirements during pregnancy

The requirements of various nutrients during pregnancy are briefly discussed below:

Calories: Hytten and Leitch (1964) have estimated the average net changes in the amount of protein and fat deposited during pregnancy. The total calorie cost of supplying and maintaining the new tissues approximates to 80,000 Kcal per pregnancy of which more than 40,000Kcal is accounted for by fat storage. It is important to note that these calculations are referred to a weight gain of 12.5kg. It has already been mentioned that women in developing countries may have body weight gains of only 6 kg.

It is probable that women under these conditions would deposit much less fat. In the above case, the calorie requirements per pregnancy will be reduced by 40,000 Kcal. Further allowance has to be made for the spontaneous decrease in physical activity that occurs particularly during the third trimester. The second FAO Committee (1957) on 'Calorie Requirements' therefore, was of the opinion that the additional calorie requirements per pregnancy will be 40,000 Kcal. The NRC, USA (1968) recommended an extra allowance of 200 Kcal per day while the ICMR Nutrition Expert Group (1968) recommended an extra allowance of 300 Kcal per day.

Proteins: The joint FAO/WHO Expert Group (1965) on 'Protein Requirements' accepted evidence that about 910 g. of protein is deposited in the body during pregnancy in the foetus and maternal tissues. The average daily increment is computed to be about 5 g. (as tissue protein) during the last six months of pregnancy. This will work out to 10 g. additional protein in terms of dietary protein of N.P.U. 50. The N.R.C. of U.S.A. and the I.C.M.R. Nutrition Expert Group recommended an extra allowance of 10 g. per day.

Calcium: The joint FAO/WHO Expert Group (1962) on 'Calcium Requirements' accepted evidence that about 30 g. of calcium is deposited in the foetus during pregnancy. The same group recommended an extra daily allowance of 500 mg to 600 mg. calcium to meet the needs of the third trimester of pregnancy. The NRC of U.S.A. recommended an extra allowance of 400 mg. per day, while the I.C.M.R. Nutrition Expert Group recommended an extra allowance of 500 to 600 mg. per day.

Iron: At term, the foetus contains about 300 mg and the placenta about 70 mg of iron. Early in pregnancy, the maternal red blood cell mass begins to increase. At term, this increase may represent, on the average, an increment of 290 mg. of iron. Due to cessation of menstruation, iron loss is minimised to the extent of 120 mg for the period of pregnancy. The additional iron requirements will be 540 mg when all the above factors are taken into account. A large part of the iron must be made available in the second half of pregnancy and would amount to 2-3 mg of iron per day. Since the iron from diets is utilized only to an extent of about 20 per cent, the extra iron requirements in terms

of dietary iron will be about 10 to 15 mg. per day. The I.C.M.R. Nutrition Expert Group recommended an extra allowance of 10 mg/day.

Vitamin A: The liver of the infant at birth will contain about 5,400 to 7,200 μg. of retinol. In order to deposit this amount, about 25 μg. of retinol per day will be drawn from the maternal stores during pregnancy. This figure will be increased by the quite unknown but probably limited additional amounts resulting from any inefficiency in transfer across the placenta. Assuming that the habitual diet of the woman provides 750 μg. of retinol per day, the FAO/WHO Expert Group (1967) on 'Vitamin Requirements' assumed that increase in the total daily requirements associated with pregnancy can be met without increasing the recommended intake for the adult. The N.R.C. of U.S.A., however, recommended an extra allowance of 330 μg. vitamin A during pregnancy, while the I.C.M.R. Nutrition Expert Group has not recommended any additional allowance.

Thiamine: The FAO/WHO Expert Group on 'Vitamin Requirements' did not recommend any extra allowance for thiamine during pregnancy. In view of the fact that small amounts of thiamine are stored in the tissue of new born infants, it is desirable to give an extra allowance of thiamine. The NRC of U.S.A. has recommended an extra allowance of 0.1 mg. while the I.C.M.R. Nutrition Expert Group has recommended an allowance of 0.2 mg/day to meet the additional needs of pregnancy.

Riboflavin: The FAO/WHO Expert Group on 'Vitamin Requirements' did not recommend any extra allowance for riboflavin during pregnancy. In view of the fact that small amounts of riboflavin are found in the tissues of new born infants, it is desirable to provide an extra allowance of riboflavin during pregnancy. The N.R.C. of U.S.A. recommended an extra allowance of 0.3 mg. riboflavin, while the I.C.M.R. Nutrition Expert Group recommended an extra allowance of 0.2 mg/day.

Nicotinic acid: Since nicotinic acid and co-enzymes I and II containing icotinamide are found in the tissues of new born infants, it is desirable to give an extra allowance to meet the needs of pregnancy. The FAO/WHO Committee on 'Vitamin Requirements' did not recommend any additional allowance for nicotinic acid in pregnancy. The NRC of U.S.A. recommended

an extra allowance of 2 mg. nicotinic acid/day and the I.C.M.R. Nutrition Expert Group also recommended 2 mg/day.

Folic acid: The NRC of U.S.A. recommended an extra allowance of 400 μg. as *total* folic acid determined by *L-casei* while the I.C.M.R. Nutrition Expert Group recommended an extra allowance of 50 to 200 μg. of *free* folic acid determined by *L-casei.*

Vitamin B_6: The NRC of U.S.A. has recommended an extra allowance of 0.5 mg. over and above 2.0 mg. vitamin B_6 required for normal women.

Vitamin B_{12}: The NRC of U.S.A. has suggested an additional requirement of 3 μg. over the normal requirement of 5 μg. for an average woman to meet the needs of pregnancy. The recommendations of the I.C.M.R. Nutrition Expert Group are very much less than the above recommendations—normal women 1.0 μg. and pregnant women 1.5 μg.

Ascorbic acid: The NRC of U.S.A. has suggested an additional allowance of 5 mg. over the requirements of 55 mg. for a normal woman. The I.C.M.R. Nutrition Expert Group is of the opinion that the allowance of 50 mg. for a normal woman would meet the additional requirements of pregnancy. The nutritional requirements for expectant and nursing mothers suggested by various Expert Groups are given in Table 20.3.

Effect of malnutrition and socio-economic factors on the nutritional status of pregnant women

A large majority of pregnant women in India and other developing countries consume inadequate and ill-balanced diets. The adverse effects of consuming such diets on the nutritional status of the women and of the new born are briefly discussed below:

Diet and nutrition surveys: Diet and nutrition surveys carried out in India have shown that the diets of pregnant women of low income groups are deficient in almost all dietary essentials. The calorie intake ranged from 1200 to 1800 Kcal and the protein intake from 30 to 40 g/day. The diets were deficient in proteins, essential minerals and vitamins. Iron deficiency anaemia was widely prevalent. Manifestations of signs and symptoms due to deficiencies of vitamin A and B vitamins were common.

Table 20.3 **Recommended allowances for pregnancy and lactation**

Country	Weight	Calories (Kcal)	Protein (g)	Calcium (g)	Iron (mg.)	Vitamin A (I.U.)	Vitamin D (I.U.)	Thiamine (mg.)	Riboflavin (mg)	Niacin (mg)	Ascorbic acid (mg)
1	2	3	4	5	6	7	8	9	10	11	12
United States (1968)						(a)				(b)	
Non-pregnant (18–22 yrs)	128 lb.	2000	55	0.8	18	5000	400	1.0	1.5	13	55
Non-pregnant (22–35 yrs)	128 lb.	2000	55	0.8	18	5000	—	1.0	1.5	13	55
Non-pregnant (35–55 yrs)	128 lb.	1850	55	0.8	18	5000	—	1.0	1.5	13	55
Pregnancy		+ 200	65	+0.4	18	6000	400	+0.1	1.8	15	60
Lactation		+1000	75	+0.5	18	8000	400	+0.5	2.0	20	60
Canada (1963)						(c)					
Non-pregnant	124 lb.	2400	40	0.5	10	3700	—	0.7	1.2	7	30
Pregnancy		2900	50	1.2	13	4200	400	0.9	1.5	8.5	40
Lactation		2900–3400	60–70	1.2	13	5200	400	1.0	1.7	10	50
Great Britain (1969)											
Non-pregnant	121 lb.					(d)					
Most occupations		2200	55	0.5	12	2500	100	0.9	1.3	15	30
Very active		2500	63	0.5	12	2500	100	1.0	1.3	15	30
Pregnancy		2400	60	1.2	15	2500	400	1.0	1.6	18	60
Lactation		2700	68	1.2	15	4000	400	1.1	1.8	21	60

Table **20.3** (*Contd.*) **Recommended allowances for pregnancy and lactation**

Country	Weight	Calories (Kcal)	Protein (g)	Calcium (g)	Iron (mg.)	Vitamin A (I.U.)	Vitamin D (I.U.)	Thiamine (mg.)	Riboflavin (mg)	Niacin (mg)	Ascorbic acid (mg)
Japan (1961)						(e)					
Non-pregnant		2100	60	0.6	10	2000	400	1.1	1.1	11	60
Pregnant 1–5 months		2400	75	0.7	12	2500	400	1.5	1.5	15	80
6–9 months		2700	90	1.4	15	2500	400	1.8	1.8	18	100
Lactation		2700	90	1.4	15	2500	400	1.8	1.8	18	100
Central America (INCAP) (1955)											
Non-pregnant	50 kg.	2000	50	0.7	10	1.3 mg.		1·0	1.2	10	45
Pregnant 7–9 months		2500	75	1.3	14	1.6 ,,		1.2	1.8	12	65
Lactation		3000	90	1.8	14	2.1 ,,		1.5	2.2	15	95
India (1968)											
Women: Sedentary work	45 kg.	1900	45	0.4–0.5				1.0	1.0	13	50
Women: Moderate work		2200			30	750 μg 3000		1.1	1.2	15	
Pregnancy (second half of pregnancy)		+300	+10	1.0	40	750 ,, 3000	200	+0.2	+0.2	+2	50
Lactation (upto 1 yr.)		+700	+20		30	1150,, 4600		+0.4	+0.4	+5	80

(a) 1/3 vitamin A: Vitamin A: Carotene=2:1
(b) Expressed as 'Niacin equivalents'.
(c) ½ vitamin A: Vitamin A: Carotene activity=3:1
(d) 1/3 vitamin A: Vitamin A: Carotene activity=3:1
(e) all as vitamin A

Gain in body weight and body composition in pregnancy: The mean gain in body weight during pregnancy in under-nourished women ranged from 6.0 to 6.5 kg in India and other developing countries. This is very much less than that (12.5 kg) reported for pregnant women in Western countries.

Biochemical assessment of the nutritional status of pregnant women: Most of the constituents in the blood showed decrease in concentration between the first and third trimesters and serum albumin levels were reduced. The levels of vitamin A, vitamin C and folic acid were reduced. Anaemias due to iron and folic acid deficiencies were widely prevalent.

Nitrogen balance studies: Nitrogen balance studies were carried out at four levels of protein intake—60, 84, 106 and 118 g/day. On the lowest level, 50 per cent of the women were in negative balance. There was a general trend towards an increase in the amount of nitrogen retained with increase in protein intake. The study showed that the optimal protein intake for maximum nitrogen retention was between 84 and 106 g daily.

Iron and calcium absorption: The efficiency of iron absorption improved with progress of pregnancy. Calcium retention also increased, the retention during the third trimester being much higher than that observed in the first and second trimesters.

Nutritionl deficiency syndromes: Anaemias due to deficiency of iron and less commonly due to the deficiencies of folic acid and vitamin B_{12} are prevalent among pregnant women in the developing countries. Manifestations of vitamin B complex and vitamin A deficiencies were faily common.

Diets of pregnant women in different countries

The results of surveys show that the diets consumed by pregnant women in Europe and America meet the nutritional requirements recommended by the N.R.C. of U.S.A., while the diets consumed by pregnant women in the developing countries are deficient in calories, proteins, some minerals and vitamins. The results are briefly discussed below:

Calories: The mean daily calorie intakes of pregnant women in the developed countries range from 2400 to 2700 Kcal as compared with values of 1500 to 2000 Kcal in the developing

countries which are very much less than the requirements of 2300 to 2800 Kcal.

Proteins: The mean daily protein intakes of pregnant women in developed countries range from 70 to 85 g while in the developing countries, the protein intakes range from 31 to 45 g among the low income groups. The protein intakes in the developing countries are very much less than the recommended allowances of 65 to 75 g.

Calcium: The mean daily intakes of calcium by pregnant women in the developed countries range from 800 to 1200 mg, while in the developing countries the calcium intakes are low ranging from 300 to 400 mg as compared with requirements of 1000 to 1200 mg.

Iron: The mean intakes in pregnant women in the developing countries range from 8 to 18 mg. Further, the availability of iron from the diet is poor as the diet contains large amounts of phytic acid which interfere in the absorption of iron. This may account for the high incidence of iron deficiency anaemia in pregnant women in the developing countries.

Vitamin A: The intakes of vitamin A by pregnant women in the developed countries range from 6000-8000 I.U. while the corresponding values for women in developing countries are 900 to 2000 I.U. It is obvious that the mean intakes of vitamin A are much below the requirements.

B-Vitamins: The mean daily intakes of B-vitamins by pregnant women are very much less than the recommended allowances. The diets have been found to be deficient in riboflavin, folic acid and vitamin B_{12}.

Balanced diets for pregnant women

Balanced diets for pregnant women at high, moderate and low costs are given in Tables, 20.4, 20.5 and 20.6.

Balanced diets at high costs: These diets contain large amounts of protein rich and protective foods such as milk, eggs, meat and fish which are costly.

Balanced diets at moderate costs: These diets contain moderate amounts of milk and other animal foods.

Table 20.4 **Balanced diets (g) for normal, pregnant and lactating women (High cost)**

Foodstuffs	(Normal women) Sedentary work		Moderate work		Heavy work		Additional allowances during Pregnancy		Lactation	
	V (g)	NV (g)	V (g)	NV (g)	V (g)	NV (g)	V (g)	NV (g)	V (g)	NV (g)
Cereals	230	220	280	270	410	400	—	—	70	70
Pulses	60	50	60	50	60	50	—	—	—	—
Green leafy vegetables	100	100	100	100	100	100	—	—	—	—
Other vegetables	75	75	75	75	100	100	—	—	—	—
Roots and tubers	50	50	75	75	100	100	—	—	—	—
Fruits	100	100	100	100	100	100	50	50	50	50
Milk	600	400	600	400	600	400	500	200	700	300
Cheese	40	—	40	—	40	—	20	—	20	—
Fats and oils	30	35	35	40	40	45	—	—	20	20
Sugar and jaggery	30	30	30	30	40	40	—	—	20	20
Meat and fish	—	100	—	100	—	100	—	25	—	40
Eggs	—	60	—	60	—	60	—	—	—	—
Groundnuts	—	—	—	—	40	40	—	—	—	—

Table 20.5 **Balanced diets (g) for normal, pregnant and lactating women (Moderate cost)**

Foodstuffs	(Normal women) Sedentary work		Moderate work		Heavy work		Additional allowances during Pregnancy		Lactation	
	V (g)	NV (g)	V (g)	NV (g)	V (g)	NV (g)	V (g)	NV (g)	V (g)	NV (g)
Cereals	260	250	310	300	440	425	—	—	110	100
Pulses	60	50	60	50	60	50	20	—	40	—
Green leafy vegetables	100	100	100	100	100	100	—	—	—	—
Other vegetables	75	75	75	75	100	100	—	—	—	—
Roots and tubers	50	50	75	75	100	100	—	—	—	—
Fruits	60	60	60	60	60	60	50	50	50	50
Milk	400	250	400	250	400	250	400	200	600	300
Fats and oils	30	35	35	40	40	45	—	—	20	20
Sugar and jaggery	30	30	30	30	40	40	—	—	20	20
Meat and fish	—	60	—	60	—	60	—	25	—	40
Eggs	—	30	—	30	—	30	—	—	—	—
Groundnut	—	—	—	—	40	40	—	—	—	—

Table 20.6 **Balanced diets for normal, pregnant and lactating women (Low cost) I.C.M.R. Expert Group 1968**

Foodstuffs	(Normal women) Sedentary work		Moderate work		Heavy work		Additional allowances during Pregnancy	Lactation
	V (g)	NV (g)	V (g)	NV (g)	V (g)	NV (g)	(g)	(g)
Cereals	300	300	350	350	475	475	50	100
Pulses	60	45	70	55	70	55	—	10
Green leafy vegetables	125	125	125	125	125	125	25	25
Other vegetables	75	75	75	75	100	100	—	—
Roots and tubers	50	50	75	75	100	100	—	—
Fruits	30	30	30	30	30	30	—	—
Milk	200	100	200	100	200	100	125	125
Fats and oils	30	35	35	40	40	45	—	15
Sugar and jaggery	30	30	30	30	40	40	10	20
Meat and fish	—	30	—	30	—	30	—	—
Eggs	—	30	—	30	—	30	—	—
Groundnuts	—	—	—	—	40*	40*	—	—

*An additional 25 g. of fats and oils can be included in the diet in place of groundnuts.

Balanced diets at low costs: These diets contain minimal amounts of milk and other animal foods which are costly. The diets, however, include fair amounts of legumes and green leafy vegetables.

LACTATION

Though lactation is a normal physiological process, it makes considerable nutritional demands on the mother. The physiological developments for lactation begin during later part of pregnancy. Apart from the growth and development of mammary glands energy reserves are laid down in the form of fat in the body of the mother and this may become available in part to provide the extra energy required during lactation.

Lactation in relation to the growth and health of infants

Mother's milk in sufficient quantity is probably the best food for infants in the first months of life. Its chemical composition seems to be the most appropriate for the needs of the infant. Breast feeding brings about a close relationship between mother and infant and promotes the optimal physical and mental development of the infant; furthermore, by reducing the hazard to infection associated with artificial feeding, it helps to protect the infant's health.

Indicators of the adequacy of lactation: The simplest indicator of the adequacy of breast feeding is the normal rate of increase in body weight of the infant. Comparison of growth curves from different countries and communities is useful, particularly when based on longitudinal data. It is difficult to define the ideal growth curve. However, for practical purposes a gain in weight of 800 ± 20 g. per month during the first six months of life or the doubling the birth weight by about the beginning of fifth month may be regarded as optimal.

Milk yield: Many factors may influence the volume of milk produced. These include age, parity, state of health, nutrition of mother and the duration of lactation period. The average daily milk yield in successful lactation in Western women is about 500 ml. in the first month. This rises to about one litre in the fifth month and steadily declines later. Two techniques exist for measuring the milk yield by the mother (1) to weigh the child before and after each feed and (2) to empty the breast by hand or mechanically. Both the methods have been used in assessing milk yields by mothers.

Effect of mother's diet on the composition and output of milk: The mother's diet and state of nutrition are likely to affect the composition and output of milk. The feeding studies on lactating mothers carried out by Deem (1931) are of considerable interest as they were carried out under controlled conditions. Deem studied five healthy lactating women living in a home for unmarried mothers who were given for a week at a time each of the seven diets in succession. The results given in Table 20.7 indicate that (1) high fat diet increased slightly the fat content and output of milk and (2) a high protein diet increased slightly the milk output.

Table **20.7 Effect of diet on milk yield and composition**

Milk	Diet A	B	A	C	D	E	F
Yield (ml) ...	927	1037	1002	1091	1064	1075	1017
Protein (g/100 ml) ...	1.16	1.26	1.08	1.19	1.11	1.09	1.08
Fat (g/100 ml) ...	3.8	3.4	3.5	3.7	3.6	4.3	4.2
Lactose (g/100 ml) ...	7.67	7.41	7.53	7.40	7.41	7.28	7.36

Diet		Calories (Kcal)	Proteins (g)	Fat (g)	Carbohydrates (g)
A.	Institution ...	3132	87	96	458
B.	High protein ...	3203	147	88	388
C.	High protein+vitamin B (3 g Marmite) ...	2899	144	85	370
D.	High sugar ...	3905	90	98	641
E.	High fat ...	4315	88	251	399
F.	Low protein ...	2826	61	123	350

Composition of human milk: It is generally recognised that the chemical composition of human milk will be influenced to some extent by the following factors (*i*) composition of mother's diet and (*ii*) period of lactation. There is less information on the composition of mature milk during substantially prolonged lactation. Values given in Table 20.8 (WHO, 1965) indicate the wide variations in the literature values for iron and ascorbic acid. The Table also gives the nutrients present in 850 ml. of human milk which has been accepted by the WHO Committee as a realistic basis for estimating the physiological cost of milk production.

Nutritional requirements of nursing mothers

The WHO Expert Committee assumed the optimal daily milk output of mother's milk to be 850 ml. This would provide 600 Kcal., 10.2 g. protein, 290 mg. calcium, 0.25 to 3.1 mg. iron, 420μg. vitamin A, 22 to 44 mg. ascorbic acid, 1.6 mg. niacin, 0.52 mg. riboflavin, 0.12 mg. thiamine, 9.0 μg. folic acid, and 0.2 μg. vitamin B_{12}. The efficiency of conversion of food energy into milk solids is believed to be about 80 per cent while that of protein about 50 per cent and that of calcium about 30 per cent. The efficiency of absorption and secretion of dietary vitamins in milk

Table 20.8 **Nutrients in mature human milk**

Nutrients	Conc./100 ml.	Content in 850 ml.[1]
Protein (g)	1.2	10.2
Calcium (mg)	34	290
Iron[2] (mg)	0.03	0.25
	0.36	3.1
Vitamin A (μg)	49	420
Ascorbic acid[3] (mg)	2.6	22
	3.7	32
	5.2	44
Niacin (mg)	0.18	1.6
Riboflavin (μg)	37.3	320
Thiamine (μg)	14	120

[1] Assumed average daily volume for whole of lactation.

[2] The difference between these two averages is probably a result of the methods used, and the common practice of quoting the averages of the two is not justifiable.

[3] The first figure cited relates to poor Indian women, the third to women living in U.S.A.

is not known. It is therefore evident that the lactating mother will need extra amounts of all the nutrients at levels somewhat greater than those present in milk depending on the efficiency of the incorporation in milk of the nutrients present in the diet. The I.C.M.R. Nutrition Expert Committee assumed the average amount of milk secreted by lactating women in India and other developing countries to be 600 ml. According to this estimate, the nutritional needs of lactation will be less than those of the WHO Expert Committee. The nutritional requirements of nursing mothers suggested by various Expert Committees are given in Table 20.3 and are briefly discussed below:

Calories: The FAO Committee on 'Calorie requirements' used an efficiency coefficient of 60 per cent for the conversion of diet calories into milk calories. Hytten and Thompson (1961) proposed a higher coefficient of 80 per cent. According to the above estimates the dietary calorie requirements for the production of 850 ml. of milk will be 1000 Kcal. and 750 Kcal. respectively.

Proteins: The quantity of proteins present in 850 ml. of human milk is about 10.2 g. Since human milk proteins possess NPU of

about 87 and that of dietary proteins consumed in Western counries is about 60 and in the developing countries about 50, the additional dietary protein requirements will be about 15 g. for Western diets and 18 g. for diets consumed in developing countries. The FAO/WHO Committee recommended an additional allowance of 15 g. while the NRC of USA and I.C.M.R. Nutrition Expert Group recommended 20 g. additional protein during lactation.

Calcium: The quantity of calcium present in 850 ml of milk will be 290 mg. On the assumption that the retention of dietary calcium in lactating women to be about 30 per cent, the extra dietary calcium will be 0.970 g. daily. Duckworth and Warnock (1942) concluded from a review of calcium balance data that the calcium requirements of lactating women lie between 1.5 and 2.0 g daily. The FAO/WHO Expert Committee recommended an additional allowance of 0.5 to 0.6 g., the N.R.C. of U.S.A., 0.5 g. and I.C.M.R. Nutrition Expert Group, 0.6 g.

Iron: The iron content of human milk reported in the literature varies from 0.03 to 0.36 mg/100 ml. Eight hundred and fifty ml of milk will contain about 0.25 mg to 3.1 mg of iron according to available data. Assuming that the retention of dietary iron in lactating women to be about 30 per cent, the extra dietary iron requirements will be about 10 mg. This is in accordance with the recommendations of the I.C.M.R. Nutrition Expert Group.

Vitamin A and Carotene: The WHO Expert Committee on 'Nutrition in Pregnancy and Lactation' assessed the vitamin A content of 850 ml. of human milk to be 420 μg. and suggested that an additional allowance of 450 μg vitamin A to meet the above needs. Since a greater part of vitamin A activity in diets consumed in India and other developing countries is contributed by β-carotene, the extra needs of β-carotene for lactation for providing 450 μg. vitamin A will be 1600 μg. as suggested by the I.C.M.R. Nutrition Expert Group.

Thiamine: According to the FAO/WHO Expert Group on "Vitamin Requirements" 0.4 mg. of thiamine will be required per 1000 Kcal. Since the extra calories required during lactation is about 1000 Kcal/day the thiamine needs will be met by an extra quantity of 0.4 mg/day. This is in agreement with the recommendations of the I.C.M.R. Nutrition Expert Group.

Riboflavin: The riboflavin content of 850 ml of human milk has been estimated to be 0.32 mg. According to the FAO/WHO Expert Group on "Vitamin Requirements" the riboflavin requirements per 1000 Kcal. will be 0.55 mg. Since the extra calori eneeds of lactation are 1000 Kcal, riboflavin needs will be met by an additional 0.55 mg. The I.C.M.R. Nutrition Expert Group recommended 0.4 mg.

Niacin: According to the FAO/WHO Expert Group on 'Vitamin Requirements,' the requirements of niacin will be 6.6 mg/1000 Kcal. The I.C.M.R. Nutrition Expert Group recommended 5.0 mg. additional niacin to meet the needs during lactation.

Ascorbic acid: The ascorbic acid content of 850 ml of human milk will range from 22 to 44 mg. The I.C.M.R. Nutrition Expert Group recommended an additional allowance of 30 mg. of ascorbic acid.

Folic acid: Folic acid deficiency is common among expectant and nursing mothers in developing countries. According to the I.C.M.R. Nutrition Expert Group the needs of lactation will be met by an additional 50 μg. of free folic acid.

Vitamin B_{12}: According to the I.C.M.R. Nutrition Expert Group the additional vitamin B_{12} requirements during lactation will be 0.5 μg. while the N.R.C., U.S.A. has recommended 1.0 μg.

The effect of malnutrition on the nutritional status of nursing mothers

Studies on the dietary and nutritional status of poor nursing mothers and on the chemical composition and the output of milk have been carried out in India. The results are briefly discussed below:

Breast-feeding and weaning practices: Breast-feeding is usually started with in 2 or 3 days after delivery and is continued into the second year in some countries. In a survey covering different parts of South India, it was found that among poor communities, 92 per cent of the infants were breast-fed at the age of six months and one child in every five was at the breast beyond two years of age. In a recent survey of 3100 pre-school children in the rural community of Andhra Pradesh, more than 50 per cent of the infants were breast-fed even at the age of $1\frac{1}{2}$ years. Supplementary feeding was started around six months.

Diets of nursing mothers in developing countries: The diets consumed by nursing mothers in India have been found to be inadequate both in quantity and quality and deficient in calories, proteins, several vitamins and minerals. The mean body weight of nursing mothers of the low socio-economic group was found to be about 40 to 42 kg. Serial body weight changes recorded in 14 mothers throughout the period of lactation showed that while some of the mothers did not show any change, many lost weight to the extent of 2 to 7 kg. in a period of one year of lactation. Anaemia was fairly common. The levels of vitamin A, carotene and ascorbic acid in blood were low.

Output of milk: The output of milk was assessed by test feeding technique. The studies revealed that the output of milk ranged from about 600 ml daily during the first six months to 500 ml. in the first year and about 350 ml during the second year (Table 20.9). When the calorie and protein intakes of the mothers were increased to 2900 Kcal and 100 g. respectively, an appreciable increase in the output of milk was observed.

Table **20.9 Output of breast milk at different stages of lactation**

Weeks of Lactation	0-2	3-4	5-6	7-8	9-10	11-12	12-15	16-18	19-24	25-36	37-48
Number examined	20	18	14	14	17	30	11	29	14	12	4
Mean output of breast milk (ml)	530	640	730	660	600	525	515	440	400	425	345

Someswara Rao *et al* (1959), W.H.O. Bulletin **20**, 603.

Nitrogen and calcium metabolism: Nitrogen balance studies were carried out with nursing mothers at three levels of protein intake, 61 g., 99 g. and 114 g. and at the same level of calorie intake. The women were in positive nitrogen balance on 60 g. protein intake and nitrogen retention was optimal at 99 g. protein intake. Calcium balance studies were carried out on nursing mothers at calcium intakes of 420 to 480 mg. daily. All the mothers were in negative calcium balance.

Chemical composition of milk: The mean concentrations of total solids, calcium, phosphorus, proteins, vitamin A, riboflavin and vitamin C were significantly higher in early milk samples (within one month after delivery) than those observed in the later

stages of lactation (after one year). The levels of thiamine and folic acid were lower in early samples than in later samples. Supplementation of the mother's diet with large doses of vitamin A, ascorbic acid, thiamine and riboflavin brought about appreciable increases in the concentration of the respective vitamin in human milk.

Improvement of maternal nutrition in developing countries

Effect of food supplements on the nutritional status of malnourished expectant mothers and the new born: The diets consumed by a large majority of the population in the developing countries are lacking in protein-rich and protective foods. The most adversely affected are the vulnerable groups i.e., pregnant and lactating women and weaned infants and pre-school children. Public health nutritional measures should, therefore, be directed towards improvement of the diet of the family as a whole. The following studies carried out in India on the effect of maternal nutrition on the condition of the new born, are of considerable interest. A number of pregnant women belonging to the low income groups of the population were admitted to the hospital during the last trimester and received a good hospital diet for a period of 4 to 6 weeks. The diets supplied 2400 to 2500 Kcal. and 60 to 90 g. protein including a fair proportion of animal proteins. The average birth weight of infants born to these women was significantly higher than that of infants born to women of the same socio-economic group not receiving additional food. The general health and nutritional status of the women also improved as a result of additional food. The above studies have clearly shown the importance of adequate nutrition in improving the health and nutritional status of the pregnant mothers and the new born babies.

Methods for improving the health and nutritional status of expectant and nursing mothers in developing countries

Since milk and other animal foods are in short supplies in the developing countries, there is urgent need for the large scale production of processed protein foods based on oilseed meals and legumes and fortified with calcium and iron salts, vitamin A, riboflavin, folic acid and vitamin B_{12}. Studies carried out in India

and other countries have shown the possibilities of using such foods for overcoming malnutrition among mothers and pre-school children. Steps should, therefore, be taken urgently for the large scale production and distribution of processed protein foods as stressed in a recent report of the United Nations (1968). Well balanced diets at varying costs for nursing mothers are given in Tables 20.4-20.6. The composition of typical ill-balanced diets consumed by poor pregnant and nursing women in India are given in Table 20.10. The deficiencies in the diets could be made

Table **20.10 Diets of poor pregnant and nursing women in India and their improvement**[1] (g/day)

	Pregnant women	Nursing women
Habitual diet		
Cereals	276	333
Pulses	21	15
Leafy vegetables	8	9
Other vegetables	12	23
Roots and tubers	8	3
Fruits	26	4
Milk and milk products	41	23
Fats and oils	20	12
Flesh foods	22	20
Sugar and jaggery	14	15
Supplements		
Ragi	100	100
Other cereals	100	200
Indian Multi purpose food	25	50
Leafy vegetables	100	100
Vegetable oils and fats	—	50

Nutritive Value	Calories (Kcal)	Proteins (g)	Iron (mg)	Calcium (g)	Vit. A (I.U.)	Riboflavin (mg)
Pregnant women						
Habitual diet	1420	37	18	0.2	1210	0.41
Habitual diet+supplements	2500	69	40	1.0	6250	1.52
Nursing women						
Habitual diet	1425	39	1'	0.2	1220	0.42
Habitual diet+supplements	2900	71	4(	1.0	6310	1.64

[1]Diet Atlas of India, National Institute of Nutrition, pp. 53-54.

up by supplementing with extra cereals, Indian multipurpose food and green leafy vegetables as indicated in the same Table.

Large scale production and distribution of supplementary foods

The Governments of developing countries should take immediate steps to set up units for the large scale production of processed protein foods such as Indian multipurpose food. A daily supplement of 60g of the Indian multipurpose food would provide about 20 g. protein, 0.4 g. calcium, 1500 I.U. vitamin A and 1.5 mg. riboflavin and will make up the deficiencies in the diets of mothers and children. The protein foods should be distributed through (1) M.C.H. centres and (2) Out-patient departments of women's and children's hospitals. Feeding studies should be held periodically to demonstrate the value of these foods in improving the health of the child and the mother.

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CHAPTER 21

NUTRITION OF INDUSTRIAL WORKERS

The importance of adequate nutrition for maintaining good health and normal physical efficiency among the industrial workers was realised during the second world war by the Western Nations. The tremendous industrial expansion during the above period and the anxiety of the different nations to maintain maximum industrial efficiency and output focused their attention to improving the nutrition and health of the worker. During the last 15 years, the number of workers employed in the industry and in many irrigation projects in India has increased enormously. Increasing interest has been evinced both by the Government and the employers in improving the nutrition and health of the workers with a view to achieve the maximum output of work. In the present Chapter the following aspects of the subject are discussed: (1) Nutritional requirements of workers (2) Malnutrition among industrial workers and its causes (3) Diet and working efficiency (4) Measures for improving nutrition of the workers and (5) Balanced diets, lunches, snacks for industrial workers and their families in developing countries.

Nutritional requirements for different activities

The requirements of calories, proteins, vitamins and minerals for workers engaged in different types of physical activity suggested by different Nutrition Committees are given in Tables 16.1-16.3 (Chapter 16). The requirements of various dietary essentials are briefly discussed below:

Calories: Muscular work needs energy. The energy needed for various types of muscular activity constitutes the most important variable in the calculation of total energy expenditure. A large amount of work has been carried out in Europe and America on the energy needs of industrial workers. The results have been summarised by Passmore and Durnin (1955) and by Spitzer and Hettinger (1958). Further, the energy requirements of workers in cold climates will be about 15 to 20 per cent greater

and for those working in warm climates about 10 per cent less than the recommended allowances.

Proteins: Available evidence would indicate that muscular work does not affect to an appreciable extent the protein requirements of workers, provided the diet meets adequately the energy requirements. It is, however, desirable to provide about 20 per cent more protein in the diet for industrial workers as their diets are not usually adequate in calorie intake and as more protein is required to maintain N equilibrium in calorie deficiency. Further, industrial workers in temperate climates who are constantly exposed to cold environments will require more proteins (i.e., about 50 per cent more) than the recommended allowances, as exposure to cold increases the metabolic loss of body proteins and hence the protein requirements.

Vitamins

Vitamins must be present in adequate amounts in the diets of industrial workers, as vitamins are concerned in the utilisation of food energy and the maintenance of the body in good health. The requirements for various vitamins are given in Tables 16.1-16.3 (Chapter 16).

Vitamin A: There is no evidence so far indicating that increased muscular activity increases vitamin A requirements. Since vitamin A is required for proper vision in dim light and for the proper functioning of the nervous and epithelial tissues, the diets of workers should contain adequate amounts of vitamin A. Those exposed to bright light during work may need more vitamin A.

Thiamine: Thiamine plays an important part in energy metabolism. It is essential for the maintenance of good appetite and normal digestion. It is also required for the normal functioning of the nervous tissue. The requirements for thiamine for varying types of physical activity are given in 16.1-16.3 (Chapter 16).

Riboflavin: Riboflavin is concerned with the utilisation of energy from carbohydrates, fats, and proteins in the body. It is also essential for the maintenance of normal vision and the mucous membrances in good health. Diets consumed by industrial workers should provide adequate amounts of riboflavin.

The riboflavin requirements for different physical activities are given in Tables 16.1-16.3 (Chapter 16).

Niacin: Niacin is concerned with the utilisation of energy from carbohydrates, fats and proteins in the body. It is also essential for the normal functioning of the nervous tissue and mucous membranes. The requirements of niacin for different physical activities are given in Tables 16.1-16.3 (Chapter 16).

Ascorbic acid: Ascorbic acid is essential for maintaining good health. It helps in the healing of wounds and bone fractures and takes part in several important physiological functions. The diets of workers should contain adequate amounts of ascorbic acid. A high concentration of ascorbic acid has been recommended as a protection against benzol and other aromatic hydrocarbons. Ascorbic acid has been shown to be present in low concentrations in the blood of workers exposed to T.N.T. and a high intake of ascorbic acid has been recommended for them. The ascorbic acid requirements are given in Tables 16.1-16.3 (Chapter 16).

Folic acid: Folic acid is essential for the formation of red and white blood corpuscles. Deficiency of folic acid in the diet results in macrocytic anaemia which is likely to reduce the working capacity of the individual. The requirements of folic acid can be easily met by including green leafy vegetables in the diet.

Vitamin B_{12}: Vitamin B_{12} is identical with the anti-pernicious anaemia factor occurring in liver. It stimulates the formation of red blood cells. Vitamin B_{12} deficiency causes macrocytic anaemia. This disease occurs commonly in women workers during pregnancy. Since vitamin B_{12} occurs only in foods of animal origin, diets should include some animal foods.

Other vitamins: Other vitamins such as pantothenic acid, pyridoxin, vitamins E, K, etc. are essential in human nutrition. Well-balanced diets provide adequate amounts of the above vitamins.

Minerals

The most important changes in mineral metabolism caused by work in warm climates are the losses of sodium chloride, calcium and iron through sweat. Hence, the requirements for the above minerals increase markedly for workers in warm climates.

Sodium chloride: The loss of sodium chloride in sweat may be as much as 10 to 20 g. per 8 hours of hard work in warm climates. Continuous loss of sodium chloride may result in sodium chloride deficiency characterised by muscular cramps. The approximate sodium chloride requirements of workers in the tropics are given below:

Type of work	*Sodium chloride* (g/day)
Light work	10-15
Hard work	16-20
Very hard work	25-30

Calcium, Phosphorus and Iron: Workers engaged in lead industry need larger amounts of calcium as calcium helps in the excretion of lead from the body. The losses of calcium, phosphorus and iron in sweat in workers in warm climates are greater than those in temperate climates. The requirements of calcium and iron are given in Tables 16.1-16.3 (Chapter 16).

Malnutrition among industrial workers and its causes

Types of malnutrition prevalent: Only a few studies on the nutritional status and the extent of incidence of malnutrition among industrial workers have been carried out so far. Even in an advanced country like U.S.A. a survey carried out among 1100 aircraft workers revealed that the diets consumed by them were inadequate in several dietary essentials and signs and symptoms of malnutrition due to deficiencies of vitamin A, riboflavin and thiamine were present in a fair percentage of the workers (Borsook *et al* 1943). The results of two surveys carried out in India are summarised below: De Mello *et al* (1950) conducted a nutrition survey amongst factory workers in Bombay. The investigation included diet survey of 29 families. The average protein intake was 75 g. a day but only 9 per cent of the calories came from the proteins. Body weights were low and 50 per cent of the adult males had anaemia. A large number of children suffered from vitamin A deficiency. Unlike most other reports which have exclusively concentrated on children, this study has included a good number of adults. The higher incidence of vitamin A deficiency signs in children compared to that in adults is in keeping with the observations of other workers. Ramalinga-

swami and Patwardhan (1949) carried out a survey of dietary and health conditions of plantation workers in South India. The diets were found to be low in calories, proteins, calcium, vitamins A and B_1. A nutrition survey of 461 persons drawn at random from ten estates showed that 79 per cent were underweight by 10 per cent. According to these authors, 81.2 per cent of adults and 61.3 per cent of children showed signs attributable to early vitamin A deficiency. Bitot's spots, however, were observed to an extent of 7.5 per cent in both the groups. About 31 per cent of adults were reported to show signs of vitamin B deficiency. Nearly one-third of the subjects had haemoglobin values below 10.0 g. per cent. Stool examination carried out in one locality revealed a high incidence of hook-worm infestation.

Causes of malnutrition among industrial workers

Malnutrition exists in the working population chiefly because of the following reasons: (1) Inadequate income (2) Poor food habits (3) Unavailability and high cost of protective foods (4) Physiological stress and metabolic disorders, and (5) Infections and infestations (Fig. 21.1). These are discussed below:

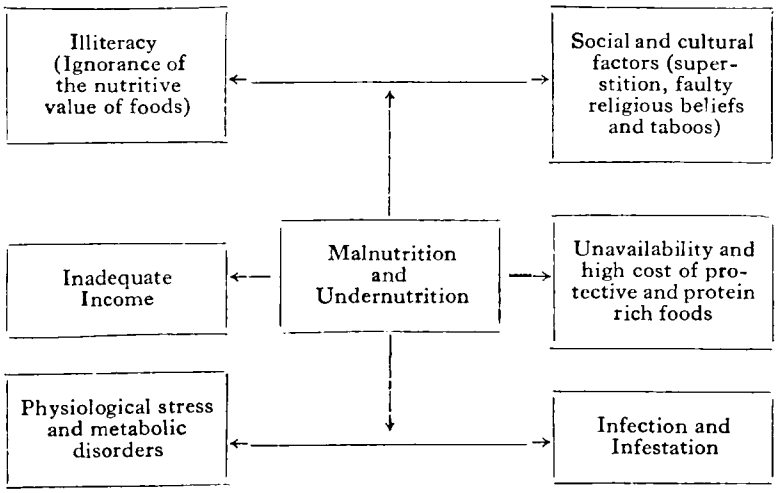

Fig. 21.1 Causes of malnutrition among industrial workers

Inadequate income: It is now generally agreed that the present rate of wages of industrial workers in India is inadequate to enable them to purchase the foodstuffs required for a balanced

diet. Under the existing economic conditions of the industry, it is also realised by all concerned that the employers find it difficult to increase the wages of workers above the present levels. It is, therefore, essential to develop low cost food products for supplementing the diets of industrial workers.

Poor food habits

Ignorance: Foods vary considerably in nutritive value. Some foods that cost little may be nutritionally rich while foods costing much may be nutritionally poor. For example, green leafy vegetables which are cheaper than other vegetables are rich sources of vitamins and minerals. A knowledge of foods which can give adequate nutrition at low cost is necessary if a worker and his family are to be properly fed on a limited budget. Hence, education of industrial workers regarding the importance of balanced diet for maintaining good health is essential.

Indifference: Indifference to scientific knowledge on the nutritional qualities of foods is one of the important causes of malnutrition. This can be overcome only by persistent education of the workers periodically through films on health and food.

Improper selection and preparation of foods: Industrial workers should be taught about the classification of foods into different groups based on their nutritional qualities and the need for including different categories of foods in the required amounts for obtaining a balanced diet. They should also be taught the right methods of cooking rice and vegetables without discarding the cooked water.

Unavailability and high cost of protective foods. Protective foods such as milk, eggs, fish and meat are costly. They are needed mostly by infants, young children and expectant mothers. Normal adults can consume a diet based on cereals, pulses and vegetables and containing less milk. Nutrition education should emphasise on (1) the importance of milk and other protective foods in the diets of infants and children, (2) the need for more quantities of Indian multipurpose food, pulses, nuts and green-leafy vegetables in the diets of older children and expectant and nursing mothers and (3) the importance of low cost protein foods such as Indian multipurpose food, pulses and green leafy vegetables as supplements to the diets.

Physiological stress and metabolic disorders: Strenuous physical exertion and hot or cold environments and exposure to toxic vapours may increase the requirements of certain nutrients. All the above conditions may cause digestive and metabolic disorders e.g. gastro-intestinal upsets, liver dysfunction etc. which may interfere with the digestion and absorption of food.

Infections and infestations: As workers live under insanitary conditions in most of the developing countries, the incidence of diseases due to infections and infestations are very high. Consequently, the health of the workers is affected and larger quantities of nourishing food are required during convalescence to recoup lost health. The children of the workers are prone to suffer more than their parents as a result of frequent illnesses. The incidence of protein malnutrition is fairly high among weaned infants.

Diet and working efficiency

One of the most prominent symptoms of consuming inadequate and ill-balanced diets is the reduction in working capacity. The workers are tired out easily after working for short periods. The beneficial effects of extra calories, proteins and vitamins upon working efficiency have been demonstrated in controlled studies with workers indicating the need for adequate intake of all nutrients for maintaining good health and full working efficiency. A few examples are given below:

Calories: During and immediately after the second world war, a remarkable correlation between calorie intake and work output was observed in the Ruhr District of Germany, in relation to production of coal. (FAO, 1962). In 1939, the miners' rations provided an average of 4,500 Kcal, of which 2,200 Kcal were required for the maintenance of metabolism and for the various activities not directly related to work. The remaining 2,300 Kcal were available for work (work calories). The daily output per miner was 1.9 tons of coal mined, i.e. about 1,200 work calories per ton. As the war proceeded and the food situation became worse, only 1,700 work calories were available in 1942, and the coal output diminished slowly. When, for some time only 900 Kcal per ton were available, the workers lost weight. In 1944, with 1,900 work calories, 1.65 tons of coal per man were mined

daily which gain corresponded tc 1,200 work calories per ton of coal. On the average of all food consumed, the amount of coal produced per 1,000 Kcal fell from 0.42 ton in 1939 to 0.40 ton in 1944.

The development of production in a German steel works during the last war provided another example of the close relation between work calories and production. The establishment was spared from air raids and the production programme remained exactly the same throughout the war. In 1939, the workers' rations supplied 1,900 work calories and the monthly steel production amounted to more than 120 tons per man. However. when food rations became smaller, the output fell more and more, until 1944, when only 1250 Kcal were available for work, production was less than 80 tons monthly per man. Again output for 1,000 Kcal of total food consumption fell from 29 tons per month in 1939 to 26 tons in 1944. In both cases, decline in the food consumption level resulted in reduced output per unit of food consumed.

The following observations made in war-time Germany under semi-experimental conditions have yielded interesting results. The influence of dietary supplements on the work output of a group of coal miners in the Ruhr district was investigated with the aim of finding whether the low production could be raised by increasing the food rations. A first supplement of 400 Kcal increased the coal output from 6.7 to 9.4 tons per person, which the workers knew was expected of them. The resulting fall in body weight indicated that energy expenditure was slightly in excess of calorie intake. A further increase in calories resulted in a small further increase in output, but body weights rose again. Evidently, 177 Kcal were required per ton of coal, and for the desired increase in output, 600 extra calories were necessary (Table 21.1).

Table **21.1 Increased output in a coal mine after improvement of rations**

Calories available for work (Kcal)	Output (tons)	Calories/ton	Body weight
1200	6.7	177	Constant
1600	9.4	169	Loss of 1.2 kg in six weeks
2000	10.8	192	Slow rise

A controlled group of 30 workers engaged in building railway tracks moved about 1½ tons of earth per hour on a food ration supplying a total of 2,300 Kcal. After ten weeks, additional food was given which brought the Kcal upto 3,000 and raised the output to 2½ tons per hour. Output per 1,000 Kcal increased almost 30 per cent. In the course of a year, the work output rose and fell with the inevitable minor fluctuation of food rations, while body weights remained constant or even increased slightly. It may be of interest in this context to note that a further increase of work output was achieved by a bonus of cigarettes for each additional ton, which was temporarily given for reasons of comparison, but as there was no increase in food intake, the increased output proved to be at the expense of body weight.

Proteins: Since maintenance of muscle mass requires adequate intake of proteins, protein deficiency will affect adversely the working capacity. The results of studies on the effect of varying intakes of proteins on working efficiency are summarised in Table 21.2. Only when the protein intake was increased considerably

Table **21.2 Protein intake and capacity for work**

Year	Occupational groups	Nutritional status	Calorie intake Kcal/day	Intake of protein g/kg	Performance capacity
1939–41	Miners	moderate	3,800	1.0–1.2	Rising
				below 1.0	Falling
1942	Gardeners	moderate	3,000	1.0	Unchanged
				0.7	Falling
1946	Scientists	moderate	3,000	0.8	Unchanged
				1.6	Steep rise after 6 weeks
1951	Students	good	4,000	2.0	Doubling of muscle powers in 12 weeks training period
				1.0	Slight increase in 12 weeks
				0.8	No change in 8 weeks

Data from Kraut and Lehmann (1948), *Biochem. Z.*, 319, 228; Lehmann and Michaelis (1948) *Biochem. Z.*, 319, 247; Kraut and Muller (1950), *Biochem. Z.*, 320, 302; Kraut, Muller-Wecker (1953), *Biochem. Z.*, 324, 280.

over the minimum, there was a continuous rise of muscle power and N-retention. The importance of the size of the protein stores for trainability, which is also largely influenced by constitutional factors, is shown by the fact that in the first experiment which was conducted shortly after the end of several years of extreme food shortage, subjects did not respond to training on 1 g. protein/kg body weight, while in the second experiment there was a definite response. Two grammes of protein/kg. body weight, when given for three months led to a deposition of 8.3 kg. protein (calculated from nitrogen retention), the figure corresponding well to the 8 kg. increase of muscle mass estimated from the gained muscle power. In the first experiment, subjects on 1 g. protein/kg lost part of the muscle power they had previously attained with 1.7-2.1 g. protein/kg. In the second series, when body stores were repleted, nitrogen balance was achieved on 0.8g protein/kg. without loss of muscle power. That, these figures are influenced by the biological value of the proteins in the diet, as was shown by Kraut *et al.*, (1958) seems not surprising in view of our present concepts of protein metabolism. In a group of prison inmates doing heavy work, Kraut *et al* (1951) found the work output (which was stimulated by incentives) unchanged when the percentage of animal proteins in the diet was lowered from 40 to 14 per cent at an over-all protein intake of 90 to 100 g. and a calorie intake of 3,800 to 4,000 Kcal/day. The practical conclusion to be drawn from all this seems to be that, while protein catabolism is not increased by work, protein requirements are greater for the development of the musculature necessary to a heavy worker, as well as for the maintenance of such muscle power. Therefore, Kraut *et al* (1951) recommended a daily intake of more than 1 g. protein per kg. body weight for heavy workers.

Vitamins and minerals: Vitamins are concerned in the maintenance of good health and vigour. Even a mild deficiency of vitamins generally results in loss of vigour (FAO, 1962). Iron deficiency will lead to anaemia which will affect seriously the working capacity. A rigorously controlled large-scale experiment on the effect of a 'vitamin-mineral' supplement on the working efficiency of air-craft workers was carried out in U.S.A. The study extended for a year during which time the men received a 'vitamin-mineral' supplement in capsules and pills

twice a day while they were at work, for five days a week. The supplement contained Vitamin A, 50,000 I.U.; Vitamin D, 800 I.U.; Vitamin B_1 10 mg; Vitamin B_2 10 mg; Niacinamide, 100 mg; Vitamin C, 250 mg; and Calcium (as carbonate) 500 mg. A conservative estimate of the over-all gain to production from the physiological effect of the 'vitamin-mineral' supplement, after all psychological effects were eliminated, was 10.5 working days per man per year. This will correspond to a gain in manpower of 4.1 per cent. Of this total gain, 78.4 per cent was due to better general work performance and reduced absenteeism contributed 21.6 per cent. The estimates of the results obtained are based on performance data and not on subjective reports of the subjects. The data are consistent in showing a physiological benefit from the 'vitamin-mineral' supplement not ascribable to any psychological influence.

Effect of dietary improvement on work output

The results of studies carried out in different countries have shown that provision of lunches for workers at the work spot increased to a marked extent the output of work. Some examples are given below:

An impressive example is the experience gained in the Central American Public Road Programme in Costa Rica (FAO, 1962). For the road work which was done by United States contracts, local labour was employed and proved to be extremely inefficient at work. After some time the organisation of the construction camps was changed, sanitation was improved and the management began to supply substantial meals, including large portions of meat to the workers who had formerly been subsisting on a poor and mainly vegetarian diet. The resulting improvement in working efficiency was striking. When the work was started in 1943, a labour force consisting of 30 per cent United States labourers and 70 per cent Costa Ricans moved 240 cubic metres of earth per day, with modern equipment. A year later with 33 per cent United States labourers and 67 per cent Costa Ricans, the daily average had risen to 388 cubic metres per day. By January 1945, with 28 per cent United States labourers and 72 per cent Costa Ricans, 1,025 cubic metres of earth were moved per man per day and by January 1946, with only 12 per cent United States

labourers and 88 per cent Costa Ricans, the average had risen to 1,157 cubic metres per man per day.

The benefits to be gained from the supply of a balanced cooked meal to the workers have long been recognised in the mining industry in South Africa. A survey carried out in 1956 revealed that the average calorie value of the rations supplied to each worker in the mines in South Africa, was approximately 4,500 Kcal per day. It was claimed that the balanced cooked meals led to a marked improvement in physical condition after a spell of mining employment. Hard work was performed and weight in most cases increased.

Similarly, in Ruanda Urundi, an employer estimated that by providing a balanced cooked meal to his workers, the productivity was raised by 30 per cent. In Madagascar, a sugar refiner is reported to have reduced the turnover rate of migrant labour from 60 to 6 per cent by the introduction of a balanced cooked meal. In Zanzibar, it was reported in 1937 that after well-balanced meals were introduced on an experimental basis by the Clove Growers' Associations, the employees' capacity for work was greatly increased, provided the worker remained in regular employment for some weeks; also, the increased efficiency more than compensated for an addition of a little over 50 per cent in the average wages of the labourer. Experience gained during the last war in a rubber plantation in South Vietnam showed an increase of 50 per cent in work output after the opening of canteen facilities providing a liberal diet to the workers. Such surprisingly high rates of production increases are largely due to the fact that workers' diet had been grossly inadequate before the introduction of feeding facilities on the work site.

The following two examples, one from Canada and the other from the United States show the kind of beneficial effects which may be expected to result even in well-to-do countries from the establishment of such facilities.

The Canadian example (I.L.O. 1946) is based on a study carried out after the management had set up a lunch room for the workers. The study showed that the establishment of the lunch room was followed by a significant reduction in the number of accidents. The results before and after the opening of the room, per million man hours were as follows:

	Before opening of lunch room	After opening of lunch room
	(Three years average)	
First aid treatment	3000	2130
Lost time accidents	49	42

A survey (FAO, 1962) carried out by the United States Department of Agriculture in later 1955 and early 1956 showed that half of the manufacturing plants in the United States employing 250 or more workers had facilities for serving hot food to the employees, and approximately nine out of ten plants had at least one kind of vending machine dispensing food or beverages. The great majority of the executives when questioned specifically, considered the employee food services beneficial to employee morale, labour-management relations, employee health, and employee productivity. These proportions regarded the services as having a good effect or bad effect or no effect at all (Table 21.3).

Table 21.3 **Effect of serving lunches on the productivity and health of workers**

	Good effect	No effect at all	Bad effect
	Percentage		
Employee morale	92	8	(1)*
Productivity	63	36	1
Recruiting	48	52	(1)*
Health	70	30	(1)*
Labour-management relations	77	21	2

*(1)—less than 0.5 per cent.

Haggard and Greenberg (1935) studied the rate of production in relation to frequency of meals in a rubber footwear factory in which the rate of production represented, as nearly as possible, a measure of the actual effort of the individual operator. Forty operators were divided into two groups of twenty each. In one—the control group, the operators ate their accustomed three meals a day, in the other—the experimental group, the operators ate three, five, three, five, three meals respectively for periods of two weeks. The mean hourly output of the operators in the

control group was 183, 184, 183, 184 and 184 for five periods of two weeks each. The mean hourly outputs of the operators in the experimental group during the three periods in which they ate three meals a day were 175, 176 and 176 and during the two periods in which they ate five meals a day were 192 and 194. From the above results, they concluded that the extra meals unquestionably increased the daily productivity.

According to Katsuki (1962), Japanese farmers obtain about 93 per cent of their calorie intake and 72 per cent of their proteins from vegetable foodstuffs—mostly rice. Katsuki's group noted that single-crop farmers retire about 10 years earlier than double-crop farmers and suffer from lack of muscle strength, nervous disorders and metabolic disturbances to a greater extent than double-crop farmers. They found double-crop farmers consumed more calories and more nutritious diet than single crop farmers.

Measures for improving workers' nutrition

It was during the last war that the importance of nutrition in industry was realised by the Western nations. Steps were taken to provide nutritious lunches and snacks to workers in the plant premises and these measures brought about highly significant increases in the work output. The various measures which are likely to improve the nutrition of the workers are (1) Establishment of non-profit food stores (2) Provision of free or low-cost nutritious lunches and snacks in canteens and (3) Nutrition education (Fig. 21.2). The need for providing adequate wages is obviously essential for any programme designed to improve the health of the workers.

Non-profit stores: The establishment of non-profit stores is likely to give workers and their families, facilities for buying their food requirements at controlled rates. The essential foods should be subsidised and sold against coupons. Non-profit food stores offer the chance to promote the consumption in adequate amounts of certain basic and supplementary foods such as cereals, millets, legumes and other supplementary foods. In view of the shortage and high cost of milk, meat, fish and eggs, there is need for the production of low-cost nutritious foods for use as supplements to the diets of industrial workers

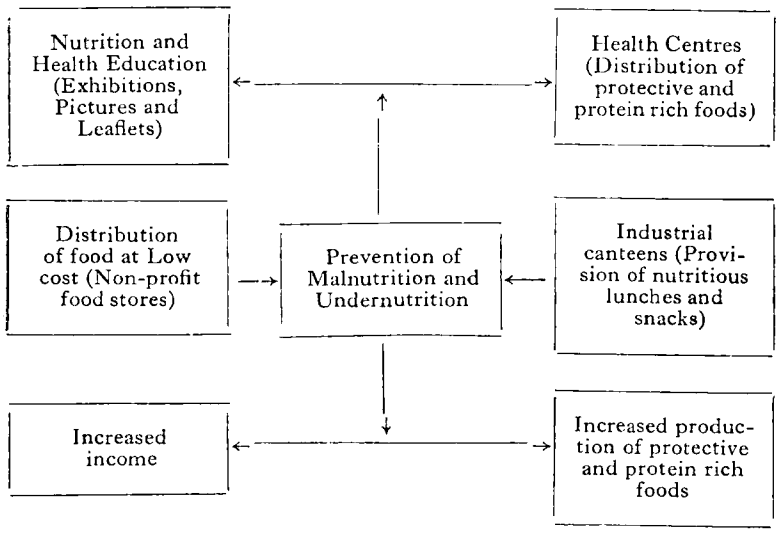

Fig. 21.2 Methods of prevention of malnutrition among industrial workers

and their families. Low-cost protein-rich foods based on oilseed meals e.g. Indian multipurpose food and legumes can be used as supplements to the diets of industrial workers and their families and should be sold at subsidised rates. Milk foods should also be distributed for the feeding of infants.

Canteens: The most effective measure for improving the workers' diets in a relatively short time is the provision of free lunches and snacks at the work spot. This is absolutely necessary as workers who live far away from the work spot find it difficult to bring their own lunches, as they have to leave their homes early in the morning. Night-shift workers will need snacks during the night. The provision of free meal and snacks is of great economic benefit to employees and also helps to increase production by avoiding waste of time, and preventing fatigue and nervousness which may occur when workers have to go outside the work spot for meals. Experience gained in several Western countries have proved beyond doubt that the provision of nutritious lunches and snacks helps to improve the general health and psychological outlook of workers and acts as an incentive for hard work resulting in increased production (Table 21.3).

Nutrition and health education: Nutrition and health education will help considerably in improving the diets of the workers

and their families. Industrial organisations have an excellent opportunity to disseminate information about the role of balanced diets in maintaining good health and the value of low-cost supplementary foods i.e., Indian multipurpose food, pulses, green leafy vegetables, roasted groundnuts etc., in making up deficiencies in the diets. The important methods of educating workers are as follows:

Exhibitions: Permanent exhibits relating to health and nutrition of the workers and their families should be organised and the workers encouraged to visit them. Display of charts and photographs showing the effects of supplementary foods in improving the health and nutritional status of children and in curing protein malnutrition in children will help effectively in creating interest in food and nutrition. Charts showing how infectious diseases are caused and can be prevented should also be displayed.

Pamphlets: Pamphlets on health and nutrition education should be prepared in regional languages and distributed to the workers.

Motion pictures: Motion pictures are effective in educating the workers. Educational films relating to health and nutrition should be screened periodically.

Balanced diets, lunches and snacks for workers and their families

The problem of providing balanced diets for industrial and agricultural workers and their families at low cost has been engaging the attention of nutrition workers in India and other developing countries. In view of their scarcity and high cost, protective and protein-rich foods such as milk, eggs, meat, fish and fruits cannot be included in adequate amounts in diets of the low income groups. The diets should, therefore, include liberal amounts of pulses, groundnut and green leafy vegetables.

Balanced diets for male workers: When recommending balanced diets for men, the degree of activity has to be borne in mind. A sedentary or light worker needs less calories than a moderately hard worker and a very hard worker needs much more than the former two. Balanced diets, at varying costs for men of varying degrees of activities are given in Tables 21.4-21.6.

Table 21.4 **Balanced diets for adult male and female workers (High cost) (g/caput/day)**

Foodstuffs	Male workers						Female workers						Additional allowances during			
	Sedentary work		Moderate work		Heavy work		Sedentary work		Moderate work		Heavy work		Pregnancy		Lactation	
	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV
Cereals	350	350	420	420	600	600	230	230	280	280	400	400	—	—	100	100
Pulses	70	55	80	65	80	65	60	50	60	50	60	50	—	—	—	—
Green leafy vegetables	100	100	125	125	125	125	100	100	100	100	100	100	—	—	—	—
Other vegetables	75	75	75	75	100	100	75	75	75	75	100	100	—	—	—	—
Roots and tubers	75	75	100	100	100	100	50	50	75	75	100	100	—	—	—	—
Fruits	100	100	100	100	100	100	100	100	100	100	100	100	50	50	50	50
Milk	600	400	600	400	600	400	600	400	600	400	600	400	500	300	700	400
Cheese	40	—	40	—	40	—	40	—	40	—	40	—	20	—	20	—
Fats and oils	35	40	40	40	50	50	30	35	35	40	40	45	—	—	20	20
Sugar and jaggery	30	30	40	40	55	55	30	30	30	30	40	40	—	—	20	20
Meat and fish	—	100	—	100	—	100	—	100	—	100	—	100	—	25	—	40
Eggs	—	60	—	60	—	60	—	60	—	60	—	60	—	—	—	—
Groundnut (roasted)	—	—	—	—	50	50	—	—	—	—	40	40	—	—	—	—

V = Vegetarian NV = Non-vegetarian

Table 21.5 **Balanced diets for adult male and female workers (Moderate cost) (g/caput/day)**

Foodstuffs	Male workers						Female workers						Additional allowances during			
	Sedentary work		Moderate work		Heavy work		Sedentary work		Moderate work		Heavy work		Pregnancy		Lactation	
	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV
Cereals	380	380	450	450	630	630	260	260	310	310	440	425	—	—	100	100
Pulses	70	55	80	65	80	65	60	50	60	50	60	50	—	—	—	—
Green leafy vegetables	100	100	125	125	125	125	100	100	100	100	100	100	—	—	—	—
Other vegetables	75	75	75	75	100	100	75	75	75	75	100	100	—	—	—	—
Roots and tubers	75	75	100	100	100	100	50	50	75	75	100	100	—	—	—	—
Fruits	60	60	60	60	60	60	60	60	60	60	60	60	50	50	50	50
Milk	400	250	400	250	400	250	400	250	400	250	400	250	400	200	600	300
Fats and oils	35	40	40	40	50	50	30	35	35	40	40	45	—	—	20	20
Sugar and jaggery	30	30	40	40	55	55	30	30	30	30	40	40	—	—	20	20
Meat and fish	—	60	—	60	—	60	—	60	—	60	—	60	—	25	—	40
Eggs	—	30	—	30	—	30	—	30	—	30	—	30	—	30	—	30
Groundnut (roasted)	—	—	—	—	50	50	—	—	—	—	40	40	—	—	—	—

V = Vegetarian NV = Non-vegetarian

Table 21.6 **Balanced diets for adult male and female workers (Low-cost)—Nutrition Expert Group, ICMR, (1968) (g/caput/day)**

Foodstuffs	Male workers						Female workers						Additional allowances during	
	Sedentary work		Moderate work		Heavy work		Sedentary work		Moderate work		Heavy work			
	V	NV	V	NV	V	NV	V	NV	V	NV	V	NV	Pregnancy	Lactation
Cereals	400	400	475	475	650	650	300	300	350	350	475	475	50	100
Pulses	70	55	80	65	80	65	60	45	70	55	70	55	—	10
Green leafy vegetables	100	100	125	125	125	125	125	125	125	125	125	125	25	25
Other vegetables	75	75	75	75	100	100	75	75	75	75	100	100	—	—
Roots and tubers	75	75	100	100	100	100	50	50	75	75	100	100	—	—
Fruits	30	30	30	30	30	30	30	30	30	30	30	30	—	—
Milk	200	100	200	100	200	100	200	100	200	100	200	100	125	125
Fats and oils	35	40	40	40	50	50	30	35	35	40	40	45	—	15
Sugar and jaggery	30	30	40	40	55	55	30	30	30	30	40	30	10	20
Meat and fish	—	30	—	30	—	30	—	30	—	30	—	30	—	—
Eggs	—	30	—	30	—	30	—	30	—	30	—	30	—	—
Groundnut (roasted)	—	—	—	—	50†	50†	—	—	—	—	40†	40†	—	—

V=Vegetarian NV=Non-Vegetarian

† An additional 25g of fats and oils can be included in the diet in the place of groundnut.

Balanced diets for working women: In the case of women, in addition to the degree of physical activity, other physiological factors such as pregnancy and lactation have also to be taken into consideration. An expectant mother needs more proteins, calcium, iron and vitamins, especially during the second half of pregnancy. A nursing mother needs more of all the nutrients than a normal woman as she has to feed the baby. Hence, diets have to be modified according to their needs. Balanced diets at varying costs for women are given in Tables 21.4-21.6. These include a low cost protein supplement (i.e.) Indian multipurpose food.

Balanced lunches: Provision of balanced lunch to industrial workers is a measure for improving their general health and working efficiency. Almost all industrial concerns in Western countries provide lunches for their workers. The lunches should be acceptable to the workers, besides being nutritious. They should meet about one third the daily requirements of calories, proteins, vitamins and minerals. The composition of model lunches based on locally available foodstuffs is given in Table 21.7. The lunches should be based on locally available foodstuffs and the recipes should be varied to avoid monotony.

Table **21.7 Balanced lunches at varying costs for industrial workers (g/day/caput)**

Foodstuffs	High cost		Moderate cost		Low cost	
	V	NV	V	NV	V	NV
Cereals and millets	140	140	150	150	170	170
Pulses	40	20	40	20	50	30
Green leafy vegetables	70	70	70	70	70	70
Other vegetables	50	50	50	50	50	50
Fruits	100	100	50	50	30	30
Meat, fish and eggs	—	100	—	70	—	30
Cheese	30	—	15	—	—	—
Milk and curds	300	150	200	100	100	50
Oils and fats	30	30	30	30	20	20
Sugar and jaggery	20	20	20	20	20	20

V=Vegetarian NV=Non-vegetarian

Balanced snacks: Balanced snacks help to make up the deficiencies in the daily diet. They should meet about one fifth the daily requirements of calories, proteins, vitamins and minerals.

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CHAPTER 22

NUTRITION OF ATHLETES

Few areas of nutrition are as infiltrated with food fads as nutrition of athletes. Diet fads are dangerous to the athletes on the following grounds (*i*) They induce the athletes to consume some foods which are assumed to possess special nutritional properties as against foods of proven nutritive value (*ii*) Special foods are costly while being not superior to common articles of diet and hence drain away the financial resources of the individual and (*iii*) The practice of diet faddism makes the individual less receptive to sound scientific concepts of nutrition.

Requirements of nutrients

The recommended dietary allowances for athletes are given in Table 22.1.

Table 22.1 **Recommended dietary allowances for athletes**

Nutrient	Daily requirements
Calories (Kcal)	3000-5000
Proteins (g)	60-90
Fat (g)	80-150
Calcium (g)	0.6-0.8
Iron (mg)	20-30
Vitamin A (Retinol) (μg.)	750-1000
Thiamine (mg)	2.0-3.0
Riboflavin (mg)	2.0-3.2
Nicotinic acid (mg)	26-36
Ascorbic acid (mg)	50-80
Folic acid (μg)	50-100
Vitamin B_{12} (μg)	2-4
Vitamin D (I.U.)	400

Calories: The calorie requirements are increased considerably during athletic activities. Passmore and Durnin (1955) have

reviewed the energy requirements for various types of activities. A summary of the data taken from their paper is given in Table 22.2. Depending on the intensity and duration of the daily

Table 22.2 **Energy expenditure in various types of athletic activities**

Sr. No.	Activity	Calorie expenditure per minute (Kcal)
1.	Sitting in a chair	1.40
2.	Standing	1.65
3.	Running 7 Km/hr.	9.0
4.	Running 12 Km/hr.	20.0
5.	Climbing ladder	8.0-11.0
6.	Cycling (5.5 mph)	4.5
7.	,, (9.4 mph)	7.0
8.	,, (13.1 mph)	11.1
9.	Canoeing (2.5 mph)	3.0
10.	,, (4.0 mph)	7.0
11.	Horse riding (trot)	8.0
12.	,, (gallop)	10.0
13.	*Gymnastic exercises*	
	Balancing exercises	2.5
	Abdominal exercises	3.0
	Arms swinging, hopping etc.	6.5
14.	Volleyball	3.5
15.	Bowls	4.4
16.	Golf	5.0
17.	Cricket, fielding	3.9
18.	,, bowling	5.2
19.	,, batting	6.0
20.	Tennis	7.1
21.	Football	8.9
22.	Swimming, back stroke	11.0
23.	Swimming, back crawl	11.5
24.	Swimming, breast stroke	10.0
25.	Playing squash rackets	10.2
26.	Cross country running	10.6
27.	Skiing	9.9-10.8

exercises of the athlete, the total daily calorie requirements may range from 3000 to 5000 Kcal as compared with the calorie requirements for very hard work of 3600-3900 Kcal per day. It is evident that the calorie requirements of athletes may be as high as or higher than those doing very hard work. About 20-25 per cent of the calories should be derived from fats as otherwise, the diet will be too bulky and difficult to consume.

Carbohydrates: About 30 per cent of the carbohydrates in the diet should be in the form of sucrose and glucose, as these are readily assimilable to provide energy. Sucrose and glucose should be given in quantities of 50-80 g. at a time in the form of drinks once in 2 hours before and after the athletic practice. Carbohydrates are superior to fats as a source of energy for athletes.

Fat: Fat is a concentrated source of energy and hence about 20 to 25 per cent of the calorie intake should be derived from fats. Vegetable oils rich in essential fatty acids should constitute about 50 per cent of the fat in the diet. The daily fat intake should be between 80-150 g. consisting about 20-25 per cent of the daily calorie requirements.

Proteins: One of the persisting misconceptions is the increased need for proteins for persons doing hard physical work. The extra proteins required for the formation of new muscle, are met by the proteins provided by well-balanced diets. Darling and co-workers (1944) studied the performance of three groups of persons engaged in the same type of manual work and receiving daily high protein (160g) moderate protein (80-90g) and low protein (50-55g) diets providing the same amount of calories and other nutrients, over a period of 2 months. There was no significant difference in the work efficiency and output of the three groups.

Calcium and iron: The calcium and iron requirements of athletes will be the same as for hard working persons.

Vitamin A: Vitamin A intake is of the same order as for persons doing hard work.

Thiamine: Since thiamine needs are related to the calorie requirements, the requirements for athletes will be about 2.0-3.0 mg. per day.

Riboflavin and niacin: Since both riboflavin and niacin requirements are related to the calorie requirements, the recommended allowances for riboflavin will be approximately 2.2-3.2 mg. and of niacin 26-36 mg. per day.

Ascorbic acid: The ascorbic acid requirements of athletes are of the same order as for persons doing hard work.

Folic acid and vitamin B_{12}: The requirements of folic acid and vitamin B_{12} for athletes are of the same order as those required for persons doing hard work.

Vitamin D: Vitamin D requirements are the same as for ordinary adults.

Gelatin, glycine and creatine: Since muscle contains creatine and glycine is the precursor of creatine, it has been postulated by some workers that a diet supplemented with glycine or gelatin (as it contains 25 per cent glycine) might increase the concentration of creatine in muscle and hence muscular efficiency. Studies carried out by several workers have shown, however, that supplements of glycine or gelatin have no beneficial effect in increasing the creatine content or in improving physical performance.

Vitamin E and wheat germ oil: Supplements of vitamin E or wheat germ oil (rich source of vitamin E) have been reported to increase the physical performance of athletes by some workers, while some others have not been able to confirm the above findings.

Meal composition and athletic performance

The optimal proportions of carbohydrates to fat in the diets of athletes have been studied by several workers. Two types of studies have been conducted: (1) Those in which experimental subjects practise following the ingestion of single meals of desired composition and (2) those in which the subjects are fed for several days on a special diet containing high levels of a particular component and low levels of others. A high carbohydrate diet has been reported to increase physical efficiency to a significant extent as compared with a high fat diet.

Timing and relative size of meals

The influence of number, relative size and spacing of meals on physical performance has been studied by various workers. The results have shown that (*i*) Frequent small meals improved overall physical performance more than few large meals and (*ii*) A pattern of 5 meals a day led to a total work output greater than that observed with 3 meals a day.

'Crash' diet

In sports such as boxing and wrestling, present day athletes —professionals and amateurs—often place themselves on 'crash' diets combined with dehydration to bring down their weight, to become eligible for lower weight classification. The intention of these athletes is not to attain their most desirable weight from the view point of health and fitness but rather to compete with an unfair advantage against an opponent who really does belong to a lower weight bracket. This practice should be strongly discouraged.

Nutrition of athletes immediately preceding and during competition

Water balance: The replacement of salt and water lost from the body is of great importance in warm climates. This can be achieved by consuming a salty dish at least 3 hrs. before the competition and drinking fruit juice frequently.

Carbohydrate loading: Drinking of fruit juices and soft drinks containing glucose, sugar, ascorbic acid and citric acid frequently during the contest helps to improve the physical performance of the athletes.

Tea, coffee and alcohol

These beverages should be avoided during a contest. Alcohol may produce depression and incoordination of movements. Tea and coffee may have an immediate stimulating action but have a depressing effect three or four hours later and thus may impair physical performance.

Table 22.3 **Diets* for athletes (g/caput/day)**

Foodstuffs	Calories 3000 V	3000 NV	3500 V	3500 NV	4000 V	4000 NV	4500 V	4500 NV	5000 V	5000 NV
Cereals	150	150	175	175	200	200	250	250	300	300
Legumes	50	50	50	50	50	50	50	50	50	50
Nuts (cashew, peanut etc.)	80	70	100	90	120	110	120	110	160	150
Milk	1000	500	1000	500	1000	500	1000	500	1000	500
Cheese	50	—	50	—	50	—	50	—	50	—
Meat and fish	—	100	—	100	—	100	—	100	—	100
Eggs	—	50	—	50	—	50	—	50	—	50
Fats and Oils										
Butter or margarine	20	20	30	30	30	30	40	40	40	40
Vegetable oil	30	30	40	40	60	60	70	70	90	90
Sugar: Cane sugar	80	80	100	100	110	110	120	120	140	140
Glucose	80	80	100	100	110	110	120	120	140	140
Vegetables	200	200	200	200	200	200	200	200	200	200
Fruits	200	200	200	200	200	200	200	200	200	200
Fruit juice	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

* One multi-vitamin tablet providing the daily requirements of different vitamins should be included in the diet.

Diets for athletes

Diets providing 3000-5000 Kcal per day are given in Table 22.3. They include liberal amounts of fruits and fruit juices, cane sugar and glucose, as these serve as readily available sources of energy immediately before and after the athletic practice. They also provide adequate amounts of proteins, vitamins and minerals to meet their requirements (Table 22.1). It will be desirable to supplement the diets with a multi-vitamin tablet which will meet the daily *additional* vitamin needs of the athletes.

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CHAPTER 23

METABOLISM IN STARVATION AND UNDERNUTRITION

Starvation and severe undernutrition occur in human beings during famine and food shortage resulting from various causes such as floods, drought etc. The great Irish famine of the nineteenth century was caused by the destruction of the potato crops by fungal disease. The famine which occurred in the province of Bengal, in India in 1943 was due to drought. The term 'starvation' is used to denote conditions in which food is not available to the individuals due to acute shortage, while the term 'undernutrition' denotes conditions in which the individuals are semi-starved.

STARVATION

Studies have been carried out by some workers on the biochemical and physiological changes occurring during starvation in human volunteers. During starvation, the entire energy requirements of the body are obtained by the oxidation of fat, proteins and carbohydrates present in the tissues. The carbohydrate reserves are small and will meet only one day's energy requirements while the fat reserves are large and can meet the energy requirements for several days. The breakdown of tissue proteins occurs continuously during starvation. The quantity of tissue proteins lost daily in an adult may range from 60-70g, providing about 240-280 Kcal per day while the rest of the energy requirements are derived from oxidation of fat present in adipose tissues. The number of days, an adult could fast will depend on his physical condition (fat stores and general health) and may normally be about 9-10 weeks. Terence MacSwiney, Mayor of Cork, after his arrest in 1820, went on a fast which lasted 74 days, terminating in his death in coma.

Loss of weight of body and tissues

During starvation, there is steady loss of body weight (Table 23.1), the loss of weight during the first 10 days being 10-12 per

cent of the initial weight. This loss of body weight does not affect all the tissues and organs alike. Muscle, liver, skin lose much weight while heart and brain lose very little (Table 23.2).

Table 23.1 **Changes in body weight, nitrogen, mineral and energy metabolism in a fasting adult male (Benedict)**

	Period of fasting (days)			
	1st	11th	21st	31st
Body weight (kg)	59.6	53.9	50.5	47.4
Urine				
Total solids (g)	43.5	42.1	31.9	27.1
Total N (g)	7.1	10.3	7.9	6.9
Ammonia N (g)	0.41	1.58	1.57	1.14
Creatinine+creatine N (g)	0.48	0.49	0.38	0.32
Calcium as Ca (g)	0.22	0.22	0.24	0.14
Phosphorus as P (g)	0.37	0.43	0.35	0.29
Potassium (g)	1.63	1.01	0.64	0.61
Sodium (g)	2.07	0.10	0.07	0.05
Sulphur (g)	0.46	0.62	0.51	0.49
β-hydroxy butyric acid (g)	—	1.4	5.0	4.5
R.Q.	0.78	0.72	0.73	0.72
Energy metabolism at complete rest (Kcal/24 hrs.).	1441	1193	1052	1025
Energy metabolism at complete rest (Kcal/square meter body surface)	843	732	653	661

Table 23.2 **Percentage loss of weight of different organs and tissues after prolonged starvation (Voit)**

Tissue	Loss of weight of organ or tissue (%)
Adipose tissue	97
Spleen	67
Liver	54
Muscles	31
Blood	27
Kidneys	26
Skin and hair	21
Intestine	18
Lungs	18
Pancreas	17
Heart	3
Brain and spinal cord	3

Basal energy output in starvation

There is a steady decline in the basal metabolic rate probably due to loss of muscle tissue. In an adult (Table 23.1) the energy metabolism at rest decreases steadily from the initial value of 1441 Kcal/24 hrs. to 1025 Kcal/24 hrs. on the 31st day of fast.

The metabolism of carbohydrate, fat and proteins during starvation

During the first and second days of starvation, the carbohydrate reserves are used up. From the third day onwards energy is derived mainly from fat (80-90 per cent) and to a small extent (10-20 per cent) from proteins.

Loss of protein from the body and different tissues

Addis and co-workers (1936) found that in rats fasted for 7 days, the loss of protein from different tissues varied widely (Table 23.3). Of the total protein lost from the body, muscle, skin and skeleton contributed 62 per cent, liver 16 per cent, alimentary tract, pancreas and spleen, 14 per cent and blood, 6 per cent. The total loss of protein from the body was 3.8 g in a 7 day period in adult rats weighing 200g and containing about 32-36g protein in the body. An adult human subject (Table 23.1) weighing about 60 kg may lose about 1550g of protein from the body in a 31 day fast. The protein content of the body of the adult will be about 9600g (on the basis of 16 per cent of proteins in the body). Thus the tissue proteins lost in a 31 day fast will amount to about 16 per cent of the total proteins present in the body.

Carbohydrate metabolism

Even in the later stages of fasting in animals, glycogen is found in the liver and the level of blood sugar is maintained within normal limits. Glucose is formed in the body from the glycerol of fat and from neo-glucogenesis from amino acids. The quantity of glucose formed from the above sources may be about 30-50g per day in an adult human subject.

Table **23.3 Loss of proteins from some tissues during a 7 day fast—(The values are measured in gm. per rat with an original body surface of 310 sq. cm. (200 gm. of body weight)**

Organ or tissue	Fed rats (g)	Fasted rats (g)	Protein lost (g)	Proportion of total protein lost (%)
Muscle, skin and skeleton	28.9	26.5	2.4	62
Liver	1.53	0.92	0.61	16
Alimentary tract, pancreas and spleen	1.81	1.30	0.51	14
Blood	1.19	0.95	0.24	6

Fat metabolism

Adipose tissue fat serves as the main source of energy during starvation. In an adult with a resting calorie requirements of 1200-1400 Kcal, about 80-90 per cent of the calories is derived from fat. The quantity of fat oxidised daily may vary from 100-120g daily. The R. Q. is about 0.72, indicating that fat is the main nutrient oxidised for the production of energy.

Ketosis

Owing to lack of carbohydrates, ketosis develops due to the oxidation of excessive amounts of fat in the body. The ketone bodies i.e. acetoacetic acid, β-hydroxybutyric acid and acetone circulate in the blood and are excreted in urine. Ammonia is excreted in larger amounts for neutralising the acids formed.

Minerals

There is continuous loss of calcium, phosphorus from the bones and potassium from the cells. Sulphur compounds derived from the oxidation of tissue proteins are excreted in urine.

Physiological and clinical changes in starvation

Loss of adipose tissue fat and muscle, gives a emaciated appearance to the subject. The subject becomes very weak.

The blood pressure is low and the pulse rate falls slowly. Anaemia develops gradually. Due to loss of plasma proteins, oedema develops in the legs, hands and face. Ketosis develops and death occurs due to coma.

UNDERNUTRITION

Undernutrition is due to inadequate calorie intake. It occurs in many individuals in developing countries, due to poverty, diseases etc. The effects of undernutrition are (1) loss of body weight (2) emaciation due to loss of tissue proteins and adipose tissue (3) reduction in plasma proteins leading to the development of oedema and (4) general weakness, apathy and lack of interest in surroundings. Severe undernutrition causes the condition known as marasmus which has already been discussed in Chapter 4 in this volume. The effects of undernutrition on adults have been studied in two experiments *viz.* (i) The Carnegie experiment and (ii) The Minnesota experiment. The results of these studies are briefly discussed below:

Effects of moderate calorie deficiency

The Carnegie experiment: These studies were carried out by Benedict and co-workers (1919). The object of these studies was to effect a reduction of 10 per cent of body weight in a period of 5 weeks. It was believed that such a study will yield data simullating semi-starvation conditions without causing any discomfort and disability to the individuals. Twelve healthy young adult males whose normal calorie intake was about 3100 Kcal/day were fed on diets providing 1600-1800 Kcal/day for 5 weeks. The diets were adequate in other nutrients. During this period, they lost about 10 per cent of their body weight on an average. From the sixth week onwards, the calorie intake was increased to 1970 Kcal/day. On these diets, the subjects maintained their body weight at a stationary level for several months. Their general health and mental state had, however, deteriorated to a considerable extent. The subjects were weak and got tired easily. They were unable to work hard or work for long periods. This chronic state of undernutrition decreased the basal metabolic rate. They had no initiative or interest in doing work. A vicious circle was thus established in which undernutrition

led to weakness which, in turn, aggravated the degree of malnutrition due to inaction.

The Minnesota experiment: This study was carried out by Keys and co-workers (1950). Thirty-two healthy young adult males whose average daily energy requirement was about 3490 Kcal served as experimental subjects. They were fed on a diet providing 1570 Kcal for 24 weeks. The diets were adequate in other nutrients. They lost on an average 25 per cent of their body weight. The subjects were emaciated due to loss of adipose tissue and muscle. The skin became rough, thin and pigmented. Oedema appeared after 12 weeks. Tolerance to heat was increased while tolerance to cold was decreased. They were very weak and evinced no interest in doing any work. The subjects complained of hunger immediately after consuming food, as the food eaten did not meet the calorie needs. Data regarding the changes in weight and blood constituents are given in Table 23.4. The effect of varying calorie intakes on nitrogen balance in adult human subjects is given in Table 23.5.

Table 23.4 **Changes in weight and blood constituents in adult males due to undernutrition**

Constituent	Initial	After 24 weeks Semi-starvation
Calorie intake (Kcal/day)	3490	1570
Body weight (kg)	70	52.5
Body fat (kg) (Calculated)	9.8	3.0
Haemoglobin (g/100ml)	15.1	11.7
Plasma protein (g/100 ml)	6.7	6.0
Plasma albumin (g/100 ml)	4.3	3.9
Plasma cholesterol (mg/100 ml)	169	151
Basal O_2 consumption (ml/minute)	228	139
Period for which work test could be done (seconds)	242	50
Strength of handgrip (kg)	58	42

Table 23.5 **Nitrogen balance in adult males on low calorie intake**

Period	Calorie intake (Kcal/day)	N Intake (g/day)	N balance (g/day)
I	894	9.4	-3.4
II	1296	10.1	-2.1
III	1620	11.6	-1.2
IV	3353	15.2	2.0

Rehabilitation of under-nourished subjects

The under-nourished subjects from the Minnesota experiment were placed on well-balanced diets providing progressively larger amounts of calories and adequate amounts of other nutrients. The general health and oedema improved slowly. Some of the subjects complained of flatulence and discomfort in the stomach.

Even after rehabilitation for a period of 3 months, the physical condition and general health of the subjects were not restored to normal levels. The subjects had to be fed for a further period of 3 months with well-balanced diets, before they regained their physical capacity and general health to the initial pre-experimental levels.

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CHAPTER 24

INTER-RELATIONSHIP BETWEEN NUTRIENTS

During recent years considerable amount of work has been carried out by several workers on the inter-relationship between different nutrients. It has been found that excess or deficiency of certain nutrients affects the requirements of some other nutrients. The different aspects are discussed under the following heads: (*i*) Protein-energy inter-relationship (*ii*) Hormones and protein metabolism (*iii*) Effect of carbohydrates, fats and proteins on vitamin requirements (*iv*) Vitamin-vitamin inter-relationship (*v*) Vitamin-mineral inter-relationship and (*vi*) Mineral-mineral inter-relationship.

Protein-energy inter-relationship

Restriction in calorie intake causes breakdown of tissue proteins and negative nitrogen balance. If the calorie restriction is severe, the animal body is depleted of depot fat and catabolism of tissue proteins continues, eventually leading to the death of the animal. The extent to which protein is catabolised for energy purposes under conditions of calorie restriction has been studied by some workers. The results (Morrison and Narayana Rao, 1967) given in Table 24.1 indicate that when the calorie restriction was 50 per cent of the requirements, about 74 per cent of the dietary protein was oxidised and used to meet energy needs and only 20 per cent of the dietary protein was used to meet the protein needs of the body.

Table 24.1 **The effect of calorie restriction on the utilisation of dietary protein in rats**

Calorie intake (Kcal/day)	Total protein intake (g/10 days)	Protein used for caloric needs (g)	(%) of intake	NPU
32	9.96	3.14	31.5	59.3
24	9.78	4.60	47.0	44.8
16	10.00	7.36	73.6	19.8

Effect of calorie restriction on protein content of the body: The effect of calorie restriction on the protein content of the body and liver of albino rats has been studied by some workers. The results have shown that both the protein contents of the body and liver are reduced when the caloric intake is restricted to 50 per cent when the protein intake was about 1g/day/rat.

Effect of carbohydrate and fat on protein metabolism: A difference in the effects of equicaloric amounts of carbohydrates and fats as a source of calories on protein metabolism has been demonstrated in fasted rats (Table 24.2). Administration of carbohydrate brought about a marked reduction in the catabolism of tissue proteins as seen by decrease in the urinary N while equicaloric quantity of fat was less effective. (Munro, 1951).

Table 24.2 **Effect of feeding carbohydrate or fat on urinary N output in fasting adult rats**

Diet during 24 hr. period	Urinary N (mg/24 hrs)
Fasted	135
Glucose (12 g)	93
Olive oil (5 ml)	111

Hormones and protein metabolism

Action of anabolic hormones on protein metabolism: Retention of N is caused by (*a*) administration of carbohydrates along with insulin (*b*) administration of androgens and (*c*) administration of growth hormones.

Action of catabolic hormones on protein metabolism: The catabolic hormones include thyroxine, cortisone, estrogens and progesterone. These, on administration increase the catabolism of tissue proteins and nitrogen loss in urine.

Fat and galactose inter-relationship

Incorporation of lactose or galactose in large amounts in a fat-free diet produces diarrhoea and cataract in rats. These have been prevented by the addition of fat in the diets. It has been shown that fat helps in the utilization of galactose.

Amino acid inter-relationship

The presence of excessive amounts of certain amino acids may cause depression in the growth of rats and may produce toxic effects. Three types of effects *viz.* (*i*) amino acid imbalance (*ii*) amino acid toxicity and (*iii*) amino acid antagonism have been observed. These have been discussed in Chapter 4 in this Volume.

Effect of carbohydrates, fats and proteins on vitamin requirements

Dietary carbohydrates, fats and proteins influence the requirements of some vitamins. These are discussed below:

Thiamine: Carbohydrates present in the diet require thiamine for their metabolism, as thiamine pyrophosphate is a coenzyme for the oxidative decarboxylation of pyruvic acid, a breakdown product of carbohydrate. Thiamine is not required for the oxidation of fat. Hence, the presence of excess fat in diet decreases, while the presence of excess carbohydrates increases thiamine requirements.

Riboflavin: The riboflavin requirements have been found to be proportional to the protein content of the diet in experimental animals.

Nicotinic acid: Tryptophan present in proteins is converted to niacin in the body. A part of the niacin requirements is met by the above process. Hence, proteins rich in tryptophan can serve as a source of nicotinic acid.

Pyridoxine (Vitamin B_6): The requirements of vitamin B_6 are influenced by both the quantity and quality of dietary proteins and fats. The pyridoxine requirements are directly proportional to the protein content of the diet. Supplementation of the dietary proteins with methionine or glycine increases pyridoxine requirements. On the other hand, addition of essential fatty acids to a low pyridoxine diet, offers protection against the development of the deficiency signs due to pyridoxine deficiency in albino rats.

Vitamin A and carotene: It has been reported that the mobilisation of the vitamin A stores in liver is directly proportional to the protein content of the diets, low protein diets helping to

maintain the liver stores for longer periods. The absorption of α, β and γ-carotenes (provitamin A) requires fat. In the absence of fat in the diet, they are not absorbed. Rancid fats destroy the vitamin A and β-carotene present in the diet.

Choline: Administration of choline enables the rat to utilize homocystine for growth in place of methionine. It has been shown that methyl group is transferred from choline to homocystine leading to the formation of methionine and *vice-versa*. Vitamin B_{12} and folic acid are involved in the above reactions.

Intestinal synthesis of B-vitamins: It has been found that intestinal synthesis of certain B-vitamins takes place in certain experimental animals. The quantity of vitamin synthesised depends on the nature of carbohydrate in the diet. Dietary fat has been reported to inhibit the intestinal synthesis of riboflavin and folic acid in the rat. Children fed high carbohydrate diet excrete more riboflavin in the urine than those fed low carbohydrate diets, when the intakes of proteins, calories and vitamins are the same in the two groups.

Vitamin-vitamin inter-relationship

Inter-relationship between various vitamins has been studied by several workers and is discussed below:

Vitamins A, E and K: It has been shown that albino rats fed on a vitamin K deficient diet develop signs of vitamin K deficiency when vitamin A intake is increased. Considerable amount of work has been carried out by different workers on the role of vitamin E in influencing storage and mobilisation of vitamin A in liver. Administration of vitamin E helps to increase the storage of vitamin A in liver.

Ascorbic acid and other vitamins: The albino rat can synthesise ascorbic acid. This synthetic capacity, however, is diminished considerably in thiamine and riboflavin deficiencies. This indicates that both these vitamins play an important role in the biosynthesis of ascorbic acid. A depletion of the ascorbic acid content of tissues of rats occurred when the animals were fed on diets deficient in vitamin A or thiamine or riboflavin.

Thiamine and other vitamins: Thiamine deficiency is often accompanied by disturbance of riboflavin metabolism, causing considerable excretion of riboflavin in urine.

Vitamin B_6 and other vitamins: The absorption and storage of vitamin B_3 are adversely affected in rats fed on vitamin B_6 deficient diets. Administration of thiamine, riboflavin and pantothenic acid offered partial protection from spontaneous seizures in experimental animals fed vitamin B_6 deficient diet.

Pantothenic acid and other vitamins: A deficiency of pantothenic acid affects adversely the mobilisation of riboflavin from the liver. Administration of pantothenic acid to deficient animals, increases the mobilisation of riboflavin from liver and raises the levels of riboflavin in blood.

Niacin and other vitamins: The livers of rats fed on thiamine deficient or riboflavin deficient diets contained smaller amounts of niacin than normal controls. Treatment of pellagra with niacin precipitates symptoms of thiamine and riboflavin deficiencies, indicating that niacin deficiency is accompanied by secondary deficiencies of thiamine and riboflavin.

Folic acid and other vitamins: For the conversion of folic acid to folinic acid (*Citrovorum factor*), vitamin B_{12} is essential. Folic acid therapy of patients suffering from sprue, causes an increase in plasma vitamin E content.

Vitamin B_{12} and folic acid: Vitamin B_{12} deficiency causes a rise in unconjugated folates and a marked depletion of intracellular conjugated folates.

Vitamin-mineral inter-relationship

Certain vitamins influence the metabolism of some minerals.

Vitamin D in calcium and phosphorus metabolism: In vitamin D deficiency, the absorption of calcium and phosphorus from the intestines is very low. Administration of vitamin D corrects the defect.

Ascorbic acid and iron: Ascorbic acid reduces ferric iron to ferrous iron and thus helps in the absorption of iron.

Vitamin E and selenium: An organic compound of selenium (known as factor 3) appears to function in the same way as vitamin E in preventing necrosis of the liver in animals fed on low protein diets. It also prevents lipid peroxidation occurring in tissues of vitamin E deficient animals.

Mineral-mineral inter-relationship

Studies carried out by several workers have shown that certain minerals affect the metabolism of other minerals.

Copper and Iron: Copper is essential for the incorporation of iron in haemoglobin.

Calcium, Magnesium, Iron and Phosphorus: The presence of excess of calcium in the diet affects adversely the intestinal absorption of phosphorus and *vice-versa*. Presence of excessive amounts of phosphates and phytates in the diet interferes with the absorption of magnesium and iron.

Copper and Molybdenum: The presence of excess of molybdenum prevents the absorption of dietary copper and causes copper deficiency in experimental animals.

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CHAPTER 25

ENERGY AND PROTEIN REQUIREMENTS

The report of the Joint FAO/WHO *Ad hoc* Expert Committee on 'Energy and Protein requirements' has recently been published (FAO/WHO 1973). The recommendations of the Committee are summarized in this Chapter.

ENERGY REQUIREMENTS

The Committee defined energy requirements as 'The energy intake that is considered adequate to meet the energy needs of the average healthy person in a specified age/sex category'. The energy requirements of individuals are influenced by the following factors (*a*) Physical activity (*b*) Body size and composition (*c*) Age and (*d*) Climate and other ecological factors.

Infants

The energy requirements of infants during the first 6 months of life can be estimated from the observed intakes of breast milk by healthy infants from healthy mothers. An intake of 850 ml of human milk per day will provide on an average 120 Kcal per day during the first 3 months. From the 3rd month to 6th month, the energy intake from breast milk is slightly less. After 6 months of age, the quantity of breast milk secreted will be inadequate to meet the energy needs of the infant. The average energy requirements of the infant are shown in Table 25.1.

Table 25.1 **Energy requirements of infants**

Age	Kcal per kg.
<3 months	120
3-5 months	115
6-8 months	110
9-11 months	105
Average during first year	112

Children and adolescents

The energy requirements of children and adolescents are given in Table 25.2. The figures given in the Table are based on actual energy consumption data obtained with active healthy children in U.S.A. and U.K. whose growth rates have been found to be normal.

Table **25.2 Energy requirements of children and adolescents**

Age (years)	Males			Females		
	Weight (kg)	Energy/kg/day (Kcal)	Energy/person/day (Kcal)	Weight (kg)	Energy/kg/day (Kcal)	Energy/person/day (Kcal)
<1	7.3	112	820	7.3	112	820
1	11.4	103	1180	11.1	106	1180
2	13.6	100	1360	13.4	100	1350
3	15.6	100	1560	15.4	99	1520
4	17.4	99	1720	17.5	96	1670
5	20.7	91	1870	20.0	90	1790
6	23.2	87	2010	22.4	85	1900
7	25.9	83	2140	25.0	80	2010
8	28.6	79	2260	27.6	76	2110
9	31.3	76	2380	30.4	73	2210
10	33.9	74	2500	33.8	68	2300
11	36.7	71	2600	37.7	62	2350
12	40.2	67	2700	42.4	57	2400
13	45.5	61	2800	47.0	52	2450
14	51.7	56	2900	50.3	50	2500
15	56.6	53	3000	52.3	48	2500
16	60.3	51	3050	53.6	45	2420
17	62.4	50	3100	54.2	43	2340
18	63.7	49	3100	54.6	42	2270
19	65.0	47	3020	55.0	40	2200
Reference adult (moderately active)	65.0	46	3000	55.0	40	2200

Adults

The Committee expressed the energy requirements of adults in terms of 'Reference man' and 'Reference woman' doing mode-

rate work. The definitions of 'Reference man' and 'Reference woman' given by the Committee are reproduced below.

Reference man: He is between 20 and 39 years of age and weighs 65 kg. He is healthy, that is, free from disease and physically fit for active work. On each working day, he is employed for 8 hours in an occupation that usually involves moderate activity. When not at work, he spends 8 hours in bed, 4-6 hours sitting or moving around in only very light activity and 2 hours in walking, in active recreation, or in household duties.

Studies on energy expenditure and food intake of moderately active healthy adult males with an average weight of 65 kg show that 3000 Kcal per day (12.5 MJ) adequately cover this average expenditure and this figure is taken as the requirement.

Reference woman: She is between 20 and 39 yeais of age, similarly healthy, and weighs 55 kg. She may be engaged for 8 hours in general household work, in light industry, or in other moderately active work. Apart from 8 hours in bed, she spends 4-6 hours sitting or moving around in only very light activity and 2 hours in walking, in active recreation, or in household duties.

Studies on the energy expenditure and food intake of such women show that 2200 Kcal per day (9.2 MJ) cover average energy expenditure and this figure is taken as the requirement.

Effect of physical activity on energy requirement

The energy requirements of 'Reference man' and 'Reference woman' for varying degrees of physical activity are given in Tables 25.3 and 25.4.

The energy requirements of 'Reference man' engaged in various types of physical activity are as follows: Light activity 2700 Kcal; moderately active 3000 Kcal; very active 3500 Kcal; exceptionally active 4000 Kcal. The corresponding values for 'Reference woman' are 2000 Kcal, 2200 Kcal, 2600 Kcal and 3000 Kcal respectively.

Adjustment for body weight

The energy requirements of adults of varying body weight and engaged in varying degrees of physical activity are given in

Table 25.3 **Illustrations of how the energy expenditure of the 65-kg reference man may be distributed over the 24 hours and the effect of occupation**

	Light activity (Kcal)	Moderately active (Kcal)	Very active (Kcal)	Exceptionally active (Kcal)
In bed (8 hours)	500	500	500	500
At work (8 hours)	1100	1400	1900	2400
Non-occupational activities (8 hours)	700-1500	700-1500	700-1500	700-1500
Range of energy expenditure (24 hours)	2300-3100	2600-3400	3100-3900	3600-4400
Mean (24 hours)	2700	3000	3500	4000
Mean (per kg body wt)	42	46	54	62

Table 25.4 **Illustrations of how the energy expenditure of the 55-kg reference woman may be distributed over the 24 hours and the effect of occupation**

	Light activity (Kcal)	Moderately active (Kcal)	Very active (Kcal)	Exceptionally active (Kcal)
In bed (8 hours)	420	420	420	420
At work (8 hours)	800	1000	1400	1800
Non-occupational activities (8 hours)	580-980	580-980	580-980	580-980
Range of energy expenditure (24 hours)	1800-2200	2000-2400	2400-2700	2800-3200
Mean (24 hours)	2000	2200	2600	3000
Mean (per kg body wt)	36	40	47	53

Tables 25.5 and 25.6. The Committee expressed energy requirements, in proportion to body weight as in the case of protein.

Table 25.5 **The effects of body weight and occupation on energy requirements of men**

Body weight (Kg)	Light activity (Kcal)	Moderately active (Kcal)	Very active (Kcal)	Exceptionally active (Kcal)
50	2100	2300	2700	3100
55	2310	2530	2970	3410
60	2520	2760	3240	3720
65	2700	3000	3500	4000
70	2940	3220	3780	4340
75	3150	3450	4050	4650
80	3360	3680	4320	4960

Table 25.6 **The effects of body weight and occupation on energy requirements of women**

Body weight (Kg)	Light activity (Kcal)	Moderately active (Kcal)	Very active (Kcal)	Exceptionally active (Kcal)
40	1440	1600	1880	2200
45	1620	1800	2120	2480
50	1800	2000	2330	2750
55	2000	2200	2600	3000
60	2160	2400	2830	3300
65	2340	2600	3055	3575
70	2520	2800	3290	3850

Effect of age on energy requirements

The energy expenditure of adult, will decrease gradually as age advances due mainly to decline in physical activity. Energy requirements of moderately active adults of varying ages are given in Table 25.7.

Table **25.7 Average energy requirement of moderately active adults of reference body weight at different ages**

Age (years)	65-kg man (Kcal)	55-kg woman (Kcal)	%of reference adult (Kcal)
30-39	3000	2200	100
40-49	2850	2090	95
50-59	2700	1980	90
60-69	2400	1760	80
70-79	2100	1540	70

Energy requirements during pregnancy and lactation

The Committee recommended an additional allowance of 80,000 Kcal for the entire pregnancy distributed as follows: (*i*) 150 Kcal/day in the first trimester and (*ii*) 350 Kcal/day in the 2nd and 3rd trimesters. The Committee has estimated the additional requirements, during the first 6 months of lactation as 100,000 Kcal or about 550 Kcal per day.

Protein Requirements

The Committee expressed the protein requirements in terms of egg or milk proteins. The Committee defined 'safe level of protein intake as the amount necessary to meet the physiological needs and maintain the health of nearly all the individuals in a specified age/sex group'.

The Committee followed two procedures in arriving at the protein requirements, (*i*) The factorial method in which the obligatory nitrogen losses and N-required for growth, pregnancy and lactation are estimated and (*ii*) Measurement of minimum protein intake required for satisfactory growth and N-balance in infants and children and N-equilibrium in adults.

Estimation of nitrogen requirements by the factorial method

(*a*) *Obligatory N-losses.* The Committee estimated the total obligatory nitrogen losses through faeces, urine, skin, and other miscellaneous routes in adult men as 2.0 mg N/ basal Kcal (Table 25.8).

Table 25.8 **Obligatory nitrogen losses in adult men on a protein-free diet**

Route	N per kg of body weight (mg)	N per Kcal of basal energy (mg)
Urine	37	1.4
Faeces	12	0.4
Skin	3[1]	0.13
Miscellaneous	2	0.08
Total[2]	54	2.0

[1] Under temperate conditions; under tropical conditions or in heavy work situations this figure is somewhat higher.

[2] These are approximate average figures. Since the co-efficient of variation i.e., (standard deviation divided by the mean) X 100, is 15 per cent, a value 30 per cent larger should encompass the losses of nearly all healthy persons in a normal population.

The Committee used the same figure of 2 mg N per basal Kcal foi the total obligatory losses in women, infants and children.

(*b*) *N-requirements for growth:* The nitrogen requirements for growth of infants and children as estimated by the Committee are given in Table 25.9.

N-requirements in pregnancy and lactation: The nitrogen accretion in the pregnant woman, estimated by the Committee is given in Table 25.10. During the first 6 months of normal lactation, the average volume of milk secreted will be 850 ml per day. This amount of milk will contain 10g of protein. Hence, the average additional N-requirements in lactation will be 1.6g per day.

Safe level of intake of egg or milk proteins

The procedure used by the Committee for calculating the safe level of intake of egg or milk proteins was as follows (*i*) Estimation of the obligatory losses of nitrogen on a protein-free diet and the quantities of nitrogen retained in the body during the growth of infants and children and during pregnancy and lactation in women, and (*ii*) Addition of 30 per cent of the above values to allow for the losses in digestion and metabolism of egg or milk proteins in the body.

Table 25.9 **Computed obligatory nitrogen loss and nitrogen increment for growth of children**

Age	Obligatory N loss[1] (mg/kg/day)		Nitrogen (mg/kg/day) Growth[2]		Total N requirements[3] (mg/kg/day)	
	M	F	M	F	M	F
(months)						
6-8	112		42		154	
9-11	110		26		136	
(years)						
1	104		16		120	
2	100		12		112	
3	96		10		106	
4	92		8.5		100	
5	86		9.5		96	
6	83		9		92	
7	73		8.5		88	
8	76		7		83	
9	73		7		80	
10	72	68	6	9	78	77
11	70	64	7	8	77	72
12	66	60	8	10	74	70
13	62	57	11	7	73	64
14	59	55	9	4	68	59
15	57	54	6	2	63	56
16	57	54	4	1	61	55
17	56	53	2	1	58	54
Adult	54	49	0	0	54	49

[1] Calculated as 2 mg of N per basal Kcal. Basal metabolic rate data given in Annex 4 are from Talbot (89), with values at intermediate ages obtained by interpolation.

[2] Weight increment from data for 50th percentile given in Annex I and the composition of gain according to references 87 and 88.

[3] These are approximate average figures. Co-efficients of variation are assumed to be 15 per cent for obligatory loss and 10 per cent for growth, so a value 30 per cent higher should encompass the values of nearly all healthy individuals in a normal population.

Table 25.10 **Predicted nitrogen accretion during pregnancy assuming that the foetus weighs 3.30 kg at term**

Stage of gestation	N accretion/day[1] (mg)
First quarter	80
Second quarter	400
Third quarter	740
Fourth quarter	860

[1] These are approximate average figures from reference 37. The co-efficient of variation is assumed to be 15 per cent, so a value 30 per cent higher should encompass nearly all healthy individual in a normal population.

The values so obtained are estimates of the average physiological requirements in terms of egg or milk proteins. To derive safe levels of protein intake, an allowance is needed for individual variations. The co-efficient of variations of mean values in human metabolic studies has been found to be approximately 15 per cent. A value twice that of the co-efficient of variation i.e. 30 per cent above the mean would thus meet the needs of individual variations and will represent the safe level of nitrogen requirements. The nitrogen requirements thus obtained are converted into protein requirements by multiplying by the factor 6.25. The safe levels of intake of egg or milk proteins estimated by the Committee are given in Tables 25.11 and 25.12.

Adjustments for the quality of protein in the diet

Since the net protein utilization (NPU) values of milk or egg proteins are higher than those of proteins of average diets consumed in different countries, a correction has to be made for this variation in the NPU of dietary proteins as shown below:

$$\text{Dietary protein requirements (g)} = \text{Safe level of intake of egg or milk proteins} \times \frac{\text{NPU of egg or milk proteins}}{\text{NPU of dietary proteins}}$$

For example, if the NPU of the dietary proteins is 45 and egg proteins is 90, the correction factor will be $\frac{90}{45} = 2.0$.

Table **25.11 Safe levels of intake of egg or milk proteins**

Age	A		B		C		D	
	Total N-requirements (mg/kg/day)		Adjusted N-requirements (A+30% excess) (mg/kg/day)		Safe level of intake B+30% excess			
					Nitrogen (mg/kg/day)		Proteins (g/kg/day)	
(months)								
3					384[1]		2.40[1]	
3–6					96[1]		1.85[1]	
6–9	154		200		260		1.62	
9–11	136		177		230		1.44	
(years)								
1	120		156		203		1.27	
2	112		146		190		1.19	
3	106		138		179		1.12	
4	100		130		169		1.06	
5	96		125		162		1.01	
6	92		120		156		0.98	
7	88		114		148		0.92	
8	83		108		140		0.87	
9	80		104		135		0.85	
	M	F	M	F	M	F	M	F
10	78	77	101	100	132	130	0.82	0.81
11	77	72	100	94	130	122	0.81	0.76
12	74	70	96	91	125	118	0.78	0.74
13	73	64	95	83	123	108	0.77	0.68
14	68	59	88	77	115	100	0.72	0.62
15	63	56	82	73	107	95	0.67	0.59
16	61	55	79	71	103	93	0.64	0.58
17	58	54	75	70	98	91	0.61	0.57
Adult	54	49	70	64	91	83	0.57	0.52

(A) Total nitrogen requirements; obligatory losses and growth (mgN/kg/day). (B) Adjusted N requirements increased by 30% in accordance with balance and growth data (mg N/kg/day). (C) Safe level of intake. (D) Protein = N intake × 6.25 (adjusted requirement + 30% to allow for individual variability).

[1] Based on observed intakes (mean + 2 standard deviations) of healthy infants.

Table 25.12 **Additional requirement of egg or milk proteins during pregnancy and lactation**[a]

	Average N increment or loss (mg N per day)	Adjusted N (+30%) for utilization of dietary protein (mg N per day)	Adjusted N +30% for individual variability: safe level of intake [b]	
			(mg N per day)	(g protein/day)
Pregnancy: 1st quarter	80	104	135	1
2nd quarter	400	520	675	4
3rd quarter	740	960	1250	8
4th quarter	860	1120	1460	9
Lactation: 1st 6 months	1600 [c]	2080	2700	17

[a] To be added to the predicted safe level of daily intake of egg or milk protein derived from Table 25.11.

[b] Expressed as egg or milk protein; must be adjusted for quality of dietary protein.

[c] Nitrogen content of milk secreted per day.

The safe levels of protein requirements in terms of egg or milk proteins and also in terms of diets containing proteins of varying qualities i.e. 60 per cent, 70 per cent and 80 per cent relative to milk or egg proteins are given in Table 25.13. The calculation of the relative protein quality scores is explained below: Example: If the NPU value for egg protein is 90 and NPU values obtained for diets A, B and C are 72, 63 and 54, then the relative protein scores of the dietary proteins in terms of egg proteins (score 100) will be as follows:

$$\text{Diet A} = 100 \times \frac{72}{90} = 80\%$$

$$\text{Diet B} = 100 \times \frac{63}{90} = 70\%$$

$$\text{Diet C} = 100 \times \frac{54}{90} = 60\%$$

Table 25.13 **Safe level of protein in terms of diets of protein qualities of 60 % 70 % and 80 % relative to milk or eggs**

Age group	Body weight (kg)	Safe level of protein intake (egg or milk protein)	Adjusted level for dietary proteins of different quality		
		Score 100	Score[a] 80	Score 70	Score 60
		(g/kg/day) (g/day)	(g/person/day)		
Infants					
6-11 months	9.0	1.53 14	17	20	23
Children					
1-3 years	13.4	1.19 16	20	23	27
4-6 years	20.2	1.01 20	26	29	34
7-9 years	28.1	0.88 25	31	35	41
Male adolescents					
10-12 years	36.9	0.81 30	37	43	50
13-15 years	51.3	0.72 37	46	53	62
16-19 years	62.9	0.60 38	47	54	63
Female adolescents					
10-12 years	38.0	0.76 29	36	41	48
13-15 years	49.9	0.63 31	39	45	52
16-19 years	54.4	0.55 30	37	43	50
Adult man	65.0	0.57 37	46[b]	53[b]	62[b]
Adult woman	55.0	0.52 29	36[b]	41[b]	48[b]
Pregnant woman (latter half of pregnancy)		+9	+11	+13	+15
Lactating woman (first 6 months)		+17	+21	+24	+28

[a] Scores are estimates of the quality of the protein usually consumed relative to that of egg or milk. The safe level of protein intake is adjusted by multiplying it by 100 divided by the score of the food protein, for example, 100/60=1.67 and for a child of 1-4 years the safe level of protein intake would be 16×1.67 or 27 g of protein having a relative quality of 60.

[b] The correction may over estimate adult protein requirements.

Growth and N-balance studies with infants and children and N-balance studies with adults

The Committee considered the available data on the minimum milk or egg proteins required for promoting optimal growth

and N-retention in infants and pre-school children and for maintenance of N-equilibrium in adults.

Infants: Studies carried out by Fomon *et al* (1971) showed that infants receiving cow's milk formula providing 2.0-1.5 g/kg body weight promoted good growth and optimal N-balance in infants aged 1-5 months.

Children: Studies carried out by Arroyave (1971) have shown that pre-school children (1-3 years) receiving 1g of egg protein per kg body weight per day maintained good growth and optimal N-balance. Chan and Waterlow (1966) reported that weaned infants (6-24 months) receiving daily 1.25 g cow's milk protein per kg body weight per day showed good growth and optimal N-balance. Begum *et al* (1970) reported that pre-school children receiving 2 g of proteins per kg body weight per day on diets based on rice and wheat, maintained good growth and optimal N retention.

Adults: Bricker *et al* (1945) found that adults receiving 0.42 g of milk protein per kg body weight per day maintained N-equilibrium. Calloway and Margen (1971) found that adults maintained N-equilibrium in a diet providing 0.46 g of egg protein per kg body weight per day.

The protein requirements of infants, children and adults obtained by direct feeding experiments mentioned above, are in accordance with the safe levels of protein intake in terms of proteins of milk or egg or of diets of varying protein qualities recommended (Table 25.13) by the Joint FAO/WHO Expert Committee.

REFERENCE

FAO/WHO (1973) 'Energy and Protein Requirements', Report of a Joint FAO/WHO *Ad Hoc*. Expert Committee. WHO. Tech. Rep. Ser. No. 522. WHO, Geneva.

APPENDICES

Composition of foods

The chemical composition of foods has been determined by several workers in different countries. The following compilations of food values are widely used: (1) The composition of British Foods by McCance and Widdowson (2) The composition of American Foods by Watt and Merrill (3) The composition of Indian Foods by Aykroyd *et al* (1963) revised recently by Gopalan *et al* (1971) and (4) Composition of Foods for International use by Chatfield (1949, 1954). The data given in Table 1 A were taken mainly from the booklet 'Chemical Composition of Indian Foods and the Planning of Satisfactory diets' by Aykroyd, revised by Patwardhan and Ranganathan, Health Bulletin No. 23, Government of India (1957).

Amino acid content of proteins

Data regarding the amino acid content of proteins in foods are given in the following publications: (1) Amino Acid Hand Book by Block and Weiss (1956) (2) Amino acid content of Foods by Orr and Watt (1957) (3) Proteins in Foods by Kuppuswamy, Srinivasan and Subrahmanyan (1958) and (4) Amino acid composition of Foods by Harvey (1970).

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Table **1 A: The chemical**

Name of foodstuff	Moisture (g)	Protein (g)
1	2	3
Cereals		
Bajra or Pearl millet (Pennisetum typhoides)	12.4	11.6
Barley (Hordeum vulgare)	12.5	11.5
Cholam or Kaffircorn (Sorghum vulgare)	11.9	10.4
Italian millet (Setaria Italica)	11.2	12.3
Maize, tender (Zea Mays)	79.4	4.3
Maize, dry (Zea Mays)	14.9	11.1
Oat meal (Avena sterilis)	10.7	13.6
Pani varagu (Common millet) (Panicum miliaceum)	11.9	12.5
Ragi (Eleusine coracana)	13.1	7.1
Rice, raw home pounded (Oryza sativa)	12.2	8.5
Rice Parboiled, home pounded ,,	12.6	8.5
Rice, raw milled ,,	13.0	6.9
Rice, Parboiled, milled ,,	13.3	6.4
Rice, flakes ,,	12.6	6.6
Rice, puffed ,,	14.7	7.5
Rice, polishings	11.0	13.5
Samai (Little millet) (Panicum miliare)	11.5	7.7
Sanwa millet (Echinochloa frumantacea)	11.9	6.2
Vermicelli	11.7	8.7
Varagu or kodu millet (Pospalum Scorbiculatum)	12.8	8.3
Wheat, flour, whole (Triticum aestivum)	12.8	11.8
Wheat flour, refined	13.3	11.0
Wheat bread (brown)	40.8	8.0
Wheat bread (white)	42.0	7.0
Wheat germ	5.2	29.2
Pulses		
Bengal gram, whole (Cicer arietinum)	9.8	17.1
Bengal gram dhal	9.9	20.8
Bengal gram, roasted (without outer husk)	11.2	22.5
Black gram dhal (Phaseolus mungo)	10.9	24.0
Cow gram (Vigna catiang)	12.0	24.6
Field bean, dry (Dolichos lablab.)	9.6	24.9
Green gram, whole (Phaseolus aureus Roxb)	10.4	24.0

* Taken from the Health Bulletin No. 23. 'The nutritive value of Indian Patwardhan and S. Ranganathan—Published by the Manager of Publications,

composition of foods* (values per 100g.)

Fat (g)	Fibre (g)	Carbo-hydrate (g)	Calcium (Ca) (g)	Phosphorus (P) (g)	Iron (Fe) (mg)	Calories (Kcal)	Carotene (μg)	Thiamine (mg)	Niacin (mg)	Riboflavin (mg)	Ascorbic acid (mg)
4	5	6	7	8	9	10	11	12	13	14	15
5.0	1.2	67.1	0.05	0.35	8.8	360	132	0.33	2.3	0.25	...
1.3	3.9	69.4	0.03	0.23	3.7	335	10	0.47	4.7	0.20	...
1.9	1.6	72.4	0.03	0.28	6.2	349	47	0.37	1.8	0.13	...
4.7	8.0	60.6	0.03	0.29	6.3	334	32	0.59	0.7	0.11	...
0.05	...	15.1	0.01	0.10	0.7	82	32	0.11	0.6	0.17	4
3.6	2.7	66.2	0.01	0.33	2.1	342	90	0.42	1.4	0.10	...
7.6	3.5	62.8	0.05	0.38	3.8	374	0	0.54	1.1	0.16	...
1.1	2.2	68.9	0.01	0.33	5.7	336	0	0.20	2.3	0.18	...
1.3	3.6	72.7	0.33	0.27	5.4	331	42	0.42	1.1	0.19	...
0.6	0.6	77.4	0.01	0.17	2.8	349	4	0.21	2.4	0.16	...
0.6	0.2	77.2	0.01	0.28	2.8	348	9	0.27	4.0	0.12	...
0.4	0.2	79.0	0.01	0.11	1.0	347	0	0.06	1.2	0.06	...
0.4	0.2	78.9	0.01	0.15	2.2	345	0	0.21	3.8	0.05	...
1.2	0.7	77.5	0.02	0.22	8.0	347	...	0.21	4.0	0.05	...
0.1	0.3	74.0	0.02	0.16	6.2	327	...	0.21	4.1	0.04	...
16.2	4.3	48.4	0.07	1.41	35.0	393	0	2.70	28.0	0.48	...
4.7	7.6	63.7	0.02	0.36	7.1	328	0	0.30	1.5	0.09	...
2.2	9.8	65.5	0.02	0.28	2.9	307	0	0.31	1.5	0.08	...
0.4	0.3	78.4	0.02	0.08	0.3	357	0	0.19	1.8	0.05	...
1.4	9.0	65.6	0.04	0.24	5.2	308	0	0.33	2.0	0.09	...
1.5	1.2	71.2	0.05	0.32	5.3	348	64	0.45	5.0	0.17	...
0.9	0.3	74.1	0.02	0.09	1.0	349	29	0.12	1.2	0.07	...
1.1	0.8	49.0	0.02	0.20	3.0	238	50	0.21	1.4	0.07	...
0.7	0.2	52.0	0.01	0.07	1.0	238	25	0.04	0.7	0.04	...
7.4	1.4	53.3	0.04	0.85	6.0	397	64	1.40	2.9	0.54	...
5.3	3.9	61.2	0.19	0.24	9.8	361	190	0.30	2.6	0.35	...
5.6	1.2	59.8	0.06	0.33	9.1	372	129	0.48	2.4	0.18	...
5.2	1.0	58.9	0.07	0.31	8.9	372	129	0.20	1.3	0.15	...
1.4	0.9	60.3	0.20	0.37	9.8	350	38	0.42	2.0	0.37	...
0.7	3.8	55.7	0.07	0.49	3.8	327	12	0.50	1.3	0.20	...
0.8	1.4	60.1	0.06	0.45	2.0	347	0	0.52	1.8	0.16	...
1.3	4.1	56.6	0.14	0.28	8.4	334	94	0.47	2.0	0.39	...

Foods and the planning of Satisfactory Diets' by W. R. Aykroyd, V. N. Government of India, Delhi.

Table 1A: (Cont.)

Name of foodstuff	Moisture (g)	Protein (g)
1	2	3
Green gram dhal	10.1	24.5
Horse gram (Dolichos biflorus)	11.8	22.0
Lathyrus pea (Lathyrus sativus)	10.0	28.2
Lentil (Lens culinaris Medic)	12.4	25.1
Peas, dried (Pisum sativum)	13.0	19.7
Peas, roasted	9.9	22.9
Red gram dhal (Cajanus cajan)	12.2	22.3
Soybean (Glycine Max. Merr.)	8.1	43.2
Leafy vegetables		
'Agathi' (Sesbania grandiflora)	73.1	8.4
Amaranth, tender (Amaranthus tricolor)	85.8	4.9
Amaranth, spined (Amaranthus, spinosus)	85.0	3.0
Bamboo, tender shoots (Bambusa bambos)	87.1	3.9
Bengal gram leaves (Cicer arietinum)	77.8	7.0
Brussels sprouts (Brassica oleracea var gemmifera)	84.6	4.7
Cabbage (Brassica oleracea var capitata)	90.2	1.8
Carrot leaves (Daucus Carota)	83.3	5.1
Celery leaves (Apium graveolens Var. dulce)	81.3	6.0
Coriander (Coriandrum sativum)	87.9	3.3
Curry leaves (Murraya koenigii)	66.3	6.1
Drumstick leaves (Moringa oleifera)	75.0	6.7
Fenugreek leaves (Trigonella foenum-graecum)	81.8	4.9
Garden cress (Lepidium sativum)	82.3	5.8
'Gogu' or red sorrel (Hibiscus cannabinus)	86.2	1.7
Ipomoea leaves (Ipomoea reptans)	90.3	2.9
Khesari leaves (Lathyrus sativus)	84.2	6.1
Lettuce (Lactuca sativa)	92.9	2.1
'Manathakkall' leaves (Solanum nigrum)	82.1	5.9
Mint (Mentha spicate)	83.0	4.8
Parsley (Petroselinum crispum)	68.4	5.9
'Ponnanganni' (Alternanthera sessilis)	77.4	5.0
Rape leaves (Brassica napus)	84.9	5.1
Safflower leaves (Carthamus tinctorius)	89.9	3.3
Spinach (Spinacia oleracea)	91.7	1.9
Soy leaves (Glycine Max. Merr)	79.5	6.0
Water cress (Nasturtium officinale)	89.2	2.9

Fat (g)	Fibre (g)	Carbo-hydrate (g)	Calcium (Ca) (g)	Phosphorus (P) (g)	Iron (Fe) (mg)	Calories (Kcal)	Carotene (μg)	Thiamine (mg)	Niacin (mg)	Riboflavin (mg)	Ascorbic acid (mg)
4	5	6	7	8	9	10	11	12	13	14	15
1.2	0.8	59.9	0.07	0.41	8.5	351	49	0.52	2.4	0.25	...
0.5	5.3	57.3	0.28	0.39	7.6	322	71	0.42	1.5	0·20	...
0.6	2.3	55.9	0.11	0.50	5.6	342	120	0.39	2.9	0.17	...
0.7	0.7	59.0	0.13	0.25	2.0	343	270	0.45	2.5	0.20	...
1.1	4.5	56.6	0.07	0.30	4.4	315	39	0.45	2.3	0.19	...
1.4	4.4	59.1	0.03	0.36	5.0	340	18	0.47	2.5	0.21	...
1.7	1.5	55.7	0.14	0.26	8.8	327	132	0.45	2.4	0.19	...
19.5	3.7	20.9	0.24	0.69	11.5	432	426	0.73	3.2	0.39	...
1.4	2.2	11.8	1.13	0.08	3.9	93	5400	0.21	1.2	0.09	169
0.5	...	5.7	0.50	0·10	21.4	47	5560	0.03	1.2	0.30	103
0.3	...	8.1	0.80	0.05	22.9	47	3660	0	...	...	35
0.5	...	7.5	0.02	0.09	0.1	47	0	0.08	0.2	0.19	5
1.4	2.0	9.7	0.34	0.12	23.8	79	978	0.09	0.6	0.10	61
0.5	1.2	8.0	0.05	0.08	2.3	55	128	0 05	0.4	0.16	72
0.1	1.0	6.3	0.08	0.05	0.8	33	1200	0.06	0.4	0.09	124
0.5	1.9	6.4	0.34	0.11	8.8	50	5700	0.04	2.1	0.37	79
0.6	1.4	8.6	0.23	0.14	6.3	64	3900	0	1.2	0.11	62
0.6	1.2	5.3	0.14	0.06	10.0	40	6900	0.05	0.8	0.06	135
1.0	6.4	16.0	0.81	0.60	3.1	97	7560	0.08	2.3	0.21	4
1.7	0.9	13.4	0.44	0.07	7.0	96	6780	0.06	0.8	0.05	220
0.9	1.0	9.8	0.47	0.05	16.9	67	2340	0.04	0.8	0.31	52
1.0	...	8.7	0.36	0.11	28.6	67	...	0.15	...	...	...
1.1	...	10.0	0.18	0.04	5.4	57	2900	0.07	1.1	0.39	20
0.4	1.2	3.1	0.11	0.05	3.9	27	1980	0.05	0.6	0.12	137
1.0	2.1	5.5	0.16	0.10	7.3	56	3000	0.01	...	0.03	41
0.3	0.5	3.0	0.05	0.03	2.4	23	990	0.09	0.4	0.12	15
1.0	...	8.9	0.41	0.07	20.5	68	...	...	0.9	0.59	11
0.6	2.0	8.0	0.20	0.08	15.6	57	1600	0.05	1.0	0.16	27
1.0	1.8	19.7	0.39	0.20	17.9	111	1900	0.04	0.5	0.18	281
0.7	2.8	11.6	0.51	0.06	16.7	73	1930	0	1.2	0.14	17
0.4	1.2	5.9	0.37	0.11	12.5	47	1400	0.01	0.9	0.03	65
0.7	...	5.1	0.18	0.06	7.6	40	3540	0.04	0	0.10	15
0.9	0.6	3.4	0.06	0.01	5.0	30	5600	0.05	0.5	0.16	28
0.5	...	10.8	0.18	0.19	8.0	72	...	...	...	0.16	...
0.2	0.6	4.9	0.29	0.14	4.6	33	2800	0.12	0.8	0.38	13

Table 1A: (Cont.)

Name of foodstuff	Moisture (g)	Protein (g)
1	2	3
Roots and Tubers		
Beet root (Beta vulgaris)	83.8	1.7
Carrot (Daucus carota)	86.0	0.9
Colocasia (Colocasia antiquorum)	73.1	3.0
Onion, big (Allium cepa)	86.8	1.2
Onion, small	84.3	1.8
Parsnips (Pastinaca sativa)	72.4	1.3
Potato (Solanum tuberosum)	74.7	1.6
Radish (Pink) (Raphanus sativus)	90.8	0.6
Radish (White) (Raphanus sativus)	94.4	0.7
Sweet potato (Ipomoea batatas)	68.5	1.2
Tapioca (Manihot esculenta)	59.4	0.7
Yam (elephant) (Amorphophallus camapanulatus)	78.7	1.2
Yam (ordinary) (Typhonium trilobatum)	69.9	1.4
Other vegetables		
Amaranth stem (Amaranthus gangeticus)	92.5	0.9
Artichoke (Cynara scolymus)	77.3	3.6
Ash gourd (Benincasa hispida)	96.0	0.4
Bitter gourd (Momordica charantia)	92.4	1.6
Bitter gourd (small variety) (Momordica charantia)	83.2	2.9
Brinjal (Solanum melongena)	91.5	1.3
Broad beans (Vicia faba)	82.4	4.5
Calabash cucumber (Lagenaria siceraria)	96.3	0.2
Cauliflower (Brassica oleracea botrytis)	89.4	3.5
'Cho-cho' marrow (Sechium edule)	92.5	0.7
Cluster beans (Cyamopsis tetragonoloha)	82.5	3.7
Cucumber (Cucumis sativus)	96.4	0.4
Double beans (Faba vulgaris)	73.8	8.3
Drumstick (Moringa oleifera)	86.9	2.5
French beans (Phaseolus vulgaris)	91.4	1.7
Jack, tender (Artocarpus hetero-phyllus)	84.0	2.6
Jack fruit seeds (Artocarpus heterophyllus)	51.6	6.6
'Kovai' fruit, tender (Coccinia cordifolia)	93.1	1.2
Knol-khol (Brassica caulorapa)	92.1	1.1
Ladies fingers (Abelmoschus esculentus)	88.0	2.2
Leeks (Allium porrum)	78.9	1.8

0.1	1.2	3.5	0.26	0.03	1.8	19	260	0.01	...	0.18	10
0.1	1.2	16.0	0.12	0.10	2.3	79	37	0.23	...	0.01	0
0.1	0.8	3.2	0.03	0.02	0.5	15	0	0.06	0.4	0.01	1
0.2	0.8	4.2	0.02	0.07	2.2	25	125	0.07	0.5	0.09	88
1.0	1.7	9.8	0.05	0.14	9.4	60	125	0.07	0.4	0.06	96
0.3	1.3	6.4	0.02	0.06	1.3	34	75	0.05	0.8	0.10	12
0.1	2.0	10.0	0.05	0.06	1.6	59	10	0.08	0.8	...	12
0.1	0.4	2.9	0.02	0.01	0.7	13	0	0.03	0.2	0.01	7
0.4	1.2	5.3	0.03	0.06	1.3	39	38	0.10	0.9	0.10	66
0.1	0.6	6.3	0.14	0.03	0.6	29	0	0	0.3	0.04	4
0.2	2.3	9.9	0.13	0.05	5.8	56	200	0.09	0.6	0.03	49
0.1	0.4	2.8	0.01	0.03	1.5	14	0	0.03	0.2	0.01	7
0.3	4.3	12.3	0.04	0.14	2.3	85	...	...	...	...	22
0.1	4.8	3.7	0.03	0.11	5.3	26	110	0.05	0.2	0.07	120
0.1	1.8	4.5	0.05	0.03	1.7	26	130	0.08	0.3	0.06	24
0.3	2.8	9.4	0.03	0.04	1.7	51	0	0.05	0.2	0.04	14
0.4	1.5	38.4	0.05	0.13	1.2	184	...	...	...	...	...
0.1	1.6	3.5	0.04	0.03	1.4	20	160	0.07	0.7	0.08	15
0.2	1.5	5.9	0.02	0.04	0.4	30	36	0.05	0.5	0.09	85
0.2	1.2	7.7	0.09	0.08	1.5	41	58	0.07	0.6	0.10	16
0.1	1.3	17.2	0.05	0.07	2.3	77	30	0.23	...	...	11

Fat (g)	Fibre (g)	Carbo-hydrate (g)	Calcium (Ca) (g)	Phosphorus (P) (g)	Iron (Fe) (mg)	Calories (Kcal)	Carotene (μg)	Thiamine (mg)	Niacin (mg)	Riboflavin (mg)	Ascorbic acid (mg)
4	5	6	7	8	9	10	11	12	13	14	15
0.1	0.8	13.6	0.20	0.06	1.0	62	0	0.04	0.4	0.09	10
0.2	1.1	10.7	0.08	0.05	1.5	47	1890	0.04	0.4	0.02	3
0.1	1.7	22.1	0.04	0.14	2.1	101	40	0.09	0.4	0.03	...
0.1	0.4	11.6	0.18	0.05	0.7	51	15	0.08	0.4	0.01	11
0.1	0.6	13.2	0.04	0.06	1.2	61	25	0.08	0.5	0.02	2
0.3	1.7	23.2	0.05	0.04	0.4	101	18	0.06	0.4	0.05	16
0.1	0.4	22.9	0.01	0.03	0.7	99	24	0.10	1.2	0.01	17
0.3	0.6	7.4	0.05	0.02	0.5	35	5	0.06	0.4	0.02	17
0.1	0.8	4.2	0.05	0.03	0.4	21	5	0.06	0.5	0.02	15
0.3	0.8	31.0	0.02	0.05	0.8	132	10	0.08	0.7	0.04	24
0.2	0.6	38.7	0.05	0.04	0.9	159	...	0.05	0.3	0.10	25
0.1	0.8	18.4	0.05	0.02	0.6	79	260	0.06	0.7	0.07	0
0.1	1.0	27.0	0.06	0.02	1.3	115	78	0.07	0.7	...	...

Table 1A: (Cont.)

Name of foodstuff	Moisture (g)	Protein (g)
1	2	3
Mango, green (Mangifera indica)	90.0	0.7
Indian gooseberry (Emblica officinalis)	81.2	0.5
Onion stalks (Allium cepa)	87.6	0.9
'Parwar' (Trichosanthes dioica)	92.3	2.0
Peas English (Pisum sativum)	72.1	7.2
Plantain flower (Musa sapientum)	90.2	1.5
Plantain, green (Musa sapientum)	83.2	1.4
Plantain, stem (Musa sapientum)	88.3	0.5
Pumpkin (Cucurbita maxima)	92.6	1.4
Rhubarb stalks (Rheum rhaponticum)	92.7	1.1
Ridge gourd (Luffa acutangula)	95.4	0.5
Waterchest-nut (Trapa bispinosa)	70.0	4.7
Snake-gourd (Trichosanthes anguina)	94.1	0.5
'Sundakai' dry (Solanum torvum)	12.3	8.3
Sword beans (Canavalia gladiata)	88.6	2.7
'Tinda' tender (Cirtrullus vulgaris)	92.3	1.7
Tomato, green (Lycopersicon esculentum)	92.8	1.9
Turnip (Brassica rapa)	91.1	0.5
Vegetable marrow (Cucurbita pepo)	94.8	0.5
Nuts and oilseeds		
Almond (Prunus amygdalus)	5.2	20.8
Cashewnut (Anacardium occidentale)	5.9	21.2
Coconut, fresh (Cocos nucifera)	36.3	4.5
Coconut, dry	3.5	6.6
Groundnut (Arachis hypogea)	7.9	26.7
Groundnut, roasted (Arachis hypogea)	4.0	31.5
Linseed seeds (Linum usitatissimum)	6.5	20.3
Mustard seeds (Brassica nigra)	8.5	22.0
Pistachio nut (Pistacia vera)	5.6	19.8
Sesame seeds (Sesamum indicum)	5.1	18.3
Walnut (Juglans regia)	4.5	15.6
Condiments, Spices, Etc.		
'Arisithippili' (Piper clusii)	12.5	13.2
Asafoetida (Perula foetida)	16.0	4.0
Cardamom seeds (Elettaria cardamomum)	8.3	10.3

Fat (g)	Fibre (g)	Carbo-hydrate (g)	Calcium (Ca) (g)	Phosphorus (P) (g)	Iron (Fe) (mg)	Calories (Kcal)	Carotene (μg)	Thiamine (mg)	Niacin (mg)	Riboflavin (mg)	Ascorbic acid (mg)
4	5	6	7	8	9	10	11	12	13	14	15
0.1	1.2	8.8	0.01	0.02	4.5	39	90	0.04	0.2	0.01	3
0.1	3.4	14.1	0.05	0.02	1.2	59	...	0.03	0.2	...	600
0.2	1.6	8.9	0.05	0.05	7.5	41	600	0	0.3	0 03	17
0.3	3.0	1.9	0.03	0.04	1.7	18	155	0.05	0.5	0.06	30
0.1	4.0	19.8	0.02	0.08	1.5	109	83	0.25	0.8	0.01	9
0.2	1.9	5.0	0.03	0.05	0.1	28	27	0.05	0.6	0.02	16
0.2	0.7	14.7	0.01	0.03	0.6	66	30	0.05	0.3	0.02	24
0.1	0.8	9.7	0.01	0.01	1.1	42	0	0.02	0.2	0.01	7
0.1	0.7	5.3	0.01	0.03	0.7	28	50	0.06	0.5	0.04	2
0.5	0.3	3.7	0.12	0.01	2.2	24	...	...	...	...	37
0.1	0.5	3.7	0.04	0.04	1.6	18	33	0.07	0.2	0.01	5
0.3	...	23.9	0.02	0.15	0.8	117	20	0.05	0.6	0.07	9
0.3	0.8	4.4	0.05	0.02	1.3	22	100	0.04	0.3	0.06	0
1.7	17.6	55.0	0.37	0.18	22.2	269	450	...	...	...	0
0.2	1.5	6.4	0.06	0.04	2.0	38	24	0.08	0.5	0.08	12
0.1	1.0	5.3	0.02	0.03	0.9	29	13	0.04	0.3	0.08	18
0.1	...	4.5	0.02	0.04	2.4	27	190	0.07	0.4	0.01	31
0.2	...	7.5	0.03	0.04	0.4	34	0	0.04	0.5	0.04	43
0.1	0.8	4.3	0.01	0.03	0.6	20	0	0.02	0.4	0	18
58.9	1.7	10.5	0.23	0.49	3.5	655	0	0.24	4.4	0.57	0
46.9	1.3	22.3	0.05	0.45	5.0	596	60	0.63	1.2	0.19	0
41.6	3.6	13.0	0.01	0.24	1.7	444	0	0.05	0.8	0.10	7
62.0	3.9	22.6	0.02	0.16	3.6	628	0	0.08	3.0	0.04	1
40.1	3.1	20.3	0.05	0.39	1.6	549	37	0.90	14.1	0.30	0
39.8	3.1	19.3	0.05	0.44	0.3	561	0	0.39	22.1	0.13	...
37.1	4.8	28.8	0.17	0.37	2.7	530	30	0.23	1.0	0.07	...
39.7	1.8	23.8	0.49	0.70	17.9	541	160	0.65	4.0	0.26	0
53.5	2.1	16.2	0.14	0.43	13.7	626	140	0.67	2.3	0.28	0
43.3	2.9	25.2	1.45	0.57	10.5	564	60	1.01	4.4	0.34	0
64.5	2.6	11.0	0.10	0.38	4.8	687	10	0.45	1.6	0.40	0
4.7	5.2	58.4	0.46	0.28	13.5	329	...	...	...	...	0
1.1	4.1	67.8	0.69	0.05	22.2	297	4	0	0.3	0.04	0
8.3	9.2	48.9	0.13	0.16	5.0	244	0	0.22	0.8	0.17	0

Table 1A: (Cont.)

Name of foodstuff	Moisture (g)	Protein (g)
1	2	3
Chillies, green (Capsicum frutescens)	82.6	2.9
Chillies, dry (Capsicum frutescens)	10.0	15.9
Cloves, dry (*Syzygium aromaticum*)	23.3	5.2
Coriander seeds (Coriandrum sativum)	11.2	14.1
Cumin seeds (Cuminum cyminum)	11.9	18.7
Fenugreek seeds (Trigonella foenum graecum)	13.7	26.2
Garlic (Allium sativum)	62.8	6.3
Ginger, fresh (Zingiber officinale)	80.9	2.3
'Kandanthippilli' (Piper roxburghii)	12.2	6.4
Lime peel (Citrus medica var acida)	66.5	1.8
Mace (Myristica fragrans)	15.9	6.5
Mustard (Brassica juncea)	8.5	22.0
Nutmeg (Myristica fragrans)	14.3	7.5
Omum (Trochyspermum ammi)	8.9	15.4
Pepper, dry (Piper nigrum)	12.9	11.5
Tamarind pulp (Tamarindus indica)	20.9	3.1
Turmeric (Crucuma domestica)	13.1	6.3
Fruits		
Amla or Indian gooseberry (Emblica officinalis)	81.8	0.5
Apple (Malus sylvestris)	85.9	0.3
Banana (Musa Paradisiaca)	61.4	1.3
Bilimbi (Averrhoa bilimbi)	93.9	0.5
Bread fruit (Artocarpus altilis)	79.5	1.5
Bullock's heart (Anona reticulata)	76.8	1.4
Cape gooseberry (Physalis peruviana)	82.7	1.8
Cashew fruit (Anacardium occidentale)	87.9	0.2
Custard apple (Annona squamosa)	73.5	1.6
Dates, Persian (Phoenix dactylifera)	26.1	3.0
Figs (Ficus carica)	80.8	1.3
Grapes, blue variety (Vitis vinifera)	85.5	0.8
Grapes, pale green variety	79.2	0.6
Grape fruit (Triumph) (Citrus paradisi)	92.0	0.7
Grape fruit (Marsh, seedless) (Citrus paradisi)	88.5	1.0
Guava, country (Psidium guajavo)	76.1	1.5
Guava, hill (Psidium cattelianum)	85.3	0.1
Jack fruit (Artocarpus heterophyllus)	77.2	1.9

0.1	3.4	13.7	0.05	0.02	1.2	58	9	0.03	0.2	0.01	600
0.1	1.0	13.4	0.01	0.02	1.7	56	0	0.12	0.2	0.03	2
0.2	0.4	36.4	0.01	0.05	0.4	153	80	0.05	0.3	0.08	7
0.2	0.4	4.8	0.01	0.01	0.6	23	18	0.09	0.6	0.04	32
0.2	2.1	17.9	0.04	0.03	0.5	79	15	0.04	...	0.07	21
0.2	5.2	20.9	0.01	0.01	0.6	91	70	...	0.6	0.07	5
0.2	3.2	11.5	0.01	0.06	1.8	55	1428	0.05	0.3	0.02	49
0.1	0.9	11.6	0.01	0.06	0.2	48	25	0.02	0.4	0.05	180
0.3	3.1	23.9	0.02	0.04	1.0	105	0	0.33	1.3	0.44	37
0.2	2.1	67.3	0.07	0.08	10.6	283	600	0.09	0.8	0.03	0
0.2	2.2	17.1	0.06	0.03	1.2	75	16	0.06	0.6	0.05	5
0.1	3.0	10.1	0.03	0.02	0.4	45	15	0.04	0.3	0.03	3
0.4	2.8	13.2	0.02	0.02	0.5	71	0	0.04	0.3	0.03	1
0.1	...	7.1	0.02	0.02	0.2	32	...	0.12	0.3	0.02	31
0.1	...	10.0	0.03	0.03	0.2	45	...	0.12	0.3	0.02	...
0.2	6.9	14.5	0.01	0.04	1.0	66	0	0.03	0.2	0.03	212
0.2	4.8	8.1	0.05	0.02	1.2	38	0	0.02	0.3	0.02	15
0.1	1.1	18.9	0.02	0.03	0.5	84	175	0.03	0.4	0.13	10

Fat (g)	Fibre (g)	Carbo-hydrate (g)	Calcium (Ca) (g)	Phosphorus (P) (g)	Iron (Fe) (mg)	Calories (Kcal)	Carotene (μg)	Thiamine (mg)	Niacin (mg)	Riboflavin (mg)	Ascorbic acid (mg)
4	5	6	7	8	9	10	11	12	13	14	15
0.6	6.8	6.1	0.03	0.08	1.2	41	175	0.19	0.5	0.29	111
6.2	30.2	31.6	0.16	0.37	2.3	246	345	0.93	9.5	0.43	50
8.9	9.5	47.9	0.74	0.10	4.9	293	250	0.08	0	0.13	0
16.1	32.6	21.6	0 63	0.37	17.9	288	950	0.22	1.1	0.35	0
15.0	12.0	36.6	1.08	0.49	31.0	356	520	0.55	2.6	0.36	3
5.8	7.2	44.1	0.16	0.37	14.1	333	100	0.34	1.1	0.29	0
0.1	0.8	29.0	0.03	0.31	1.3	142	0	0.06	0.4	0.23	13
0.9	2.4	12.3	0.02	0.06	2.6	67	40	0.06	0.6	0.03	6
2.3	8.5	65.8	1.23	0.19	62.1	310	...	...	...	...	0
0.5	...	29.4	0.71	0.06	2.7	129	...	...	...	...	...
24.4	3.8	47.8	0.18	0.10	12.6	437	3027	0.25	1.4	0.42	0
39.7	1.8	23.8	0.49	0.70	17.9	541	270	...	4.0	0.08	0
36.4	11.6	28.5	0.12	0.24	4.6	472	0	0.33	1.4	0.01	0
18.1	11.9	38.6	1.42	0.30	14.6	379	...	0.21	2.1	0.28	...
6.8	14.9	49.5	0.46	0.20	16.8	305	1080	0.09	1.4	0.14	...
0.1	5.6	67.4	0.17	0.11	10.9	283	60	...	0.7	0.07	3
5.1	2.6	69.4	0.15	0.28	18.6	349	30	0.03	2.3	0	0

Table 1A: (Cont.)

Name of foodstuff	Moisture (g)	Protein (g)
1	2	3
Jambu fruit (Syzigium cuminii)	78.2	0.7
'Korukkapalli' (Pithecolobium dulce)	80.8	2.6
Lemon (Citrus limon)	85.0	1.0
Lime (Citrus aurantifolia)	84.6	1.5
Loquat (Eribotrya japonica)	87.4	0.7
Mango, green (Mangifera Indica)	90.0	0.7
Mango, ripe (Mangifera Indica)	86.1	0.6
Mango 'Ankola' (Mangifera Indica)	85.9	1.0
Mangosteen (Garcinia mangostana)	84.9	0.5
Melon, water (Citrullus vulgaris)	95.7	0.1
Orange (Citrus urantium)	87.8	0.9
Palmyra fruit, tender (Borassus flabellifer)	92.7	0.6
'Pannir Koyya' or Rose apple (Syzygium jambos)	89.1	0.7
Papaya, ripe (Carica papaya)	89.6	0.5
Passion fruit (Passiflora dulis)	76.3	0.9
Peaches (Amygialis persica)	90.1	1.5
Pears, country (Prunus persica)	86.9	0.2
Pears, English (Pyrus achras)	85.8	0.9
Pears, avocado or Butter fruit (Persea americana)	73.6	0.7
Persimmon (Diospros kaka)	79.6	0.8
Pine apple (Ananas comosus)	86.5	0.6
Plantain (ordinary) (Musa paradisiaca)	73.4	1.1
Plantain, hill variety (Musa paradisiaca)	79.9	1.2
Plantain, red variety (Musa, rubrum)	74.1	1.6
Plum (Prunus domestica)	89.6	0.7
Pomegranate (Punica granatum)	78.0	1.6
Pomeloe (Citrus maxima)	88.0	0.6
Quince (Cydonia oblonga)	85.7	0.3
Raisins (Vitis vinifera)	18.5	2.0
Strawberry (Frangaria vesca)	87.8	0.7
Tamarind pulp (Tamarindus indicus)	20.9	3.1
Tomato, ripe (Lycopersicum esculentum)	94.5	1.0
Wood apple (Limonia acdissima)	69.5	7.3

Fat (g)	Fibre (g)	Carbo-hydrate (g)	Calcium (Ca) (g)	Phosphorus (P) (g)	Iron (Fe) (mg)	Calories (Kcal)	Carotene (µg)	Thiamine (mg)	Niacin (mg)	Riboflavin (mg)	Ascorbic acid (mg)
4	5	6	7	8	9	10	11	12	13	14	15
0.1	0.9	19.7	0.02	0.01	1.0	83	50	0.03	0.2	0.01	18
0.3	...	15.9	0.01	0.04	0.4	77	0	0.22	1.6	0.06	108
0.9	1.7	11.1	0.07	0.01	2.3	57	0	0.02	0.1	0.01	39
1.0	1.3	10.9	0.09	0.02	0.3	59	15	0.02	0.1	0.03	63
0.3	0.9	10.2	0.03	0.02	0.7	46	559	...	...	0	0
0.1	...	8.8	0.01	0.02	4.5	39	150	...	...	0.03	3
0.1	1.1	11.8	0.01	0.02	0.3	50	2540	0.08	0.9	0.09	16
0.1	...	12.5	0.01	0.02	0.5	55	1860	...	...	...	24
0.1	...	14.3	0.01	0.02	0.2	60	...	...	...	...	...
0.2	...	3.8	0.01	0.01	0.2	17	0	0.02	0.2	0.04	1
0.3	...	10.6	0.05	0.02	0.1	49	350	0.12	0.3	0.06	68
0.1	0.3	6.2	0.01	0.02	0.5	27	...	0.01	0.2	0.01	4
0.2	...	9.7	0.01	0.03	0.5	43	...	...	...	0.05	...
0.1	...	9.5	0.01	0 01	0.4	40	666	0.04	0.2	0.25	57
0.1	...	22.0	0.01	0.06	2.0	93	54	0.07	1.6	0.14	25
0.2	1.2	6.4	0.01	0.03	1.7	33	0	0.02	0.2	0.02	6
0.1	1.0	11.5	0.01	0.01	0.7	47	28	0.02	0.2	0.03	0
0.2	1.1	11.8	0.01	0.02	0.8	53	80	0.09	0.2	0.05	4
22.8	...	0.8	0.01	0.08	0.7	215	...	...	...	...	13
0.2	0.9	18.1	0.01	0.01	0.3	77	2268	0.03	0	0.01	33
0.1	0.3	12.0	0.02	0.01	0.9	50	18	0.20	0.1	0.12	39
0.1	..	24.7	0.01	0.03	0.5	104	124	0.05	0.3	0.17	6
0.1	...	18.0	0.01	0.03	0.3	78	124	0.04	0.3	0.14	9
0.1	...	23.4	0.01	0.02	0.6	101	350	0.04	0.3	0.15	8
0.2	...	8.9	0.02	0.02	0.5	40	166	0.04	0.3	0.03	1
0.1	5.1	14.6	0.01	0.07	0.3	65	0	0.06	0.3	0.10	16
0.1	0.6	10.2	0.03	0.03	0.1	44	120	0.03	0.2	0.03	20
0.1	1.7	11.9	0.01	0.02	0.4	49	...	0.02	0.2	0.02	10
0.2	1.1	76.2	0.10	0.08	4.0	315	0	0.06	0.5	0.09	0
0.2	1.1	9.8	0.03	0.03	1.8	44	18	0.03	0.2	0.02	52
0.1	5.6	67.4	0.17	0.11	10.9	283	100	0.06	0.7	0.05	3
0.1	...	3.9	0.01	0.02	1.0	21	350	0.12	0.4	0.06	32
0.6	5.2	15.5	0.13	0.11	0.6	97	61	0.04	0.8	0.17	3

Table 1A : (Cont.)

Name of foodstuff	Moisture (g)	Protein (g)	Fat (Ether Extractives) (g)
1	2	3	4
Fish and other sea foods			
Bombay Duck	89.3	9.1	0.7
Cat fish	77.0	17.6	3.1
Chela (fresh)	77.6	14.6	4.3
Chela (dried)	9.7	54.8	17.0
Crab (muscle)	83.5	8.9	1.1
Herring (Indian)	74.8	20.3	3.2
Lobster	76.3	20.5	0.9
Mackerel	77.3	18.9	1.7
Prawn	77.4	19.1	1.0
Rohu (Labeo rohita)	76.7	16.6	1.4
Sardine { Low fat	78.1	21.0	1.9
Sardine { High fat	63.7	20.5	14.3
Shark	76.0	21.6	0.4
Snail (big)	74.1	10.5	0.6
Vajra	76.2	19.9	1.5
Whitebait	79.1	14.5	1.4
Meat, Egg and Poultry			
Beef (muscle)	74.3	22.6	2.6
Duck (meat)	72.3	21.6	4.8
Egg, duck's	71.0	18.5	13.7
Egg, hen's	73.7	13.3	13.3
Fowl (meat)	72.2	25.9	0.6
Liver, sheep	70.4	19.3	7.5
Mutton (muscle)	71.5	18.5	13.3
Pork (muscle)	77.4	18.7	4.4
Pigeon (meat)	70.4	23.3	4.9
Milk and Milk Products			
Milk, cow's	87.6	3.3	3.6
Milk, buffalo's	81.0	4.3	8.8
Milk, goat's	85.2	3.7	5.6
Milk, human	88.0	1.0	3.9
Curds (from cow's milk)	90.3	2.9	2.9
Skimmed milk, liquid	90.5	3.5	0.1
Skimmed milk powder	4.1	35.0	0.1

...	0.01	0.19	0.8	114	...	trace	0.15	6.4	0.04	2
...	0.01	0.24	1.5	130	...	...	0.1	7.7	0.12	...
0.7	0.07	0.26	3.0	180	360	540	0.12	0.2	0.30	...
...	0.06	0.22	2.1	173	360	600	0.40	0.1	0.30	...
...	0.03	0.25	...	109	20	...	0.05	10.7	0.14	...
1.4	0.01	0.38	6.3	150	6690	...	0.36	17.6	1.70	20
...	0.15	0.15	2.5	194	9	...	0.18	6.8	0.27	...
...	0.03	0.20	2.3	114	trace	100	0.54	2.8	0.09	2
...	0.01	0.29	...	138	...	...	0.10	5.6	0.20	...
4.8	0.12	0.09	0.2	65	54	trace	0.05	0.1	0.20	2
5.1	0.21	0.13	0.2	117	49	trace	0.04	0.1	0.18	5
4.7	0.17	0.12	0.3	84	55	trace	0.05	0.3	0.14	1
7.0	0.02	0.01	0.2	67	62	trace	0.02	0.1	0.03	3
3.3	0.12	0.19	0.3	51	39	trace	0.05	0.1	0.06	1
5.1	0.12	0.09	0.2	35	...	...	0.05	0.1	0.2	1
54.0	1.37	1.00	1.4	357	...	...	0.45	1.0	1.64	5

Carbo-hydrates (g)	Calcium (g)	Phosphorus (g)	Iron (Fe) (mg)	Calorific value (Kcal)	Vitamin-A (μg)	Carotene (μg)	Vitamin-B_1 (mg)	Nicotinic acid (mg)	Riboflavin (mg)	Vitamin-C (mg)
5	6	7	8	9	10	11	12	13	14	15
...	0.03	0.18	2.4	43	...	...	...	...	...	...
1.0	0.01	0.23	0.4	86	...	...	0.04	2.5	0.03	...
1.5	0.59	0.34	2.0	103	...	...	...	...	...	...
5.1	2.95	1.70	10.0	392	...	.	...	...	...	...
3.4	1.37	0.15	21.2	59	...	...	...	3.1	...	...
2.2	0.43	0.31	9.3	119	...	...	...	...	...	...
1.0	0.02	0.28	...	94	...	...	...	...	...	...
0.5	0.43	0.31	4.5	93	...	...	...	...	...	...
0.8	0.32	0.28	5.3	89	...	...	0.01	4.8	0.1	...
4.4	0.65	0.18	1.0	97	...	...	0.05	0.7	0.07	22
...	0.09	0.36	2.5	101	...	...	...	2.6	...	...
...	0.08	0.32	2.2	211	...	...	...	2.1	...	...
0.8	0.36	0.26	1.4	93	...	...	...	2.5	...	...
12.4	0.87	0.12	...	97	...	...	...	...	...	...
1.0	0.04	0.38	0.7	97	...	...	0.10	2.5	0.20	...
2.5	0.64	0.44	3.8	81	...	...	0.10	2.3	0.18	...

Table 1A: (Cont.)

Name of foodstuff	Moisture (g)	Protein (g)	Fat (Ether Extractives) (g)
1	2	3	4
Cheese	40.3	24.1	25.1
'Khoa' (Whole buffalo milk)	30.6	14.6	31.2
'Khoa' (Whole cow's milk)	25.2	20.0	25.9
Milk, evaporated (Unsweetened)	73.8	7.0	7.9
Milk, condensed (Sweetened)	27.1	8.1	8.7
Milk powder (Whole)	2.0	26.4	27.5
Miscellaneous foodstuffs			
Arecanut, fresh (Areca cathecu)	31.3	4.9	4.4
Arrowroot flour (Maranta arundinecea)	16.5	0.2	0.1
Betel leaves (Piper betle)	85.4	3.1	0.8
Cocoa, dry powder	3.0	16.8	23.7
Coconut, tender	90.8	0.9	1.4
Coconut, milk	57.1	3.0	34.0
Coconut, water	95.5	0.1	0.1
Honey	20.0	0.1	...
Jaggery (Sugar cane)	3.9	0.4	0.1
Kalipakku (Arecanut processed)	13.8	6.4	8.4
Mahua flowers (Dry)	18.6	4.4	0.6
Neera (Palmyra)	84.7	0.4	...
Pappads	20.3	18.8	0.3
Mushroom	88.5	4.6	0.2
Rajkeera seeds (Amaranthus paniculatus)	8.9	15.4	5.3
Sago	12.2	0.2	0.2
Sugar cane juice	90.2	0.1	0.8
Toddy, fermented (Coconut)	98.3	0.2	0.2
Water chestnut, dry (Trapa bispinosa)	13.8	13.4	0.8
Yeast, dried (Brewer's)	13.6	39.5	0.6
Yeast, dried (Torula utilis)	7.8	35.7	1.8

47.2	0.05	0.13	1.5	248	0	3	...	...	...	...
83.1	0.01	0.02	1.0	334	0	...	...	...	...	...
6.1	0.23	0.04	6.7	44	0	5760	0.07	0.7	0.03	5
48.3	0.13	0.65	0.7	299	...	10	0.11	2.4	0.46	0
6.3	0.01	0.03	0.9	40	...	...	...	...	...	2
5.0	0.02	0.03	1.0	336	...	...	0.09	0.3	0.04	2
4.0	0.02	0.01	0.5	17	...	...	0.01	0.1	0	2
79.7	0.01	0.02	0.9	320	0	0	0.01	0.2	0.04	0
95.0	0.08	0.04	11.4	383	0	168	0.02	0.5	0.04	0
57.8	0.13	0.14	11.1	332	0	...	...	...	...	0
72.0	0.14	0.14	15.0	311	...	25	0.03	5.2	0.88	7
10.9	trace	0.14	9.1	45	...	...	0.02	0.1	0	13
52.4	0.08	0.38	17.2	288	0	0	...	...	...	0
4.3	0.01	0.11	1.5	43	...	...	0.14	2.4	0.16	12
65.7	0.22	0.65	17.6	372	...	...	0.17	3.6	0.2	0
87.1	0.01	0.01	1.3	351	...	...	...	...	...	...
9.1	0.01	0.01	1.1	39	0	6	0.02	...	0.04	...
1.3	0.1	0.01	1.3	7	0	15	0.01	0.2	0.01	...
68.9	0.07	0.44	2.4	336	...	...	...	...	0.04	4
39.1	0.44	1.49	43.7	320	...	3600	6.0	40.0	4.0	0
46.3	0.16	2.09	21.5	344	...	1920	3.2	27.0	2.2	0

Carbo-hydrates (g)	Calcium (g)	Phosphorus (g)	Iron (Fe) (mg)	Calorific value (Kcal)	Vitamin-A (μg)	Carotene (μg)	Vitamin-B_1 (mg)	Nicotine acid (mg)	Riboflavin (mg)	Vitamin-C (mg)
5	6	7	8	9	10	11	12	13	14	15
6.3	0.79	0.32	2.1	348	400	...	0.03	0.4	0.46	...
20.5	0.65	0.42	5.8	421	315	...	0.21	0.4	0.39	0
24.9	0.96	0.61	6.1	413	497	...	0.23	0.4	0.41	0
9.7	0.25	0.21	0.1	137	96	...	0.04	0.2	0.34	1
54.3	0.26	0.21	0.1	321	108	...	0.08	0.2	0.38	1
38.2	0.91	0.71	0.5	502	339	...	0.31	0.7	1.46	6

Table 2A: **Vitamin B_6, Pantothenic acid, biotin, choline, and inositol contents of edible portions of common foods (values per 100g.)**

Foods	Vitamin B_6 (mg)	Pantothenic acid (mg)	Biotin (μg)	Choline (mg)	Inositol (mg)
Cereals and their products					
Barley (whole)	0.55	0.73	31.0	139	392
Corn (yellow)	0.46	0.64	21.0	61	50
Oats	0.20	1.50	24.0	156	269
Rice (brown)	0.62	1.52	12.0	112	119
Rice polishings	2.0	3.33	57.0	102	454
Rice (parboiled)	0.10	1.37	10.0	98	25
Rice (white)	0.03	0.75	5.0	59	10
Wheat (whole)	0.52	1.37	16.0	94	370
Wheat, flour (refined)	0.33	0.54	1.0	52	110
Wheat (germ)	0.91	2.20	—	406	770
Bread, white	0.10	0.44	1.1	—	51
Bread, whole wheat	0.42	0.79	1.9	—	67
Pulses (Legumes)					
Cow pea	0.21	1.24	21.0	257	240
Kidney bean	—	0.65	—	—	—
Split pea	0.32	2.18	18.4	201	150
Lentil	0.49	1.50	13.2	223	130
Lima bean	0.55	1.30	9.8	—	170
Mung bean (Green gram)	0.57	2.50	7.5	209	70
Navy bean	—	1.21	—	—	500
Soybean	0.64	1.68	61.0	340	200
Vegetables					
Asparagus	0.13	0.62	—	10	—
Lima bean	0.17	0.45	—	—	—
Snap bean, green	0.06	0.20	—	42	—
Beet root	0.03	0.17	1.9	—	21
Cabbage	0.12	0.26	2.4	23	95
Carrot	0.12	0.27	1.4	13	48
Cauliflower	0.17	1.01	17.0	—	95
Cow pea	0.11	0.40	21.0	97	240
Peas, green	0.15	0.82	9.4	75	162
Potato	0.22	0.40	—	29	29
Sweet potato	0.32	0.93	4.3	11	66
Spinach	0.19	0.31	6.9	22	27

Table 2A: (Contd.)

Foods	Vitamin B^6 (mg)	Pantothenic acid (mg)	Biotin (μg)	Choline (mg)	Inositol (mg)
Tomato	—	0.38	4.0	—	46
Turnip	0.10	0.38	—	27	46
Nuts and Oilseeds					
Almond	0.10	0.58	18.0	—	—
Cashewnut	—	1.62	—	—	—
Coconut (fresh)	—	0.33	—	—	—
Peanut (roasted)	0.30	2.14	34.0	162	180
Walnut	0.96	0.97	37.0	—	—
Fruits					
Apple	0.03	0.10	0.9	—	24
Apricot	0.07	0.29	—	—	—
Banana	0.32	0.31	4.4	—	34
Raspberry	0.03	0.21	—	—	—
Strawberry	0.06	0.16	4.0	—	60
Figs	0.13	0.34	—	—	—
Grape fruit	0.02	0.24	3.0	—	150
Grapes	0.08	0.05	1.6	—	—
Orange	0.03	0.22	1.9	—	210
Peaches	0.02	0.12	1.7	—	96
Pineapple	0.02	0.07	—	—	—
Watermelon	0.03	0.30	3.6	—	64
Dates	—	0.78	—	—	—
Raisins	0.32	0.10	4.5	—	120
Flesh foods					
Lamb: Muscle	0.32	0.62	5.9	84	58
Liver	0.64	7.10	127.0	508	53
Beef: Muscle	0.49	0.52	2.6	68	12
Liver	0.66	9.34	96.0	510	51
Pork: Bacon	0.55	0.44	7.6	80	43
Ham	0.44	0.64	5.0	122	31
Liver	0.65	7.1	100.0	552	52
Chicken, meat	0.13	0.80	11.3	—	48
Crab	0.36	—	—	—	—
Shrimp	0.11	0.21	—	—	—
Mackerel	0.27	0.47	18.0	—	—
Salmon	0.45	0.58	15.0	—	17

Table 2A: (Contd.)

Foods	Vitamin B_6 (mg)	Pantothenic acid (mg)	Biotin (μg)	Choline (mg)	Inositol (mg)
Sardines	0.28	0.60	24.0	—	—
Egg (whole)	0.25	1.59	22.5	504	33
Milk and Milk Products					
Milk (cow's)	0.03	0.31	4.7	15	13
Cheese	0.06	0.40	3.6	48	25
Miscellaneous					
Chocolate	0.02	0.19	32.0	—	85
Molasses	0.27	0.46	9.0	86	150
Honey	0.01	0.06	—	—	—
Yeast (Brewer's)	2.41	11.0	200.0	240	—
Yeast (Torula)	3.50	10.0	100.0	250	270

Table 3A: **Magnesium, Copper, Sodium, Potassium and Oxalic acid contents of some foodstuffs (Values per 100g)**

Name of Foodstuff	Magnesium (mg)	Copper (mg)	Sodium (mg)	Potassium (mg)	Oxalic acid (mg)
Cereals					
Bajra or pearl millet	125	0.55	10	402	14.4
Barley	127	0.34	4	179	2.0
Cholam or Kaffir corn	140	0.55	7	321	10.0
Maize or corn	144	0.19	6	290	6.0
Rice, raw (home pounded)	—	—	10	116	4.6
Rice, raw (polished)	48	0.72	3	110	3.0
Rice (parboiled, milled)	38	0.33	9	161	1.0
Bread, white	—	—	28	120	—
Wheat flour (refined)	55	0.49	14	101	—
Wheat (whole)	139	0.49	18	349	8.0
Pulses					
Bengalgram (with husk)	168	0.76	71	322	5.0
Bengalgram dhal	138	0.98	72	386	5.0
Blackgram (with husk)	—	—	80	523	27.8
Blackgram dhal	185	0.72	75	643	16.0
Cow gram	230	0.75	95	323	9.0
Field bean	—	—	89	402	—
Green gram (with husk)	171	0.97	57	583	2.0
Green gram dhal	189	1.13	49	643	0
Horse gram	172	1.03	46	322	417.0
Lentil (Masur dhal)	94	0.66	29	402	14.0
Peas (dried)	124	0.85	20	980	0
Red gram dhal	133	1.25	66	482	9.0
Leafy vegetables					
Brussels sprouts	26	0.07	12	400	4.0
Cabbage	10	0.08	7	277	3.0
Celery	52	0.30	91	293	37.0
Coriander leaves	64	0.53	4	453	5.0
Lettuce	30	0.08	9	329	13.6
Spinach	84	0.01	89	570	658.0
Roots and Tubers					
Beetroot	9	0.20	60	300	40.4
Carrot	14	0.13	47	482	5.6
Colocasia	—	—	25	464	133.4
Onion	—	—	5	385	1.0
Potato	20	0.20	18	249	15.0

Table 3A: (Contd.)

Name of Foodstuff	Magnesium (mg)	Copper (mg)	Sodium (mg)	Potassium (mg)	Oxalic acid (mg)
Radish	9	0.19	41	325	9.2
Sweet Potato	—	—	10	373	—
Tapioca	66	0.15	2	10	17.1
Yam	34	0.16	22	422	15.0
Other vegetables					
Ash gourd	13	0.17	2	211	3.4
Bitter gourd	17	0.18	14	382	0.5
Brinjal	16	0.17	3	229	18.0
Cucumber	11	0.10	7	149	15.0
Cauliflower	20	0.05	12	285	6.8
Drumstick	24	0.60	6	463	101.0
French beans	29	0.21	10	282	31.2
Knol-khol	18	0.09	12	199	10.0
Ladies fingers	43	0.19	54	332	8.0
Indian gooseberry (Amla)	—	—	3	201	296.0
Peas, green	34	0.23	2	346	6.0
Plantain, green	33	0.16	3	365	480.0
Pumpkin	14	0.20	2	259	—
Tomato	15	0.19	3	360	2.0
Turnip	—	—	6	243	0.8
Vegetable marrow	13	0.22	5	220	56.0
Fruits					
Apple	7	0.13	3	94	1.5
Banana	34	0.40	2	141	2.2
Custard apple	24	0.15	2	140	—
Dates	59	0.21	13	537	—
Figs (Fresh)	20	0.06	2	190	—
Grapes	4.0	0.08	4	249	—
Grape fruit	10	0.06	1	200	3.4
Guava	—	—	15	106	—
Lemon	12	0.26	1	148	—
Peaches	21	0.06	1	212	1.0
Pears	7	0.40	2	161	4.0
Pine apple	20	0.36	1	86	5.0
Plums	147	0.23	1	198	1.0
Pomegranate	12	0.17	4	171	14.0
Papaya, ripe	11	0.20	5	211	1.0
Raspberries	22	0.21	1	224	—

Table 3A: (Contd.)

Name of Foodstuff	Magnesium (mg)	Copper (mg)	Sodium (mg)	Potassium (mg)	Oxalic acid (mg)
Raisin, dry	42	0.24	67	629	—
Strawberries	12	0.13	1	161	—
Water melon	13	0.04	1	115	—
Nuts and oil seeds					
Almonds	257	0.14	6	856	407.3
Cashew nut (unsalted)	—	—	41	129	318.0
Groundnut, roasted (unsalted)	181	0.27	4	700	—
Coconut, fresh	52	0.32	15	336	—
Coconut, dry	30	0.04	30	700	—
Sesame seeds	—	—	60	725	708.0
Dairy Products					
Milk, fresh (whole)	14.0	0.02	34	130	0.2
Milk, fresh (skimmed)	14.0	0.02	32	116	0.2
Milk (condensed)	36.0	0.08	140	340	0.4
Milk (malted dry)	—	—	440	720	0.2
Skim milk powder	111.0	1.39	450	1210	0.8
Whole milk powder	112.0	0.16	410	1100	0.8
Butter (unsalted)	2.0	0.03	4	5	—
Egg, whole	12.0	0.03	129	145	0.2
Egg, white only	10.0	0.03	184	154	0.2
Egg, yolk only	14.0	0.02	57	129	0.2
Flesh Foods					
Fish (Rohi)	—	—	69	225	0.6
Mutton	27.0	0.16	63	244	0.2
Chicken	29.0	—	58	322	0.3
Liver	20.0	5.8	83	296	0.4
Lamb	—	—	90	327	0.5
Pork	26.0	—	55	260	0.2
Beef	24.0	—	65	333	0.2
Miscellaneous					
Coffee	235	0.82	74	2020	15.4
Dried yeast	—	—	3	670	—
Sago	0.7	0.01	2	4	—
Tea	254	1.59	44	2160	375.0 —1450.0
Cocoa	420	0.92	6	1522	442

Table 4 A: **Amino Acid Composition of some**

Foodstuffs	Try	Thr
1	2	3
Cereals and millets		
Amaranth seeds (Amaranthus paniculatus)	0.9	4.4
Barley (Hordeum vulgare)	1.2	3.2
Buckwheat (Fagopyrum esculentum)	1.4	3.8
Canihua (Chenopodium pallidiculae)	0.8	4.8
Common Millet (Panicum miliaceum)	1.1	1.1
Corn (Zea mays)	0.6	4.0
Corn germ	1.0	4.3
Kaffir corn (Sorghum vulgare)	1.1	3.6
Italian millet (Seteria italica)	1.0	3.1
Job tears (Coiz lacryma-jobi)	0.5	4.2
Kodu millet (Paspalum scrobiculatum)	0.7	3.8
Little Millet (Panicum miliare)	0.6	3.4
Oats (Avena sterilis)	1.2	3.1
Quinoa (Chenopodium quinoa)	1.1	4.8
Ragi (Eleusine coracana)	1.3	4.1
Rice (Oryza sativa)	1.0	3.7
Wheat, whole (Triticum vulgare)	1.2	2.7
Wheat germ	1.0	4.9
Legumes (Dry seeds)		
Black gram (Phaseolus mungo)	1.0	3.4
Broad bean (Vicia faba)	0.9	3.3
Chick-pea or Bengal gram (Cicer arietinum)	0.8	3.6
Cowpea (Vigna app)	1.0	3.9
Field bean (Dolichos lab lab)	0.5	3,3
Green gram (Phaseolus radiatus)	0.7	3.1
Horse gram (Dolichos biflorus)	1.0	3.9
Lathyrus pea (Lathyrus sativus)	0.8	2.2
Kidney bean (Phaseolus vulgaris)	1.1	3.5
Lentil (Lens esculentus)	0.9	3.9
Lima bean (Phaseolus lunatus)	0.9	4.7
Navy bean (Phaseolus vulgaris)	—	5.2
Pea (Pisum sativum)	1.1	3.9
Pinto bean (Phaseolus vulgaris)	1.0	4.3
Red gram or Pigeon pea (Cajanus cajan)	0.5	3.8

important Food Proteins (g/16 gN)

Iso	Leu	Lys	Met	Cys	Met + Cys	Phe	Tyr	Val	Arg	His
4	5	6	7	8	9	10	11	12	13	14
6.9	8.0	8.2	2.5	2.2	4.7	4.8	3.3	6.1	14.8	2.9
4.0	6.5	3.2	1.3	1.9	3.2	4.8	3.4	4.7	4.8	1.7
3.8	5.8	5.9	1.8	2.0	3.8	3.8	2.1	5.2	8.0	2.2
6.8	5.8	6.0	1.8	1.1	2.9	3.6	2.0	4.6	7.9	2.5
6.4	13.4	4.2	2.9	—	—	—	—	6.9	1.1	0.5
4.6	13.0	2.9	1.9	1.3	3.2	4.5	6.1	5.1	3.5	2.6
3.9	7.1	5.5	1.6	0.9	2.5	3.3	2.3	5.4	7.8	3.2
5.4	16.1	2.7	1.7	1.7	3.4	5.0	2.8	5.7	3.8	1.9
7.6	16.2	2.1	2.8	—	—	6.7	—	6.9	3.6	2.1
5.6	23.7	2.5	3.1	1.8	4.9	4.8	—	—	3.5	2.1
6.5	9.7	2.7	3.0	—	—	8.0	—	6.5	4.4	1.9
6.7	10.9	1.8	3.2	—	—	4.8	—	6.1	4.7	1.9
4.8	7.0	3.4	1.4	2.0	3.4	4.9	3.4	5.6	6.1	1.7
6.6	7.1	6.6	2.5	1.0	3.5	3.6	2.3	4.1	7.5	2.7
6.0	9.3	3.0	3.0	2.2	5.2	4.0	5.3	7.1	1.5	1.2
4.5	8.2	3.8	1.7	1.3	3.0	4.8	4.4	6.7	5.5	1.6
4.1	6.3	2.6	1.4	2.1	3.5	4.6	3.5	4.3	4.5	1.9
4.3	6.3	5.7	1.5	1.1	2.6	3.3	3.3	5.0	6.7	2.5
5.9	8.7	6.4	1.4	1.2	2.6	5.3	2.3	6.1	6.6	2.4
6.3	8.7	5.6	0.4	0.7	1.1	4.2	4.7	5.0	7.0	2.9
5.7	7.4	6.9	1.3	1.4	2.7	4.9	3.3	4.9	7.5	2.7
4.9	7.5	6.5	1.5	1.3	2.8	5.2	3.0	5.7	6.4	3.0
6.0	8.9	8.1	0.7	1.0	1.7	5.3	—	5.6	9.2	2.8
5.5	9.0	6.8	1.1	0.6	1.7	4.8	1.6	5.9	5.6	2.2
6.7	7.9	7.9	1.4	2.2	3.6	6.9	2.6	5.9	5.7	3.0
6.6	6.6	7.5	0.5	1.1	1.6	4.2	—	4.0	7.8	2.6
4.9	7.7	9.2	1.0	—	—	5.3	—	5.9	5.9	2.4
5.3	7.0	6.1	0.7	0.8	1.5	4.4	2.7	5.4	7.6	2.2
5.8	8.3	6.6	1.6	1.5	3.1	5.9	2.6	6.3	6.4	3.2
4.8	8.0	7.9	—	1.2	—	6.1	—	6.4	6.9	2.9
5.6	8.3	7.3	1.2	1.3	2.5	5.0	4.0	5.6	8.8	2.7
5.7	8.2	6.8	1.0	—	—	6.2	—	6.2	5.4	2.9
6.1	7.8	7.1	1.2	1.4	2.6	8.6	3.3	5.3	6.8	2.8

Table 4 A: (Contd.)

Foodstuffs	Try	Thr
1	2	3
Oilseeds and nuts		
Almond (Prunus amygdalus)	0.8	2.7
Brazil nut (Bertho lettia excelsa)	1.1	2.6
Cashewnut (Anacardium occidentale)	1.2	3.4
Coconut (Cocos mucifera)	0.8	3.2
Cotton seed (Gossypium herbaceum)	1.2	6.3
Filbert nut (Corylus avellana)	1.4	2.8
Peanut (Arachis hypogea)	1.1	2.7
Pecans nut (Carya peacan)	1.3	3.5
Rapeseed (Brassica napus)	1.4	3.4
Safflower seed (Carthamus tinctorius)	1.4	2.9
Sesame seed (Sesamum indicum)	1.5	3.1
Soy bean (Glycine max)	1.4	3.9
Sunflower seed (Helianthus annus)	1.3	3.4
Walnut (Juglans regia)	1.0	3.3
Roots and tubers		
Carrot (Daucus carota)	0.8	3.6
Colocasia (Taro)	1.4	4.7
Potato (Solanum tuberosum)	1.1	3.9
Sweet potato (Ipomoea batatas)	1.7	4.7
Tapioca (Cassava)	1.3	2.8
Leafy and other vegetables		
Amaranth leaves (Amaranthus gangeticus)	1.0	1.6
Cabbage (Brassica cleraces capitata)	0.8	2.8
Spinach (Spinacia oleracea)	1.6	4.4
French bean (Phaseolus vulgaris)	1.4	3.8
Peas, green (Pisum sativum)	0.8	3.7
Leaf and grass protein concentrates		
Clover (Trifolium subterraneum)	1.6	5.1
Turnip (Brassica rapa)	1.3	5.3
Rye grass (Secale cereale)	1.8	5.1
Lucerne (Medicago sativa)	1.9	5.1
Wheat grass (Triticum aestivum)	1.6	5.3
Corn leaf (Zea mays)	1.5	5.1

Iso	Leu	Lys	Met	Cys	Met + cys	Phe	Tyr	Val	Arg	His
4	5	6	7	8	9	10	11	12	13	14
3.9	6.5	2.6	1.2	1.7	2.9	5.1	2.8	5.0	12.2	2.3
3.6	6.9	2.7	3.7	2.1	5.8	3.7	2.9	5.0	13.6	2.2
5.6	7.0	3.6	1.6	2.4	4.0	4.3	3.3	7.3	9.6	2.0
4.5	6.7	3.8	1.8	1.6	3.4	4.3	2.5	5.3	12.1	1.7
3.8	5.9	4.3	1.4	1.6	3.0	5.2	2.7	4.9	11.2	2.7
5.7	6.3	2.8	0.9	1.1	2.0	3.6	2.9	6.2	14.5	1.9
4.1	6.1	3.6	0.9	1.5	2.4	3.1	3.6	5.0	10.7	2.4
5.0	7.2	3.9	1.4	2.0	3.4	5.1	2.9	4.7	10.7	2.5
5.2	3.1	4.9	1.5	1.2	2.7	6.5	—	6.2	7.2	2.0
3.9	5.5	3.1	1.5	—	—	5.3	—	4.9	9.3	2.0
4.2	7.4	2.6	2.8	2.2	5.0	6.4	4.1	3.9	8.8	1.9
5.4	7.7	6.3	1.3	1.8	3.1	4.9	3.2	5.3	7.2	2.4
4.7	6.4	3.2	1.6	1.7	3.3	4.5	2.4	5.0	8.7	2.2
3.3	5.3	2.5	1.7	1.8	3.5	4.3	3.3	5.5	9.7	2.3
3.8	5.4	4.3	0.9	2.5	3.4	3.5	1.7	4.7	3.5	1.4
5.2	8.9	5.8	1.1	—	—	5.2	—	6.0	6.2	1.7
4.4	5.0	5.3	1.3	1.0	2.3	4.4	1.8	5.3	4.9	1.4
4.8	5.7	4.7	1.9	1.6	3.5	5.6	4.5	7.4	5.2	2.0
2.8	4.1	4.1	0.6	1.1	1.7	2.8	1.9	3.0	10.0	1.5
4.7	6.0	4.0	0.7	0.7	1.4	2.8	3.0	3.9	3.8	2.0
2.9	4.1	4.7	0.9	2.0	2.9	2.3	2.1	3.2	7.5	1.8
4.6	7.7	6.2	1.7	2.0	3.7	4.0	3.2	5.5	5.1	2.1
4.5	5.8	5.3	1.4	1.0	2.4	2.4	2.1	4.8	4.2	1.9
4.6	6.2	4.7	0.8	1.1	1.9	3.8	2.4	4.1	8.9	1.6
5.5	9.2	6.5	1.9	0.9	2.8	6.2	4.4	6.5	6.5	1.8
5.3	9.7	6.6	1.6	0.6	2.2	6.5	4.4	6.5	6.6	2.9
5.2	9.1	6.6	2.1	0.9	3.0	6.1	4.5	6.4	6.9	2.5
6.6	9.6	6.3	1.9	0.6	2.5	6.4	4.5	6.3	4.8	2.1
5.0	9.4	6.1	2.0	0.6	2.6	6.1	3.8	6.4	6.7	2.8
4.9	10.0	4.6	2.1	0.7	2.8	6.0	3.9	6.5	5.8	1.9

Table 4 A : (Contd.)

Foodstuffs	Try	Thr
1	2	3
Milk		
Milk, Buffalo's	1.4	5.2
Milk, Cow's	1.4	4.7
Milk, Goat's	1.3	4.3
Milk, Human	1.7	4.5
Eggs		
Egg, whole (Hen)	1.7	5.0
Egg, white (Hen)	1.7	4.9
Egg, yolk, (Hen)	1.7	5.1
Egg, whole (Duck)	1.4	7.0
Egg, white (Duck)	1.4	7.5
Egg, yolk (Duck)	1.5	6.4
Fish and Shell fish		
Crab	1.0	3.3
Mackeral	1.0	4.4
Prawn	1.0	4.0
Sardine	1.2	4.7
Shrimp	0.7	4.3
Meat		
Beef	1.2	4.1
Chicken	1.2	4.3
Mutton (Lamb)	1.3	4.6
Pork	1.3	4.6
Veal	1.1	4.7
Liver (Beef)	1.5	4.8
Liver (Lamb)	1.5	4.8
Liver (Pork)	1.5	4.8
Isolated Proteins		
Casein	1.3	4.3
Egg, Albumin	1.4	4.9
Gelatin	0	1.9
Lactalbumin	2.3	5.4
Wheat gluten	1.0	2.4
Zein	0.1	3.2

Key to abbreviations: Try=Tryptophan; Thr=Threonine; Iso=Isoleucine; Phe=Phenylalanine; Val=Valine; Arg=Arginine ; His=Histidine.

Iso	Leu	Lys	Met	Cys	Met + cys	Phe	Tyr	Val	Arg	His
4	5	6	7	8	9	10	11	12	13	14
5.0	9.1	8.1	2.7	1.4	4.1	4.3	5.1	6.2	3.3	2.1
6.5	10.0	7.9	2.5	0.9	3.4	4.9	5.2	7.0	3.7	2.7
5.2	8.7	6.3	2.2	0.7	2.9	4.4	4.9	5.8	2.2	2.0
5.5	9.1	6.6	2.4	2.0	4.4	4.4	5.2	6.3	4.1	2.2
6.6	8.8	6.4	3.1	2.4	5.5	5.8	4.2	7.3	6.6	2.4
6.5	8.8	6.2	3.4	2.4	5.8	6.4	4.2	7.5	6.3	2.2
6.7	8.8	6.6	2.9	2.4	5.3	5.2	4.2	7.2	6.9	2.5
5.5	9.6	7.9	4.9	1.7	6.6	6.2	5.4	7.5	5.0	2.9
5.0	9.6	7.1	5.5	1.9	7.4	6.7	5.5	7.9	4.0	2.5
6.1	9.7	8.7	4.3	1.5	5.8	5.7	5.3	7.1	5.9	3.3
3.3	3.3	8.3	2.3	1.2	3.5	3.4	2.8	3.3	6.3	1.9
5.1	7.5	8.8	2.9	1.3	4.2	3.7	2.7	5.3	5.6	2.8
5.2	9.0	7.2	2.9	2.2	5.1	4.6	3.8	5.0	8.8	3.7
4.5	6.9	8.4	2.8	1.5	4.3	3.4	2.8	5.1	5.1	2.9
3.2	6.7	6.3	3.0	2.8	5.8	3.8	3.7	4.0	5.0	3.1
5.2	8.2	8.7	2.5	1.3	3.8	4.1	3.4	5.6	6.5	3.5
5.3	7.2	8.8	2.6	1.3	3.9	3.9	3.5	4.9	6.3	2.9
5.2	7.7	8.1	2.4	1.3	3.7	4.1	3.8	4.9	6.5	2.8
5.1	7.4	8.2	2.5	1.2	3.7	3.9	3.6	5.2	6.1	3.5
5.2	7.9	8.8	2.2	1.1	3.3	4.4	3.7	4.8	7.8	3.5
5.2	9.2	7.5	2.3	1.2	3.5	5.0	3.7	6.3	6.1	2.7
5.2	9.2	7.5	2.4	1.2	3.6	5.0	3.7	6.3	6.1	2.7
5.2	9.2	7.5	2.3	1.2	3.5	5.0	3.7	6.3	6.1	2.7
6.6	10.1	8.1	2.7	0.4	3.1	5.4	5.9	7.2	4.1	3.0
6.0	8.7	6.7	3.6	2.4	6.0	6.5	3.0	8.3	5.9	2.5
1.4	3.0	4.3	0.8	0.1	0.9	2.1	0.5	2.6	8.2	0.8
6.5	12.8	9.4	2.3	3.0	5.3	4.5	4.0	5.9	3.6	2.0
4.0	7.5	1.9	1.6	1.9	3.5	5.5	3.2	4.8	4.3	2.4
5.1	19.2	0	1.7	1.1	2.8	10.4	6.1	4.1	1.8	1.3

Leu=Leucine; Lys=Lysine; Cys=Cystine; Met=Methionine;

Table **5A: Normal values of blood constituents**

Haemoglobin
Men 14-16g/100ml
Women 13-15g/100ml.

Erythrocytes
Men 5-5.5 million/cu.mm.
Women 4.5-5.0 million/cu.mm.

Reticulocytes: 0.8-1.0 per cent

Hematocrit
Men 44.0-48.0 per cent
Women 40.0-44.0 per cent

Mean corpuscular volume: 84-90 cu. microns.
Mean corpuscular haemoglobin concentration: 34-36 per cent.
Serum iron: 50 to 180 μg/100 ml.
Total protein: 6 to 8 g/100 ml of serum.
Albumin: 4 to 5.5 g/100 ml of serum.
Non-protein nitrogen (NPN): 25 to 38 mg/100 ml. of plasma
Urea nitrogen: 8 to 20 mg/100ml. of plasma.
Uric acid: 2 to 6 mg/100 ml of serum.
Glucose: 80 to 120 mg/100 ml of blood.
Cholesterol, total: 120 to 260 mg/100 ml of serum.
Cholesterol, esters: 60 to 80 per cent of total cholesterol.
Total lipids: 570 to 820 mg/100 ml of serum.
Calcium: 9 to 11 mg/100 ml serum (4.5 to 6.0 mEq/l).
Chlorides: 355-376 mg/100 ml serum. (98-106 mEq/l)
Copper: 100 μg/100 ml of blood.
Ceruloplasmin: 15 to 30 mg/100 ml. plasma.
Iron binding capacity: 300 to 450 μg/100 ml.
Protein bound iodine (PBI): 4 to 8 μg/100 ml. of serum.
Magnesium: 1.2 to 3.0 mg/100 ml of serum (0.4 to 1.0 mEq/l).
Manganese: 2.5 μg/100 ml. of serum.
Phosphorus (Inorganic): 3.0 to 4 mg. (adults); 4 to 6 mg (children)/100 ml of serum.
Potassium: 14 to 20 mg/100 ml serum (3.6 to 5.0 mEq/l).
Sodium: 315 to 340 mg/100 ml serum (136 to 145 mEq/l).
Zinc: 120 to 130 μg/100 ml of serum.
Vitamin A: 25 to 90 μg/100 ml of serum.
Carotene: 40 to 250 μg/100 ml of serum.
Tocopherol: 0.5 to 2.02 mg/100 ml of plasma.
Folate activity for *lactobacillus casei:* 6 to 10 mμg/ml of serum.
Vitamin B_{12} (*euglena gracilis*): 100 to 800 $\mu\mu$g/100 ml of serum.
Ascorbic acid: 0.3 to 1.4 mg/ml of serum.
Serum alkaline phosphatase: 1 to 4 Bodansky units (5 to 14, children)
5-10 King-Armstrong Units (12 to 25, children)
Prothrombin time (Quick): 10 to 15 seconds.
Bleeding time: 1 to 3 minutes.
Coagulation time: 6 to 12 minutes.

Table **6A: Some common units and measures with conversion factors**

	Mass
1 gram (g)	= 1000 milligrams (mg)
1 mg.	= 1000 micrograms (μg)
1 μg	= 1000 milimicrograms (m μg) (Nanogram)
1 m μg	= 1000 micromicrograms ($\mu\mu$g) (Picogram)
1 ounce (oz)	= 28.4 g
1 pound (lb)	= 16 oz=453.6g
1 g	= 15.4 grains
1 grain	= 65 mg.
	Volume
1 litre (l)	= 1000 millilitres (ml)
1 millilitre (ml)	= 1000 microlitres (μl)
1 litre	= 35.196 fluid ounces (oz)
1 litre	= 1.76 pint
1 pint	= 568 ml
1 pint	= 20 fluid ounces
1 quart	= 2 pints
1 fluid ounce	= 28.4 ml.
1 gallon	= 4.55 litres.
	Length
1 metre (m)	= 1000 millimetres (mm)
1 millimetre (mm)	=1000 microns (μ)
1 micron	= 1000 millimicrons (mμ)
1 mμ	= 1000 micromicrons ($\mu\mu$)
1 mμ	= 10 Angstrom units (A).

Table 7A: **Normal heights and weights of infants and pre-school children* (birth to 5 years)**

Age Year Month	Weight (kg)		Height (cm)	
	Boys	Girls	Boys	Girls
Birth	3.40	3.36	50.6	50.2
0–3	5.72	5.62	60.4	59.5
0–6	7.58	7.26	66.4	65.2
0-9	9.07	8.71	71.2	70.1
1–0	10.07	9.75	75.2	74.2
1–3	10.75	10.43	78.5	77.6
1–6	11.43	11.11	81.8	80.9
2–0	12.56	12.29	87.5	86.6
2–6	13.61	13.43	92.1	91.4
3–0	14.61	14.42	96.2	95.7
3–6	15.56	15.38	99.8	99.5
4–0	16.51	16.42	103.4	103.2
4–6	17.42	17.46	106.7	106.8
5–0	18.37	18.37	108.7	109.1

* 50th percentile data from studies of Child Health Development, Department of Maternal and Child Health, Harvard School of Public Health.

Table 8A: **Normal weights and heights of children and adolescents* (5-18 yrs)**

Age Years	Weight (kg)		Height (cm)	
	Boys	Girls	Boys	Girls
5	19.41	18.78	111.3	109.7
6	21.91	21.09	117.5	115.9
7	24.54	23.68	124.1	122.3
8	27.26	26.35	130.0	128.0
9	29.94	28.94	135.5	132.9
10	32.61	31.89	140.3	138.6
11	35.25	35.74	144.2	144.7
12	38.26	39.74	149.6	151.9
13	42.18	44.95	155.0	157.1
14	48.81	49.17	162.7	159.6
15	54.48	51.48	167.8	161.1
16	58.83	53.07	171.6	162.2
17	61.78	54.02	173.7	162.5
18	63.05	54.39	174.5	162.5

* 50th percentile data from studies by Howard V. Meredith, Iowa Child Welfare Research Station, The State University of Iowa.

ERRATA

Page 60: Lysine-Read α instead of β.

,, 100: Under clinical pictures; 3rd line read "loss of subcutaneous fat" instead of "Subcutaneous fat."

,, 134: 5th line, Read 1.2 Kcal instead of 1.2 cal.

,, 185: Table 9.10; Read "Alpha tocopherol" instead of "Alpha".

,, 196: Last but one line; Read infant "lies" instead of infant "line."

SUBJECT INDEX