D.P.H.

FOOD VALUES.

In this lecture the chemical and physiological properties of the carbohydrates, fats and proteins will be considered - with a short reference to salts. The subject of accessory food factors (vitamins) will be considered in a later lecture.

CHEMICAL CONSTITUTION of various foodstuffs.

(A) CARBOHYDRATES. Substances containing only C, H and O. H and O are always in the same proportion as in H_2O.

CRYSTALLOIDAL.

(1) Monosaccharides. $C_6H_{12}O_6$ (M.W. = 180 gms.), e.g. Glucose, fructose, galactose.

Soluble in H_2O, very slightly soluble in alcohol, insoluble in ether.

Reduce alkaline copper solutions, such as Fehling's solution and Benedict's solution.

(2) Disaccharides. $C_{12}H_{22}O_{11}$ (M.W. = 342 gms.).

Consist of two monosaccharide molecules linked together, one molecule of H_2O being removed in the condensation; e.g., glucose + fructose --> Cane sugar + H_2O

Cane sugar, lactose and maltose are soluble in H_2O, almost insoluble in alcohol, insoluble in ether.

Lactose is a condensation of 1 molecule of glucose with 1 molecule of galactose.

Maltose is a condensation of 2 molecules of glucose.

Cane sugar will not reduce alkaline copper solution until after hydrolysis (by acid or invertase) to glucose and fructose. This is because the two monosaccharides of which cane sugar is constituted are linked together by their reducing groups, and so no reduction occurs; hydrolysis breaks up this link and so after hydrolysis the two reducing groups, being free, the hydrolysed cane sugar will reduce copper solutions.

In the case of both lactose and maltose, the linkages are only partly by reducing groups, so that before hydrolysis both lactose and maltose effect some reduction, but after hydrolysis the power of reduction of lactose and maltose is considerably increased. The reducing capacity of each of these unhydrolysed sugars is adequate for their quantitative determination.

COLLOIDAL. Polysaccharides.

Consist of many monosaccharide molecules linked together.
Reduce copper solutions only after hydrolysis.
Examples: starch, dextrins, gums, etc.

(B) FATS.

Consist of esters formed from long-chain fatty acids, and the alcohol glycerol. An example of a very simple ester is that of ethyl acetate, i.e.,

$$\underset{\text{(Acetic Acid)}}{CH_3\,COOH} + \underset{\text{(Ethyl alcohol)}}{HO\,CH_2\cdot CH_3} \longrightarrow \underset{\text{(Ethyl acetate)}}{CH_3\,COOC_2H_5} + H_2O$$

Similarly a typical fatty ester is represented by:-

$$\begin{array}{l} CH_3\,(CH_2)_{16}\cdot COOH \\ CH_3\cdot(CH_2)_{16}\cdot COOH \\ CH_3\cdot(CH_2)_{16}\cdot COOH \end{array} + \begin{array}{l} HO-CH_2 \\ HO-CH \\ HO-CH_2 \end{array} \longrightarrow \begin{array}{l} CH_3\cdot(CH_2)_{16}\cdot COOCH_2 \\ CH_3\cdot(CH_2)_{16}\cdot COOCH \\ CH_3\cdot(CH_2)_{16}\cdot COOCH_2 \end{array} + 3H_2O$$

(3 molecules of stearic acid) (1 molecule of glycerol) (Tristearin)

Besides the saturated acids palmitic and stearic acids, fats nearly always contain unsaturated acids, such as oleic acid

$/CH_3\ (CH_2)_7\ CH = CH\ (CH_2)_7\ COOH/$

also linked to glycerol to form an ester. A high proportion of unsaturated fatty acids in a fat increases the energy value of combustion of a fat.

Fats are insoluble in H_2O or cold dilute NaOH. They are not easily soluble in alcohol, but are soluble in ether, CS_2, CCl_4, etc.

Fats are hydrolysed by boiling in H_2O under pressure, or by acids or alkalis, or by certain enzymes (lipases) to yield free fatty acid and glycerol. If the hydrolysis occurs in an alkaline medium a salt of the acid is formed - this is a soap:-

e.g., Tristearin + 3NaOH ——> 3 Na-stearate + 1 glycerol.

If mineral acid be added to a soap the fatty acid is displaced and forms a solid layer,

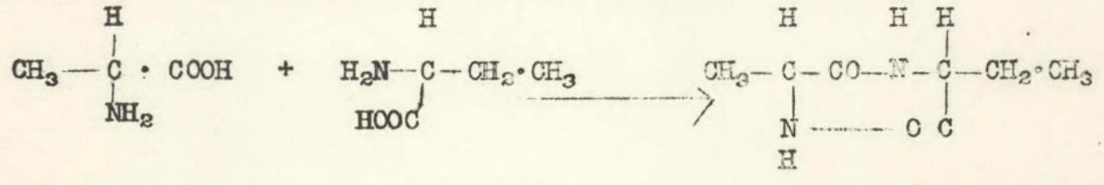
e.g., Na-stearate + HCl ——> H-stearic acid + NaCl.

The fat content of a foodstuff is best estimated by a method of continuous extraction using a suitable solvent, usually ether. A given weight of the foodstuff is dried and put into a special extractor apparatus, usually of the Soxhlet pattern; prolonged extraction with ether removes fats, leaving all else behind. The ether with dissolved fats is collected in a weighed flask (which is part of the Soxhlet apparatus), the ether evaporated and the flask, which now contains all the fat from the foodstuff, is re-weighed.

Other estimations made on fats include (1) Free acid; (2) Volatile acids; (3) Non-volatile acid; (4) Saponification value; (5) Iodine value for degree of unsaturation.

(C) PROTEINS.

Are compounds consisting of condensations of numerous amino-acids linked in a certain manner. An amino-acid is both acidic on account of its -COOH (carboxyl) group, and basic on account of its $-NH_2$(amine) group. For this reason, solutions of amino-acids in water are nearly neutral. An illustration of the linkage of two amino-acids is as follows:-



The linkage -CO-NH- is known as the peptide linkage. Proteins, then, consist of large numbers of amino-acids of various kinds linked together chiefly by this peptide linkage, though other linkages are known.

Proteins can be hydrolysed by acids, alkalis or enzymes (Pepsin, Trypsin, Erepsin) to form firstly proteoses and peptones, compounds of amino-acids less complex than the proteins, and later free amino-acids.

Proteins contain amino-acids which partly consist of benzene (and other) ring compounds, as well as non-ring (i.e., entirely straight chain) compounds. Important in the ring group are Tyrosine and Tryptophane, and in the non-ring group, lysine, arginine, and the sulphur containing amino-acid cystine.

Certain tests for presence of Proteins are based on some of the chemical properties just mentioned:-

(1) Biuret test: is due to presence of peptide linkage, and so indicates presence of protein, proteoses and peptones. The reagents used are 5% NaOH and 1% $CuSO_4$. A violet or pink colour is a positive reaction.

(2) Xanthoproteic test: with strong HNO_3, giving a yellow colour, followed by adding NH_3 when colour changed to orange. Test depends upon the presence of benzene ring in the protein, i.e. due to presence of such amino-acids as Tryptophane and Tyrosine.

(3) Mercuric-nitrite test (Modified Millon's): protein is boiled with mercuric sulphate, and after cooling, with $NaNO_2$. A red solution or precipitate shows a positive reaction, which depends upon the presence of the phenol-ring in the protein, i.e., tyrosine, which has a phenol ring and which is the only known protein derivative to give the reaction, is present.

(4) Aldehyde reaction for Tryptophane. Formalin and mercuric sulphate give a deep violet solution. This reaction is specific for tryptophane.

(5) Sulphur Reaction for Cystine. By boiling with NaOH and lead acetate a black precipitate (of lead sulphide) indicates the presence of cystine in the protein.

Determination of Protein in a Foodstuff.

The protein content of a solution, or of a foodstuff, is determined by the Kjehdahl process. A known amount of the foodstuff is digested at high temperature with concentrated H_2SO_4, which converts practically all the nitrogen of the protein into ammonium sulphate. The digestion mixture is cooled, diluted and made alkaline, NH_3 being thus released and distilled into a known volume of decinormal acid. The NH_3 neutralizes its equivalent of N/10 acid. The remaining N/10 acid is estimated by N/10 NaOH. From this the amount of NH_3 and hence N from a given amount of foodstuff can be estimated, and from this the protein content found.

Example.

Suppose 0.5 gms foo dstuff were digested, and the NH_3 distilled with 50 cc.s N/10 HCl. Suppose at end of distillation 40 cc.s of N/10 NaOH were required to neutralize the excess N/10 HCl.

Then the NH_3 from 0.5 gm. foodstuff = [50-40] = 10 cc.s N/10 HCl
= 10 cc.s N/10 HC_3
= 10 cc.s (N/10 (Nitrogen
= $\frac{10}{1000}$ X 1.4 gms. Nitrogen

Protein contains 16% N, so that 0.5 gm. foodstuff contains

$$\frac{10 \times 1.4 \times 100}{1000 \qquad 16}$$ gms. Protein.

= 0.088 gms. Protein.

Since 0.88 gms. of Protein are contained in 0.5 gms of foodstuff:

Percentage of Protein in the foodstuff is

$$\frac{0.088}{0.5} \quad 100 = 17.6$$

PHYSIOLOGICAL.

(A) Energy Considerations.

The purpose of food is to supply energy, both for doing work and for maintenance of temperature of the body. The amount of energy which complete combustion of a given weight (usually 1 gram) of a foodstuff will yield, may be calculated by means of the Bomb Calorimeter. The amount of heat required to raise 1000 grams of water by 1°C (at about 15°C) is known as the 'kilo-gram-calorie', i.e., K (or C).

Whilst a furnace or bomb-calorimeter is capable of liberating energy from a great many materials, the human body is only able to oxidise 3 main groups:-

(1) The Carbohydrates, i.e., Sugars and Starches.

They are completely oxidised in the body: 1 gram yields 4·1 K.

(2) The Fats.

Also completely oxidised in the body: 1 gram yields 9·3 K. This high energy yield by fats is due to their high proportion of hydrogen, and low proportion of oxygen; thus much oxygen from outside is required to combust a fat.

(3) The Proteins.

In the bomb-calorimeter 1 gram yields 5·8 K, but in the body 1 gram yields only 4·1 K. This is because the whole protein is not oxidised in the body, certain protein derivatives such as urea, uric acid, creatinine, etc. being excreted as such and are not oxidised.

A man, doing ordinary physical work, requires a certain minimum daily supply of energy (about 3500 K). This energy may theoretically be supplied by almost any combination of foodstuffs, but a degree of balance in a diet is essential, since besides energy, wastage of tissue can only be repaired by protein, and further requirements of palatability and digestibility must be met. A fair example of an adequate daily ration of the 3 main classes of foodstuffs for an average man doing moderate physical labour is:-

Carbohydrates	500 grams	yielding	2050 K
Fats	100 "	"	930 K
Proteins	120 "	"	492 K
	Total		3472 K

(B) Certain physiological features of the 3 main classes of foodstuffs.

Proteins,- alone can replace the daily wastage of the various tissues. The only possible substitutes are the amino-acids from the protein. The true value of any protein as a tissue builder lies in the amino-acids it contains, quite apart from mere energy yielding properties. Tryptophane and lysine are essential for growth, and it appears that the former is always essential for adult animals (including man). Should a protein lacking tryptophane and tyrosine be the sole protein of a diet, wastage will occur and recovery from a wasting disease will be delayed. Other amino-acids including cystine must be supplied. Gelatin is a protein lacking both tryptophane and tyrosine; nevertheless it is often given to convalescents more on account of its palatibility and easy digestion and its energy yielding properties, despite its serious deficiency in two most essential amino-acids.

Proteins have the additional valuable property of accelerating the whole metabolic processes of the body. This "specific dynamic action" is especially valuable in resisting cold, and in enabling a man to put forth extra effort, as in exploration work, warfare, etc.

It is possible to live fairly comfortably and efficiently on much smaller daily protein allowances than are customary, but undoubtedly the particular advantages of proteins just mentioned are then unnecessarily neglected. The supposed evil effects, physical or moral, of a diet rich in protein, are now almost universally regarded as being mythical.

Biological values of some proteins, as estimated by the percentage quantity of body protein which their ingestion will spare from loss when energy requirements are adequately furnished by carbohydrates and fats:-

Ox Meat	Protein	104	Yeast	Protein	71
Cow's Milk	"	100	Spinach	"	64
Fish	"	95	Pea	"	56
Rice	"	88	Wheat flour	"	40
Cauliflower	"	84	Cornmeal	"	30
Potato	"	79			

Carbohydrates and Fats.

Neither can replace proteins as tissue repairers, but each can, theoretically, supply all the energy needs of the body by itself. The proportion of carbohydrates to fats is of minor importance; however, a certain amount of carbohydrates is necessary for without them fats are not properly combusted, and if only a very small amount is taken very large and indigestible quantities of fat must be taken to meet energy requirements. On the other hand, very large quantities of carbohydrates are difficult to digest, and a certain amount of fat should be taken. Apparently, in spite of the fact that fat can be formed in the body from carbohydrates, nutrition will suffer unless the food contains a certain amount of fat.

Foods not yielding energy.

(1) Water; but is, of course, absolutely essential. All living matter has a water content of $>75\%$.

(2) Salts. The ordinary mixed diet of man is usually deficient in sodium salts - hence the use of common salt NaCl, generally in very excessive amounts. Most of the salts required are derived from the foods eaten.

References to Literature.

Science of Nutrition. Lusk.

Newer Knowledge of Nutrition. McCollum and Simmonds.

Physiology of Protein Metabolism. Cathcart.

Practical Exercises.

(1) Determine the Protein Content of the Sample of Milk.

(i) Pipette 1 cc. of milk into a long-necked flask.

(ii) Add a few crystals of K_2SO_4, and then 15 cc.s of concentrated H_2SO_4, being careful that all the milk is washed into the flask.

(iii) Heat gently in a fume cupboard over a small flame for 5-10 minutes.

(iv) Heat vigorously till the black liquid becomes straw yellow.

(v) Turn off the burner, and when cool enough to handle, remove the flask to another fume cupboard, and allow flask to cool thoroughly.

(vi) When cool, add 50 cc.s of NH_3-free water.

(vii) Transfer the liquid to a distillation flask, washing out with NH_3-free water till there are about 250 cc.s altogether in the distillation flask.

(viii) Add one cc. of phenol-phthalein, and then add strong NaOH till distinctly alkaline.

(ix) Into the receiving flask pipette 40 cc.s of N/10 HCl, and a few drops of methyl-red.

(x) Distil over 150-180 cc.s into the receiving flask, i.e., until the distillate is no longer alkaline to litmus paper.

(xi) Finally - determine the amount of HCl which remains unneutralized by NH_3, by titrating with N/10 NaOH. Calculate the Nitrogen and protein content of the milk, assuming that 1 cc. of milk weighs 1·030 grams. (See example given earlier).

(2) Solution (A) contains glucose.

(1) Perform the Molisch reaction for carbohydrates.

To 2 cc.s of the solution add 1 or 2 drops of α-naphthol solution.
Run in concentrated H_2SO_4 below the solution.
Agitate very slightly - a purple ring will appear at the junction of the two liquids.

(2) Determine the percentage of Glucose by Benedict's Method.

(i) Pipette 25 cc.s of Benedict's solution into a conical flask.

(ii) Add 5-10 grms of anhydrous Na_2CO_3 and a few pieces of porous-pot. (Na_2CO_3 increases the alkalinity of the heated solution).

(iii) Boil vigorously, and run in glucose solution from a burette till a heavy white precipitate is formed and the blue colour begins perceptibly to diminish. Then run in the glucose solution a few drops at a time with constant vigorous boiling until the blue colour has quite disappeared. (Sometimes the end-point is not exact decolorisation, but a greenish colour).

An interval of 30 seconds between the additions of sugar solution at the end should be allowed.

(iv) Read burette and calculate the percentage of glucose.

25 cc.s Benedict solution = 0·05 gms. glucose.

(3) Solutions (B) and (C) contain casein and gelatin respectively.

Carry out the following protein tests on each of them.

(1) Biuret Reaction.

To about 3 cc. of the protein solution add an equal volume of 5% NaOH. Add a single drop of 1% $CuSO_4$ solution. A violet colour indicates the presence of protein.

(2) Xanthoproteic.

To 3 cc. of the protein solution add about 1 cc. of strong HNO_3. A white precipitate may appear. Boil for half a minute. The precipitate turns yellow and partly dissolves to give a yellow solution. Cool under the tap, and add strong ammonia till the reaction is alkaline. The yellow colour is changed to orange.

(3) Mercuric Nitrite.

To about 1 cc. of the protein solution add an equal volume of mercuric sulphate in 10% H_2SO_4. Boil gently for half a minute. A precipitate may appear which clings to the side of the tube and turns yellow. Cool under the tap. Add a drop of 1% $NaNO_2$ solution and warm gently. The precipitate, or the solution, changes to red.

(4) Aldehyde reaction for Tryptophane.

To 1 cc. of protein solution add 1 drop of formalin solution (1:500 dilution of 40% formaldehyde). Add 1 drop of 10% mercuric sulphate solution. Mix and add at least 1 cc. of concentrated H_2SO_4. Agitate gently: a deep violet or purple solution indicates presence of tryptophane.