CHAPTER X

METABOLISM

Introduction. The different food materials (mostly complex and unsuitable for absorption) are transformed into their simple and soluble form by digestion prior to the entry from the lumen of the intestine into the body through its gateway (absorption).

After their entry into the body, they undergo a **series of biochemical reactions in different organs** catalysed by enzymes, coenzymes, catalysts, and governed by hormones and vitamins to serve the following purposes: (1) Synthesis of many specialised substances for the organisation of these substances into the types of characteristic protoplasm of different varieties of cells and tissues forming up the organism, resulting development, growth and maintenance of a living organism. Those reactions which are included in the process of synthesis of larger protoplasmic molecules from lower ones for building up tissues are collectively known as **synthesis** or **anabolism.** (2) Production of energy required for the protoplasmic synthesis and other process of the body, viz.,.maintenance of temperature, movement, generation of electrical potentials, the secretion and excretion of fluid and transport of substances against concentration gradient. Those reactions which are included in the process of breakdown of larger protoplasmic molecules to smaller ones for the supply of energy are collectively called **analysis** or **catabolism.**

Definition. Thus the term **metabolism of a food substance** is meant by a series of specific biochemical reactions occurring within the living organism from the time of its incorporation into the cell or tissue till its excretion, of which some are concerned with tissue synthesis and others with tissue breakdown what are termed as anabolism and catabolism respectively. Although both anabolism and catabolism are reversible biochemical reactions but growth and loss of tissue mass (breakdown of tissue) depends on predominance of one over the other opposite reaction

$$\text{small molecules} \underset{\text{loss of tissue mass}}{\overset{\text{growth}}{\rightleftharpoons}} \text{large molecules.}$$

METHODS OF METABOLIC STUDY

The chemical reaction of the tissue is so numerous and fast that the study of these reactions, either individual or overall pictures of these reactions had been encountered with much difficulty due to lack of suitable equipment and methods in the past. But recent advancement in researches in the field of development of different techniques and methods at modern times, it has been possible today in unfolding the total picture of metabolism.

The general principles of the different methods employed in the study of metabolism are as follows:

1. **Study of the metabolic process.** Few steps in the path of metabolism may be known by the biochemical analysis of body fluids of patients suffering from diseases either congenital or acquired whose metabolic reactions checked or inhibited or deviated abnormally without passing to its final end products, e.g., Diabetes mellitus, ketosis, alcaptonuria, give some information of glucose, fat and protein metabolism respectively. These abnormal metabolic conditions may be produced experimentally in animals and the results may be confirmed.

2. **Study of metabolism by arresting few metabolic steps with certain metabolic poisons.** This is an important method of studying the breakdown of fatty acids (*vide* oxidation of fatty acids).

3. **Study of metabolism under the influence of endocrines.** This may be observed by extirpation of the gland and introduction of the hormone, and the results are compared and confirmed with the picture obtained in diseases suffering from hypoactivity and hyperactivity of the gland.

4. **Study of different organs in determination of site of reaction.** For instance, (*a*) Extirpation of liver (by Mann *et al*), spleen or other viscera and noting the consequent effect on general metabolism or the metabolism of a particular administered substance. (*b*) **Perfusion** of isolated organs with a solution of the test material and to note the changes therein. (*c*) **Incubation** of the test solution with thin slices of the organ or with the minced tissues of the organ. (*d*) **Fistula preparations** with the purpose of by-passing an organ, such as **Eck's fistula** in which communication is established between portal vein and inferior vena cava. The portal blood directly drains into inferior vena cava and liver is by-passed. With this preparation, the role of liver on the metabolism of many substances can be studied. (*e*) **Angiostomy experiments.** Insertion of cannulae into suitable blood vessels through which blood can be withdrawn as well as a test solution can be easily injected. Dogs with such cannulae in the hepatic, portal and renal veins have survived normally for many months.

5. **Feeding experiments.** (*a*) This is done by altering the quality and quantity of foodstuffs, vitamins, minerals, etc., and observing the consequent metabolic changes. (*b*) Administration of all theoretically possible intermediate compounds of a substance, to animals, and to see whether such compounds are metabolised in the same way as the parent substance itself. In this way the intermediate stages of metabolism of a substance may be found out.

6. **Biochemical changes.** Study of biochemical changes of blood, urine, faeces, tissues, organs, etc., is also valuable, particularly in accompaniment with other metabolic experiments.

7. **Study of basal metabolic rate** (B.M.R.), **respiratory quotients** (R.Q.), **nitrogen equilibrium, specific dynamic action,** etc.

8. **Application of isotopes.** The latest advancement in the study of metabolic processes is the application of natural and radioactive isotopes. Compound, to be studied, is labelled or marked by replacing its one of the element by isotope and administered. The changes undergone by such 'labelled' compounds, their migration from place to place and finally their excretion from the body, are observed with the help of suitable instruments and by other physico-chemical methods. Since isotopes are treated by the tissues in the same way as their normal homologue, this process is extremely valuable for metabolic studies and all disadvantages of metabolic studies can be overcome with isotopes. *Natural isotopes* remain as mixtures in all natural sources and can be isolated by careful fractionation. *Radioactive isotopes* can be estimated by the electroscope or the *Geiger-Müller* counter, that is, those instruments which can detect radiations. [*Vide* pages 116–18.]

Stable or partial stable compounds have been prepared by replacing the relevant atoms with the corresponding natural or radioactive isotopes respectively (as and when required) and have been extensively applied in the study of absorption, metabolism and excretion of proteins, fats, carbohydrates, salts, water and hormones, etc.

ENERGY METABOLISM

There is a continual exchange of energy between a living organism and its environment as in accordance with the principles of thermodynamics applied to non-living physical and chemical systems. There is a fundamental difference between plants and animals, in the way of their utilisation of energy for their life processes. Plants, unlike animals, can convert the energy of solar photons* into the energy of chemical bonds (i.e., food, e.g., carbohydrate, containing energy which is released during its breakdown) and store them in their leaves, stems, fruits, etc., which serves as food for the animals. Plants are, therefore, interposed between the sun and all animals in the *energy-flow relationship* in nature. It follows therefore that the energy required for our bodily activities is supplied by food which is stored with energy and the original source of energy is the sun.

The body's input and output of energy. The energy exchange is

* Photons = unit of energy (i.e., 1 absolute (K) Cal = $4{\cdot}184 \times 10^7$ ergs).

based on the **first law of thermodynamics** as derived by Mayer, Joule and Helmholtz and later proved applicable in case of the animal body also by Voit, Pettenkofer and Rubner, which states that energy is neither gained nor lost when it is converted from one form to the other—*mechanical, thermal, electrical, chemical*, etc.

The unit of energy is mainly expressed as heat unit or energy equivalent of foodstuffs—the **calorie (cal)**, also known as **small calorie,** *standard calorie* or *gram-calorie*. This is defined as the amount of heat energy which can raise the temperature of 1 gm of water from 15–16°C. The measurement of heat is known as **calorimetry**—the apparatus for measurement being known as the **calorimeter.** In the biological system, i.e., in physiology and medicine, the unit used is the **large calorie** or **kilo-calorie (Cal** or **K cal)** which is equal to 1000 small calories. It is defined as the amount of heat required to raise the temperature of 1 Kgm of water from 15–16°C.

The total energy obtained from food sources are utilised as:

1. for the synthesis of different types of protoplasmic constituents characteristic of different cells and tissues, e.g., phospholipid, protein, RNA, DNA, enzyme, coenzyme and other complex substances, etc.,
2. for the operation of different organs and as a whole organism, e.g., heart, lung, gland, kidney, etc.,
3. for maintenance of body temperature,
4. for generation of electrical potentials and current, viz., in central nervous system and heart, etc.,
5. for the transport of substances against the concentration gradient,
6. for growth and maintenance.

Joule verses Calorie. Amounts of heat may be expressed as equivalent amounts of energy, with the **joule** as the unit. According to the pamphlet circulated to the Nutrition Society by the Royal Society (1968) the amount of heat must be expressed in terms of energy and the relationship between the two units is 1 kilocalorie equal to 4184 or 4188·5 or 4186·8 joules or about 4·19 K joules).

Methods for determination of energy output

Energy output can be determined by measuring the heat production of an individual over a measured amount of time.

I. **Direct calorimetry.** This is done by measuring the heat output of the subject for a given period, by putting him inside a specially prepared heat proof chamber (**Atwater-Benedict's** respiration calorimeter). Heat produced is measured by changes in temperature of circulating water. This method, although very accurate, requires much elaborate apparatus and can hardly be used for ready clinical purposes.

II. **Indirect calorimetry.** Due to complications involved in direct calorimetry, heat output is calculated indirectly from O_2 consumption and CO_2 output.

(*a*) **Closed circuit method** (clinical type). Various apparatus may be used for this purpose, such as **Benedict-Roth apparatus** (Fig. 356) and other apparatus of similar type. Benedict-Roth apparatus is very useful for clinical purposes as the heat production can be calculated in this type of apparatus by the oxygen consumption only without determination of CO_2 elimination. The subject is allowed to breathe from O_2

reservoir through a mouthpiece, the nose being clipped. The CO_2 eliminated in expiration is absorbed by soda lime to keep the O_2 reservoir pure. The fall in the level of O_2 during the experiment is recorded which gives the value of O_2 consumption at the specified time. In this method, R.Q. of the subject is not determined and the average R.Q. is taken as 0·82. 4·825 Cal of heat is liberated at this R.Q. when one litre of O_2 consumed. The energy output during the experiment is calculated by multiplying litres of O_2 consumed at that time with 4·825.

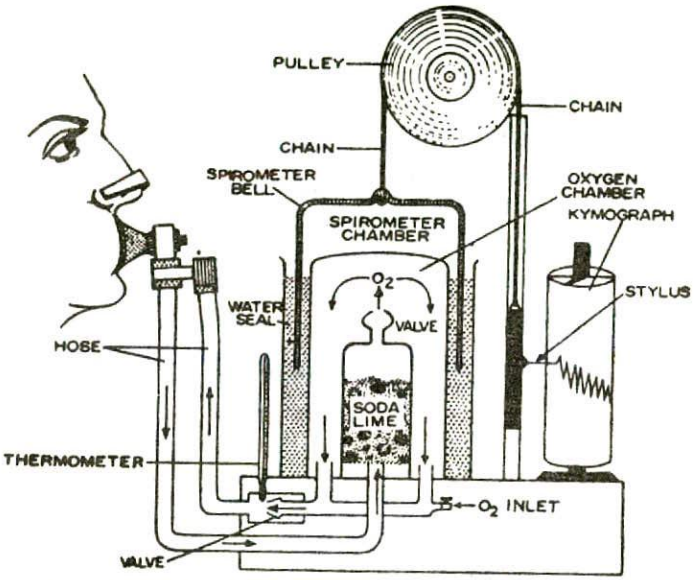


FIG. 356. Benedict-Roth apparatus for determining metabolic rate. The arrows indicate the direction of air flow during respiration.

The following table shows the amount of heat produced per litre of O_2 consumed, at different R.Q.

R.Q.	Calories evolved per litre of O_2 consumed
0·71	4·795
0·75	4·829
0·80	4·875
0·85	4·921
0·90	4·967
0·95	5·012
1·0	5·058

(*b*) **Open circuit method** (Haldane type). The subject inspires atmospheric air. The expired air is collected in a special air-tight bag known as **Douglas bag.** The total volume of expired air collected in the bag at the end of experiment is measured and the samples are analysed for CO_2 and O_2 in **Haldane gas-analysis apparatus** or in **Scholander's micro-gas analysis apparatus.** The amount of oxygen consumed and

carbon dioxide given off is calculated from the difference of percentage of O_2 and CO_2 between the atmospheric and expiratory air. The R.Q and the calorific value of O_2 can then be determined. The Douglas bag method can conveniently be used to measure the energy output during different types of activities. A **respirometer** devised at the Max-Planck Institute at Dortmund is also recently used for this purpose, and is much less cumbersome and easier to manipulate than the Douglas bag.

RESPIRATORY QUOTIENT (R.Q.)

Definition. *It is the ratio of the volume of CO_2 produced by the volume of O_2 consumed* (i.e., CO_2/O_2) *during a given time.*

It should be noted that R.Q. is simply a ratio. It gives no idea as to the absolute quantity of gaseous interchange. Proportional increase or diminution of CO_2 produced and O_2 utilised, will keep the ratio unchanged. But any disproportionate variation will be reflected by a corresponding change in the R.Q.

Normal R.Q. In a healthy adult it is 0·85 for a mixed diet.

Method of determination. This is done by measuring the volume of O_2 consumed and CO_2 produced during a given time with the help of Douglas bag (FIG. 270, *vide* page 385) and other similar instruments.

Factors affecting respiratory quotient

1. **Role of diet**

(*a*) In case of **carbohydrate** diet the R.Q. is unity. Because in carbohydrate diet the volume of CO_2 produced is same as the volume of oxygen consumed. This is due to the fact that, in the carbohydrate molecule, the amount of O_2 present is just sufficient to oxidise the H present in the same molecule. Hence, external oxygen is necessary only to convert the C of the molecule into CO_2. So that the volume of O_2 consumed and the volume of CO_2 produced will be same. This is represented in the following equation:

$$C_6H_{12}O_6+6O_2=6CO_2+6H_2O. \quad R.Q.=6CO_2/6O_2=1\cdot0.$$

(*b*) In case of **fats** the R.Q. will be lowest and is about 0·7; because fat is an oxygen-poor compound. The oxygen present in it cannot fully oxidise the H of the molecule. So that, oxygen consumed from outside, is used for two purposes: *first*, for oxidising C and producing CO_2 and *secondly*, for oxidising H giving H_2O. Consequently, the volume of CO_2 produced will be less than the volume of O_2 utilised. Hence, R.Q. will fall and will be about 0·7. This is shown in the following equations:

$$2C_3H_5(C_{18}H_{35}O_2)_3+163O_2=114CO_2+110H_2O.$$
(Tristearin) $\quad R.Q.=114CO_2/163O_2=0\cdot7.$

(*c*) In case of **proteins** the R.Q. is about 0·8.

$$2C_3H_7O_2N+6O_2=(NH_2)_2CO+5CO_2+5H_2O.$$
(Alanine) $\quad R.Q.=5CO_2/6O_2=0\cdot8.$

In any condition where fats are burnt chiefly (starvation, advanced diabetes, etc.), the R.Q. will be about 0·7. Whereas with a predominant carbohydrate combustion the R.Q. will approach 1.

2. **Effect of interconversion in the body.** When carbohydrates are converted into fats in the body, R.Q. will rise. Because in the process an oxygen-rich substance is converted into an oxygen-poor compound. So that

some amount of O_2, liberated from carbohydrate, will be utilised for purposes of oxidation. Consequently, less oxygen will be needed from outside. Hence, the amount of CO_2 produced will be more than the amount of O_2 consumed. So that R.Q. will rise. When fat is converted into carbohydrate just the opposite effects will be produced and R.Q. will fall.

It is therefore evident that R.Q. value will indicate the following: (*a*) the type of foodstuff burning in the body or (*b*) the nature of conversion of one foodstuff into another in the body.

3. **Acidosis.** During acidosis CO_2 output is greater than O_2 consumption so the R.Q. rises. The R.Q. falls due to the reverse condition of acidosis, i.e., CO_2 output is lesser than O_2 consumption.

4. **Alkalosis.** Here the R.Q. will fall, because respiration is depressed and CO_2 will be retained in the body (i.e., less CO_2 is produced).

5. **Rise of body temperature.** It may increase R.Q. as in acidosis. [Rise of body temperature, such as in fever, will cause increased breathing and thereby will wash out more CO_2.]

6. **Diabetes mellitus.** In advanced cases of diabetes, when little carbohydrate is burning, energy is supplied mainly by oxidation of fats. Hence, R.Q. will fall. In such cases, if insulin is injected, carbohydrates will start burning and R.Q. will rise.

7. **Starvation.** Here the subject has to live on its own body tissues. In the first stages (1–2 days) energy is derived mainly from the stored glycogen, so that the R.Q., although it falls below normal (0·85), is proportionately high—0·78. But later on, when energy is derived chiefly from the combustion of fats, R.Q. will fall still further and will be about 0·7.

8. **Voluntary hyperpnoea.** By this process excess CO_2 is washed out without a corresponding increase of O_2 utilised, so that R.Q. will be above unity.

9. **Muscular exercise.** (*a*) With *moderate exercise* (with a normal mixed diet) the R.Q. remains almost unaltered. Because in exercise the body uses different foodstuffs in the same proportion as at rest. (*b*) With *violent exercise* lactic acid enters blood and produces acidosis. Pulmonary ventilation will be raised washing out more CO_2. Consequently, R.Q. rises and may go above 2 even. (*c*) During *recovery* from violent exercise R.Q. falls, because less CO_2 is evolved. Gradually it goes back to normal.

Value of determining respiratory quotient

1. R.Q. acts as a guide as to the type of food burning or the nature of synthesis taking place in the whole body as well as in a particular organ.
2. R.Q. is very helpful in determining metabolic rate.
3. Non-protein R.Q. helps in finding out the proportion of the three foodstuffs that are being utilised in the body.
4. Determination of R.Q. helps in the diagnosis of various pathological conditions, such as acidosis, alkalosis, etc.

The R.Q. of various organs and tissues. The R.Q. of the individual organs, such as liver, spleen, heart, brain, etc., can be measured by noting (*a*) *the total blood flow* in a given time through the organ and (*b*) *the oxygen consumed* and *CO_2 produced* by the organ at the same time. This can be found out by determining the arteriovenous difference of these gases and then multiplying the figure by the total volume of blood flow through the organs. In this way the R.Q. of various organs can be worked out. The total gaseous interchange of an organ will show its metabolic rate and the R.Q. of the organ will indicate the nature of foodstuff burning in it.

BASAL METABOLISM

Energy, in terms of heat, produced as a by-product of total cellular metabolism is essential for the maintenance of life of the organism. Although the amount of energy, required for any individual varies directly with the degree of activity and environmental condition, but the rate of energy production in an individual by its overall cellular metabolism is more or less constant under some standard conditions known as **basal metabolism** and the rate of its energy production at basal condition per hour and per sq. metre of body surface is known as **basal metabolic rate** (B.M.R.). The **basal conditions** are as follows:

1. The person should be in awake but at complete rest, both physical and mental.
2. The person should remain in normal condition of environment, i.e., at normal temperature, pressure and humidity.
3. The person should be without food at least for 12–18 hours, i.e.. in the postabsorptive state.

Thus the B.M.R. may be **defined** as the *amount of heat given out by a subject who, though awake, is lying in a state of maximum physical and mental rest under comfortable conditions of temperature, pressure and humidity*, 12–18 *hours (postabsorptive)* after meal.*

Basal metabolic rate (B.M.R.) is usually expressed as the heat production per sq. metre of body surface per hour. In **adult male** normal B.M.R. is about 40 *calories per sq. metre of body surface per hour* and in the **adult female** *about* 37 *calories*. The surface area of an average adult is about 1·8 sq. metres, and can be calculated from the following formula by DuBois

$$S = 0{\cdot}0071 \times W^{0{\cdot}425} \times H^{0{\cdot}725}$$

when W = body weight in Kgm, H = height in cm, and S = surface area in sq. metres.

The rate of metabolism at basal conditions has been formed to vary in different individuals and therefore the B.M.R. varies with different factors.

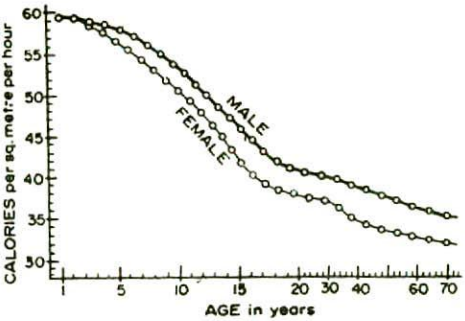


FIG. 357. Graphical tracing of normal B.M.R. at different ages (diagrammatic).

Factors affecting basal metabolic rate

1. **Age.** The B.M.R. of children is much higher than the adults. Roughly speaking it is inversely proportional to the age.** In other words, with advancing age B.M.R. gradually falls. This is due to the fact that children possess a greater surface area in proportion to their body weight. [But in newly born baby it is low—about 25 calories per sq. metre of body surface per hour. In premature infants it is still lower.]

* This period is allowed to pass for avoiding the effects of digestion and absorption, the effects of specific dynamic action (S.D.A.) of foodstuffs and also to prevent any chance of starvation.

** At the age six it is 57·5 calories; at twelve 50·4 calories; between twenty and thirty, 40 calories; between forty and seventy it is 38·5 to 35·5 calories.

2. **Sex.** The B.M.R. of the males is slightly higher than the females (FIG. 357). The figures are mentioned above.

3. **Surface area.** The B.M.R. is directly proportional to the surface area of the subject. Larger the surface area greater will be the heat loss, and equally higher will be the heat production (i.e., metabolic rate). Otherwise temperature balance will be upset. For this reason B.M.R. depends upon the surface area.

4. **Climate.** In colder climates the B.M.R. is high and in tropical climates the B.M.R. is proportionally low.

5. **Habit.** Trained athletes and manual workers have a slightly higher B.M.R. than persons leading a sedentary life.

6. **Diet.** Prolonged undernutrition lowers the metabolic rate.

7. **Hormones.** Circulating levels of hormones secreted by *adrenal medulla, adrenal cortex, thyroid and the anterior pituitary* increase B.M.R. One mgm of thyroxine increases B.M.R. by about 1,000 calories. In thyrotoxicosis, B.M.R. may increase by 50–100% above normal, but R.Q. remains unaltered since both O_2 consumption and CO_2 production increase proportionately in such cases. In myxoedema B.M.R. is diminished to 30% or even 45% below normal. Anterior pituitary through its thyroid-stimulating hormone (TSH) affects B.M.R. Growth hormone (STH) secreted by this gland also causes B.M.R. about 20% rise. Adrenaline (epinephrine) and noradrenaline (norepinephrine) increase B.M.R. by about 20% of the resting value. Male sex hormones cause a 10% increase in B.M.R., female sex hormones increase it insignificantly.

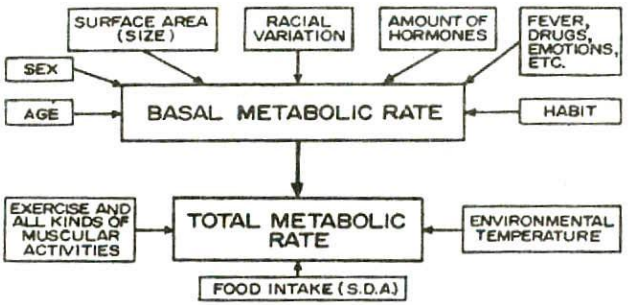


FIG. 358. Schematic representation of the different determinants of basal metabolic rate and total metabolic rate.

8. **Barometric pressure.** Moderate reduction of atmospheric pressure does not affect the B.M.R.; but a fall of pressure to half an atmosphere (viz., O_2 tension—75 mm of Hg)—as occurs in mountain climbing—increases B.M.R., but increased pressure of oxygen does not raise B.M.R.

9. **Pregnancy.** The B.M.R. of the pregnant mother, after six months of gestation, rises. It is seen that the B.M.R. of the mother is the sumtotal of her own metabolism as in her non-pregnant state and combined with that of the foetus. Hence, pregnancy exerts no specific effect upon B.M.R.

10. **Body temperature.** The B.M.R. increases by about 12% with the rise of 1°C. (such as in fever). This is due to the fact that increased temperature stimulates the chemical processes of the body and thereby increases B.M.R.

11. **Drugs.** Some drugs like caffeine, benzedrine, etc., increase the basal metabolic rate. The reverse is observed by anaesthetics.

12. **Racial variation.** Natives of Yucatan were found to have their higher metabolic rate than the Americans, whereas South-Eastern Asians were found to have much lower rate. Thus, there may be significant deviations in the metabolism according to race.

In addition to the above factors, the metabolic rate of an individual may be influenced by the following: (1) Muscular exercise—light exercise, as sitting and standing, etc., increases metabolism by 30–40%. Moderate exercise, as walking, by 50–60%, and severe exercise (hard work), by 100% of the B.M.R. (2) Mental exercise causes a slight rise. Solving mathematical problems increases by 3–4% only. But it should be remembered that the basal oxygen consumption of brain is high and amounts to about 10% of the total O_2 requirement of entire body. (3) Strong emotions increase metabolism by 5–10%. (4) Sleep reduces it by 10–13%.

DISEASE. Changes of B.M.R. are found in the following pathological conditions: (1) **Conditions increasing B.M.R.**—(*a*) Hyperthyroidism (may rise up to 100%), (*b*) Fever, (*c*) Cardiorenal disease with dyspnoea (25–50%), (*d*) Leukaemia (21–80%), (*e*) Polycythaemia (10–40%), etc. (2) **Conditions reducing B.M.R.**—(*a*) Starvation and undernutrition, (*b*) Hypothyroidism, (*c*) Addison's disease, (*d*) Lipid nephrosis, etc.

Measurement of basal metabolic rate

The apparatus most commonly employed clinically for the determination of B.M.R. is Benedict-Roth apparatus, although Tissot method and Douglas bag method are also sometimes employed.

Read's formula. This formula gives a rough estimate of B.M.R. and is often used at the bedside. It is as follows:

$$\text{B.M.R.} = 0{\cdot}75\ (\text{P.R.} + 0{\cdot}74 \times \text{P.P.}) - 72$$

where P.R. = pulse rate, P.P. = pulse pressure.

The result comes out as the percentage of the normal and is correct within a range of $\pm 10\%$, viz., if above 10% the B.M.R. is higher, if below 10% it is lower than normal.

Importance of noting B.M.R. (1) For prescribing a diet of adequate calorific value. (2) For the diagnosis of various pathological conditions specially in hypothyroidism and hyperthyroidism. (3) To note the effect of different types of food and drug on basal metabolic rate.

Mechanical work and heat

Energy. It is a physical concept which cannot be realised but measured since it is equivalent by its capacity due to the equality of the physical dimensions in both energy and work. Although there are different forms of energy, viz., chemical, electrical, mechanical, surface and radiant, etc., which are interconvertible, i.e., when one form is produced, an equivalent amount of other form disappears, but the total quantity in the universe remains same (Law of conservation of energy). The ultimate source of energy is the radiant energy coming from the sun which is converted to chemical energy and then to different forms of work in biological system, i.e., biosynthesis, osmotic, mechanical and transport work.

Of the total energy released from the breakdown of the chemical substances in the biological system, only a fraction of it (known as **free energy**) is utilised for the work **(second law of the thermodynamics).** Energy of a system unavailable to do work is termed as **entropy.** The energy which is consumed or released is termed as **enthalpy.**

When mechanical work is completely transformed into heat, or heat is completely converted into mechanical work, the amount of mechanical

work is equivalent to quantity and unit of heat which is called *mechanical equivalent of heat.*

Joule established the relation between the mechanical work and heat *mathematically,* with the help of the following formula: If W, unit of work, is converted into H, unit of heat or *vice versa,* we have,

$W = J \times H$ or $W/H = J$, where J is a constant, assuming that all the work is spent in the production of heat and no part of it is wasted by friction, radiation, etc. The constant J is known as the mechanical equivalent of heat, and the accepted value of J is $4{\cdot}184 \times 10^7$ ergs per Calorie.

1 Kilogram-Metre = 7·23 foot-pounds
= 0·002343 Calorie
= $0{\cdot}81 \times 10^7$ ergs.

Work may also be defined as the product of force and the distance through which the force acts. When a man lifts a 10 Kg weight at a height of two metres from the ground, the work done is 10 Kg $\times$ 2 M = 20 KgM. But time factor need also to be considered. If a man has lifted 10 Kg weight at a height of two metres from the ground in one second, the work done is 20 KgM/sec. If he performs the same work at same rate for 60 sec., the total work performed is 20 KgM $\times$ 60 seconds or 1,200 KgM per minute. The mechanical work done can be measured by different instruments like Bicycle Ergometer, Treadmill, etc.

Bicycle ergometer

It is a modified bicycle (Fig. 359) specially constructed for carrying out standardised work (*exercise*) whose wheel is rotated by pedalling without the actual progress of the bicycle in the ground. A brake-band is applied to the front wheel. The work done depends on the rate of pedalling and is determined by the revolution counter attached to the

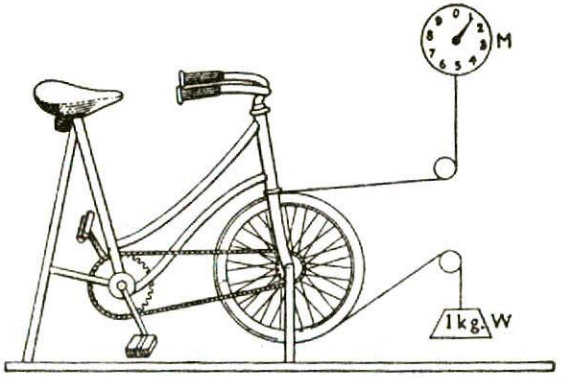


Fig. 359. Sketch of Bicycle Ergometer—Weight (W) is put with the help of string against the bicycle wheel and a recording metre M records the weight. Bicycle wheel is to be rotated 60 times a minute by pedalling. The friction of the string against the bicycle wheel will produce work according to load W. In the figure the load W is given as 1 Kg.

front wheel. The work done is calculated from the tension difference between the two sides of the bands and the linear velocity of the circumference of the wheel. A metre, calibrated in Kilogram-Metre per minute,

is kept above the front of the stationary bicycle for noting the constant work output by the subject.

With the help of the Bicycle Ergometer various physiological changes from a 'resting level' to a 'working level' can be noted. Heart rate, blood pressure, cardiac output, pulmonary ventilation, oxygen consumption, CO_2 output, body temperature, etc., change during physical activity. By measuring these variables during activity it is possible to determine in what degree the 'working level' differs from the 'resting level'. In the Bicycle Ergometer, mechanical work is imposed on the body. Even after the stoppage of mechanical work, physiological work continues above the 'resting rate' till recovery is not complete. A complete 'work cycle' indicates the 'physiological cost of work' and 'physiological cost of recovery'.

PHOSPHORYLATION

Introduction. Certain organic phosphates, formed in the tissues out of anabolic chemical reaction, play a key role in the energy transactions of living organisms. The importance of organic phosphates in metabolism was first indicated by the studies of Harden and Young, who found that fermentation of glucose by cell-free yeast juice increased rapidly when inorganic phosphorus is added to the medium, which was later converted to organically bound phosphate. The work of Embden, Meyerhof, Cori and their associates show that organic phosphates concerned with the metabolism of glucose by yeast are also intermediates in the metabolism of glycogen and glucose in muscle and other tissues.

The presence of ATP, ADP, CP and other high-energy phosphate compounds in the tissues and their participation in different metabolic processes indicate the importance of organic phosphates in metabolism. The sugar phosphates, although not high-energy compounds, are obligatory intermediates. Their formation involves phosphorylation by the high-energy compound ATP in presence of suitable enzymes.

Definition. The term **phosphorylation** includes all chemical reactions in the body which require combination with phosphoric acid. The reverse changes are called **dephosphorylation**, where phosphoric acid is dissociated from the compound.

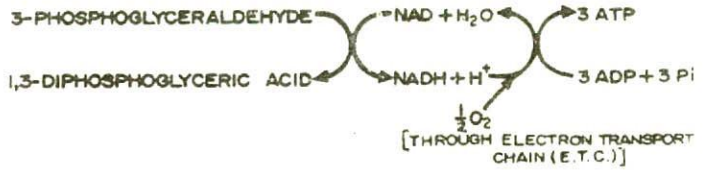


FIG. 360. Schematic representation of oxidative phosphorylation.

These two processes are probably the reversible reactions of the same enzyme system.

PHYSIOLOGICAL IMPORTANCE OF PHOSPHORYLATION

Phosphoric acid enters into the composition of cell protoplasm. Hence, phosphorylation is an essential chemical process for all cells. In addition to this it takes an essential part during absorption and metabolism of different

foodstuffs. Its effect in the different channels of metabolism are summarised below:

I. **In relation to carbohydrates.** (1) Absorption of carbohydrates through the intestinal mucosa and also reabsorption of glucose from renal tubules, are helped by phosphorylation. In both the epithelia hexose phosphate is formed. This compound undergoes dephosphorylation, hexose enters the blood stream and phosphoric acid is left behind. (2) Formation of glycogen from glucose and breakdown of glycogen into glucose in the

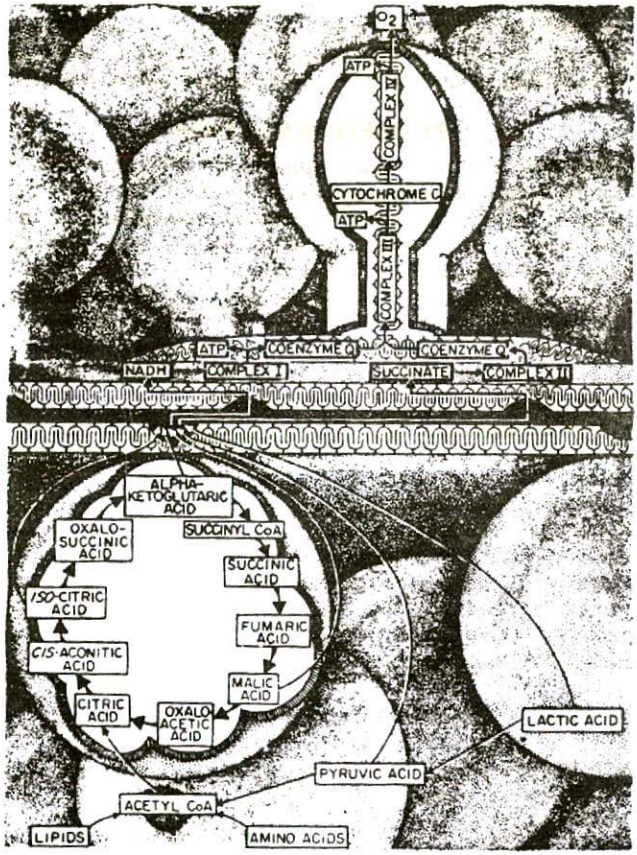


FIG. 361. Diagram showing the production of ATP by mitochondria through electron-transport chain.

liver and muscles are believed to take place through the process of phosphorylation. (3) During chemical changes accompanying muscular contraction, phosphorylation takes place at all important steps. As a matter of fact phosphorylation and dephosphorylation alternately go on in this process. Glycogen is broken down up to lactic acid through a series of phosphorylated compounds. Breakdown and synthesis of ATP and phosphagen are also the examples of dephosphorylation and phosphorylation. The phosphorylation

of carbohydrate metabolites takes place in two stages: In the *first stage* ADP is phosphorylated by Pi to ATP (Fig. 360). This reaction can take place only in presence of intact oxidation process and the energy-yielding (oxidation) mechanism is coupled with energy-harnessing (phosphorylation) mechanism. Uncoupling agents like 2,4-dinitrophenol inhibit phosphorylation but not oxidation. They elevate the oxygen consumption rate. The *second stage* in phosphorylation process is transfer of phosphate group from ATP to other intermediates. Oxidative phosphorylation takes place in the cristae of the mitochondria (Fig. 361).

II. **In relation to fats.** (1) During absorption of fats, neutral fats and phospholipids are synthesised in the absorbing epithelium. Phosphorylation is carried out by the enzyme, *phosphorylase*. (2) Liver synthesises phospholipids, specially lecithin. It is a very important step in the transport of fat. It also acts as a primary stage in the further oxidation of fatty acids. Fatty acid oxidation is a function of mitochondria (Fig. 362). (3) Phospholipids

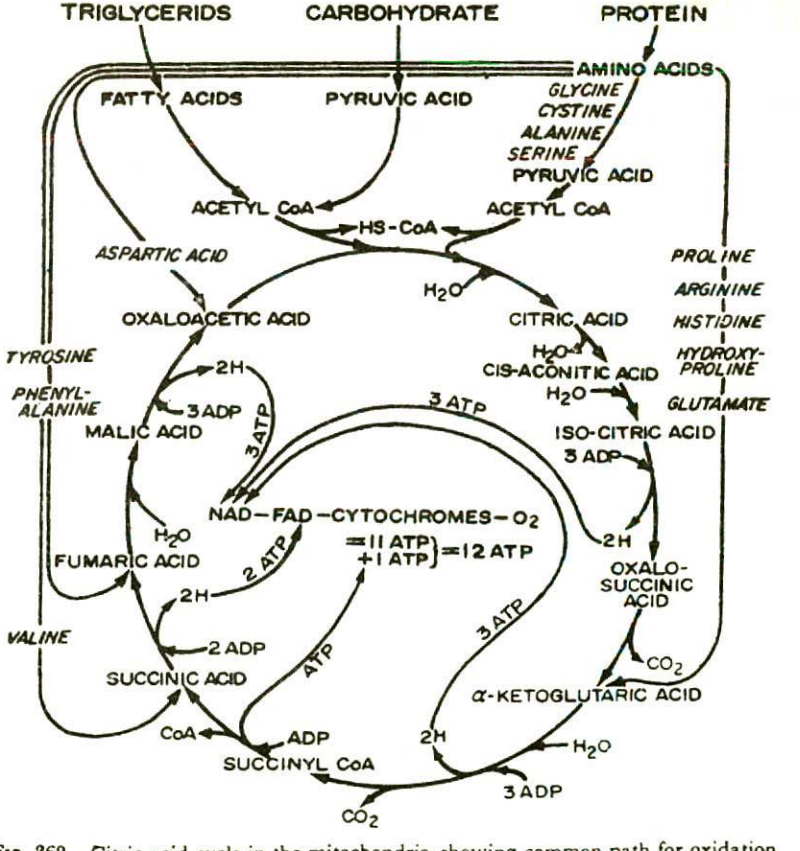


Fig. 362. Citric acid cycle in the mitochondria showing common path for oxidation of carbohydrate-protein-fat and oxidative phosphorylation, both *via* NAD-linked dehydrogenase and FAD-linked dehydrogenase (integration of carbohydrate, fat and protein metabolism).

enter into the composition of element constant of the cell. Hence, it is essential for cell life. It is believed that each cell can synthesise its own phospholipids locally by a process of phosphorylation. (4) Kephalin or cephalin, one of the phospholipids, is built up by phosphorylation which initiates blood clotting.

III. **In relation to proteins.** (1) It is an important step by which all the phosphoproteins are synthesised, such as, nucleoproteins, caseinogens, etc. (2) It is also probable that phosphorylation takes an important part in tissue oxidation during which proteins, fats and carbohydrates are finally broken down.

IV. **In relation to vitamins.** Some members of the vitamin B group are phosphorylated compounds, e.g., *thiamine pyrophosphate*, *riboflavin phosphate*, etc. They are believed to act as coenzymes in the oxidation and reduction processes in the cell.

V. **In relation to inorganic salts.** (1) Sodium plays an important part in phosphorylation. Its role is described below. (2) The acid and alkaline phosphates, which should be regarded as phosphorylated compounds, act as a very important buffer system in the body. (3) The complex interrelation between calcium and phosphorus may be primarily a process of phosphorylation and dephosphorylation.

VI. **In relation to bone formation.** Formation of bone, i.e., deposition of inorganic phosphoric acid compounds from organic phosphates—is also probably another example of phosphorylation.

CONTROL OF PHOSPHORYLATION

1. The **enzymes** that take part in this process are phosphorylase, phosphatase, etc.

2. **Hormones.** (*a*) **Adrenal cortex** is believed to be directly responsible for phosphorylation. Glucocorticoids inhibit phosphorylation, adrenalectomy accelerate it. (*b*) **Anterior pituitary,** by its adrenocorticotrophic hormone, may exert a superior control on phosphorylation, through adrenal cortex. The marasmic condition, that developes in diseases of adrenal cortex, may be partly explained by the consequent disturbance of phosphorylation, which affects absorption, metabolism and nutrition of the body. Growth hormone affects phosphorylation in a similar way as glucocorticoids.

3. **Inorganic salts.** Sodium may have some effects on phosphorylation (*vide* below).

It is believed that adrenal cortex controls phosphorylation by helping the enzymes responsible for the process. But it is also known that many of the defects, seen in diseases of the adrenal cortex, are rectified by giving enough NaCl. For instance, the defective fat absorption in adrenalectomised animals improves by giving NaCl. It is interesting to note that it is the same adrenal cortex which also controls Na metabolism. From these observations it may be suggested that adrenal cortex controls phosphorylation not by a direct action on the enzymes but by an indirect influence on Na metabolism.

It is also known that phospholipids are antagonistic to cholesterol. These two compounds are always found to remain together. It is also interesting to note that adrenal cortex which controls phosphorylation and herefore phospholipid formation, also controls metabolism of sterols.

CARBOHYDRATE METABOLISM

Introduction. Although the products of carbohydrate digestion, absorbed into the blood, are mainly hexose monosaccharides, i.e., glucose, fructose, galactose as well as partly pentose sugars but animal cells utilise mostly glucose so monosaccharides are converted into glucose by the cells mainly for their oxidation and partly for their storage as glycogen (through UDPG cycle). As fructose and galactose are utilised to a lesser extent in the body so they are reconverted from glucose as evident from the following. (1) Large quantities of galactose are found in the brain tissue. (2) The *brain probably uses carbohydrates as galactose only.* (3) During the normal process of glucose utilisation, fructose diphosphate is formed as an intermediary step. (4) When liver function is deficient, fructose and galactose fail to be converted into glucose and are turned out into the blood stream as such. Due to their low renal threshold they are excreted in the urine. The **fructose** and **galactose tolerance tests** *of liver function* depends upon this principle.

Since carbohydrate is utilised by the cells of all animals including man mainly as glucose so carbohydrate metabolism is meant essentially as metabolism of glucose and other substances, convertible to glucose or *vice versa.*

Pentose sugars, viz., xylose, arabinose and ribose present in the diet may be absorbed but their fate is not known whereas α-ribulose and α-2-deoxyribose after absorption are utilised in the body for the synthesis of nucleoproteins.

Normally glucose metabolism not only supplies major amount of energy for the body but also (1) other forms of monosaccharides (viz., fructose, galactose, α-ribulose and α-2-deoxyribose, etc.), disaccharides (lactose) and polysaccharides (glycogen); (2) reserve depot fats, (3) tissue glycolipids, mucopolysaccharides, and (4) amino acids are formed from glucose and reversely protein and lipids are metabolised through glucose pathway.

Thus it appears likely that glucose metabolism holds a central key position in carbohydrate metabolism which is closely associated with the metabolism of protein and fat.

The metabolism of carbohydrate, i.e., mainly of glucose, in the mammalian organism is discussed under the following points (fate and functions). (1) **Storage (by the process of glycogenesis)**—When glucose is not immediately required by the tissues, it remains stored. The chief form of storage is glycogen. Total about 500–700 gm of glucose may remain stored in this form. The main storehouses are liver and muscles in which glycogen is almost equally distributed. Some glucose may remain stored as such temporarily in the skin and subcutaneous tissue. (2) **Sources of energy (by the process of glycolysis and oxidation of pyruvic acid through TCA cycle)**—Carbohydrates are oxidised in the tissues and supply energy. One gram yields 4 C of energy. The major part of daily energy requirement under normal conditions is derived from carbohydrate oxidation. It is the most readily available source of energy and its combustion involves the least oxygen requirement. (3) **Maintenance of blood sugar (homeostasis)**—Glucose remains in blood and its level is always kept within a narrow range. Normal blood sugar varies from 80–120 mgm per 100 ml. Whenever it tends to fall, glucose is mobilised from glycogen and

blood sugar is maintained. (4) **Synthesis of hexose phosphate**—This is an intermediary step formed during oxidation of glucose, absorption of glucose from intestine and reabsorption from kidney tubules. It is a very important form in which hexose exists in the body. It is present in large quantities in the muscles, intestine, liver, etc. (5) **Synthesis of lactose**—In the lactating mother lactose is synthesised from blood glucose. The mammary glands convert *glucose into galactose* and then unite the latter with another molecule of glucose to form lactose. (6) **Synthesis of glycoproteins**—[*Vide* 'Conjugated Proteins'—page 74.] (7) **Synthesis of complex fats containing sugar**—For instance, **cerebrosides** are synthesised by the nerve cells from galactose and fats. Galactose is locally synthesised from blood glucose. (8) **Synthesis of fat (lipogenesis)**—It is an established fact that the body can convert carbohydrates into fats. (9) **Synthesis of proteins**—Simple amino acids may be formed by uniting ammonia with pyruvic acid, etc., which may easily be derived from carbohydrates.

Excretion of glucose. Glucose is not excreted from the body in normal health and Fehling's test is negative. It is a high threshold substance (170 mgm per 100 ml of blood). In metabolic disorders (e.g., diabetes mellitus), glucose appears in the urine and Fehling's test is positive. When blood sugar level gives higher value than renal threshold value, the glucose appears in the urine, the condition is known as **glycosuria.** In lactating mother, lactose may be found in the mother's urine. Pentose is found in a condition known as **pentosuria.** Glucose may be found in urine in higher quantities in some pathological conditions (*vide* 'Glycosuria' under the chapter of *Excretion*).

GLYCOGEN

Chemistry and properties. It is called *animal starch;* because it is in this form that glucose remains stored in the liver and muscles. It is branched polysaccharides (amylopectin type) consisting of hundreds of glucose units linked together by **glucosidic linkages,** i.e., α-1,4′ linkage and 1,6′ linkage which are formed by specific enzymes—uridine diphosphate glucose (UDPG)-pyrophosphorylase, glycogen synthetase and amylo-(1,4′→1,6′)-transglucosidase respectively. It is *soluble in water* and makes an *opalescent solution* and gives *red colour with iodine.* Glycogen liberates more energy than the corresponding weight of glucose. It does not diffuse into the intracellular fluid, as it exerts no osmotic pressure. It may be easily broken down into glucose by enzymes present in the liver.

Amount and distribution. In a normal adult about 700 gm of glycogen is present in the body, about 300 gm in liver and 400 gm in muscles. Liver and muscles are the chief storehouses. All growing tissues can store glycogen. Consequently, they are present in large amounts in the placenta in its early stage, the foetal muscles, and the yeast, etc. In the *foetal* muscles it may be as much as 40% of the total dried solids. Oyster is very rich in glycogen and is a good source for its manufacture. Glycogen in any tissue is not a static quantity. It is being constantly used up and resynthesised. So that at any time the glycogen of the tissue should be considered as a balance between the constant production and loss. *Liver glycogen is most mobile.* It is the first to be formed and is also the first to be mobilised. *Muscle glycogen is much slower to move.* There are remarkable differences between the metabolism of liver glycogen and muscle glycogen.

GLYCOGENESIS, GLYCOLYSIS AND CITRIC ACID CYCLE

Formation and mobilisation of glycogen. Glycogen is formed both in the liver and muscles (FIG. 363). When blood sugar tends to fall, liver glycogen is converted into glucose and mobilised in the blood stream (FIG. 364). Thus blood sugar is maintained. In muscular exercise, starvation, exposure to cold and such other conditions, in which extra energy

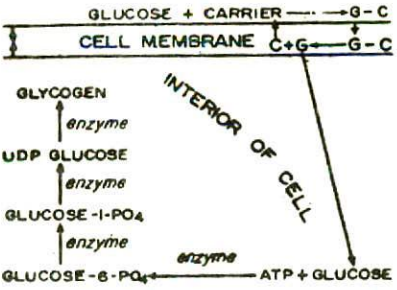


FIG. 363. Schematic representation of glycogenesis in a cell (simplified).

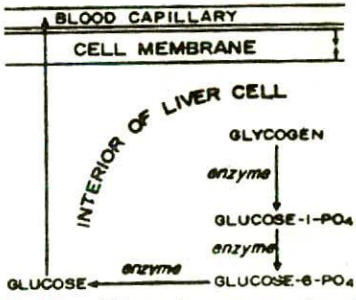


FIG. 364. Schematic representation of glycogenolysis in a liver cell (simplified).

is demanded, liver glycogen is mobilised. This action is helped by certain hormones such as adrenaline (epinephrine), glucagon, thyroxine, growth or somatotrophic hormone (STH) of anterior pituitary, etc. Stimulation of the sympathetic has same function. It is antagonised by insulin. Insulin helps glycogenesis in liver and prevents glycogenolysis.

Formation of glycogen (glycogenesis). In the process of glycogenesis, glucose is phosphorylated to glucose-6-phosphate by *hexokinase* (*glucokinase*) in presence of a phosphate donor, ATP a common to the first reaction in the glycolytic path of glucose metabolism. Glucose-6-phosphate is transformed into glucose-1-phosphate, catalysed by the enzyme *phosphoglucomutase*. In the next step glucose-1-phosphate reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (activated glucose units as UDPG) and inorganic pyrophosphate (PPi). This reaction is catalysed by enzyme *UDPG-pyrophosphorylase*. An enzyme *UDPG-glycogen transglucosylase* (*glycogen synthetase*) helps in the addition of glucose residue present in its activated form (UDPG) to a pre-existing glycogen chain at non-reducing outer end of the molecule (glycogen) so that glycogen tree is elongated successively due to the 1,4′ linkage formation. Thus uridine diphosphate (UDP) is liberated and resynthesised with the help of ATP: $UDP + ATP \rightleftharpoons ADP + UTP$.

A second enzyme, called **branching enzyme** (amylo-(1,4′→1,6′)-transglucosidase) transfers a part of the 1,4′-chain to adjacent chain to form α-1,6′ linkage and helps in glycogen synthesis by forming a branch point (1,6′ linkage) in the molecule.

Mobilisation of glycogen (glycogenolysis and glycolysis). The former process is the breakdown of glycogen to glucose whereas the latter is the process of breakdown of glycogen or glucose to pyruvic acid (anaerobic) which is further oxidised to CO_2 and H_2O (aerobic) through TCA cycle. In both the processes, glycogen is converted to glucose-6-phosphate and in the process of glycogenolysis glucose-6-phosphate is splitted into glucose and Pi by phosphatase whereas in the process of

glycolysis glucose-6-phosphate is converted further into fructose-6-phosphate by phosphohexose isomerase.

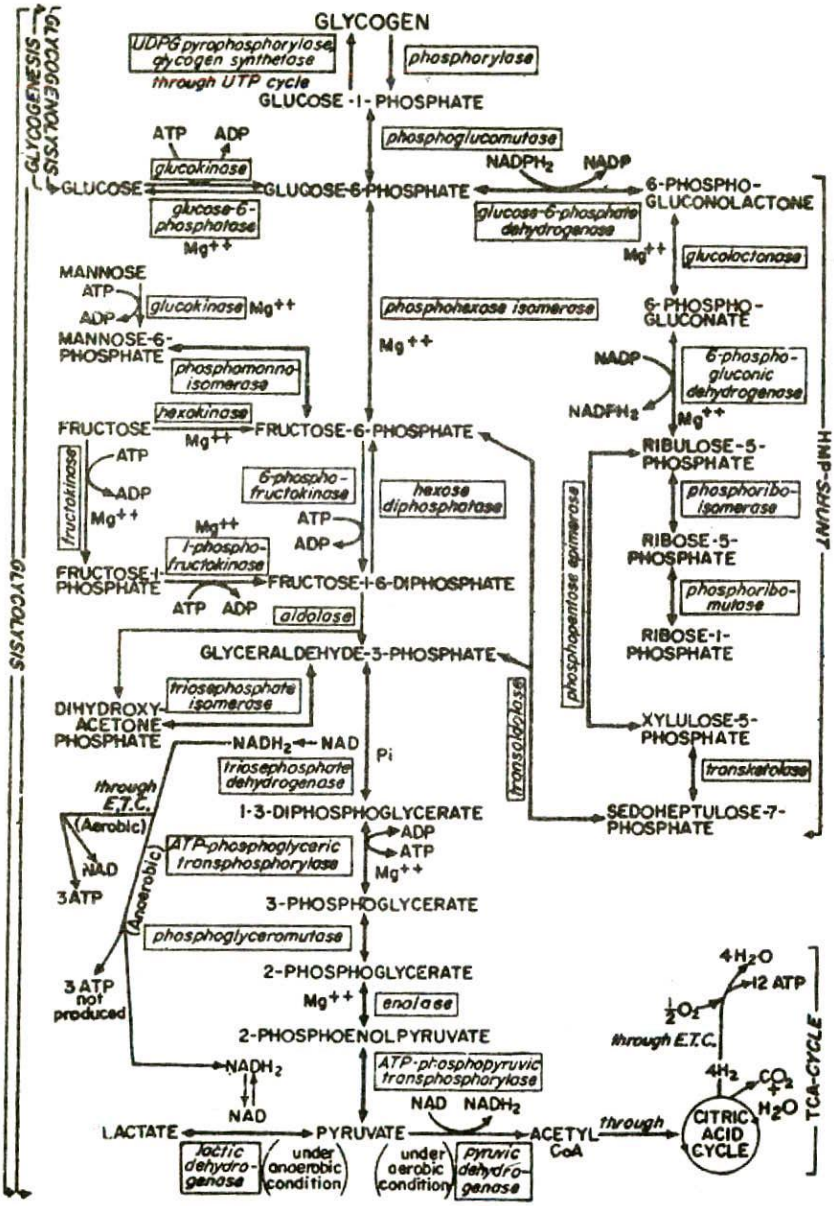


FIG. 365. Schematic representation of paths of glycogenolysis, glycogenesis and glycolysis, oxidation of glucose through hexose monophosphate (HMP) shunt (PPP = pentose phosphate pathway) and oxidation of acetyl CoA through TCA cycle.

Glycogen is broken down to glucose-1-phosphate, catalysed by the enzyme **phosphorylase a** (active form). Phosphorylase exists in an inactive form, **phosphorylase b.** Cyclic AMP (CAMP or 3′-5′-AMP) donates a phosphate group and converts it into an active form, phosphorylase a. An enzyme, *adenyl cyclase,* helps in the formation of cyclic AMP from ATP which is accelerated by glucagon and adrenaline (epinephrine). The glucose-1-phosphate is converted into glucose-6-phosphate, catalysed by the enzyme **phosphoglucomutase.** The enzyme *phosphohexo isomerase* converts glucose-6-phosphate to fructose-6-phosphate.

Pathway of formation of pyruvic acid. Fructose-6-phosphate accepts another phosphate group from ATP and is transformed into fructose-1-6-diphosphate. This reaction is influenced by the enzyme 6-*phosphofructokinase.* Another enzyme *aldolase* breaks down the above hexose diphosphate into dihydroxyacetone phosphate and glyceral dehyde-3-phosphate each containing 3 carbon atoms (triosephosphate). Enzyme *triosephosphate isomerase* keeps the above two triosephosphates in equilibrium. Glyceraldehyde-3-phosphate is then dehydrogenated by *triosephosphate dehydrogenase* into 1-3-diphosphoglycerate, the hydrogen being accepted by NAD. Phosphorylation also takes place at this stage and requires inorganic phosphate (Pi). Diphosphoglycerate then donates one high-energy phosphate to ADP to convert it into ATP and it itself is transformed into 3-phosphoglycerate. This reaction is catalysed by the enzyme *ATP-phosphoglyceric transphosphorylase* (*phosphoglyceric acid kinase*). *Phosphoglyceromutase* then transforms 3-phosphoglycerate to 2-phosphoglycerate and *enolase* converts it into 2-phosphoenolpyruvate. Phosphoenolpyruvate is spontaneously converted to pyruvate which is oxidised further to CO_2 and H_2O through TCA cycle if aerobic condition exists or otherwise reduced to lactic acid. *ATP-phosphopyruvic transphosphorylase* (*pyruvic acid kinase*) then transfers one energy-rich (~) phosphate bond from phosphoenolpyruvate to ADP to form ATP and pyruvic acid.

Other monosaccharides (galactose, fructose, mannose) gain their entrance in the glycolytic pathway as indicated in the figure 365.

Citric acid cycle or Krebs cycle. The citric acid cycle is one of the most important biochemical mechanism of oxidation of the activated metabolites and it is perhaps the major terminal pathway of biological oxidation in all animal tissues. The activated metabolites, which are few in number derived from carbohydrate, protein, and fat, are oxidised by the electron-transport chain and most of the utilisable energy (ATP) are produced for the organism. The activated metabolites, which are derived from different foodstuffs (Fig. 366), are given below:

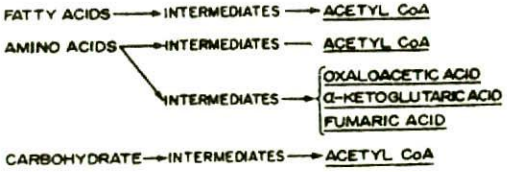


Fig. 366. The underlined substances are the activated compounds oxidised in TCA cycle.

The components, included in this cycle, are interrelated by oxidation and reduction, and other reactions which produce $2CO_2$, H_2O and energy ATP In case of carbohydrate, the pyruvic acid which is formed by the glycolytic path of oxidation, enters this cycle by first being transformed into acetyl CoA. This cycle is known as Krebs (citric acid) cycle after the English biochemist H. A. Krebs who first formulated and proposed the mechanism. Citric acid being one of the member of the cycle and some of the members contains these carboxylised groups so this cycle is also known as *citric acid cycle* and **tricarboxylic acid (TCA) cycle.**

Acetyl CoA or Active acetate formation. In presence of six factors, i.e., Mg^{++}, NAD, thiamine pyrophosphate, lipoic acid, FAD and coenzyme A, the pyruvic oxidase along with enzyme complex converts pyruvate to active acetate as a result of oxidative decarboxylation and as a result the $NADH_2$ is formed which is reconverted to NAD by electron-transport chain. It enters into the TCA cycle. A condensing enzyme, *citrate synthetase*, helps in the condensation of acetyl CoA with oxalo-acetate to form *citrate*. Citrate first by a process of dehydration is converted into *cis-aconitate* which again by a process of rehydration is transformed into *iso-citrate*. The enzyme *aconitase* catalyses the reaction at both the two steps. *Iso*-citrate in presence of the enzyme *iso-citrate dehydrogenase* is then dehydrogenated to oxalosuccinate. NAD or NADP acts as a hydrogen-acceptor and is converted into $NADH_2$ or $NADPH_2$. An enzyme *oxalosuccinate decarboxylase* in presence of Mn^{++} removes CO_2 from oxalosuccinate which is thus converted into α-ketoglutarate. A process of oxidative decarboxylation, similar to that in the conversion of pyruvate to acetyl CoA, transforms α-ketoglutarate to succinyl CoA catalysed by α-ketoglutaric oxidase which also requires coenzyme A, lipoic acid and NAD acting as hydrogen-acceptor. Succinyl CoA, while it being converted into succinate, provides energy for synthesis of GTP (guanosine-5'-triphosphate) from GDP (guanosine-5'-diphosphate). So GTP in turn supplies energy for synthesis of ATP from ADP while it is reconverted to GDP. Thus succinyl CoA supplies ultimately energy for the synthesis of ATP.

[The enzyme *thiophorase* present in tissues, other than liver, can help in the conversion of succinyl CoA⟶succinate.] Enzyme *succinate dehydrogenase* converts succinate to fumarate, the hydrogen is transferred directly to flavoprotein (FAD) converting it into $FADH_2$. *Fumarase* helps in the addition of water to fumarate, *malate* is formed in this process. Oxaloacetate is regenerated from malate under the influence of *malate dehydrogenase*, NAD again is the hydrogen-acceptor (Fig. 367).

Pentose phosphate pathway (PPP) or pentose cycle or hexose monophosphate (HMP) shunt or phosphogluconate oxidative path or Warburg-Dickens-Lipmann path. This pathway of glucose metabolism takes place in liver, mammary gland, testis, adrenal cortex and leucocytes. Glucose-6-phosphate derived from different sources is dehydrogenated by *glucose-6-phosphate dehydrogenase* into 6-phospho-gluconolactone which, through several steps described in the figure 365, is ultimately converted into sedoheptulose-7-phosphate and enters again into the main glycolytic pathway at fructose-6-phosphate and glyceraldehyde-3-phosphate. The formation of sedoheptulose-7-phosphate is catalysed by *transketolase* whereas the breakdown is catalysed by the enzyme *transaldolase*.

The transketolase and transaldolase reactions are important in this path which is responsible for conversion of aldehydes to ketones and *vice versa*,

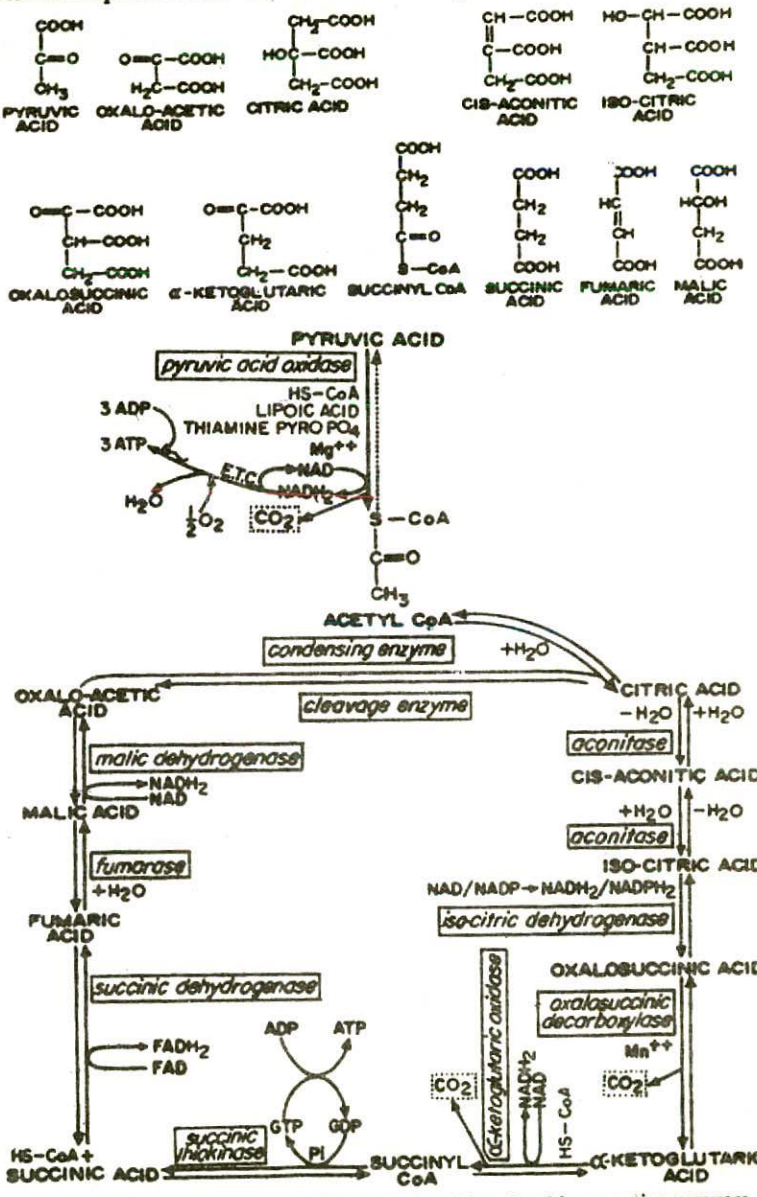


Fig. 367. Schematic representation of citric acid cycle with respective enzymes.

as well as lower sugars to higher sugars and *vice versa*. The physiological benefits in the cycle are as follows:

1. Synthesis of pentose to be required for the synthesis of nucleotides.
2. Pentoses can enter into the glycolytic path and it itself may be oxidised in pentose phosphate pathway (PPP).
3. Hexose (glucose-fructose) may be burnt in this PPP or may supply pentoses.
4. $NADPH_2$, formed in the PPP, is utilised in the fat and steroid synthesis.
5. Energy formed in this path is 36 ATP per molecule of glucose if all the $NADPH_2$ are oxidised in the mitochondria to NADP.
6. The oxidation of glucose (FIG. 368) in this path is independent of the TCA cycle components.
7. Components of PPP may enter into the path of formation of glucuronic acid and ascorbic acid (vitamin C).

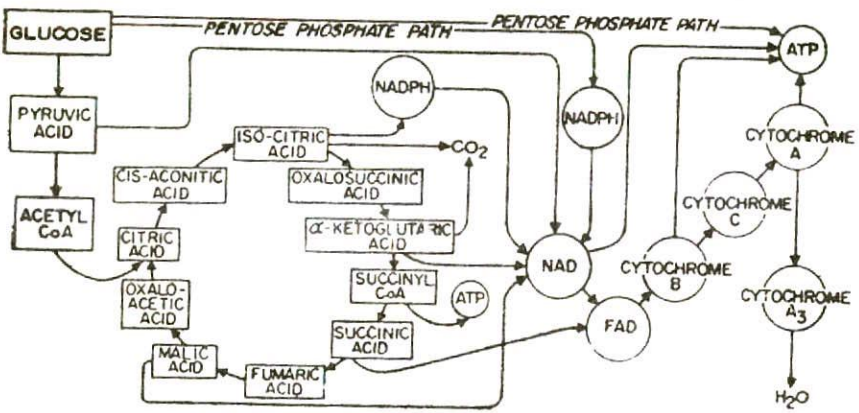


FIG. 368. Schematic representation of chain of oxidation of glucose.

I. METABOLISM OF GLYCOGEN IN LIVER

Sources of liver glycogen. *Glycogenesis* (formation of glycogen) in the liver can take place from the following sources: (*a*) **From carbohydrates and the related substances**—For instance, glucose, galactose, fructose, mannose, lactic acid (from muscles or otherwise), pyruvic acid, methyl glyoxal, etc. Lactic acid of muscles is carried through blood stream to the liver where it is converted into glycogen very readily. It is believed that pentose does not form glycogen. (*b*) **From proteins**—The *anti-ketogenic amino acids* (e.g., glycine, alanine, aspartic acid and glutamic acid, etc.) can readily form glucose

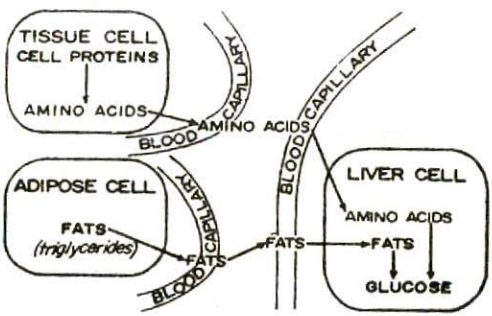


FIG. 369. Schematic representation of neoglucogenesis in liver from mobilised tissue proteins and fats (simplified).

through TCA cycle or reversible glycolytic path or both, as the case may be, as seen in diabetes mellitus. In diabetes mellitus the G : N ratio increased indicating the glucose is formed from protein (neoglucogenesis). It is reasonable to believe that this glucose may be available for glycogen formation. (*c*) **From fats**—The glycerol part of fats is converted into glucose from which glycogen may be derived.

Functions of liver glycogen. (*a*) Liver glycogen is a ready source of glucose supply in the blood. (*b*) It helps in the detoxicating mechanism in the liver. (*c*) It protects the liver from the toxic effects of *arsenic, carbon tetrachloride*, etc. (*d*) If the liver glycogen level is high, ketone body formation and rate of deamination of amino acids are depressed.

II. METABOLISM OF GLYCOGEN IN MUSCLES

[*Vide* pages 217–18, 225, 265–66.]

Muscle contains about 0·5%–1·0% of glycogen as opposed to 5% in the liver. But due to greater amount of muscles in the body, the total quantity is higher and is about 400 gm or approximately equal depending on the total muscle mass of the body.

GLYCOGENESIS IN MUSCLES

Source. Muscle glycogen can be derived from the following sources: (*a*) **From glucose**—which is obviously taken from the blood stream. (*b*) **From lactic acid**—which is produced in the muscle during muscular contraction. The major part (4/5ths) of the lactic acid produced during exercise is reconverted into glycogen. A small part of it (1/5th) is oxidised into

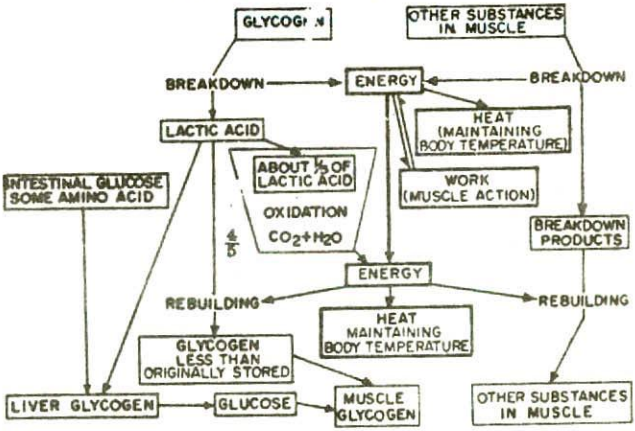


FIG. 370. Schematic representation of metabolism during muscle activity.

carbon dioxide and water through TCA cycle. The conversion of lactic acid into glycogen in the muscle is comparatively much slower than in the liver. So that during heavy muscular exercise, a large amount of lactic acid is produced in the muscles. A good part of it diffuses into the blood stream and is brought to liver where it is readily reconverted into glycogen. Probably glycogen is not produced in the muscles from proteins and carbohydrates.

GLYCOLYSIS IN MUSCLES

Glycolysis is the process of breakdown of glycogen or glucose in muscles and other tissues into pyruvic and lactic acids (**Embden-Meyerhof glycolytic pathway**). Glycogen leaves the liver in the form of glucose, but it leaves the muscles in the form of pyruvic and lactic acids. The difference is probably due to the fact that, the enzyme systems and the chemical reaction in the liver and muscles are not the same. Lactic acid that emerges from the muscles, is carried to liver through blood stream where it is reconverted into glycogen. This glycogen is again mobilised in the form of glucose which enters into the blood stream. Muscles take up this glucose from the blood stream and recovers its lost glycogen. This cyclic process of circulation of carbohydrate in different forms in different tissues is known as **Cori cycle** through which muscle and liver glycogens become readily interchangeable (FIG. 371).

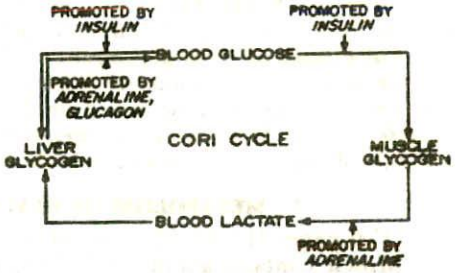


FIG. 371. Schematic representation showing circulation of carbohydrate in different forms in different tissues (Cori cycle) and effects of adrenaline, insulin and glucagon upon it.

Glycogen, in other tissues excepting liver, exhibits the same pattern of breakdown as in the muscles

DIFFERENCE IN GLYCOGEN METABOLISM IN LIVER AND MUSCLES

LIVER	MUSCLE
1. *Glycogenesis*—from carbohydrates, muscle lactic acid and other related compounds, as well as from proteins and fats.	1. *Glycogenesis*—from blood glucose and muscle lactic acid. Not from protiens and fats.
2. Liver glycogen is most mobile, i.e., first formed and first mobilised.	2. Muscle glycogen is less mobile. The process is comparatively slower.
3. Glycogen breakdown is mostly up to glucose and the glucose then enters the blood stream (*glycogenolysis*).	3. Glycogen breakdown is up to pyruvic and lactic acids (*glycolysis*)—1/5th of lactate is broken down into CO_2 and H_2O, and 4/5ths reconverted into glycogen in liver.
4. Liver glycogen is utilised for maintaining blood sugar. From this glucose, muscle glycogen is replenished.	4. Muscle glycogen is not available for maintaining blood sugar. It comes out as lactic acid from which liver glycogen is formed.

UTILISATION OF GLUCOSE BY THE TISSUES

Although glucose is used up by all tissues, yet the different tissues do not use the carbohydrate in the same manner. The peculiarities of carbohydrate metabolism of some of the important tissues are given below:

I. **In the skeletal muscles.** [*Vide* pp. 217–18, 225.] Recent evidence suggests that pyruvic acid and not lactic acid is the end product of the glucose oxidation. It is converted into lactic acid due to certain conditions (anaerobic). Pyruvic acid is finally oxidised into CO_2 and H_2O through an elaborate cycle of chemical reactions known as **citric acid cycle** or **Krebs cycle** (FIG. 365).

II. **In the cardiac muscle.** Although in broad principles, it is similar to that in voluntary muscles yet it varies in certain details. [*Vide* pp. 225, 265.] In starvation and in depancreatised animals glycogen in heart increases in amount, whereas, that in the liver and muscles fall. Cardiac muscle utilises lactic acid directly and completely in preference to glucose.

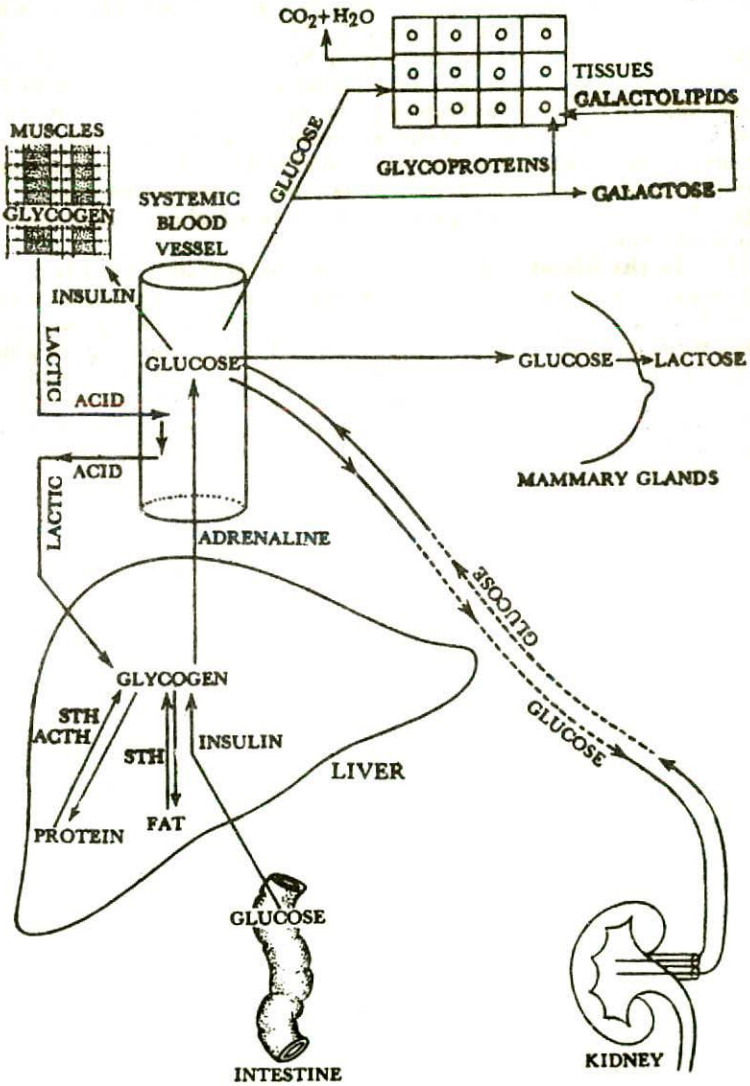


FIG. 372. Diagrammatic representation of the fate and utilisation of glucose in the body.

Experimentally, it is seen that the dog's heart can take up about three times more lactic acid than glucose from blood. Adrenaline (epinephrine)

has got no effect on cardiac glycogen. Glucose utilisation by the heart of a diabetic animal is less than normal, but as regards lactate, the diabetic heart uses it almost as readily as the normal heart. The rate of usage of glucose by the diabetic heart is increased by insulin whereas it is not in case of lactate.

III. **In the brain.** The R.Q. value of brain indicates that carbohydrates are utilised by the brain exclusively. The brain forms lactic acid from sugar and oxidises further into lactic acid. Since there is very little glycogen in brain, it was formerly believed that lactic acid was formed directly from blood sugar. But recent findings indicate that *brain uses sugar in the form of galactose only* which is locally synthesised from blood glucose, and it is from the breakdown of galactose that lactic acid is derived. It is interesting to note that brain tissue is very rich in fat, but it derives its energy from sugar. Brain can synthesise galactose from blood glucose and convert it into cerebroside.

IV. **In the blood.** The red blood corpuscles readily form lactic acid from sugar. The blood lactic acid in resting subjects is supposed to be formed in this way. Lactic acid from muscles enters the blood stream only in heavy muscular exercise. If a sample of blood is kept standing, its glucose content rapidly falls due to the conversion of glucose into lactic acid along with the rise of concentration of lactic acid in blood (*glycolysis*). From this fact it is obvious that the blood sample, whose glucose content to be estimated, should be (preferably) freshly collected or otherwise antiglycolytic preservative treated (in case of old sample).

V. **In other tissues.** [*Vide* page 545.]

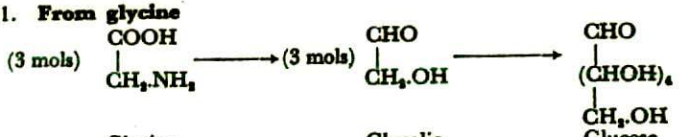
SYNTHESIS OF CARBOHYDRATES

I. **From fats.** It is certain that the glycerol, portion of fat, which makes up about 10% of the fat molecule, is converted into glucose in the body but the conversion of fatty acid portion of fat molecules to glucose is a matter of dispute specially in animal body as contrast to plants. Some of the evidences are given below: (*a*) During hibernation the marmot shows a very low respiratory quotient—about 0·3–0·4. The excess oxygen intake is explained on the assumption that oxygen-poor substance (fat) is being converted into an oxygen-rich substance (carbohydrate). But these findings and conclusions are not beyond question. (*b*) The same type of conversion is believed to take place in diabetic subjects where a low respiratory quotient is found. (*c*) When fatty acids containing *odd number of carbon atoms*, such as, propionic, valeric and heptoic acids, etc., are administered to starving rats they are converted into glycogen in the liver. But these fatty acids are not found in the natural fats. *Natural fats*, which contain fatty acids with *even number of carbon atoms* only, do not produce this effect. From this it can be concluded that the synthesis of carbohydrates from fats is not beyond possibility but it takes place indirectly.

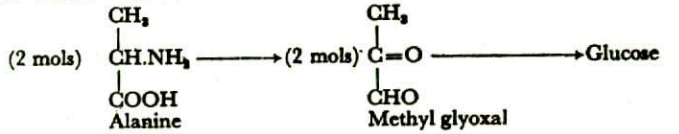
II. **From proteins.** There is ample evidence to show that formation of glucose and glycogen may take place from proteins. The administration of certain amino acids in a depancreatised dog raises the urinary glucose. These amino acids are called antiketogenic amino acids (*vide* under *protein metabolism*). On the whole it is generally accepted that about 60% of the food proteins can form sugar. As to the chemical process of synthesis of

carbohydrates from proteins, nature of the mechanism is different with different amino acids. Some examples are given below:

1. **From glycine**



2. **From alanine**



EFFECT OF BLOOD SUGAR LEVEL ON THE FUNCTIONS OF VARIOUS TISSUES AND ORGANS

The sugar content of blood is not a passive constituent. Blood sugar level is found to exert enormous influence in regulating the functions of various tissues in different ways. The following is a brief summary: (1) **On stomach**—Low blood sugar stimulates gastric secretion and gastric movements. Hunger contractions are always stimulated by low sugar (as also by insulin injections). They stop when the blood sugar rises. (2) **On liver**—Glycogenesis in liver is stimulated when blood sugar level is high as contrast to the hypoglycaemic condition when instead of glycogenesis, glycogenolysis occurs. (3) **On muscles**—Muscle glycogen is formed when its glycogen content is low whereas it does not follow this principle when its glycogen content is high in spite of high blood sugar level. Muscle glycogen has got no glycostatic action. (4) **On heart**—Fall of blood sugar level results lactic acid accumulation in muscle but these are converted into glycogen in cardiac muscle. (5) **On central nervous system**—Since sugars are the main fuel of central nervous system activity, the hypoglycaemia results interference, i.e., hypoactivity of central nervous system, e.g., feeling of fatigue, abnormal behaviour, i.e., anxiety and irritability, appearance of tremour and vasomotor disturbances, etc. (6) **On autonomic nervous system**—Low blood sugar stimulates the sympathetic and raises blood sugar level. It acts in two ways: (*a*) *by stimulating adrenaline secretion*, (*b*) *by acting directly on the liver*, through its sympathetic nerves and thus mobilising its glycogen content. Hyperglycaemia stimulates the parasympathetic which increases insulin secretion and reduces blood sugar. (7) **On adipose tissue**—When sugar supplied is more than can be utilised, blood sugar tends to rise. This extra amount is converted into fat by the adipose tissue and is deposited. (8) **On the utilisation of glucose by other tissues**—Hypoglycaemia reduces the rate of glucose oxidation in the tissues. Hyperglycaemia, on the other hand, stimulates glucose utilisation in the tissues. When viewed from this standpoint it seems that high blood sugar in diabetes mellitus is a protective phenomenon which tries to maintain the rate of glucose utilisation by the tissues. (9) **On endocrines**—(*a*) **Adrenaline (epinephrine)**—Low blood sugar stimulates adrenaline secretion (probably not a direct action but through the sympathetic). (*b*) **Insulin**—Hypoglycaemia depresses and hyperglycaemia stimulates insulin secretion by directly acting upon the islets of Langerhans. As already mentioned these factors also act through the parasympathetic. (10) **On neoglucogenesis**—When blood sugar tends to fall, neoglucogenesis is stimulated in the liver. As blood sugar rises neoglucogenesis is inhibited in a normal subject. Thus in a normal subject the rate of neoglucogenesis is controlled by blood sugar level. (11) **On kidney threshold**—In a chronic diabetic case, with persistently high blood sugar level, the renal threshold for glucose rises. Under such conditions blood sugar may go much above the renal threshold (180 mgm per 100 ml) and yet the urine may be free from sugar.

Some of the other important problems of carbohydrate metabolism are the following:

1. Hyperglycaemia. 2. Hypoglycaemia. 3. Glycosuria.

Above problems are physiologically satisfied by a regulating mechanism

existed in the body what is known as *regulation of normal blood sugar level.* These problems have been dealt with in their respective places.

For *Islets of Langerhans, Glucose tolerance test, Regulation of blood sugar level, Metabolism in diabetes mellitus vide* under the chapter of *Endocrine.*

ROLE OF ENDOCRINES ON CARBOHYDRATE METABOLISM

Glucose level of blood at any given time is always determined by the balance between the amount of glucose entering the blood stream and the amount leaving it. Liver takes an important part by allowing net uptake of glucose when the blood glucose is high and by allowing net release of glucose when the glucose level is low. Liver thus plays as a *glucostat* in maintaining a constant circulating glucose level by the relative activities of the above processes. These processes are not automatic but are manifested by the actions of different hormones. Relative contributions of different hormones that take part in the control of blood glucose have been presented in Fig. 376. Major hormones that take part in carbohydrate metabolism are described below:

Role of insulin—The principal effect of insulin on carbohydrate metabolism is to increase the utilisation of glucose by most tissues. The most important effect of insulin is to increase the rate of glycogen formation. It has been described earlier that the insulin is secreted from the β-cells of the islets of Langerhans. It should be borne in mind that the degree of insulin activity and probably the actual production of insulin by the β-cells of the pancreatic islets are effected by the level of blood sugar. Hyperglycaemia stimulates the pancreas to produce the increased quantity of insulin and if the hyperglycaemia is maintained for a longer period then the permanent damage to the β-cells may ensue and thus permanent diabetes prevails. But it is difficult to say that the hypoglycaemia leads to decrease in insulin secretion in same level. Because during such state adrenaline is secreted and this hormone thus masks the effect of insulin on liver glycogen. There are other factors which either suppress the production of insulin or may render its action less effective. Growth hormone, glucocorticoids (cortisone and hydrocortisone) and also thyroxine act in such process. There is evidence that growth hormone and glucocorticoids inhibit phosphorylation of glucose by affecting hexokinase activity. These two hormones have got no action on the entry of glucose into the cells.

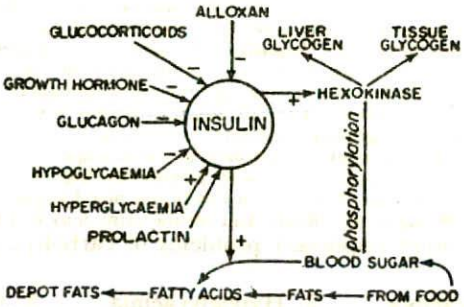


FIG. 373. Role of insulin on carbohydrate metabolism and also its interrelationship with other hormones. (−) means *inhibition* and (+) denotes *stimulation.*

Glucagon, the α-cell hormone of pancreatic islets and also of gastro-intestinal tract seems to counteract the insulin by exhaustion atrophy of β-cells. Alloxan also counteracts the insulin by damaging the β-cells.

The insulin is mostly concerned with the utilisation of glucose by the tissues and this involves the phosphorylation in which the chain of conversions of glucose and its combination is controlled by a series of enzymes of which hexokinase is an important one. Insulin stimulates the catalytic action of hexokinase. Insulin has been found to increase the glycogen synthetase activity in muscle. It is claimed that considerably more blood sugar is converted to fatty acids and eventually deposited in the fat depots than that which is turned into tissue glycogen. Insulin increases the conversion of sugar to fatty acids.

Furthermore, formation of liver glycogen is quantitatively higher than the formation of tissue glycogen.

The influence of insulin on carbohydrate metabolism has been presented schematically in Fig. 373.

Role of glucagon—Glucagon is known as hyperglycaemic-glycogenolytic factor (HGF). Main effect of glucagon on carbohydrate metabolism is to increase the breakdown of liver glycogen to glucose and hence hyperglycaemia. It does not cause the breakdown of muscle glycogen. Glucagon is secreted from the α-cells of the islets of Langerhans, walls of the duodenum and stomach. If glucose is placed in the gastro-intestinal tract then glucagon is secreted from the gastro-intestinal tract directly in the circulation. Glucagon raises the blood glucose level by stimulating the adenyl cyclase in the liver leading to the formation of cyclic AMP that activates the phosphorylase. Glucagon has got no effect on muscle phosphorylase. Due to action of glucagon on adenyl cyclase, cyclic AMP is formed from ATP. The cyclic AMP thus activates the phosphorylation process of liver glycogen and thus glucose is formed.

Besides this, glucagon also stimulates the process of neoglucogenesis from available amino acids in the liver. Thus increased activity of glucagon increases the blood glucose level which may indirectly stimulate the β-cell activity for the production of excess insulin. Thus prolonged treatment with glucagon causes exhaustion of β-cell and diabetes is produced. Role of glucagon on carbohydrate metabolism has been presented schematically in Fig. 374.

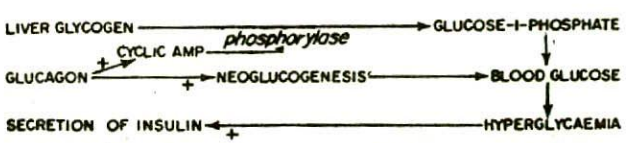


FIG. 374. Role of glucagon on carbohydrate metabolism. (+) means *stimulation*.

Role of growth hormone—It is established that growth hormone opposes the hexokinase mechanism, so that the phosphorylation of glucose is depressed causing hyperglycaemia. This hyperglycaemia causes secretion of insulin from the β-cells. Prolonged effect of growth hormone may eventually exhaust the β-cells. Histologically it is proved that α-cells remains unaffected when β-cells are damaged due to prolonged glucagon

therapy. Role of growth hormone on carbohydrate metabolism has been presented schematically in Fig. 375.

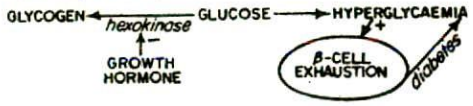


FIG. 375. Effects of growth hormone on carbohydrate metabolism. (−) denotes *inhibition* and (+) means *stimulation.*

Role of adrenal glucocorticoids—Like growth hormone adrenal glucocorticoids also elevate the blood sugar level. It is claimed that these hormones produce the hyperglycaemic effect by increased neoglucogenesis in the liver. It also produces hyperglycaemia by decreasing the glucose utilisation in the liver and peripheral tissue possibly through the inhibition of phosphorylation. In patients with adrenal insufficiency, the blood glucose-lowering effect of insulin is greatly enhanced. In experimental diabetes, adrenalectomy may markedly ameliorate the diabetic state.

In cats and rats after bilateral adrenalectomy, the carbohydrate reserves of the liver and muscles are depleted and hypoglycaemia is produced. But this hypoglycaemic condition is corrected only when corticoids and glucose together, but not glucose only, are provided.

Role of epinephrine (adrenaline)—Epinephrine increases the blood sugar level and this is one of the most important factors in the normal organism for counteracting the hypoglycaemic action of insulin. Epinephrine causes rapid breakdown of liver glycogen to glucose with the production of hyperglycaemia. In muscle, the epinephrine causes the breakdown of glycogen to lactic acid. Epinephrine is released as an emergency in response to emotional excitement, injury, fright, stress, exercise, etc., and consequently augments blood sugar. Hypoglycaemia from any cause leads to secretion of epinephrine from adrenal medulla and brings the blood glucose level back to normal. Epinephrine exerts its hyperglycaemic effects by increasing the rate of glycogenolysis in the liver and muscles.

Muscle glycogen is not directly available for the replenish of glucose. By the action of epinephrine, both liver and muscle glycogen are converted into hexose phosphate. In the liver, glucose is formed by the action of phosphatase on the hexose phosphate. But the enzyme, phosphatase, is lacking in muscle and for this reason it has to complete the whole glycolytic process with the formation of lactic acid. A some amount of lactic acid may be transformed into liver glycogen which under the action of epinephrine or glucagon, may be converted into glucose. So the ultimate action of epinephrine on the muscle glycogen is the increased deposition of liver glycogen. The breakdown of the liver and muscle glycogen under the action of epinephrine takes place through the activation of adenyl cyclase that catalyses the formation of cyclic AMP.

Epinephrine also influences the carbohydrate metabolism indirectly by stimulating the adenophysis in releasing the ACTH. ACTH, on the other hand, augments the release of glucocorticoids from the adrenal cortex. This is observed in emergency, stress, fright, exercise, hypoglycaemia, etc. Insulin and epinephrine play important part in the homeostatic regulation of blood sugar. Because hypoglycaemia stimulates the secretion of epinephrine whereas hyperglycaemia stimulates the secretion of insulin.

Summarily, epinephrine elevates the blood sugar in three ways: (1) by mobilising the carbohydrate stores of the liver; (2) by indirect formation of glucose from muscle glycogen; (3) by excessive formation of glucocorticoids indirectly through liberation of ACTH.

Role of posterior pituitary hormones (vasopressin and oxytocin)—A large doses of vasopressin and oxytocin raise the blood sugar level temporarily. In rabbits vasopressin is more effective in raising the blood sugar level, whereas in dogs oxytocin has greater hyperglycaemic effect.

Role of thyroid hormones—Thyroid hormones increase the glucose absorption from the intestine. The rate of glucose absorption from the intestine is decreased in hypothyroidism. The principal diabetogenic effect of thyroid hormones is possibly due to this increased absorption of glucose from the gut. The hormone also depletes some liver glycogen. Administrations of thyroid hormones to normal animals do not cause immediate effect on blood sugar but liver glycogen is depleted within six to eight hours.

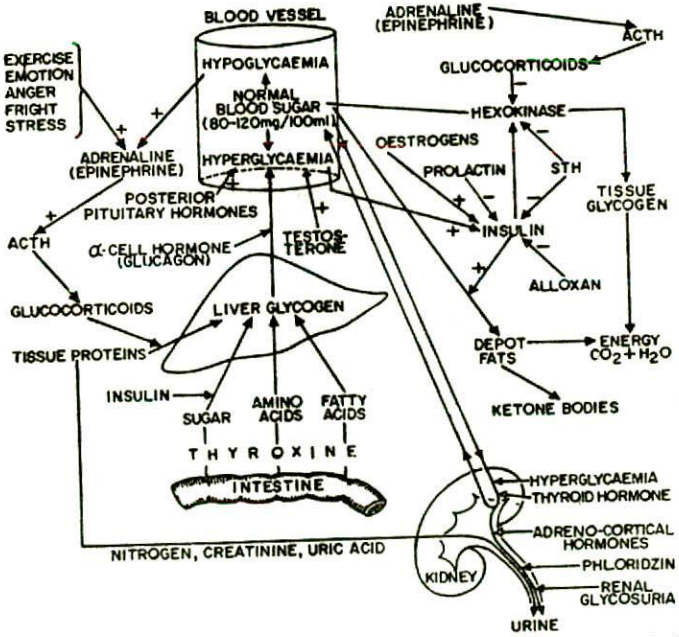


FIG. 376. Hormonal control of carbohydrate metabolism and their interrelationship. (−) means *inhibition* and (+) denotes *stimulation*.

In hyperthyroidism the diabetic condition is aggravated but thyroidectomy markedly decreases the intensity of the diabetes. Rate of protein catabolism is increased by excessive thyroid hormone and for this reason increased hyperglycaemia is observed due to neoglucogenesis from amino acids. Besides this, the thyroid hormones sensitise the adrenaline and the hepatic depletion of glycogen may be the indirect effect of adrenaline by thyroid hormones. Thyroid hormones also raise the renal threshold for glucose.

Role of anterior pituitary hormones—Like growth hormone, the anterior pituitary hormones ACTH and TSH may have some indirect role on the glucose metabolism through acting on the respective target organs. Direct role on the metabolism is possibly lacking.

Role of prolactin has got some anti-insulin effect. It reduces the sensitivity of the animals to insulin. The diabetogenic action of prolactin is probably due to this desensitisation of animals to insulin. After hypophysectomy, the blood sugar level is reduced but administration of prolactin raises the level towards normal.

Role of sex hormones—*Female sex hormones*, oestrone and oestradiol, decrease the diabetic condition possibly by stimulating the secretion of insulin.

Male sex hormones, testosterone also markedly increases the severity of the diabetic condition of the castrated animals.

The endocrine control of carbohydrate metabolism has been presented schematically in Fig. 376.

ROLE OF VITAMINS ON CARBOHYDRATE METABOLISM

Thiamine (vitamin B_1). Thiamine acts as a coenzyme of the carboxylase which helps in oxidative decarboxylation of pyruvic acid. It has a potential role in the oxidation of sugar in tissues including brain. In its absence, pyruvic and lactic acids fail to be metabolised with a result of accumulation of these substances in blood and tissues. This metabolic disorders, produced by this vitamin deficiency, results *beriberi*. In the tissues thiamine exists as *thiamine pyrophosphate ester* and helps in decarboxylation of α-ketonic acid as a coenzyme.

Riboflavin. Since riboflavin is related with tissue oxidation, so it takes part in carbohydrate metabolism. In the tissues this vitamin exists as FMN and FAD. These two coenzymes in combination with apoenzyme play a great role in a number of enzyme systems. Deficiency of this vitamin results disorder of intermediary metabolism leading to condition known as *cheilosis*.

Nicotinic acid (Niacin). It remains as a prosthetic group of at least two enzyme systems: NAD and NADP, and takes part in tissue oxidation. Niacin helps in the formation of fats from carbohydrates. The deficiency of this vitamin results disorder of intermediary metabolism leading to condition known as *pallegra*.

Pantothenic acid (vitamin B_3). Since pantothenic acid is a component of coenzyme A, so it takes part in carbohydrate metabolism. The condition of *alopecia* and certain gastro-intestinal disorders are produced by the deficiency of this vitamin.

Cyanocobalamin (vitamin B_{12}). This vitamin acts as a cofactor (*cobamide*) for the enzyme methyl malonyl CoA isomerase which is concerned for the conversion of methyl malonyl CoA to succinyl CoA or succinyl CoA to methyl malonyl CoA. Thus this vitamin is essential in the biochemical conversion of carbohydrate to fat or fat to carbohydrate. After administration of B_{12} hyperglycaemia may be corrected.

Ascorbic acid (vitamin C). Ascorbic acid takes part in the tissue oxidation probably by acting as hydrogen-carrier. Deficiency of this vitamin results disorders of metabolism leading to condition known as *scurvy* (*scorbusis*). [*Vide* also under the chapter of *Vitamins*.]

LIPID METABOLISM

Although the knowledge of lipid metabolism which includes the metabolism of fats, phospholipids, sterol and glycolipids is incomplete but fat possesses its particular importance as well as higher calorigenic status than other foodstuffs, i.e., protein and carbohydrate. Some of its characteristic importance are given below:

1. Much of the carbohydrate of the diet is converted to fat prior to its utilisation for supply of energy.
2. Some organs prefer fat as a fuel in preference to carbohydrate.
3. As the calorific value of fat is over twice as great than carbohydrate and protein, i.e., 9·3 Cal/gm, so the fat is stored in the tissue (fat depots—p. 554, e.g., adipose tissue, subcutaneous fat, etc.) as principal form of energy.
4. It supplies essential fatty acid (polyunsaturated fatty acid) and fat-soluble vitamins (viz., vit. A, D, E, K).
5. It is required in diet essentially for absorption of these essential substances.
6. Although phospholipids, cholesterol and glycolipids are not stored in adipose tissue, but go to form essential structural constituent of various organs in the body.
7. 10% of fats (the glycerol part) may be converted into sugar during the synthesis of carbohydrates.
8. Amino acids can be synthesised from fatty acids and ammonia in the liver.
9. Being a poor heat conductor the subcutaneous fat helps in heat regulation.
10. The depot fats act mechanically in protecting the vital organs and also act as cushions and packing tissues.

The lipids, after absorption, remain in the blood in the following forms:

Blood fat—(1) **neutral fats,** (2) **phospholipids** (mainly lecithin and traces of other phospholipids), (3) **cholesterol esters,** (4) **free cholesterol** and (5) **free fatty acid.**

NORMAL VALUES OF LIPIDS IN BLOOD PLASMA OF MAN

LIPID FRACTION	mgm/100 ml (AVERAGE)
Total lipid	580±200
Triglycerides	148±50
Total phospholipids Lecithin Cephalin Sphingomyelins	220±125
Total cholesterol Free cholesterol	190±100 65± 30
Free fatty acid	12±7

Although the lipids are present in both cells and plasma of blood but the composition of lipids in plasma and cells widely vary. Since the

composition of plasma lipids accurately reflects the actual state of lipid metabolism, so the composition of plasma lipids is generally studied. The composition of blood lipids is not a static one but variable and the picture of composition at a particular moment is the resultant of processes of addition of lipids to the blood and removal of lipids from blood:

(*a*) Addition of lipids may be due to
1. absorption from intestine,
2. synthesis of fat and its mobilisation.

(*b*) Removal of lipids may be due to
1. deposition of fat in the depot,
2. oxidation of fat in the tissue,
3. utilisation for formation of tissue structural components.

Alimentary lipaemia. When a dog fed with a fatty meal, the concentration of its plasma lipids begins to rise within 1–2 hours and reaches maxim within 6 hours. This rise of plasma lipids after a fatty meal is termed as **alimentary lipaemia.**

The chief increase takes place in the *neutral fat* content. *Phosphatides* and *cholesterol* (both free and ester form) may also slightly increase, depending upon their amount in food and bile. *In human subjects, effects of a fatty meal on blood fat is not constant.* In most cases a slight rise is seen. But active absorption of fat may occur without any appreciable rise in blood fat, even a fall has been noted after a fatty meal.

Element constant and Element variable

The lipids in the body physiologically form two components: (1) **element constant** or **structural lipids,** (2) **element variable.** Although a sharp line of demarcation cannot be made between the two, it has been generally observed that the value of the former remains constant even under extremes of starvation whereas of the latter varies. Cytoplasm and cell membrane of all organs are composed of element constant, so that their fat content does not diminish in starvation. Element constant is composed chiefly of phospholipids. *It remains an integral part of the cell protoplasm and is essential for its life.*

Element variable is composed mainly of neutral fat. It is present mostly in the so-called fat depots or adipose tissue, viz., fatty subcutaneous layer, mesentery, omentum, intramuscular connective tissue and connective tissue around organs, viz., heart and kidney, etc. It remains as free form and represents stored energy. It has been observed that depot fat is not static but in a continuous state of change due to its continuous synthesis and breakdown in the body.

A third type of fat, **brown fat,** which has a high metabolic rate has been observed in infants but not in adults.

Liver has great metabolic activity in lipid metabolism. During starvation and certain other conditions when depot fats (element variable) mobilised, the liver fats increase. The liver acts as an intermediate station in the process of fat oxidation. Phospholipids are synthesised—in which form—they are discharged into the blood stream to be carried to the tissues for oxidation. The synthesis and breakdown of fatty acid takes place with rapidity in liver. Liver can oxidise fatty acid to CO_2 and H_2O. Liver can **lengthen,** and shorten carbon chain as well as saturate and **unsaturate** them. Free cholesterol and cholesterol ester are synthesised in liver.

Fatty degeneration. In normal tissue, a portion of the lipid is present as lipid protein complex. This masked fat cannot be demonstrated by histological methods. Treatment with protein-splitting enzymes makes these lipoproteins 'unmasked' and can

be stained by usual methods. Such unmasking, called fatty degeneration, also results from anaemia, infection, etc. That this fat is not transported from outside is proved by the fact that, the total fat content of the organ remains same.

Fatty infiltration is another condition where an organ becomes loaded with fat transported from outside. Such a condition results from poisoning of the liver with chloroform, carbon tetrachloride, etc. The total fat content of the organ increases.

Fatty liver. Excessive deposition of fat in the liver is due to the following causes: (1) **synthesis of fatty acids from carbohydrate and protein,** (2) **influx of dietary lipid** and (3) **mobilisation of fatty acids from the depots to the liver.** These may be brought about by (*a*) lack of insulin, e.g., in diabetes mellitus, (*b*) starvation, (*c*) after injection of adrenocorticotrophic hormone (ACTH) and thyroid-stimulating hormone (TSH) in fasting animals, (*d*) deficiency of choline in the diet, (*e*) poisoning by chloroform, phosphorus, etc. (*f*) reduced atmospheric pressure as in high altitude.

DEPOT FATS

Amount. About 12% of the total body weight of a man consists of fat. The major part of it remains stored in the so-called fat depots. This fat is called the **depot fats.***

Distribution. The distribution of fat, in different *fat depots*, varies. For instance, subcutaneous tissue contains 50%, the perirenal tissue 15%, mesentery 20%, omentum 10%, the intramuscular connective tissue about 5% fat, etc. (average). These are the intracellular fats which remain inside the cells of adipose tissue.

Composition. Depot fat is chiefly composed of the glycerides of various fatty acids and usually contains 75% of oleic acid, 20% of palmitic acid and 5% of stearic acid. They remain as mixed triglycerides, that is, one glycerol molecule is attached to three fatty acid molecules of either similar or dissimilar varieties. Traces of lecithin and cholesterol, as well as a little amount of polyunsaturated fatty acids, are also present. **Specificity**—*Depot fat is not identical with food fat. With the customary diet, the depot fat of each species is a characteristic one.* But if diet be altered in either quality or quantity specially after starving the animal, the depot fats may resemble food fat to a varying extent.

On the whole, depot fats are more unsaturated than food fats. One interesting fact about the depot fat is that, *they are more unsaturated under the skin than in the deeper parts.* The iodine value is found to diminish gradually from outside inwards.

Experiments with *deuterium*-labelled fatty acids indicate that fat depots are not static storehouses. They are dynamic. The stored fats are constantly being mobilised and replenished and in this way a dynamic equilibrium is maintained.

Sources of depot fats. The depot fats may be derived from the following sources: (1) **Food fat**—This is the chief source. (2) **Carbohydrates**—Carbohydrates of food may be easily converted into fats. Young pigs deposit large amounts of fat only on a barley diet. During this conversion an oxygen-rich substance (carbohydrate) is being transformed into oxygen-poor substance, so that R.Q. rises. [*Vide* pp. 523–24.] Fats formed from carbohydrates are more saturated and have a higher melting point.

* **Fat storage.** In adults, the fat is stored in the tissue comparatively (about 700 gm) much higher value than carbohydrate and protein, the protein being the least. A special type of tissue—the adipose tissue—has evolved for the purpose of storing fat only. The cells of the adipose tissue are equipped with a special enzyme system, which can take up fat from the tissue fluid and store it up in the cell and in time of necessity, the same enzyme system will help to mobilise the depot fats. In this connection it is noteworthy that fats are **typical storage compounds.**

Hence, depot fats of grazing animals are 'hard'. This is of great importance in cattle farming. (3) **Proteins**—Recently, evidence shows that carbohydrates derived from protein may be converted into fats. It has been further shown that *pyridoxine*, in collaboration of *thiamine*, catalises the formation of fats from protein.

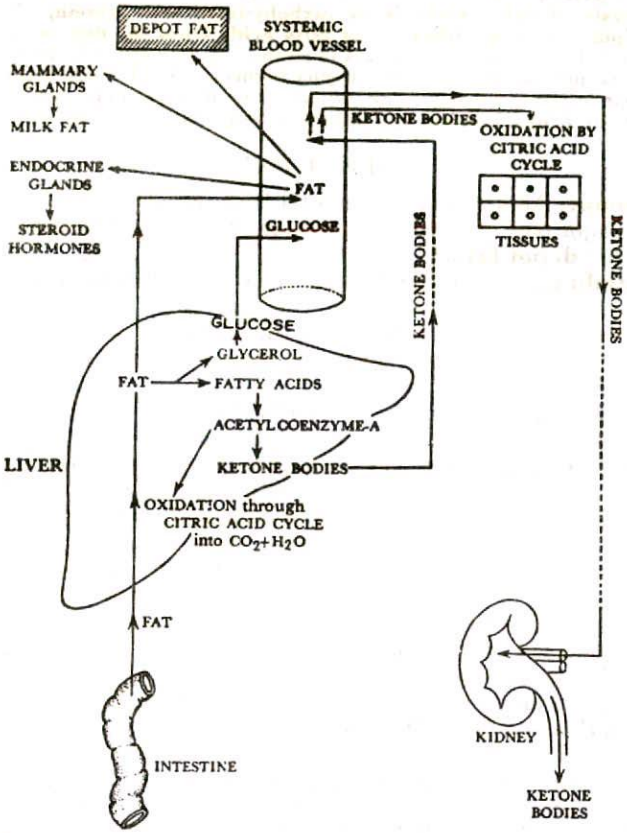


FIG. 377. Diagrammatic representation of the fate and utilisation of fat in the body.

Functions of depot fats

1. **Mechanical functions.** (*a*) While remaining under the surface, it gives shape to the limbs and body. (*b*) Remaining around the viscera it keeps them in position and acts as a mechanical buffer against injury.

2. **Physical functions.** Fat being a non-conductor of heat, the subcutaneous fat plays a good part in the regulation of body temperature.

3. **Chemical functions.** *It represents stored energy.* Large amount of energy can be stored as adipose tissue fat in concentrated water-free state. 1 gm of fat gives 9·3 Calories. Since fats constitute about 12% of body weight, it represents stored energy to the extent of 1,100 C per Kgm of body weight.

OXIDATION OF FATS

The glycerol part of fat is oxidised in the same way as carbohydrates whereas the oxidation of fatty acids is not. Since it requires relatively more oxygen, the respiratory quotient being lowest (R.Q.—0·7). In this respect carbohydrates are the ideal products for oxidation, requiring the lowest amount of oxygen (R.Q.—1·0). Protein stands midway between these values having an R.Q. of about 0·8.

MECHANISM OF OXIDATION OF FATTY ACIDS

The **process of successive β-oxidation** was described by Knoop in 1905. In this process the oxidation takes place in the carbon atom at the β-position (third from the carboxyl group). This carbon atom is converted into a carboxyl (—COOH) group and the outer two-carbon atoms are split off. Thus a fatty acid with two-carbon atoms less is formed. This substance again undergoes β-oxidation and the process is repeated successively, Thus fatty acid oxidation takes place in a process known as *alternate successive β-oxidation* where two-carbon atoms are removed at a time as acetic acid, until a four-carbon atom residue is left as butyric acid. Butyric acid is oxidised in the β-position to form aceto-acetic acid. Consequently, the original fatty acid molecule is converted into several molecules of acetic acid and one molecule of aceto-acetic acid (ketone). Both of these compounds undergo final oxidation into CO_2 and H_2O in normal health.

Subsequent discovery of coenzyme A (HS—CoA) by Lipmann and acetyl coenzyme A or *active acetate* by Lynen and his colleagues has laid a new pathway in the intermediary metabolism of fat. Coenzyme A has been found to be in large quantity in the liver. Coenzyme A is a dinucleotide and consists of 2-thiol-ethylamine and pantothenic acid linked with pyrophosphate and adenosine. Active acetate is an acetylated derivative of this coenzyme. The sulphur atom is linked to acetyl group. The neutral fat is hydrolysed in the liver into long-chain fatty acids (palmitic, stearic and oleic acids containing 16 or 18 carbon atoms) and glycerol. The fatty acid radical undergoes oxidation in the liver and breaks down into smaller fragments. Each fragment contains two-carbon atoms. In the first phase of the oxidation process, there is a chemical reaction between the fatty acid, ATP and CoA activating the fatty acid by forming a thiol ester of coenzyme A. This is the first step and is known as (I) the **activation** stage, The ester thus formed undergoes successively the following reactions: (II) **dehydrogenation,** (III) **hydration,** (IV) **dehydrogenation,** and (V) **thiolytic cleavage.**

(I) **Activation**—Fatty acid is activated by **thiokinase** in presence of coenzyme A, ATP and Mg^{++} and *activated fatty acid* or *coenzyme A ester* formed. There are at least three **kinases** that catalyse the acylation of fatty acid. **Acetic kinase** shows a high specificity for acetic acid and slightly for propionic acid. A **second kinase** acts on acids C_4–C_{12}, optimum chain length being C_8. The **long-chain kinase** (C_8–C_{18}) has little action on short-chain acids.

(II) **Dehydrogenation**—The activated fatty acid is oxidised by the removal of two hydrogen atoms by **acyl~CoA dehydrogenase** along with a coenzyme derived from flavin adenine dinucleotide (FAD) and

α-β-unsaturated activated fatty acid is produced. Just as in case of the kinases, there are at least three dehydrogenases, viz., G, Y_1 and Y_2. G is green-coloured dehydrogenase which catalyses oxidation of C_4–C_8 acids. Y_1 is yellow, having optimal action on C_8–C_{12} acids and Y_2 being more active on C_8–C_{16} acids.

(III) **Hydration**—Then α-β-unsaturated fatty acid passes through a process of hydration with the addition of H_2O under the influence of **enoyl hydrase** (*crotonase*) and β-hydroxy activated fatty acid is produced which undergoes *dehydrogenation* again.

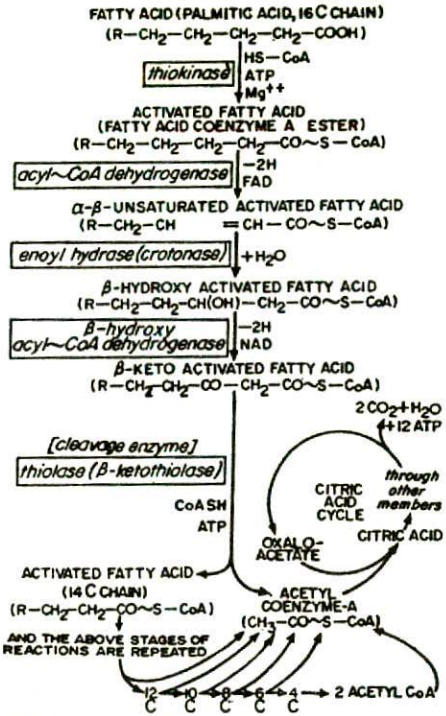


FIG. 378. Oxidation of fatty acids with respective enzymes.

(IV) **Dehydrogenation**—There is removal of two hydrogen atoms from β-carbon atoms, and β-keto activated fatty acid is produced. NAD acts as a hydrogen acceptor being converted into $NADH_2$. This reaction is catalysed by **β-hydroxyacyl CoA dehydrogenase.**

(V) **Thiolytic cleavage**—β-keto activated fatty acid is cleavaged at β-carbon atom, by **thiolase** (β-ketothiolase) in presence of CoASH and thus two-carbon fragment as acetyl CoA is separated leaving activated fatty acid less by 2C. This process is repeated and an acetyl CoA is separated off in each complete oxidation process till the fatty acid is completely splitted into two-carbon fragments (acetyl CoA), e.g., palmitic acid which contains 16-carbon atoms is broken down into eight fragments of acetyl CoA (FIG. 378).

Even-carbon fatty acids, undergoing β-oxidation, give rise to acetyl CoA whereas **odd-carbon fatty acids** also give rise to acetyl CoA and a molecule of propionyl CoA, the latter being formed from the 3 terminal carbon atoms from the methyl end of fatty acid. **Branched-chain fatty acids** (e.g., isobutyric and isocaproic acids, etc.) are also splitted into acetyl CoA and propionyl CoA during their oxidation. The **propionyl CoA** is converted to **methyl malonyl CoA** and finally isomerised to **succinyl CoA.** The succinyl CoA may be utilised for the synthesis of carbohydrate through pyruvic acid and porphyrin with glycine or may be oxidised through TCA cycle after being converted into succinic acid to supply energy as ATP. Acetyl CoA is oxidised in the TCA cycle and the coenzyme A thus set free after oxidation helps in further fatty acid oxidation. If acetyl CoA is not properly oxidised, aceto-acetic acid is produced. Fatty acid oxidation depends upon simultaneous oxidation of carbohydrate. In diabetes mellitus and starvation, etc., where carbohydrate oxidation is depressed, the fatty acid oxidation becomes incomplete. There is formation of more aceto-acetic acid and this may lead to *ketosis*.

Role of liver. Three changes occur in liver. They are as follows:

1. The fats from the fat depot at first pass to the liver. The neutral fat is hydrolised in the liver into fatty acid and glycerol. The fatty acids are oxidised and acetyl CoA (2C compounds) is formed. These units may be dissimilated in the liver through citric acid cycle, or condensed to form *aceto-acetic acid* which is transported to the peripheral tissue for oxidation. Aceto-acetic acids reach the tissues and are oxidised into CO_2 and H_2O or resynthesised to fat (Fig. 377).
2. In case of *carbohydrate deficiency*, more aceto-acetic acid is produced and *ketosis* may develop.
3. Substances which reduce liver fats are called **lipotropes** or **lipotropic factors.** They are choline, methionine, betaine and lecithin, etc. They all help in the synthesis of phospholipids in which form liver fat may be easily mobilised out. The opposite group of substances, which increase liver fat are known as **antilipotropic factors.** Cholesterol is an important member of this class.

Role of carbohydrates. It is found that oxidation of fatty acids cannot be complete unless enough carbohydrates burn at the same time. Combustion stops at the intermediate stage and aceto-acetic acid (ketone body) and β-hydroxybutyric acid are produced. These are highly toxic substances and give rise to various ill effects. If sufficient carbohydrates burn, these ketone bodies are fully oxidised and no ketosis results. Thus ketosis occurs in diabetes mellitus when sugar is not burnt even in the presence of enough sugar Similar condition is also observed in starvation where there is no sugar to be oxidised. The general pathway of catabolism of fatty acids involves the β-oxidation. However, to some extent, certain fatty acids can undergo oxidation at the carbon atom farthest from the carboxyl group—the omega (ω) carbon, producing a dicarboxylic acid. This is then subjected to β-oxidation and cleavaged to form smaller dicarboxylic acids.

α-oxidation occurs in brain microsomes where it has been found that brain **sphingolipids** contain α-hydroxy acids and odd-carbon fatty acids.

ROLE OF ENDOCRINES ON FAT METABOLISM

Certain endocrine factors take part in fat metabolism. They are as follows:

1. **Anterior pituitary.** (*a*) Hypofunction of anterior pituitary is associated with unusual deposition of fat. (*b*) The growth or somatotrophic hormone (STH) of anterior pituitary mobilises fat from depots and increases production of ketone bodies in the liver. ACTH exerts a direct action on adipose tissue increasing *lypolysis* and mobilisation of unsaturated fatty acids into the blood plasma. There is increase ketogenesis and decrease in R.Q., similar to the effect of STH.

2. **Insulin.** Administration of insulin removes the condition of ketosis in diabetes mellitus. Insulin helps complete combustion of fatty acids, probably through its indirect influence on carbohydrate metabolism. Insulin helps in the formation of fat (*lipogenesis*) from glucose and deposition in adipose tissue. Insulin decreases the cholesteremia and lipaemia. It prevents breakdown of fat in the adipose tissue.

3. **Adrenal cortex.** Glucocorticoids of adrenal cortex help fat metabolism in a number of ways: (*a*) Through its influence on sterol metabolism. (*b*) Through its probable influence on phosphorylation. (*c*) Through its effect on carbohydrate metabolism. Cortisol helps in the redistribution of fat in the body. Increased deposits of fats occur in the trunk when there is excessive secretion of cortisol.

4. **Thyroid.** Thyroxine stimulates the combustion of fats, as it also does in case of other foodstuffs.

5. **Adrenaline (Epinephrine).** It stimulates mobilisation of unesterified fatty acids from adipose tissue. The presence of ACTH and thyroid hormones is necessary for this action. There is associated increase in liver lipids and in ketogenesis.

ROLE OF VITAMINS ON FAT METABOLISM

Thiamine (vitamin B_1). Thiamine helps the enzyme system which is responsible for the synthesis of fats from carbohydrates and proteins.

Riboflavin. It helps in the conversion of sugar into fats.

Nicotinic acid (Niacin). Nicotinic acid helps in the formation of fats from carbohydrates.

Pantothenic acid (vitamin B_3). Pantothenic acid in the form of CoA takes part in the reaction of both the synthesis and metabolism of fatty acids and cholesterol.

Cyanocobalamin (vitamin B_{12}). This vitamin plays a very important role in the conversion of carbohydrate to fat (*vide* page 550).

Pyridoxine (vitamin B_6). It is related to the metabolism of unsaturated fatty acids and also helps in the synthesis of fats from carbohydrates. *Pyridoxine*, with the help of *thiamine*, accelerates the formation of fats from protein. [*Vide* also under the chapter of *Vitamins*.]

KETONE BODIES

Formation of ketone bodies (Ketogenesis). It has been observed that acetyl CoA produced during fatty acid oxidation condense with oxalo-acetic acid for oxidation in the TCA cycle. The oxalo-acetic acid formation is depressed when glucose supply is restricted so that in this condition acetyl CoA cannot be properly metabolised through citric acid

cycle. Thus acetyl CoA condenses to form aceto-acetyl CoA which in the liver produces aceto-acetic acid. The aceto-acetic acid is reduced to form β-hydroxybutyric acid which after decarboxylation forms acetones. Aceto-acetic acid, acetone and β-hydroxybutyric acid are called **ketone bodies.** The process of formation of ketone bodies is called **ketogenesis.** Normally the ketone bodies are utilised without being accumulated in the body, but they may be abnormally accumulated in body fluids known as **ketosis** and excreted through the urine called **ketonuria** (or **acetonuria**). Its accumulation in the blood is called **ketonemia.**

Conditions leading to ketosis. The following conditions produce ketosis: (*a*) diabetes mellitus, (*b*) starvation, (*c*) high fat or low carbohydrate diet, (*d*) muscular exercise.

Source of ketone bodies (Ketogenic substances). The ketogenic substances arise from (1) **all fatty acids** (i.e., 90% of food fat. Glycerol part burns as carbohydrates. Hence, it is antiketogenic.) (2) **Proteins (ketogenic amino acids, 40%).** These are the sources from which ketone bodies are formed.

Site of formation of ketone bodies. Liver is perhaps the only site where ketone bodies are normally formed since concentration of ketone bodies have been found to be higher in the hepatic vein than in other veins.

Antiketogenic substances. *These are substances which prevent the formation of ketone bodies.* They include the following:

(1) **All carbohydrates,** (2) **60% of proteins (antiketogenic amino acids)** from which sugar may be formed (*vide* page 544), (3) **10% of fats** (the glycerol part)

Utilisation of ketones (Fig. 379). It has been shown that ketone bodies are utilised rapidly and independently in many tissues with the production of CO_2 and H_2O. A significant amount of the normal energy requirement of the body is derived from this source. It has been shown that *even the tissues of the diabetic animals can oxidise the ketone bodies completely even if no sugar is burnt.* Recently it has been established that human brain can utilise appreciable amount of ketone bodies during prolonged starvation.

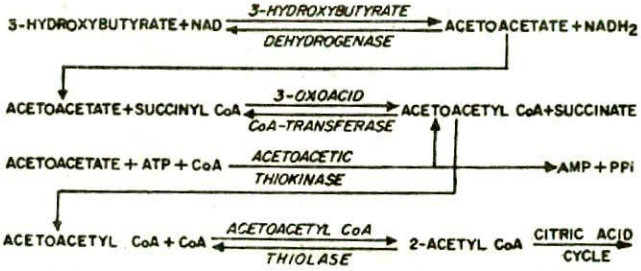


Fig. 379. Schematic representation showing enzymes and reactions involved in ketone body utilisation.

Interrelation with carbohydrate metabolism. It has been observed above that the ketone bodies only appear when enough carbohydrate is not burnt. It has also been shown that ketones can be utilised freely,

without any sugar being oxidised. The interrelation between sugar oxidation and ketone formation lies in the fact that in diabetes mellitus and starvation, the glycogen content of the liver becomes low. Since in these two conditions the fatty acids are not esterified and ketone bodies are formed. On high carbohydrate diet free fatty acid oxidation is spared. The energy requirement of the body must therefore be supplied from the oxidation of fats. For this reason fats are mobilised from the depots in large quantities and brought to the liver. Liver (whose glycogen content is already low) becomes loaded with fats. Consequently, fat oxidation takes place at a heightened rate and more ketones are formed. They come out of the cell and enter the blood stream and finally eliminated through urine. [It is due to such leakage that normal urine shows traces of ketones.] Obviously ketones are formed at a faster rate than can be utilised. Hence *ketosis may not be even due to non-utilisation of the ketone bodies but is due to their overproduction.*

Ketogenic/Antiketogenic ratio. While prescribing diets the proportion of the ketogenic and antiketogenic substances should be so regulated that ketosis may be avoided. It is found that if the *ratio between the molecules of the ketogenic substances and the molecules of the antiketogenic substances exceeds* 2, *ketone bodies appear in the urine.* The clinical rule is that the total fat (F) content of the diet must not exceed the sum of twice the carbohydrate (C) and half of the protein (P), i.e., $F = \text{or} < (2C + \frac{1}{2}P)$.

Ketonemia. Rise of ketone bodies in blood above normal value is known as ketonemia.

Ketonuria. When blood level of ketone bodies rises above the renal threshold, they are excreted in the urine. This is a condition known as ketonuria.

Ketosis. Accumulation of abnormal amount of ketone bodies in the tissue and tissue fluids is termed as ketosis where the urinary excretion of β-hydroxybutyric acid exceeds 200 mgm daily (normal, 5–10 mgm).

Relation of ketosis with blood and urine reaction. During ketosis aceto-acetic acid, β-hydroxybutyric acid, etc., are formed. Since these are all acids, ketosis is usually associated with a condition of acidosis in the body and increased acidity of the urine. Due to this relation between acidosis and ketosis, acetone bodies are generally found in highly acid urine. But it must be remembered that acidosis is the result and not the cause of ketosis. A large part of acids in the urine exists as anions. For the maintenance of neutrality cations like Na^+ are lost. As a result sodium salts are lost from plasma and other body fluids and consequently there is loss of body fluid resulting dehydration.

It is highly interesting that even in genuine cases of alkalosis (with alkaline urine) ketone bodies may be found in the urine. This can be demonstrated in subjects in which experimental alkalosis is produced by prolonged voluntary hyperpnoea. It is probable that in this condition β-hydroxybutyric acid, aceto-acetic acid, etc., (which are soluble and diffusible) migrate out of the cells to neutralise alkalis and are excreted in the urine.

In hospital cases alkaline urine containing acetone bodies is not an infrequent observation. It may be—(*a*) due to ammoniacal decomposition resulting from long standing, (*b*) due to therapeutic administration of alkalis to combat acidosis, (*c*) due to true alkalosis as mentioned above.

ROLE OF ENDOCRINES ON KETOSIS

1. **Anterior pituitary.** The growth or somatotrophic hormone (STH) of anterior pituitary produces ketosis in diabetes mellitus and starvation due to inhibition of insulin secretion and depression of glycogenesis.

2. **Insulin.** It prevents ketosis and its administration clears up the condition of ketosis in diabetes mellitus. This effect is due to the following: (*a*) It increases liver glycogen, prevents mobilisation of fats from the depots and thus reduces liver fats. Consequently fat oxidation is discouraged and

more carbohydrates burn. This leads to less ketone formation, so that ketosis disappears. Insulin is antagonistic to the growth or somatotrophic hormone of anterior pituitary.

3. **Adrenal cortex.** Glucocorticoids of adrenal glands are *adipokinaetic*, i.e., mobilise depot fat to the liver for oxidation.

4. **Thyroid.** Thyroxine increases ketosis under certain conditions. Its action is probably due to decrease in liver glycogen and its consequent loading with fat.

PHOSPHOLIPIDS (Synonym—PHOSPHATIDES)

Chemistry and classification. Phospholipids belong to the group of conjugated fats, containing sugar alcohol or complex amino alcohol, fatty acid, phosphoric acid and nitrogenous base. They may be classified as follows:

I. **Monoamino-monophospholipids.** In these compounds there are 1 molecule of phosphoric acid, 2 molecules of fatty acids, 1 molecule of glycerol and 1 molecule of nitrogenous base (choline or ethanolamine). The examples are **lecithin** and **kephalin** or **cephalin.**

II. **Diamino-monophospholipids.** In these compounds there are 1 molecule of phosphoric acid and 2 molecules of nitrogenous bases, viz., sphingosine and choline; for example, **sphingomyelin.** [For further chemistry *vide* page 84.]

Distribution. The salient facts about the distribution of phospholipids are summarised below:

1. The phospholipids are widely distributed in the body and are contained within it. They remain in the cell membrane as well as in the protoplasm. They enter into the structure of the cells and form the composition of the *element constant* of the cells.

2. Brain and nervous tissues contain the maximum amount of all the three varieties, i.e., *lecithin, cephalin* and *sphingomyelin. Sphingomyelin is present chiefly in the nervous tissue and negligible amount in other tissues.* But lecithin and cephalin are found in considerable amounts in other tissues.

3. *The phospholipid content of a particular organ or tissue, in a given species of animal, is constant both in composition and in amount.*

4. The fatty acids in the phospholipid molecule are more unsaturated than those in the neutral fat. *The degree of unsaturation seems to be more or less characteristic of a particular tissue,* because the iodine value of the phosphatides of a particular tissue is of the same order.

5. Phospholipids always remain along with cholesterol. The rise or fall of one is always accompanied by a similar change in the other.

Synthesis. The human body can synthesise phospholipids under suitable conditions provided all other constituents are available. The evidence is as follows:

1. Liver can easily synthesise phospholipids. As a matter of fact large amounts of phospholipids are daily synthesised by the liver from the intermediate products of fat oxidation.

2. The lipotropic action (reduction of fat content) of choline on liver is due to the fact that choline helps in converting neutral fat into phospholipids (*lecithin*). In this form the fat becomes highly diffusible, easily passes out of liver and thus the liver fat is reduced. It is indicated that the phospholipids are normally synthesised in the liver.

3. Phospholipids are also synthesised inside the intestinal epithelium during the absorption of fat.

4. The fact that all tissues contain a constant amount of phospholipids, having a characteristic composition which indicates that the cells of the different tissues can, at least to some extent, synthesise their own phospholipids locally.

Synthesis of a typical phospholipid like lecithin (phosphatidyl choline) is as follows: In the first step phosphate ester of choline (phosphoryl choline) is formed out of choline and ATP. Phosphoryl choline then reacts with cytidine triphosphate (CTP) forming cytidine diphosphate (CDP) choline and inorganic pyrophosphate (PPi). Then a diglyceride combines with the phosphoryl choline part of cytidine diphosphate choline to form lecithin (phosphatidyl choline) and cytidine monophosphate (CMP), the latter is rephosphorylated by ATP and thus reconverted to CTP. Diglycerides are formed from the glycerol in the liver, intestine and adipose tissue. The glycerol may be absorbed in the intestine as such or formed in the body from fructose-1-6-diphosphate (*vide* page 570). The first step in the synthesis of diglycerides from glycerol is the action of enzyme glycerol kinase on ATP and glycerol with the formation of L-α-glycerophosphate. The L-α-glycerophosphate is also formed from fructose-1-6-diphosphate. The L-α-glycerophosphate reacts with acyl CoA in presence of an enzyme to form the phosphatidic acid. The phosphatidic acid is then hydrolysed by phosphatase with the formation of D-1-2-diglyceride.

Lecithin

1. ATP + Choline —(*choline kinase*)→ Phosphoryl choline + ADP
2. Phosphoryl choline + CTP —(*PC-cytidyl transferase*)→ CDP-choline + PPi
3. CDP-choline + D-1,2-diglyceride —(*glyceride transferase*; Mg^{++} or Mn^{++})→ Lecithin + CMP

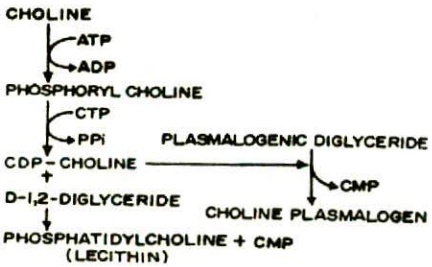


FIG. 380. Schematic representation of the path of synthesis of lecithin.

Cephalin

Reaction for the synthesis of cephalin is mostly analogous to those for the synthesis of lecithin. The serine may be formed from glycine or may come from the dietary source.

1. Serine —($-CO_2$; *decarboxylase*)→ Ethanolamine —(*ATP*; *ethanolamine kinase*)→ Phosphoethanolamine + ADP
2. Phosphoethanolamine + CTP —(*PE-cytidyl transferase*)→ CDP-ethanolamine + PPi

3. CDP-ethanolamine + D-1,2-diglyceride $\xrightarrow[\text{glyceride transferase}]{Mg^{++} \text{ or } Mn^{++}}$ CMP + Cephalin (Phosphatidyl ethanolamine)

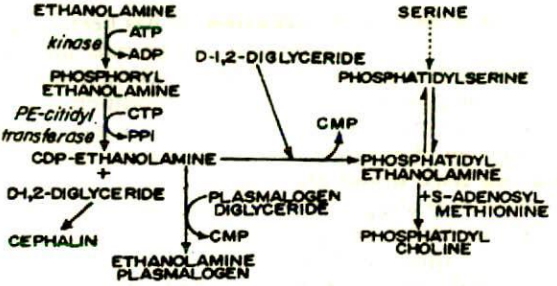


FIG. 381. Schematic representation of the path of synthesis of cephalin.

Sphingomyelin

The sphingomyelin contains fatty acids, phosphoric acid, choline and a complex amino alcohol sphingol or sphingosine. The synthesis of sphingosine has been studied in brain tissue. The first step in the synthesis of sphingosine is the reduction of palmityl CoA to the palmityl aldehyde. The palmityl aldehyde on condensation with serine in presence of pyridoxal phosphate gives rise to dihydrosphingosine. Sphingosine is formed after oxidation of dihydrosphingosine. *In vivo* the sphingomyelin is synthesised from sphingosine phosphoryl choline. Detailed pathways are as follows:

1. Palmityl CoA + NADPH + H^+ $\xrightarrow{\text{enzyme}}$ Palmityl aldehyde + NADP + CoASH
2. Palmityl aldehyde + Serine $\xrightarrow[\text{pyridoxal phosphate}]{}$ CO_2 + Dihydrosphingosine
3. Dihydrosphingosine + FAD $\xrightarrow[\text{enzyme, } Mn^{++}]{}$ Sphingosine + FADH + H^+
4. Sphingosine + CDP-choline $\xrightarrow[\text{enzyme}]{}$ Sphingosine phosphoryl choline + CMP
5. Sphingosine phosphoryl choline + Acyl CoA $\xrightarrow[\text{enzyme}]{}$ Sphingomyelin + CoASH.

FIG. 382. Reactions showing the path of synthesis of sphingomyelin.

Functions of phospholipids

1. **Essential constituent of all cells.** In the form of *element constant* phospholipids constitute an essential part of cell protoplasm.

2. **Relation with cell function.** It has been shown that the phospholipid content of an organ varies in direct proportion to the degree of its activity. From this fact it has been suggested that the phospholipid content of a tissue is intimately related to cell function and may be regarded as an index of the degree of activity of that tissue.

3. **Fat digestion and transport.** The lecithin contents of bile and food are concerned for the emulsifying action of bile salts upon food fats, and thus help their digestion. Lecithin is an important form in which fat is transported in considerable amount into blood. As phospholipids easily form emulsion with H_2O, so transport of fat from cell to cell takes place in the form of phospholipids.

4. **Protection and permeability of cells.** Being a constant constituent of cell membrane, phospholipids exert a protective action upon the cells and also regulate the passage of various substances across the cell membrane.

5. **Relation with tissue oxidation.** It has been suggested that the phospholipids being highly unsaturated compounds may act as oxygen carriers in the cell and help in tissue oxidation. Oxygen may be taken up at the unsaturated bonds and under a suitable condition it is released while required for oxidation purposes. They play a major role in regulating the respiratory group of enzymes,—cytochrome complex.

6. **Relation with blood clotting.** Phospholipid has a major role in the reaction sequence leading to the formation of blood clot. It has also been noted that rise in blood phospholipid concentration causes depression of fibrinolytic mechanism and reduces the tissue-activating factor VIII concerned for coagulation and stimulates the synthesis of prothrombinase.

7. **Donors of phosphate group.** Phospholipids donate phosphate group for various reactions in the body.

8. **'Carriers' in active transport.** It has been suggested that phospholipids act as 'carriers' for the transport of substances, e.g., hormones, enzymes, mucins, etc., through cell membrane.

Cerebrosides. Cerebrosides, found in brain, adrenal glands, liver, kidneys, etc., are glycolipids, e.g., *sphingomyelin* composed of **ceramide** (sphingosine) and phosphocholine derived from CDP-choline. In a condition known as **Gaucher's disease,** an increase in cerebroside content has been observed in spleen, liver and lymph nodes. **Gangliosides** and **sulphatides** are formed from cerebrosides. Gangliosides contain **neuraminic acid** in addition. In **Tay-Sachs disease** this lipid accumulates in ganglion cells particularly of cerebral cortex. **Cerebronic acid ester** of sulphuric acid occurs in sulphatides which have been found in many tissues including brain. Increased accumulation has been noted in a pathological condition, **metachromatic leukodystrophy.**

ROLE OF ENDOCRINES ON PHOSPHOLIPID METABOLISM

(1) **Adrenal cortex,** by its probable influence on phosphorylation exerts an important effect in the synthesis and breakdown of phospholipids. The active principles of adrenal cortex are believed to help the local enzymes (phosphatase or phosphorylase) which are directly concerned with phosphate transfer. The rise of plasma phosphatides as well as the hyperactivity of the adrenal cortex, during pregnancy, may indicate that the adrenal cortex is responsible for the synthesis of phosphatides. (2) **Anterior pituitary.** It is possible that the ultimate control of phosphorylation is carried out by anterior pituitary which regulates the activity of adrenal cortex through its adrenocorticotrophic hormone (ACTH). (3) **Insulin** decreases the phospholipid content of plasma. (4) **Thyroid.** Repeated injection of **thyroxine** increases the phospholipid content of liver. Removal of thyroid decreases it.

STEROLS (STEROIDS)

Chemistry. Sterols or steroids are not true fats. *Steroids* are derivatives of phenanthrene and have the *parent nucleus* (*perhydrocyclopentanophenanthrene, vide* p. 85) with 17 carbon atoms consisting in 3 hexagon and one pentagon rings. When the natural steroids have a methyl group at C-13 and

generally at C-10 and possess one or more alcoholic groups, then these secondary alcohols are *sterols*. Steroids are found free or in ester forms as cholesterides and can be extracted by organic solvents along with fats.

Property. Sterols cannot be hydrolysed by NaOH. Hence, they are not saponifiable. Otherwise, their physical and chemical properties are very similar to those of fats. With fatty acids they form waxes. The various members of this group can be separated by fractional distillation.

Distribution. Sterols are widely distributed in both animal and vegetable kingdoms. In the plants they exist chiefly as *phytosterols*. In the animals they are present in all cells, both in the cell membrane as well as in the cytoplasm. *They always remain along with phospholipids.* They are found both as free sterols as well as sterides. They are very rich in certain tissues, e.g., the nervous tissue, suprarenal cortex, gonads, etc.

Synthesis, destruction and excretion. Sterols can be synthesised in the body from non-sterol sources. It has been definitely proved in the case of cholesterol (*vide* page 566). The body can destroy sterols to some extent but the power is very limited. Large quantities are excreted through *sebum* of the skin secreted by the sebaceous glands. It is also excreted as *coprosterol* of the faeces, and as sex hormones and traces of cholesterol in the urine.

VARIETIES OF STEROL COMPOUNDS AND THEIR FUNCTIONS

A number of sterol compounds, synthesised in the body, are found to have immense physiological importance in the body. Many of them have been artificially synthesised. Important members of this class are enumerated below:

I. **Female sex hormones**

(*a*) **Oestrogen groups**—Oestrone, oestradiol (the natural hormones) and the various synthetic oestrogens.

(*b*) **Corpus luteum hormones**—the naturally occurring progesterone and similar synthetic products.

II. **Male sex hormones.** Testosterone and the synthetic products.

III. **Active principles of adrenal cortex.** Corticosterone and the similar products isolated from the gland.

IV. **Cholesterol and other related compounds.** Such as dihydrocholesterol, etc.

V. **Cholic acid of bile** is a sterol compound.

VI. The **vitamin D group** and the **related ergosterol** also belong to this class.

Digitalis, which is of immense pharmacological importance, is also a sterol compound. Sterols are essential for life. Moreover, it is interesting to note that sterols are intimately related to cell division. All rapidly growing tumours are very rich in sterols, specially cholesterol. The most effective cancer-producing substance is a synthetic sterol compound (*methyl cholanthrene*).

CHOLESTEROL

Chemistry and properties. It is a complex monohydric secondary alcohol being a very important member of the sterol class. With fatty acids it forms waxes. It is a stable white, crystalline substance, insoluble in water but readily soluble in chloroform, ether, alcohol and other fat-solvents. The crystals have a rhombic or rectangular shape, with one corner broken off. For its structural formula *vide* page 86.

Distribution. The broad facts about the distribution of cholesterol are as follows: (1) It is present in all cells—both in the cell membrane

and cytoplasm. It is a part of the 'element constant'. (2) All body fluids contain cholesterol excepting cerebrospinal fluid (CSF) in which the amount is negligible. (3) It may exist in the free state as well as in the form of esters, but these two forms are not equally distributed everywhere. In bile it is present only in the free form. (4) Brain contains (17%) it and it is mainly in free form and suprarenals contain the largest amount. (5) **Blood cholesterol**—Normal blood cholesterol varies between 150–200 mgm per 100 ml which is equally distributed between plasma and corpuscles. But in the corpuscles it is present chiefly in the free form, while in the plasma the major part remains as esters. (6) The free cholesterol content of a tissue is characteristic and normally remains very constant but the cholesterol esters may vary in amount. (7) The distribution of cholesterol in different types of muscles is directly proportional to their degree of activity. The cardiac and smooth muscles have a richer cholesterol content than the voluntary muscles. Moreover, the amount of cholesterol in any tissue is roughly proportional to their degree of activity. (8) Cholesterol and phospholipids always remain together. Moreover, there appears to be a definite ratio between phospholipids and cholesterol for each tissue.

Absorption. Absorption of cholesterol from the intestine follows the same principles as other lipids. Ingested cholesterol requires fat for its absorption from the lumen of the intestine into the lymphatics. These are esterified with fatty acids in the intestinal epithelium during their passage into the lymphatics.

Effects of cholesterol feeding

1. Ingestion of cholesterol-rich food, for some length of time, increases the level of cholesterol in blood and such increase is not observed when such sample of diet taken once. The rise of blood cholesterol is accompanied with the rise in bile cholesterol. There is also rise in blood cholesterol when cholesterol-rich diet is not ingested which indicates that this rise is due to increased absorption of cholesterol derived from bile.

2. Cholesterol feeding is one of the most certain means of producing excess deposition of fats, as well as cholesterol esters in the liver (anti-lipotropic action).

3. Arteriosclerosis or atherosclerosis has been observed in animals and man fed with diet containing high cholesterol but it has not been proved in man.

Synthesis of cholesterol. That it can be synthesised in the body is proved by the following facts: (1) The excretion of cholesterol may exceed the intake over long periods of time by as much as 0·3 gm per day. (2) Experiments with *heavy water* also suggests that cholesterol is built up from smaller molecules other than sterols. (3) When rats and mice are fed with acetate labelled with deuterium (D), cholesterol containing D is found in their bodies.

The **sequence of reactions in the path of cholesterol synthesis** is given below:

1. Activation of acetate to acetyl CoA.
2. Condensation of two acetyl CoA to form acetoacetyl CoA.
3. Formation of HMG CoA.
4. Formation of mevalonic acid.

Mevalonic acid is phosphorylated by ATP to form ultimately 5-diphosphomevalonic acid.

6. The diphosphomevalonic acid after losing CO_2 and H_2O gives rise to isopentenyl pyrophosphate.

7. The above compound isomerises to form 3-3-dimethylallyl pyrophosphate which combines with isopentenyl pyrophosphate to give geranyl pyrophosphate.

8. Another molecule of isopentenyl pyrophosphate combines with geranyl pyrophosphate to form farnesyl pyrophosphate.

9. Two molecules of farnesyl pyrophosphate combine to form squalene.

10. An oxidocyclase converts squalene to lanosterol.

11. The conversion of lanosterol to cholesterol involves a number of transformations, some of which are not yet clearly understood.

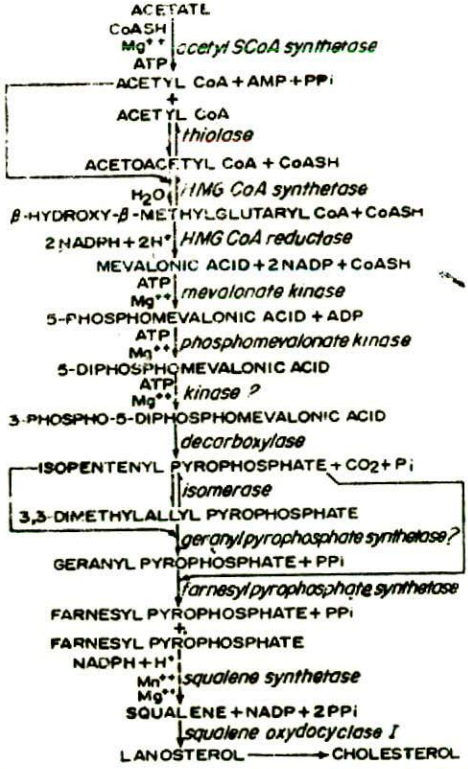


FIG. 383. Schematic representation of the path of synthesis of cholesterol.

Storage, destruction and control of cholesterol

A good deal of cholesterol, derived from the disintegration of blood cells, is stored in the cells of reticulo-endothelial system and retained in the body. Body utilises cholesterol very economically. The metabolism of

cholesterol is under the influence of hormones. Injection of *adrenocorticotrophic hormone* (ACTH) reduces cholesterol content of adrenal cortex. In hypothyroidism blood cholesterol rises whereas there is fall of blood cholesterol in hyperthyroidism. Variations in serum cholesterol has also been noted during pregnancy. In diabetes mellitus and atherosclerosis, blood cholesterol is often found to be high. Liver, tissue cells, muscles, organs like heart and brain, etc., play an important part in cholesterol metabolism.

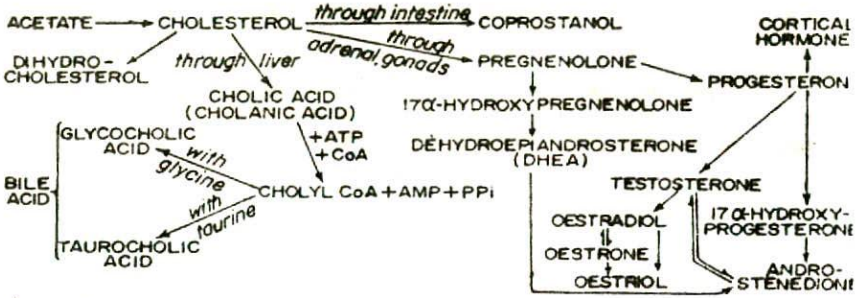


FIG. 384. Schematic representation of pathways of cholesterol metabolism.

Functions of cholesterol

(1) **Essential constituent of all cells**—It is a part of the 'element constant' of the cells. Its presence in constant amount in cells indicates that it is intimately concerned with the function of the cells. (2) **Controls cell permeability**—Being a constant constituent of cell membrane it is believed to be related to the permeability of the tissue cells. (3) **Prevents haemolysis**—In some unknown way, it protects the red cells from being easily haemolysed. Low blood cholesterol is associated with haemolysis. (4) **Defensive action**—Cholesterol is intimately related to the defensive mechanism of the body. During acute infections blood cholesterol falls and tends to rise during recovery. (5) **Fat transport**—A large part of fat is transported through blood as cholesterol esters. (6) **Formation of cholic acid (bile salts)**—It has been shown that cholesterol is the mother substance from which cholic acid is synthesised. Cholic acid is a constituent of bile salts. (7) **Antilipotropic action**—Cholesterol feeding increases the deposition of fat in the liver. This effect is due to the formation of cholesterol esters and depression of phospholipid formation in the liver. (8) **Controls cell division**—Rapidly growing tissues are very rich in cholesterol, such as, the granulation tissues of healing ulcer and rapidly growing tumours, etc. (9) **Antagonistic to phospholipids**—Its physico-chemical properties are antagonistic to phospholipids; hence, they are always found together. (10) It is a parent substance of all the steroid hormones of sex glands, adrenal cortex.

Interrelation with other sterols

Cholesterol is very closely related to many other sterols of immense physiological importance, for instance, (*a*) the **active principles of adrenal cortex,** (*b*) the **male sex hormones,** (*c*) the **female sex hormones,** (*d*) **vitamin D** and **ergosterol,** (*e*) **cholic acid,** etc. Although cholesterol has not been proved to be the mother substances for the formation of

other sterols in the body except cholic acid but there are some indications to believe that cholesterol may serve as precursor for the synthesis of other sterols since it has been possible to synthesise sex hormones, both male and female, in the laboratory from cholesterol.

Excretion of cholesterol

Excretion takes place in the following ways: (1) **In the bile**—It is found as free cholesterol but a good part of it is reabsorbed from the intestine. (2) **In the stool**—A part of the bile cholesterol undergoes bacterial putrefaction in the intestine and is excreted as *coprosterol*. But a small amount of free cholesterol is also found in the stool. (3) **In the urine**—It appears in the urine only in traces, but appears in larger amounts in different diseases namely in *hypercholesteremia* where blood cholesterol is high. (4) **In the skin**—*Sebum*, secretion of the sebaceous glands of the skin, contains large quantities of cholesterol and thus drying of the skin is prevented by sebum for its cholesterol constituent.

Blood cholesterol in pregnancy. During pregnancy blood cholesterol rises and rapidly comes back to normal after confinement. It may be that this rise is due to hyperactivity of the adrenal cortex, produced during pregnancy and the exact function is not known. It may be associated with the rapid cell division and the tissue differentiation of the foetus. It may also be that this high cholesterol ensures the supply of the mother substance from which large amounts of sex hormones are manufactured during pregnancy.

Atherosclerosis. A very common disease of advanced age, indicated by deposition of cholesterol in the damaged arterial wall. This leads to cerebral thrombosis, myocardial infarctions, etc. It has been established that abnormal lipid metabolism produces atherosclerosis. Several other factors make the system more prone to the disease. One of the principal factors is an injury to arterial wall resulting from any conditions. A high blood cholesterol level and genetic inheritance might be other contributory factors. Treatment includes administration of thyroid hormones or oestrogen in low doses. If dietary saturated fat is substituted by unsaturated one, the blood cholesterol level is lowered and atherosclerosis is prevented.

SYNTHESIS OF FATS

Fats can be synthesised from both carbohydrates and proteins. The evidence is given below:

From carbohydrates. It is an experimental fact that animals can deposit fat practically on *fat-free diets* (FIG. 385). Young pigs fed on barley deposit more fat than can be accounted for from the protein and fat content of barley. So the carbohydrates must have been used for the purpose. It has been found that palmitic and stearic acids are rapidly synthesised but the synthesis of unsaturated acids is very slow.

The body is able to introduce one or two double bonds but not more. So that *the highly unsaturated fatty acids like linoleic acid or linolenic acid or arachidonic acid* which are known as essential fatty acids *cannot be synthesised.*

Synthesis of saturated fatty acids. It is now an established fact that fatty acids are synthesised from *acetate* which is activated by reaction with CoASH and ATP in presence of acetyl CoA synthetase to form *active acetyl CoA*. This is carboxylated to form *malonyl CoA* in presence of CO_2+Mn^{++} and acetyl CoA carboxylase (dependent upon enzyme-bound *biotin*). Then acetyl and malonyl groups are transferred to the *acyl carrier*

protein (ACPSH) with the help of enzyme *fatty acyl transacylase* and the CoASH becomes free. Acetyl ACP and malonyl ACP thus formed condense by a *sulphydryl enzyme* to form *acetoacetyl ACP* with the liberation of CO_2 and one molecule of ACPSH. Acetoacetyl ACP is reduced by dehydrogenase

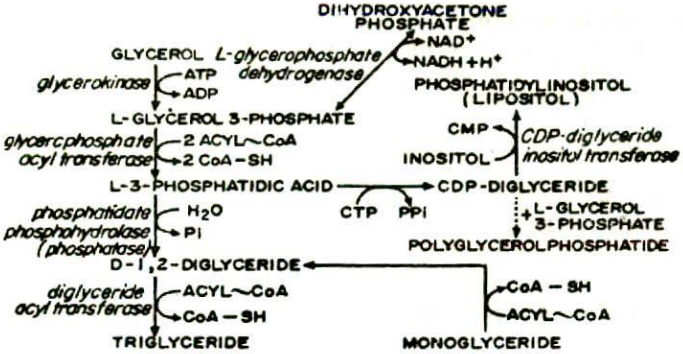


FIG. 385. Schematic representation of the path of synthesis of fat (triglycerides). Glycerol is formed from carbohydrate or absorved as such from the intestine.

in presence of NADPH+H+ (formed during glucose oxidation in hexose monophosphate shunt, HMS) to *β-hydroxybutyryl ACP* which is further unsaturated at α,β-position by the enzyme *enoyl hydrase*. Then *α-,β-unsaturated butyryl ACP* is reduced by dehydrogenase in presence of NADPH+H+ to *butyryl ACP*.

The 2C acetate is thus elongated to 4C chain, i.e., butyryl ACP which may further be elongated by 2C in each above cycle of reactions adding

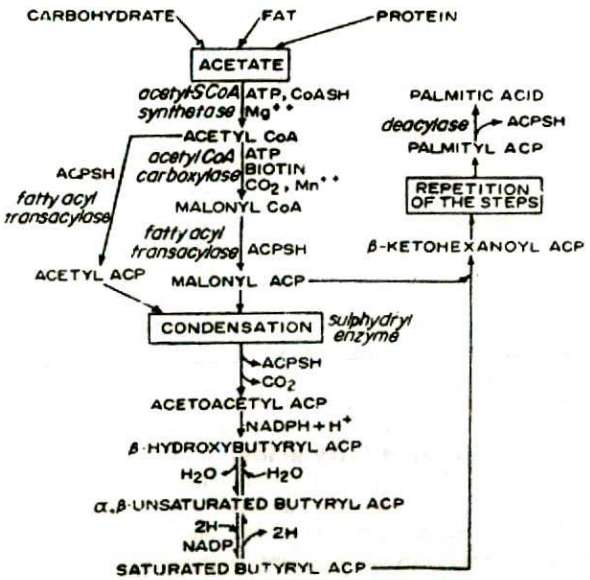


FIG. 386. Schematic representation of the path of synthesis of saturated fatty acids.

malonyl ACP in each cycle of above reaction. Finally, the fatty acid is formed by deacylase when fatty acid ACP is cleaved to form (say in case of 16C fatty acyl ACP, i.e., *palmityl ACP*) *palmitic acid* and *ACPSH*. The synthesis of saturated fatty acids may be presented schematically (Fig. 386).

It is indicated that acetic acid derived from glucose, fatty acids and amino acids go to form fatty acids in the body. The **mitochondrial** and **microsomal systems** are concerned with the synthesis of fatty acids. The detail mechanism is not known in either case but it is proved that in the former system elongation of fatty acid carbon chain and unsaturated fatty acid formation takes place which is sensitive to *avidin* and *not biotin* dependent one and in the latter system carboxylation of acetyl CoA, conversion of malonyl CoA to fatty acid and phospholipid formation takes place.

Several members of the vitamin B complex are involved *in the process. Thiamine is necessary for the conversion of sugar into fats. Riboflavin, pyridoxine and nicotinic acid play a similar but subsidiary role.*

Synthesis of unsaturated fatty acids. Schoenheimer and his associates have reported that unsaturated fatty acids are synthesised from saturated fatty acids. A microsomal system is involved for such synthesis. The liver contains such system for the synthesis of oleic and palmitoleic acids from stearyl CoA and palmitoleyl CoA respectively. NAD or NADP is required for carrying out the process.

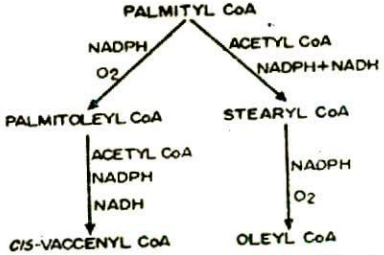


Fig. 387. Schematic representation of the path of synthesis of unsaturated fatty acids.

From proteins. Since nearly 60% of proteins can be converted into carbohydrate, it is reasonable to expect that some of this sugar may be available for conversion into fats. Recent study with isotopes has definitely shown that such conversion is possible. Certain members of vitamin B family are also important in this respect. Animals kept on pure protein diet with a liberal supply of several members of vitamin B group can deposit fats and that this synthetic fat is similar in composition to that which is synthesised from carbohydrate food.

Factors influencing lipogenesis

Lipogenesis (fatty acid synthesis) is depressed in diabetes and starvation. It is possibly due to decreased production of acetyl CoA or of NADP or NAD due to defective carbohydrate metabolism. It is postulated that in diabetic and starved rats, the activity of acetyl carboxylase is decreased. Citrate and other components of Krebs cycle have got some stimulating effects in fatty acid synthesis. Besides this, the citrate also helps lipogenesis by donating acetyl CoA units. Rate-limiting factor in fatty acid synthesis is the carboxylation of acetyl CoA.

SYNTHESIS OF GLYCEROL

Glycerol is synthesised from glucose. Glucose is first converted into two molecules of triosephosphate, i.e., dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, the latter is convertible to former which gives rise to glycerol.

Glucose → Glucose-6-phosphate → Fructose-6-phosphate → Fructose-1,6-phosphate → Dihydroxyacetone phosphate + Glyceraldehyde-3-phosphate

α-glycerophosphate

Glycerol + Pi

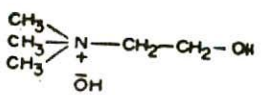
Essential fatty acids. The fats which are synthesised in the body (endogenous fat) from the fatty acid derived from non-lipid sources, i.e., carbohydrates and proteins, cannot serve all the functions of exogenous fat, e.g., maintenance of proper growth and nutrition. So it is indicated that synthesis of fat in the body is restricted to a limit where some unsaturated fatty acids are not synthesised. Since some fatty acids cannot be synthesised and, on the other hand, required by the body so they should be supplied from outside through diet and are called essential fatty acids like the essential amino acids required for growth and nutrition.

Highly unsaturated fatty acids, such as, **linoleic acid** or **linolenic acid** or **arachidonic acid,** are essential for proper growth and nutrition and may be called essential fatty acids. It has however been observed that linoleic acid might be converted into arachidonic acid and so the latter can be spared if linoleate is adequately supplied in diet. Pyridoxine (vitamin B_6) helps in the process of conversion.

Functions of essential fatty acids. (1) They help in normal growth. (2) They are structural components of cells. (3) They are in some way concerned with normal reproductive capacity. (4) They are responsible for the maintenance of normal healthy skin.

CHOLINE AND ITS PHYSIOLOGICAL IMPORTANCE

Chemistry and sources. It was first isolated by Stracker in 1849 from body fluids. It has got the following formula. It is a component of lecithin, sphingomyelin, acetylcholine, etc. Butter, nervous tissue, yolk of eggs are very rich sources. **Synthesis**—It can be easily synthesised in the human body from methionine and ethanolamine (*vide* Transmethylation —page 581).



Choline

Functions of choline

1. **Lipotropic action.** Choline prevents the deposition of fats and accelerates the rate of removal of fat from the liver. *Betaine, methionine, inositol, casein* and *certain other proteins are also lipotropic.* The lipotropic action of choline has been studied in various ways, all of which shows that *choline helps in the synthesis of phosphatides in the liver and to a lesser extent in other tissues.* In this way it accelerates the rate of phospholipid formation and mobilisation of fat. Methionine exerts its effects by transferring its methyl group to ethanolamine, with the formation of choline. Betaine also acts similarly as a methyl donor. Inositol has similar action on a fat-free diet. Under certain conditions vitamin B_{12} and folic acid have lipotropic action. The lipotropic effect of casein is due to its methionine content. Pancreatic extracts have been found to have strong lipotropic action, as they contain choline, methionine, inositol, lipotropic proteins and certain unidentified factors. This action of lipotropic substances, specially the easily available

choline, is of considerable practical importance. Because it enables us to control the amount of liver fat by dietary means, and consequently may have a useful application in the treatment of fatty liver. As found in rats, only small amount of choline is enough. A dose of 5 mgm of choline per day has been found to prevent a rise of 12% of fat in the liver. When these lipotropic factors are absent in the diet, **liver becomes fatty** (*vide* page 553) and young animals develop haemorrhagic lesions in the skin, kidney and other tissues which may be fatal. Fibrotic changes appear in the liver due to prolonged lack of choline.

It is to be noted that **cholesterol** and **cystine** exert an antilipotropic action and cause deposition of fat in the liver and various other tissues. In nature, cholesterol and lecithin (which contains choline) are constantly associated. This is important because they maintain a balance between the *lipotropic and antilipotropic factors.*

2. **Synthetic functions.** Compounds of immense physiological importance are synthesised from choline, viz., lecithin, sphingomyelin, acetylcholine, etc.. The first two belong to the group of phospholipids.

Acetylcholine is of utmost importance for the following:

(*a*) **Maintains functional integrity of the nervous system.** Formation and destruction of acetylcholine and probably of other choline derivatives—which take place normally at all synapses—are believed to be responsible for the transmission of the nerve impulses through the synapses and thereby maintain the functional continuity of the nervous system.

(*b*) **Essential for cholinergic fibres.** It is produced at the motor endings (muscle or gland) of the parasympathetic fibres and certain sympathetic fibres and initiates their peripheral activities (i.e., contraction of plain muscles or secretion of relevant glands).

(*c*) **Helps contraction of skeletal muscles.** Acetylcholine is produced at the myoneural junction of the skeletal muscles and initiates the chemical changes causing muscular contraction.

3. **Vitamin action.** Choline is supposed to be a member of the vitamin B family and acts as a methyl donor in transmethylation.

4. **Essential constituent of diet** for lower animals, specially when supply of methionine is low. It promotes growth, controls storage and mobilisation of fat and other important physiological processes in them. Although it serves the same function in man, yet it is not essential, because it can be synthesised in the human body.

PROTEIN METABOLISM

Proteins are essential constituents of all living cells. Metabolism of protein shows a number of special features. For instance—(1) The power of protein synthesis of the human body is very limited. (2) Storage of protein can only occur under certain specific conditions. (3) Distribution of protein in the body is also characteristic. (4) The method of utilisation of amino acids is also peculiar. (5) The end products of protein metabolism are also characteristic. Two other peculiarities are also noticed in connection with protein metabolism, viz., (6) Nitrogen equilibrium. (7) Specific dynamic action (S.D.A.). Each one of these characteristics has been described in proper places. (8) Each animal species and type of tissue are made up of characteristic protein and variations in the animal species and tissue type are mainly due to variation of the protein types in them.

FATE AND FUNCTIONS OF AMINO ACIDS IN THE BODY

The metabolism of protein is meant by metabolism of amino acids. Amino acids are continuously returned from the body fluids for the synthesis of tissue protein, oxidation and other uses and added into the tissue fluid by absorption from intestine, breakdown of tissue protein and synthesis. There are constant exchange of amino acid from tissue to blood, and other body fluids and reversely from body fluid to the tissue. The *size of the* **amino acid pool** *represents the balance between the removal and addition of amino acids* (total free amino acids in the body) (FIG. 388). Turnover of

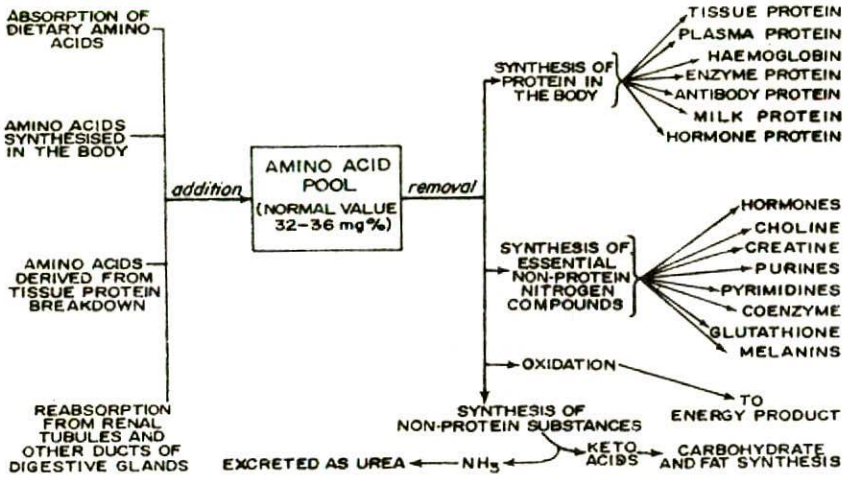


FIG. 388. Schematic representation of continual exchange of amino acid between the body fluid and tissue (protein metabolism).

the amino acids has been studied with the help of *isotopically labelled* amino acids. From the turnover of the amino acids the rate of synthesis of proteins may be calculated. The concentration of amino acids in the pool remains almost constant which is evident from the fact that even after protein ingestion there is no *variation of amino acid content* of the blood. The kidneys also help in the maintenance of the constancy of the amino acid pool. The amino acids filtered from the glomeruli are reabsorbed by the renal tubules.

The tissue cells choose their characteristic amino acids from the blood stream and utilise them in various ways. The functions of amino acids can be divided into two groups: (1) Those functions for which amino acids are used in the intact form either as such or in some derivative form. (2) Those functions for which intact amino acids are not necessary, so that they are broken down. These two groups are separately described below.

I. FUNCTIONS SERVED BY INTACT AMINO ACIDS

1. **Synthesis of cell protoplasm.** Proteins, being chief and essential constituents of all living cells, amino acids are necessary to build them up.

2. **For the repair of wear and tear.** Tissue proteins break down during metabolism. These damaged parts are repaired with the help of amino acids.

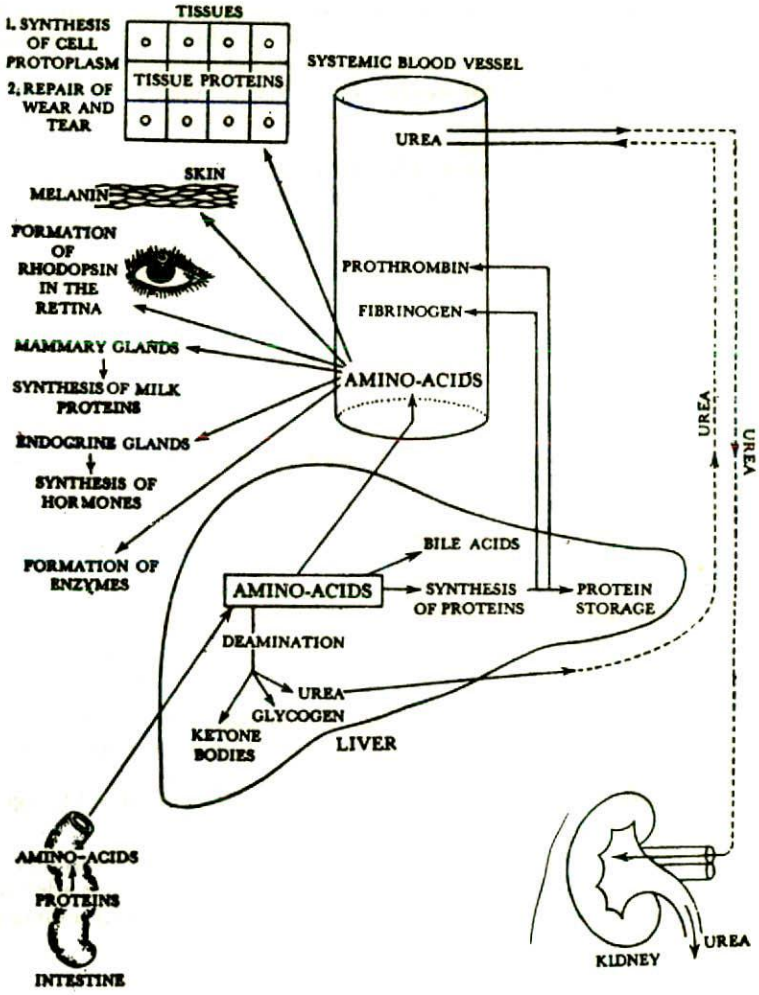


FIG. 389. Diagrammatic representation of the fate and functions of amino acids in the body.

3. **Protein storage.** In adult people, where nitrogen equilibrium is established, proteins cannot be stored. But they can be *stored in infants, children, convalescent patients, pregnant women, athletes, etc., where active growth takes place and protein synthesis exceeds protein breakdown.* In old age the protein breakdown exceeds protein synthesis so mass of protein decreases.

4. **Synthesis of bile acids.** The two bile acids—taurocholic acid and glycocholic acid, are obtained by conjugation with specific amino acids with cholic acid. Glycine and cholic acid give glycocholic acid; and taurine, which is derived from metabolism of sulphur-containing amino acid combines with cholic acid, producing taurocholic acid.

5. **Synthesis of plasma proteins.** Synthesis of prothrombin, fibrinogen, other plasma proteins and antibody products are carried out by liver, with the help of amino acids from the blood stream.

6. **Essential amino acids.** There are a number of amino acids which cannot be synthesised in the body, but are essential for growth and maintenance of life (*vide* pages 581–82).

7. **Synthesis of haemoglobin.** Glycine and succinyl CoA go to form δ-aminolevulinic acid which polymerises to form porphyrine. Then porphyrine with the incorporation of Fe^{++} forms haeme which with globin (protein) go to form haemoglobin (*vide* pages 156–57).

8. **Synthesis of hormones.** Hormones of the thyroid gland and adrenal medulla are derivatives of tyrosine. Insulin is also protein in nature. Hence, intact amino acids are required for their synthesis.

9. **Synthesis of enzymes.** The enzymes are protein in nature, so amino acids are required to build them up.

10. **Synthesis of milk proteins.** Milk proteins in lactating mothers are formed from amino acids by the mammary gland.

11. **Synthesis of glutathione and cytochrome.** The former is a tripeptide and the latter is a haemochromogen. They take part in tissue oxidation and are synthesised from amino acids.

12. **Synthesis of purine and pyrimidine.** Glycine, glutamic acid, aspartic acid, etc., help in the synthesis of purine and pyrimidine bases and thereby there is endogenous nucleoprotein synthesis.

13. **Synthesis of antibodies.** Antibodies are globulin in nature.

14. **Synthesis of melanin.** Melanin, which is the pigment of the skin, choroid, hair, substantia nigra, etc., is derived from the amino acid, tyrosine.

15. **Formation of rhodopsin.** Rhodopsin, also known as visual purple, is made up of vitamin A and another protein component. Necessarily, amino acids are required for its synthesis.

16. **Role of arginine in urea formation.** The amino acid, arginine, helps in the formation of urea (*vide* pages 577–78).

The above is a brief list of some of the important functions served by the amino acids in intact form. When the above functions are adequately done, the surplus amounts of amino acids break down and undergo the following fate and functions.

II. FUNCTIONS OF AMINO ACIDS WHILE BREAKING DOWN

1. **Supply energy.** Amino acids break down and liberate energy. One gram of protein is equivalent to 4·3 Calories.

2. **High specific dynamic action.** While breaking down, amino acids exert a specific-stimulating action on tissue metabolism. The specific dynamic action of protein is about 30% (*vide* under S.D.A.).

3. **Deamination.** Deamination is the process by which the amino radicle ($-NH_2$) is taken away from the amino acid. *It is carried out chiefly in*

the liver with the help of an enzyme—deaminase. Deamination may also take place by the enzyme *transaminase*, which transfers the amino group of the amino acid to a keto acid, converting the latter into an amino acid, and the former into a keto acid. *It is also possible to some extent in other tissues, such as kidneys.* About 5% energy of the protein molecule is lost in the process. By this process the amino acid molecule is broken down into two parts: (A) the **nitrogenous part** (ammonia), and (B) the **non-nitrogenous part** (an α-ketonic acid or an aldehyde or a hydroxy acid). The fate and functions of these two parts are described below.

A. Fate and Functions of the Nitrogenous Part

The nitrogenous part, viz., ammonia, undergoes the following fate:

1. **Formation of urea.** Most of the ammonia, under normal conditions, is converted into urea. [Because about 80% of total urinary nitrogen is found in the form of urea.] *Urea is formed mainly in the liver.* The **chief chemical process of urea formation** is as follows:

Ornithine cycle (Krebs-Henseleit cycle). *According to Krebs and Henseleit, the basic process of urea formation is as follows:* The amino acid *arginine* is broken down by the enzyme *arginase* (found in the liver) into *urea* and *ornithine*. Ornithine unites with one molecule of ammonia and carbon dioxide to produce *citrulline*. The latter combines with another molecule of ammonia to form *arginine*. Arginine again breaks down into urea and ornithine. Thus the cycle repeats, and at each cycle, one molecule of urea is formed (Fig. 390).

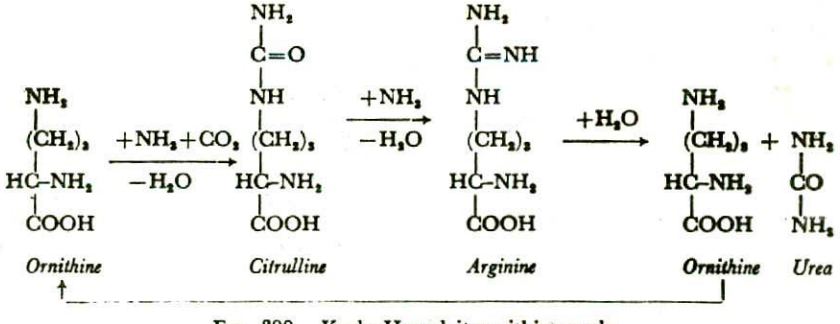


Fig. 390. Krebs-Henseleit ornithine cycle.

This theory is supported by the following main observations: (1) Arginase is present in the liver of all vertebrates (ureotelic animals) which excrete urea and only traces in the kidneys. (2) Birds which normally excrete uric acid (uricotelic animals) and not urea, do not possess arginase in the liver. (3) Perfusion of liver slices with a suitable fluid containing arginine, ornithine, etc., readily forms urea. (4) Observations with *isotopic carbon*—radioactive ^{14}C-labelled and *nitrogen* ^{15}N-labelled—in which CO_2, NH_3, arginine, etc., were used—also support this theory.

Recent observations regarding the details of the ornithine cycle are as follows: CO_2 is activated into *active* CO_2 in presence of biotin, N-acetyl glutamic acid and ATP. This *active* CO_2 reacts with ammonia donated from glutamine to form carbamyl phosphate. In the later stage also participation of both N-acetyl glutamate and ATP are necessary. Both steps are

catalysed by the enzyme carbamyl phosphate synthetase. The formed carbamyl phosphate reacts with ornithine in presence of the liver enzyme *ornithine transcarbamylase* (ornithine carbamyl transferase) to form citrulline (carbamyl ornithine). Citrulline then condenses with aspartic acid in presence of ATP and Mg^{++} to form arginosuccinate (argininosuccinate).

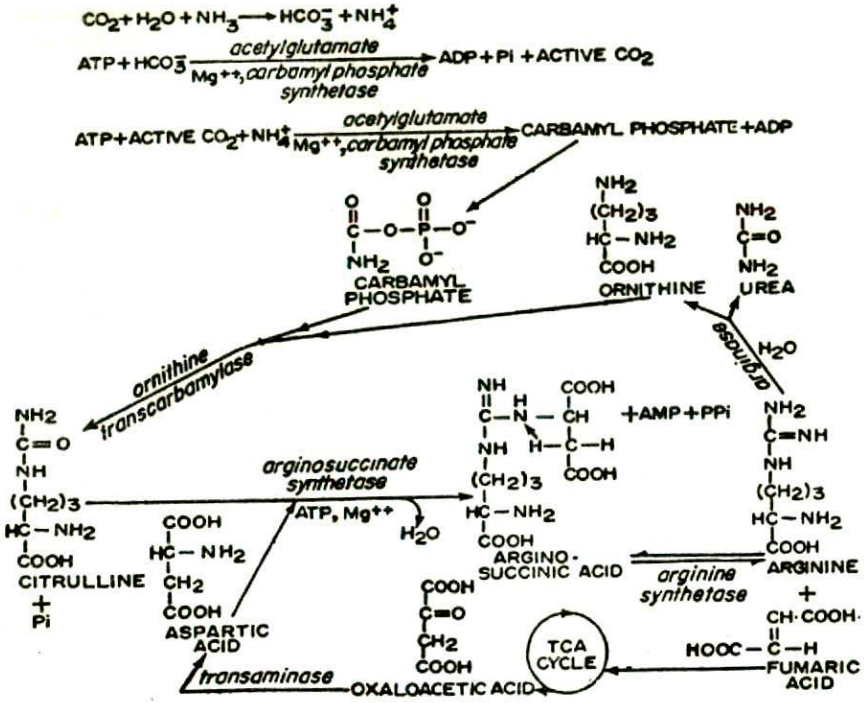


FIG. 391. Schematic representation of urea synthesis.

This reaction is catalysed by a condensing enzyme, *arginosuccinate synthetase*. Arginosuccinate then splits off into fumaric acid and arginine, influenced by the enzyme *arginine synthetase* (arginine succinase). In the liver, if arginine is not used for *protein synthesis*, then arginine is broken down by the enzyme, *arginase*, to form urea and ornithine. The liberated fumaric acid is converted into oxaloacetic acid in the tricarboxylic acid (TCA) cycle. Oxaloacetic acid is then transaminated into aspartic acid by the enzyme *transaminase*. The NH_3 is donated by glutamic acid or glutamine (FIGS. 391, 392 & 397).

2. **Formation of ammonium salts.** A small part of ammonia combines with acids, other than carbonic acid, and appears in the urine in the form of ammonium salts, such as ammonium phosphate, sulphate, urate, etc. It is obvious therefore that, the amount of ammonium salts formed will not depend upon the amount of ammonia formation but on the relative proportion of acids and bases in the body. In acidosis more ammonia is needed to neutralise the excess acids, and so there will be

proportionate increase in the amount of ammonium salts. In alkalosis opposite changes occur.*

The **function** served by the formation of ammonium salts is to neutralise acid of the body, so that the blood reaction may remain constant.

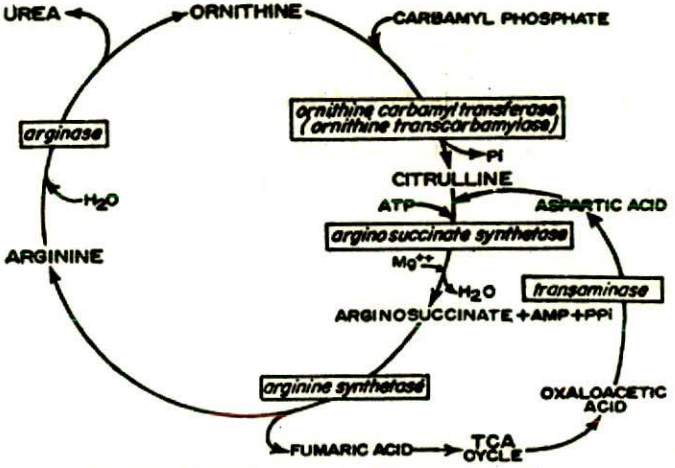


FIG. 392. Krebs-Henseleit cycle for urea formation.

3. **Ammonia may be utilised for the synthesis of simple amino acids,** such as glycine, alanine, etc., and also for amination of glutamic acid to give glutamine.

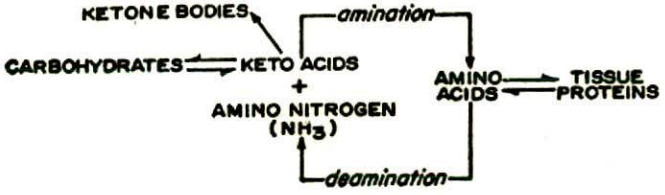


FIG. 393. Transamination showing dynamic state of amino nitrogen and proteins in the body.

4. **It may also be used for the synthesis of various nitrogenous substances,** such as creatine, purine, uric acid, pyrimidine, lecithin and such others.

B. FATE AND FUNCTIONS OF THE NON-NITROGENOUS PART

The non-nitrogenous part has the following fate:

1. **Some will have the fate of carbohydrates.** The non-nitrogenous residues of some amino acids are utilised in the body as carbohydrates. Because, when given to a diabetic animal (depancreatised or after

* **Ammonia coefficient.** Ammonia coefficient is expressed by the following ratio:

$$\frac{\text{Ammonia nitrogen of urine}}{\text{Total nitrogen of urine}} \times 100.$$

Normally it is about 5%. *It serves as a guide to the state of acidosis or alkalosis in the body.* In acidosis ammonium salts will be more, hence, the ratio will rise. In alkalosis it falls.

administration of phloridzin), these amino acids increase a corresponding amount of sugar in the urine.* For this reason, in a diabetic subject about 60% of food protein is converted into sugar. Glycine, alanine, aspartic acid, glutamic acid, serine, cysteine, arginine, proline, hydroxyproline, histidine, lysine, isoleucine, citrulline, methionine, valine, threonine, partially phenylalanine and tyrosine belong to this class. *These amino acids are called* **antiketogenic amino acids.** *Because, they are as carbohydrates in the body and prevent the formation of ketone bodies.* They are also called **glucogenic.**

2. **Some undergo the fate of fats.** There are certain amino acids, viz., leucine and in part isoleucine, phenylalanine and tyrosine, which yield no glucose, but give rise to ketone bodies when administered to a diabetic animal. It is obvious that the non-nitrogenous part of these amino acids is broken down in the body as fatty acids, from which the ketone bodies are formed. It is known that in a diabetic subject about 40% of proteins are converted into ketone bodies. *These amino acids are therefore known as* **ketogenic amino acids.**

3. The **sulphur** and **phosphorus,** derived from the non-nitrogenous part of the amino acids, are converted into various sulphur and phosphorus compounds and are excreted as such.

TRANSAMINATION

In this reaction the *nitrogen* is transferred as NH_3 from **labile nitrogen pool** to other compound which is catalised by specific enzyme originally termed as **transaminases** and recently referred as **aminotransferases.** It is reversible process as well as combined process of *deamination* and *amination.* In most cases there is transference of amino group from amino acid to keto acid which is derived from either amino acid or carbohydrate and fat. The following examples will clarify:

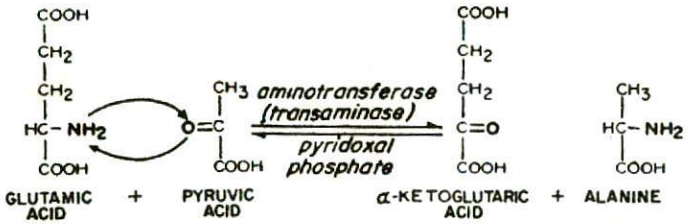


FIG. 394. Action of glutamic-pyruvic transaminase (GPT).

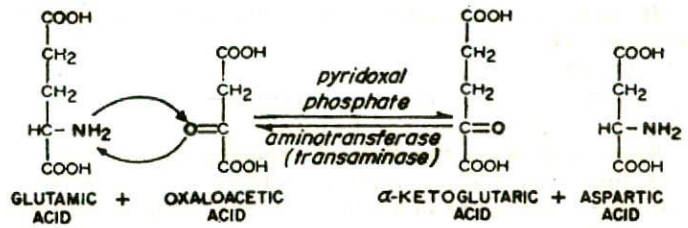


FIG. 395. Action of glutamic-oxaloacetic transaminase (GOT).

* In such cases **D/N** (i.e., dextrose/nitrogen) **ratio** in urine will be constant (about 3·6) showing that glucose and nitrogen are coming from the same source, e.g., amino acid. [*Vide* under *Metabolism in Diabetes Mellitus.*]

Pyridoxal phosphate (a vitamin B_6 derivative) acts as the coenzyme. This mechanism plays a part both in the breakdown of amino acids to yield keto acids and in the formation of new amino acids (non-essential) from keto acids. The keto acids may go to form carbohydrate (neoglucogenesis). Isotopic experiments with ^{15}N show that all natural amino acids, viz., alanine, leucine, aspartic acid, glutamic acid, histidine, proline, tyrosine, etc. take part in the transamination reaction readily where the glutamic acid and aspartic acid being most reactive. Lysine and threonine do not take part in transamination reaction.

TRANSMETHYLATION

Transfer of methyl group (CH_3) from one compound to the other is an important step in certain synthetic processes in the body. This is called **transmethylation.** Experiments with compounds containing deuterium-labelled CH_3 group support this view. Following are the examples:

(1) CH_3 from *methionine* is transferred to guanidoacetic acid to form *creatine* (*vide* page 585). (2) CH_3 from *methionine* (or betaine) is transferred to *ethanolamine* to form *choline*. (3) CH_3 can be reversibly exchanged between choline and methionine.

Methionine acting as a methyl donor in transmethylation reaction is first activated in presence of ATP, Mg^{++} and glutathione. The active methionine is termed as S-adenosyl methionine. A methionine-activating enzyme of the liver catalyses the process. The enzyme is present in other tissues also.

ESSENTIAL AMINO ACIDS

All the amino acids are required by the body but some of which are termed *indispensable* in the diet as these *cannot be synthesised* by the animal organism and others are termed *dispensable* in the diet as these amino acids *can be synthesised* in the body. Absence of the former class of amino acids in the diet results disturbances in the nitrogen equilibrium, growth, maintenance, nutrition and life span, hence this class of amino acids is otherwise termed as **nutritionally essential amino acids.**

The list of essential amino acids varies with the animal species. Some amino acids are **relatively essential.** The requirement of protein in the diet is to be considered both in its quantitative and qualitative aspects, i.e., type of amino acids.

Varieties. *Following amino acids have been found to be essential* (for their chemical formulae *vide* pages 77–78): (1) **Phenylalanine.** (2) **Tryptophan.** (3) **Methionine.** (4) **Threonine.** (5) **Valine.** (6) **Isoleucine.** (7) **Lysine.** (8) **Leucine** and (9) **Histidine.** Some are semi-indispensable and they are: (1) **Arginine.** (2) **Cystine.** (3) **Tyrosine.** (4) **Glycine.**

Apart from its other functions, phenylalanine gives rise to tyrosine in the body, which acts as the precursor for melanin, thyroxine and epinephrine (adrenaline). The **first six** amino acids are essential for the maintenance of nutrition, growth and life.

Although methionine can be converted into cystine, yet in dogs regeneration of plasma proteins fails to take place properly unless cystine is supplied in food. Hence, cystine is essential for this function in dogs, and cannot be replaced by methionine. In absence of valine rats develop severe muscular inco-ordination, and eventually die.

Lysine and **leucine** are also essential for growth.

Arginine helps in growth also, but is synthesised in the body. But the rate of synthesis cannot keep up pace with the requirement and hence it has to be supplied exogenously to supplement that which is already synthesised quantitatively. Hence, it is known as *semi-indispensable.*

Classification of proteins according to their essential amino acid contents. Proteins from different sources vary considerably in the quality and quantity of their amino acid contents. Consequently, their food values must differ. From this point of view proteins are divided into two classes: (1) **'First class' proteins** or **Proteins of 'high biological value'**—They contain all the essential amino acids and are capable of supporting growth and maintenance. Proteins of meat, fish, milk, eggs and *in general, almost all animal proteins belong to this class. Gelatin although of animal origin is an exception* (*vide* below). Of the vegetable proteins, *glutenin* of wheat and *glutelin* of maize fall in this group. (2) **'Second class' proteins** or **Proteins of 'low biological value'**—These are deficient in some of the essential amino acids and unsuitable for both growth and maintenance, e.g., *zein*, the chief protein of maize, contains no tryptophan and lysine, and *gelatin*, though an animal protein, is deficient in tryptophan, leucine, isoleucine and some other essential amino acids. Generally speaking, *most of the proteins belonging to this class are of vegetable origin.*

EXOGENOUS AND ENDOGENOUS PROTEIN METABOLISM

Definition. By the term **endogenous protein metabolism** *is meant the disintegration of those proteins which already exist as components of living cells* (*tissue proteins*). The term **exogenous protein metabolism** *implies the breakdown of food proteins which do not exist as parts of the cell protoplasm.*

The classical view of protein metabolism, originally proposed by Folin, is now challenged. Folin's view was the metabolic patterns of **exogenous (dietary)** and **endogenous (body) protein metabolism** differ, i.e., the end products of endogenous protein metabolism are uric acid, creatine and neutral sulphur whereas urea is the end product of exogenous protein metabolism. However isotopic experiment reveals that the body proteins are in a constant state of turnover and the body proteins are continually broken down and replaced by new proteins synthesised from dietary amino acids. The replacement of protein is rapid in plasma, liver, kidneys and intestinal tract and slow in haemoglobin, muscle and skin.

Dynamic state of amino nitrogen and proteins of the body. It has been established by the work of Schoenheimer and others that the amino nitrogen of amino acids (except lysine) is distributed to other amino acids of different tissues and reversibly it is withdrawn from those amino acids to the amino acids which contained amino nitrogen by the process of deamination and reamination.

Protein storage (Labile protein). Nitrogen excreted for the first few days after protein starvation was greater and then becomes more or less constant. The disorganised protein present in liver, thymus, prostate, seminal vesicle, alimentary tract, pancreas, spleen and kidneys, etc., are drawn upon to meet the need of the body and these proteins are called **labile proteins.** These are utilised for the synthesis of other proteins and may be oxidised to gain energy when required.

End products of protein metabolism. The nitrogen released from amino acid (protein) catabolism is excreted from the body in different

forms which vary in different species of animals. The considerable amount of nitrogen excreted through the urine of men, mammals and amphibians, etc., is *urea*. The birds and reptiles excrete nitrogen as *uric acid* mainly. The man also excretes nitrogen of *purine bases* as uric acid. Creatine excretion is exclusively from tissue protein breakdown. *Neutral sulphur* excretion does not indicate the nature of dietary or tissue protein breakdown.

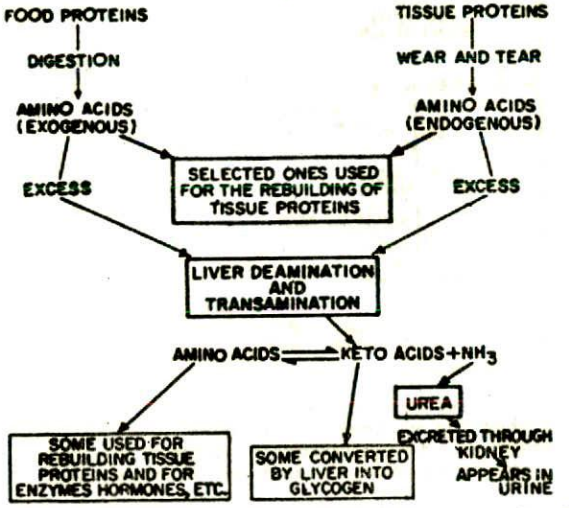


FIG. 396. Schematic representation of exogenous (dietary) and endogenous (body) protein metabolism.

Due to dynamic state of amino nitrogen of amino acid and differential pattern of metabolism of labile protein, the excretion of different nitrogenous metabolic end products does not always give real metabolic picture of protein metabolism. But some clue may be obtained if the standardised experiments were done, e.g., increased creatine excretion may indicate increased tissue protein, i.e., specially muscle protein catabolism going on. Increased uric acid excretion may be due to increased purine catabolism. Increased urea excretion may indicate increased dietary protein catabolism.

BRIEF LIFE HISTORY OF THE END PRODUCTS OF EXOGENOUS PROTEIN METABOLISM

From Folin's experiment, although challenged, it appears that the end products of exogenous protein metabolism are **urea, ammonia, inorganic sulphate** and **50% of the total uric acid** excreted. The brief life history of these products is as follows:

UREA

Chemistry—$CO(NH_2)_2$. **Sources**—(*a*) From deamination of amino acids (mainly from exogenous sources). (*b*) From salts like ammonium carbonate, lactate, etc., taken in diet or as drug. (*c*) From the amino acid arginine—It breaks down into urea and ornithine. The end product of metabolism of these bodies is urea. **Method of formation**—From arginine (*vide* pages 577–78 & Fig. 397). **Site of formation**—*The liver*

is the main site. **Amount in blood**—20–40 mgm (average 30 mgm) of urea present per 100 ml of blood. Almost equally distributed in plasma and corpuscles. **Functions served by urea formation**—Urea formation helps to maintain the reaction of blood constant. Because, in it,

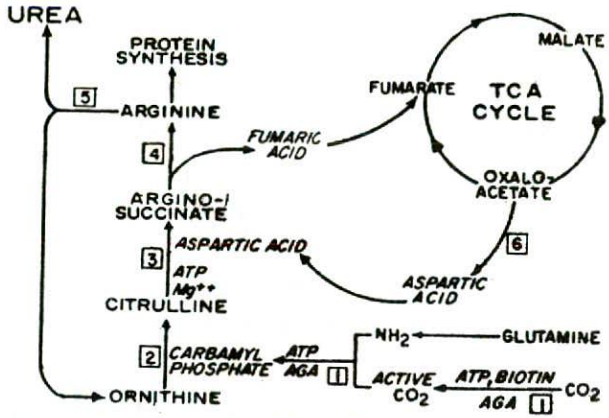


FIG. 397. Schematic representation of formation of urea in presence of enzymes: 1=carbamyl phosphate synthetase, 2=ornithine transcarbamylase (ornithine carbamyl transferase, 3=arginosuccinate synthetase, 4=arginine synthetase (arginine succinase), 5=ariginase, 6=transaminase.

one acid (carbonic acid) and two molecules of ammonia remain neutralised. **Excretion**—An adult taking a normal mixed diet excretes urea through urine an average of 30 gm daily (2%, if total urine volume be 1,500 ml). 80% of urinary nitrogen is excreted in the form of urea

AMMONIA

Sources—(1) **From deamination of amino acids,** both exogenous and endogenous. Although deamination takes place chiefly in the liver, recent observations indicate that *ammonia is also formed in the kidneys.* Ammonia formed in the liver is converted into various substances. Kidneys can deaminate amino acids normally. The amount increases during acidosis and falls in alkalosis. (2) **Certain ammonium salts taken** in food or as drug, e.g., ammonium chloride. **Fate and functions of ammonia**—(*a*) Form ammonium salts—The purpose served is to keep the blood reaction constant. (*b*) Ammonia may be utilised for the synthesis of amino acid, uric acid, nucleoproteins and other nitrogenous compounds. **Excretion**—With a mixed diet in an adult man the total daily output is about 0·7 gm. Ammonia nitrogen constitutes about 2–4% of total urinary nitrogen. **Relation with blood reaction**—In acidosis more ammonium salts will be formed. Reverse changes will take place in alkalosis. **Significance of variations**—Although ammonia is an end product of exogenous protein metabolism, yet its amount in the urine is determined by the relative proportion of acids and bases in the body. In conditions of acidosis it rises, in alkalosis it falls. Ammonia coefficient (*vide* page 579) is a reliable guide to the condition of acidosis or alkalosis of the body (*vide* 'Fate of Amino acids', page 578).

Inorganic sulphates. They are greatly produced from dietary proteins in the body. Consequently, they may be taken as the end products of exogenous protein metabolism. (The ethereal sulphates have a different life history altogether and has been discussed under 'Sulphur Metabolism'.)

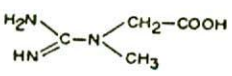
Uric acid. It is an index of both endogenous and exogenous protein metabolism. (*Vide* 'Uric Acid Metabolism'.)

BRIEF LIFE HISTORY OF THE END PRODUCTS OF ENDOGENOUS PROTEIN METABOLISM

From dietetic experiments in dogs it has been found that **creatinine** and **neutral sulphur** remain absolutely unchanged. So they are solely of endogenous origin. In this connection, the compound **creatine,** although not shown in this experiment, but when present in urine, should be regarded as derived from endogenous protein metabolism, because it is the precursor of creatinine. Thus these compounds are wholly endogenous. Half of uric acid is of endogenous origin and the other half is of exogenous origin. The life history of neutral sulphur has been discussed under *Sulphur Metabolism* and of uric acid under *Uric Acid Metabolism.* A brief summary of the life history of creatine and creatinine is given below:

CREATINE

Chemistry—Methyl guanidoacetic acid. *Total amount in the body*—90–120 gm in adult, 98% of it is present in the striated muscle as creatine phosphate. Skeletal muscles contain about 0·5% of creatine. It is also found in heart (about half of the amount in skeletal muscles, i.e., 0·25%), testes, brain and uterus, specially during pregnancy. **Amount in blood**—It is present in blood about 10 mgm per 100 ml and remain *mostly in the red cells.* As it is present inside the red cells, it is not filtered. Hence, it is usually not present in the urine.



Creatine

Origin and formation of creatine. (1) Creatine synthesis requires three amino acids, viz., *arginine, glycine* and *methionine* (as S-adenosyl methionine). (2) The compound *guanidoacetic acid is an intermediate step* in the synthesis of creatine. (3) The *methyl group of creatine is derived from methionine.* (4) *The stages* in creatine synthesis appear to be as follows: Two organs, i.e., kidneys and liver, are also involved for the complete synthesis of creatine. **In kidneys,** glycine and arginine react where amidine group ($-CNHNH_2$) of arginine is transferred to glycine with the formation of guanidoacetic acid (glycocyamine) by the enzyme *transamidinase.* Transamidinase enzyme is present only in kidneys and pancreas. But this enzymatic reaction mostly takes place in the kidneys. Methylation of guanidoacetic acid takes place **in the liver,** because the liver contains the enzyme *guanidoacetic methyl transferase.* Guanidoacetic acid is converted into creatine with the help of amino acid, methionine (activated form) in presence of enzyme *guanidoacetic methyl transferase* and *glutathione* (GSH). When methyl group of methionine is transferred to guanidoacetic acid to form creatine (methyl guanidoacetic acid), the methionine is converted into S-adenosyl homocysteine. Activation of methionine takes place by ATP when methionine is converted into S-adenosyl methionine. Recently, it has been shown that in mammals both transamidination and methylation reactions, involved in the synthesis of creatine, take place in the pancreas.

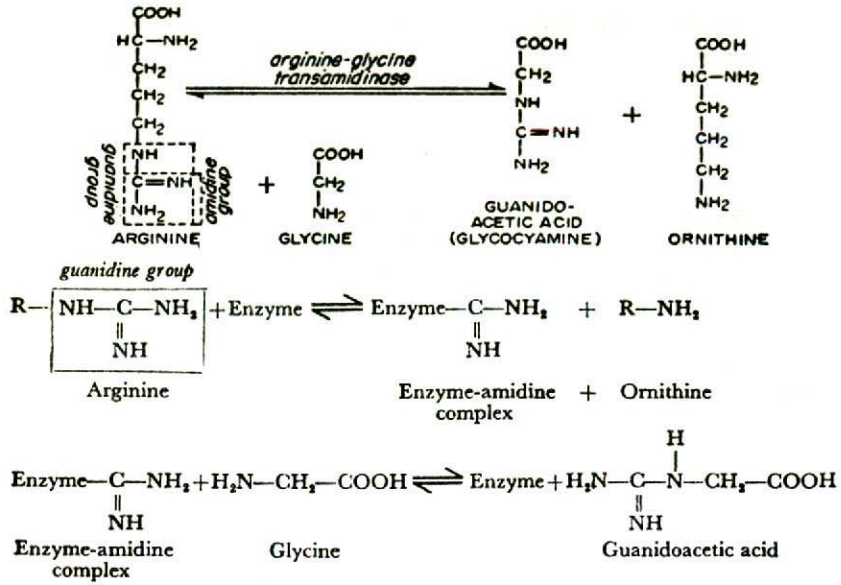


FIG. 398. Formation of guanidoacetic acid in the kidneys.

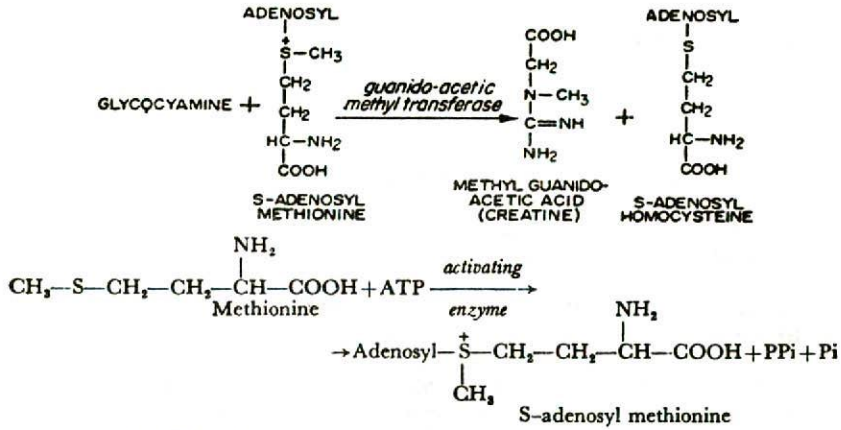


FIG. 399. Formation of creatine from glycocyamine in the liver.

Effects of creatine feeding. If creatine is ingested in *small amounts* (up to 1 gm daily), none is found in the urine, but in *moderate amounts* (up to 5 gm daily) a little is excreted as creatine and the rest is stored. But if *large amounts* (20 gm) of creatine be taken, the major part (15 gm) is excreted as such, another part (4·5 gm) is retained and a small part (0·5 gm) is excreted as creatinine in the urine. This shows that creatine is not a waste product. It is useful and there is a store for it in the body. Until this reservoir is filled up, no creatine will appear in the urine. Creatine synthesis is dependent on kidney transamidinase activity. The kidneys were thought to be

the only site of the transamidinating enzyme until recently. However, recent studies have indicated that the pancreas may play an unique role in the synthesis of creatine within the mammalian body.

Interrelation with creatinine. These two compounds are closely interrelated. *They are readily interconvertible* while in solution. Creatinine is anhydride of creatine having one molecule of water less. Acid medium favours the formation of creatinine, whereas alkaline medium favours the formation of creatine. But *in vivo* creatinine cannot be converted into *creatine*, although the reverse is the rule.

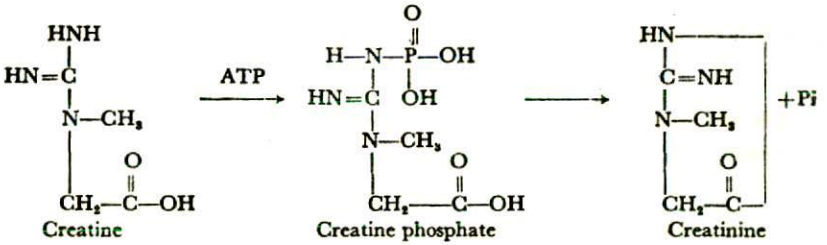


FIG. 400. Formation of creatinine from creatine.

Fate and functions. (1) Creatine is converted into creatine phosphate (phosphagen) which takes an essential part in the chemical changes underlying muscular contraction. Creatine, when given in moderate amounts by mouth, disappears completely in the body and nothing appears in the urine. This is supposed to be due to its conversion into creatine phosphate and subsequent storage in the muscles. (2) Creatine certainly has some function in tissues other than muscles but its nature is not known. (3) Creatine is the precursor of creatinine.

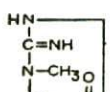
Excretion of creatine. Creatine is not generally present in the urine of normal adult males. But it may be excreted abnormally. Its excretion in the urine is determined by the following factors: (1) **Age**—Up to the age of puberty it is constantly present in the urine of both sexes. It has been suggested to be due to an increased production of creatine, induced in some unknown way, by the activity of growth impulse. It may also be due to a lower capacity of the undeveloped muscles for creatine storage. There is a third possibility in that the children possess less power to convert creatine into creatinine. (2) **Sex**—After puberty it is found intermittently in healthy females which is not related to menstruation. (3) **Pregnancy**—It is constantly present during pregnancy. It rises to a maximum of 1·5 gm daily after confinement and is probably derived from the involuting uterus. The sex difference of creatine excretion cannot be properly explained. That increased creatine excretion is not due to the less muscular development in females is proved by the fact that it occurs even in women who are highly trained physically. That sex has something to do here is supported by the observation that **creatinuria** is common in eunuchs. It may be easily induced in old people (naturally with diminished sex functions) by administration of small amount of creatine. (4) **Diet**—High protein and low carbohydrate diets increase creatine excretion. The former acts by stimulating tissue metabolism due to its high specific dynamic action. The latter acts indirectly by the absence of its sparing effects upon the breakdown of tissue protein.

(5) **Increased tissue breakdown**—In any condition that increases the breakdown of tissues, specially of striated muscles, as in starvation, prolonged diabetes mellitus, hyperthyroidism, fevers and other wasting diseases which increase the basal metabolic rate, the creatine excretion is increased. In certain diseases of muscles (myopathy) where muscles undergo degeneration, a large amount of creatine is excreted. In such conditions, 90% or more creatine appears in an unchanged form in the urine even when it is given by mouth in small amount. This is said to be due to a lower storage capacity of the muscles. *It is also probable that in this disease* (i.e., *myopathy*) *the reversible enzyme reaction, by which the broken creatine phosphate becomes resynthesised in the muscle, is absent.*

CREATININE

Chemistry. It is the anhydride of creatine.

Source. It is mostly formed from breakdown of creatine phosphate in the body. This process is not catalysed by any enzymes and is irreversible. Creatine-labelled with isotopic ^{15}N gives creatinine containing same isotopic ^{15}N. **Site of formation**—a large amount in the muscle. **Effects of creatinine feeding**—When orally administered nearly 80% is promptly excreted in the urine. Hence, it is considered to be a waste product. It is a no-threshold substance. It is filtered by the glomeruli and is also actively secreted by the tubular cells in the urine. **Amount in blood**—Normally it is present about 0·7–2·0 mgm per 100 ml. *This level is very constant* and it is considered to be pathological when its value increases about 2 mgm. Creatinine is also found in bile, sweat and in secretion of stomach and intestine. **Excretion**—about 1·2–2·0 *gm in adult males and* 0·8–1·5 *gm in adult females* are excreted *in* 24 *hours.* The amount excreted is remarkably constant for a particular individual.* It is related to the muscle bulk and is higher in muscular persons. This stands in great contrast with creatine excretion, which bears no relation to muscular development. The excretion increases during work and exercise but is immediately followed by a fall, so that the daily output remains constant.



Creatinine

Significance of variation. Creatinine represents the waste products of creatine metabolism and it arises in the body from the spontaneous breakdown of creatine phosphate. It serves practically no function in the body apparently. As its excretion is not related with food protein so its variations in the excretion indicate some of the metabolic disorders. Appearance of creatinine in urine is known as **creatinuria** when a small amount of creatine is also excreted along with creatinine. The creatine value gradually decreases as the maturity is advanced. Its excretion increases in fevers, starvation, on a carbohydrate-free diet and in diabetes mellitus. It may increase due to excessive tissue destruction releasing creatine or due to failure of creatine being properly phosphorylated. So creatinine excretion is independent of food proteins and is to be considered as an index of endogenous protein metabolism.

* **Creatinine coefficient.** It is the **creatinine and creatine** coefficient which represents the mgm of creatinine and creatine excreted per Kgm of body weight per day and the value normally averages 20–26 for men and 14–22 for women.

SYNTHESIS OF PROTEINS IN THE BODY

It is an established fact that body can synthesise protein molecules from the amino acids absorbed from the intestine. But the degree to which the amino acids themselves can be synthesised in the body is not yet fully decided. The plants and the lower animals can rapidly synthesise their body proteins from non-protein sources. But this power is very limited in the higher animals. It seems that as the animals advance in the scale of evolution, the chemical skill of synthesising amino acids becomes progressively limited. Yet, *it has been proved that human body can synthesise certain amino acids, such as glycine, alanine, glutamic acid,* etc. *The body cannot synthesise those amino acids which are known as essential amino acids.* It seems reasonable to think that if the body can deaminate amino acids, then by the reversible action of the same enzyme, it should be able to bring about synthesis of the amino acids as well. Experimentally it has been shown that amino acids can be synthesised by perfusing isolated liver tissue with ammonia and corresponding ketonic or hydroxy acids. These acids may come from the non-nitrogenous part of amino acids as well as from the breakdown products of carbohydrates and fats. The amino acids, keto acids and hydroxy acids are interconvertible in the body, the intermediate link being a keto acid, as shown by the Scheme No. 1.

1. $CH_3CHOH.COOH \rightleftharpoons CH_3CO.COOH \rightleftharpoons CH_3.CHNH_2.COOH$
(Lactic acid) (Pyruvic acid) (Alanine)

The following schemes will give a rough idea as to the mechanism of amino acid synthesis:

1. $R\text{-}\text{-}CH_2COCOOH + NH_3 \longrightarrow R\text{-}\text{-}CH_2COHNH_2COOH \xrightarrow{+H_2} R\text{-}\text{-}CH_2CHNH_2COOH + H_2O$
(Ketonic acid) (Hydroxy acid) (Amino acid)

2. $CH_3CHOH.COOH + NH_3 \longrightarrow CH_3.CHNH_2.COOH + H_2O$
(Lactic acid) (Alanine)

3. $CH_3.CO.COOH + NH_3 \xrightarrow{+H_2} CH_3.CHNH_2.COOH + H_2O$
(Pyruvic acid) (Alanine)

Synthesis of protein has been discussed under functions of DNA, mRNA and tRNA in protein synthesis (*vide* pages 15–16 and also under nucleoprotein metabolism, i.e., *functions of DNA and RNA*).

METABOLISM OF INDIVIDUAL AMINO ACIDS

Metabolisms of amino acids on physiological importance are discussed below:

GLYCINE. Glycine has the simplest chemical structure amongst the amino acids but it is one of the most versatile of them. It is α-aminoacetic acid (NH_2, CH_2· COOH). As the body can synthesise this amino acid so it is a *non-essential* amino acid. Glycine is glycogenic and antiketogenic.

Metabolic roles of glycine

1. **In the synthesis of haem.** By the use of labelled glycine, it has been observed that the α-carbon and nitrogen atoms of glycine are used in the synthesis of porphyrin moiety of haemoglobin. In such process glycine condenses with one molecule of succinyl CoA (active succinate) with the formation of α-amino-β-ketoadipic acid. β-ketoadipic acid on decarboxylation gives rise to δ-aminolevulinic acid. Two molecules of

$HOOC\text{—}\underset{\alpha\text{-amino-}\beta\text{-ketoadipic acid}}{\underset{|}{\overset{NH_2}{CH}}\text{—}CO\text{—}CH_2\text{—}CH_2\text{—}COOH}$ $\qquad$ $\underset{\delta\text{-aminolevulinic acid}}{H_2N\text{—}CH_2\text{—}CO\text{—}CH_2\text{—}CH_2\text{—}COOH}$

levulinic acid on condensation gives rise to porphobilinogen. From porphobilinogen ultimately the haem is formed (*vide* page 157).

2. **Interconversion of glycine and serine.** It has been observed that serine is produced in the body by interaction of glycine and formate

$$\underset{\text{glycine}}{H_2N{-}CH_2{-}COOH} \qquad \underset{\text{formic acid}}{H{\cdot}COOH} \qquad \underset{\text{serine}}{HO{-}CH_2{-}\overset{NH_2}{\overset{|}{C}}H{-}COOH}$$

in presence of liver enzyme *serine transhydroxymethylase*. Pyridoxal phosphate is required as coenzyme.

$$\text{Glycine} + \text{Formic acid} \underset{\text{pyridoxal phosphate}}{\overset{\text{enzyme}}{\rightleftharpoons}} \text{Serine} + H_2O$$

3. **In the formation of oxalate and formate from glycine.** The reactions (Fig. 401) are as follows:

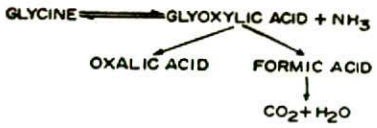


Fig. 401

Primary **hyperoxaluria** is a metabolic disease in which excess oxalic acid (HOOC—COOH) is excreted in the urine. The excess oxalate is of endogenous origin due to deficiency of alanine. Transaminase together with some impairment of oxidation of glyoxylate to formate may be the biochemical explanation for the inherited metabolic disease—the primary hyperoxaluria. In vitamin B_6 deficiency, excess oxalate is excreted in the urine if excess glycine is fed.

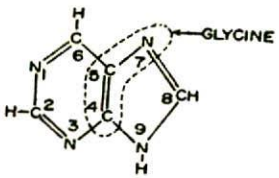


Fig. 402. Glycine in the synthesis of purine.

4. **In the synthesis of purine.** In the synthesis of purine, glycine provides carbon atoms at position 4 and 5, and nitrogen atom at position 7 of purine nucleus (Fig. 402).

5. **In the synthesis of glutathione.** Glutathione is tripeptide and contains amino acids—glutamic acid, cysteine and glycine. It is γ-glutamyl-cysteinyl-glycine and widely distributed in the tissues. The structure of glutathione (Fig. 403) is as follows:

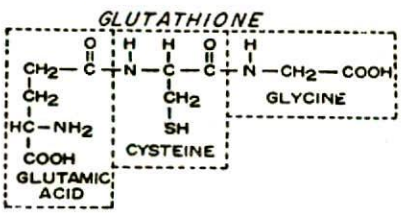


Fig. 403

The processes of synthesis of glutathione are as follows:

(*i*) Glutamic acid + ATP + Enzyme-I $\rightleftharpoons$ γ-Glutamyl phosphate enzyme-I complex + ADP

(*ii*) γ-Glutamyl phosphate enzyme-I complex + Cysteine $\longrightarrow$ γ-Glutamyl cysteine + Pi + Enzyme-I

(*iii*) γ-Glutamyl cysteine + ATP + Enzyme-II ⇌ γ-Glutamyl cysteine phosphate enzyme-II complex + ADP

(*iv*) γ-Glutamyl cysteine phosphate enzyme-II complex + Glycine ⟶ Glutathione (γ-glutamyl-cysteinyl-glycine) + Pi + Enzyme-II

6. **In the synthesis of hippuric acid.** Glycine, in conjugation with benzoyl CoA, forms the hippuric acid (benzoyl glycine).

$$\text{Benzoyl CoA} + \text{Glycine} \xrightarrow{\text{enzyme}} \text{Benzoyl glycine (Hippuric acid)}$$

7. **In the synthesis of bile acid.** Glycine, in presence of cholic acid, forms the glycocholic acid.

8. **In the synthesis of creatine.** In the synthesis of creatine, glycine takes an important part.

(*i*) Glycine + Arginine $\overset{\text{arginine-glycine transamidinase}}{\rightleftharpoons}$ Guanidoacetic acid + Ornithine

(*ii*) Guanidoacetic acid + S-Adenosyl methionine $\xrightarrow[\text{methyl transferase}]{\text{guanidoacetic}}$ Creatine + S-adenosyl homocysteine

9. Glycine may be oxidised in the TCA cycle through the succinate and glycine cycle of Shemin and Russell (Fig. 404). The pathways are as follows:

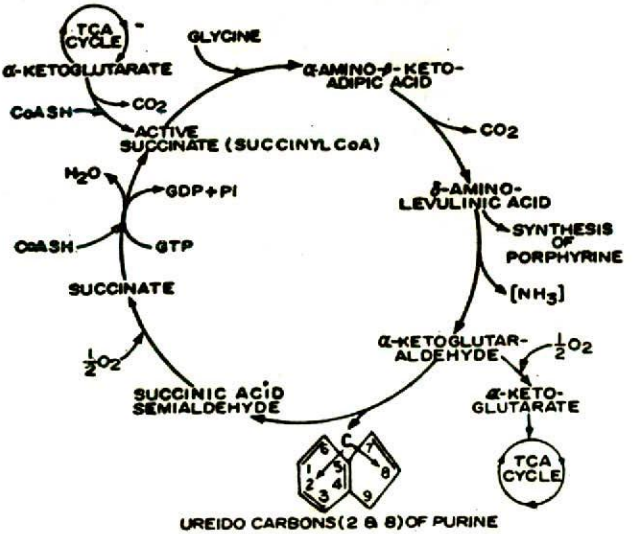


Fig. 404. Schematic pathways of succinate-glycine metabolism to the citric acid cycle.

Metabolism of glycine has been summarised in Fig. 405.

SERINE. It is α-amino-β-hydroxy propionic acid,

$$NH_2 \cdot CH \cdot COOH\ (CH_2OH),$$

non-essential and need not be included in the diet. It is glycogenic and can be easily synthesised from glycine. Interconversion of glycine and serine has been presented under glycine metabolism (page 590).

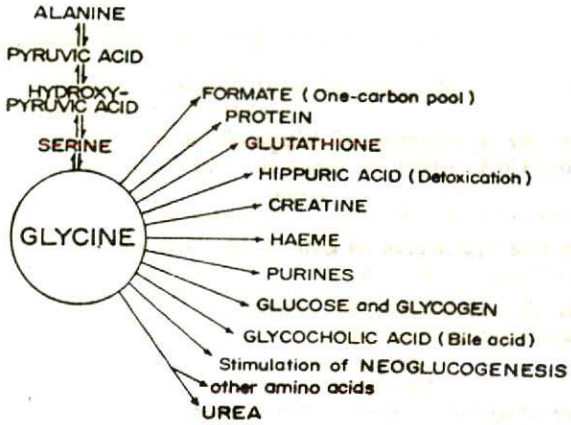


FIG. 405. Diagram showing the pathways of glycine metabolism.

Metabolic roles of serine

1. **Synthesis of serine.** Synthesis of serine from glycine and *vice versa* has been discussed (page 590). For the reaction, formate and pyridoxal phosphate are required.

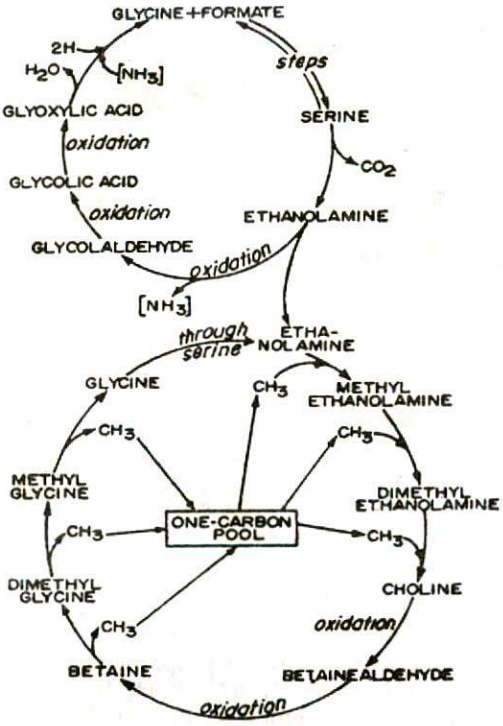


FIG. 406. Scheme shows the metabolic pathways of serine and glycine associated in the synthesis of choline and betaine with related compounds.

2. **In the synthesis of phospholipids.** Serine takes an important part in supplying the nitrogenous base of phospholipids—lecithin, sphingomyelin and cephalin. Choline, the nitrogenous base of lecithin, and ethanolamine, the nitrogenous base of cephalin are synthesised from serine. The pathways are shown in Fig. 406.

Serine is also directly involved in the synthesis of sphingosine, the complex amino alcoholic group of sphingomyelin (*vide* page 563).

3. **In the synthesis of cysteine.** Serine also takes part in the synthesis of cysteine. The pathways are as follows:

(*i*) Methionine $\xrightarrow{-CH_3}$ Homocysteine

(*ii*) Homocysteine + Serine $\xrightarrow[-H_2O]{\text{cystathionine synthetase, pyridoxal phosphate}}$ Cystathionine

(*iii*) Cystathionine $\xrightarrow[\text{cleavage enzyme, thionase}]{+H_2O}$ Homoserine + Cysteine

4. **In the synthesis of pyruvic acid.** Serine is glycogenic. It may be oxidised in the TCA cycle through the formation of pyruvic acid by deamination process (Fig. 407).

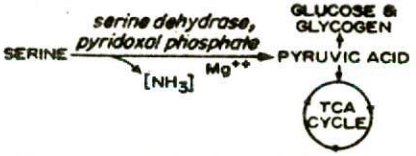


Fig. 407. Diagram shows the deaminated pathway of serine.

5. **In the synthesis of alanine.** By transamination process, serine may take part in the synthesis of alanine in presence of pyruvic acid.

Serine + Pyruvic acid $\xrightleftharpoons{\text{transaminase}}$ Alanine + Hydroxypyruvic acid

6. **In the synthesis of purine and pyrimidine.** β-carbon is the source of the methyl groups of thymine and of the carbons at positions 2 and 8 of purine nucleus (Fig. 408).

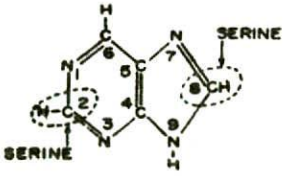


Fig. 408. Serine in the synthesis of purine.

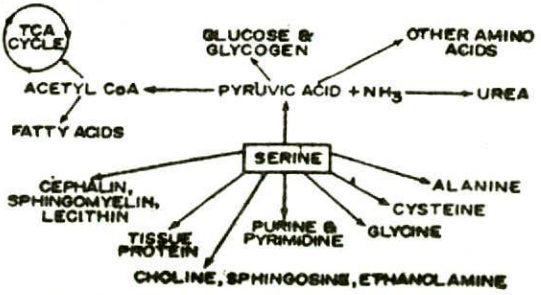


Fig. 409. Schematic representation of metabolic pathways of serine.

The metabolism of serine has been summarised in Fig. 409.

ALANINE. It is α-aminopropionic acid, $NH_2 \cdot CHMe^* \cdot COOH$. It is readily synthesised in the body and thus non-essential. It is also glycogenic and antiketogenic.

Metabolic roles of alanine

1. **In the formation of pyruvic acid.** Through the process of deamination by enzyme L-amino oxidase, the pyruvic acid is formed.

$$\text{L-Alanine} \xrightarrow[-NH_3]{\text{flavoprotein}} \text{Pyruvic acid}$$

In presence of α-ketoglutaric acid, the alanine may be transaminated with the formation of pyruvic acid and L-glutamic acid. The metabolic functions of alanine (FIG. 410) are very brief and are as follows:

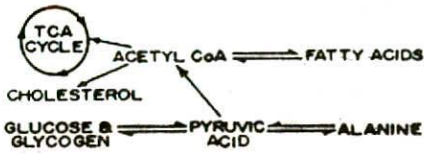
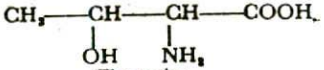


FIG. 410. Scheme showing the metabolic pathways of alanine.

THREONINE. Threonine is α-amino-β-hydroxy-*n*-butyric acid.



Threonine

It is an essential amino acid and thus indispensable in the diet of both man and rat. It is only synthesised in the micro-organism.

Metabolic roles of threonine

Synthesis of threonine. It is synthesised in micro-organism like Escherichia coli (*E. coli*) and also in yeast from (*a*) aspartic acid and (*b*) glycine and acetaldehyde. Pathways are as follows:

1. **From aspartic acid**

(*i*) L-Aspartic acid + ATP $\xrightleftharpoons{\beta\text{-aspartokinase}}$ ADP + β-Aspartyl phosphate

(*ii*) β-Aspartyl phosphate + NADPH + H^+ $\xrightleftharpoons[\text{dehydrogenase}]{\text{aspartic semialdehyde}}$ Aspartic-β-semialdehyde + $NADP^+ + Pi$

(*iii*) Aspartic-β-semialdehyde $\xrightleftharpoons{\text{homoserine dehydrogenase}}$ Homoserine

(*iv*) Homoserine $\xrightarrow[\text{pyridoxal phosphate}]{\text{ATP, enzyme}}$ Threonine

2. **From glycine and acetaldehyde**

Glycine + Acetaldehyde $\xrightleftharpoons[\text{pyridoxal phosphate}]{\text{glycinogenase}}$ Threonine

*Abbreviation for methyl, CH_3—

Catabolism of threonine. Threonine may be deaminated to form the α-ketobutyric acid by enzyme threonine dehydrase.

$$\text{Threonine} \xrightarrow[-NH_3]{\textit{threonine dehydrase}} \text{α-Ketobutyric acid}$$

α-Ketobutyric acid on transamination with glutamic acid or glutamine gives rise to α-aminobutyric acid. The threonine itself does not participate in transamination reaction.

$$\text{α-Ketobutyric acid} + \text{Glutamic acid} \underset{\textit{transamination}}{\rightleftharpoons} \text{α-Aminobutyric acid} + \text{α-Ketoglutaric acid}$$

α-Ketobutyric acid may be decarboxylated forming propionic acid.

The dehydrogenation of threonine may give rise to α-amino-β-ketobutyric acid and on spontaneous decarboxylation, aminoacetone is formed.

Threonine may split up into glycine and acetaldehyde by enzyme threonine aldolase.

$$\text{Threonine} \xrightarrow{\textit{threonine aldolase}} \text{Acetaldehyde} + \text{Glycine}$$

Threonine metabolism (Fig. 411) may be summarised thus

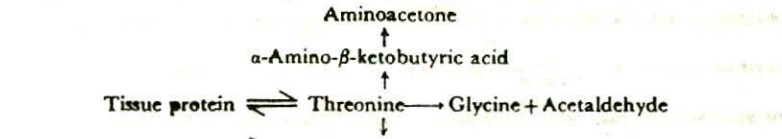


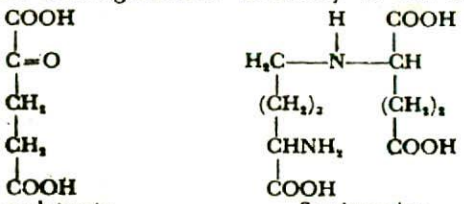
Fig. 411. Schematic representation of pathways of threonine metabolism.

LYSINE. It is α-ε-diaminocaproic acid,

$$NH_2—CH_2—CH_2—CH_2CH_2CHNH_2COOH$$

and cannot be synthesised in the animal. It is essential for growth. In micro-organism and in plants, the lysine is synthesised.

Synthesis of lysine. In the **yeast,** it is synthesised possibly from acetate and α-ketoglutarate. Recently it has been shown that



saccharopine is implicated by man and other mammals in lysine catabolism. The pathways (Fig. 412) are as follows:

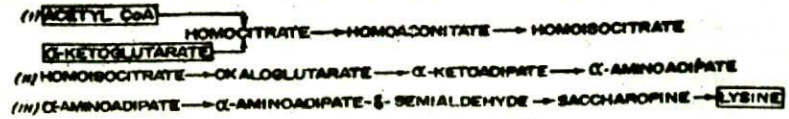


Fig. 412. Synthesis of lysine by the yeast.

In the **bacteria,** lysine is synthesised from aspartate and pyruvate (Fig. 413). The pathways are as follows:

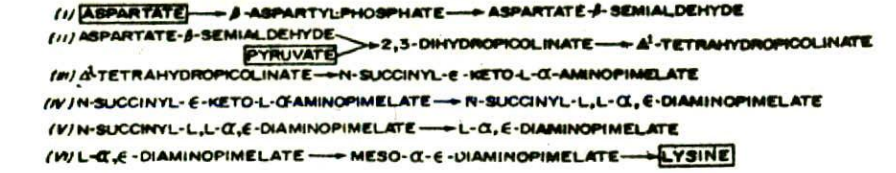


FIG. 413. Synthesis of lysine by the bacteria.

Catabolism of lysine. Lysine is ketogenic amino acids. Catabolism of lysine takes place in animal as in FIG. 414.

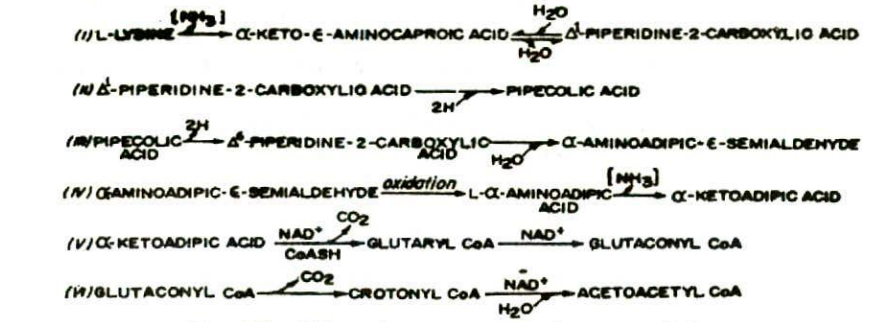


FIG. 414. Schematic representation of lysine catabolism.

In such processes, the ultimate product of lysine catabolism is acetoacetic acid. But there is other opinion of alternate pathway with direct and indirect evidences is the formation of α-ketoglutarate and its ultimate oxidation in the TCA cycle (FIG. 415).

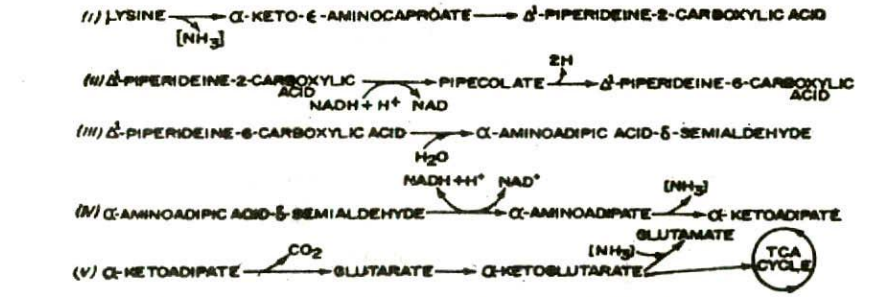
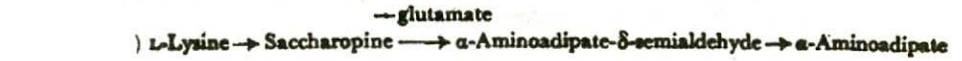


FIG. 415. Proposed pathways of lysine catabolism in the mammals.

Very recently saccharopine has been identified as a major product of ysine catabolism by rat liver mitochondria. Saccharopine is converted into -aminoadipic acid -δ-semialdehyde and then into α-aminoadipic acid. α-minoadipic acid is subsequently catabolised through the pathways as it is own in FIG. 415. It is suggested that L-lysine is catabolised *via* saccharoine and D-lysine is catabolised *via* pipecolate. Pathways of such lysine tabolism in mammals is as follows:



(ii) D-Lysine → → Pipecolate → α-Aminoadipate-δ-semialdehyde → → Saccharopine

α-Aminoadipate

The metabolism of lysine in animal system (FIG. 416) may be summarised as follows:

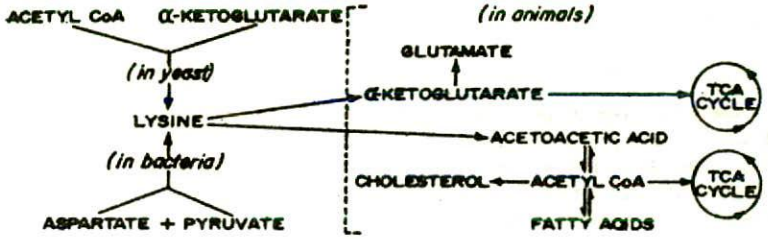
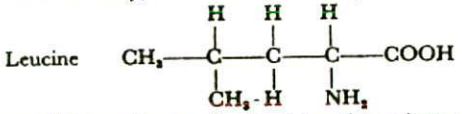


FIG. 416. Schematic representation of lysine metabolism.

LEUCINE. It is an essential amino acid and is not synthesised in mammals. Chemically, it is α-aminoisocaproic acid.



This amino acid may be synthesised by the micro-organism.

Synthesis of leucine. In micro-organism, it is synthesised from α-ketoisovaleric acid and acetyl CoA. The pathways of its synthesis (FIG. 417) are as follows:

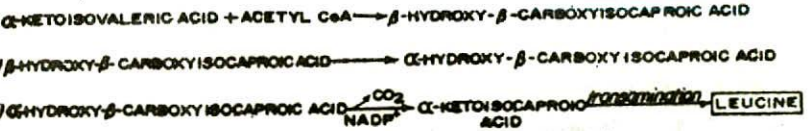


FIG. 417. Scheme depicts leucine biosynthesis.

Catabolism of leucine. Metabolic degradations of leucine (FIG. 418) in the animal body are as follows:

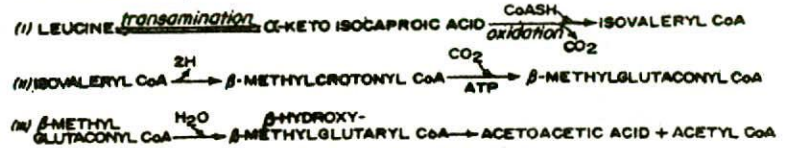
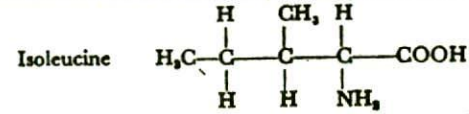


FIG. 418. Catabolism of leucine.

Acetyl CoA is convertible to ketone bodies and thus the leucine is ketogenic amino acid.

ISOLEUCINE. It is α-amino-β-methylvaleric acid. It is essential and



is not synthesised in the mammal. This amino acid is also synthesised in the micro-organism.

Synthesis of isoleucine. The pathways of the synthesis of isoleucine (Fig. 419) in the micro-organism are given below:

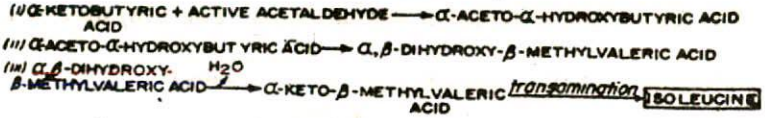


Fig. 419. Schematic representation of isoleucine biosynthesis.

Catabolism of isoleucine. Metabolic degradations of isoleucine in the animal body are the ultimate formations of acetyl CoA and propionyl CoA. The acetyl CoA may ultimately give rise to acetoacetyl CoA whereas the propionyl CoA gives rise to glucose. So the isoleucine is both ketogenic and antiketogenic. Metabolic pathways (Fig. 420) are as follows:

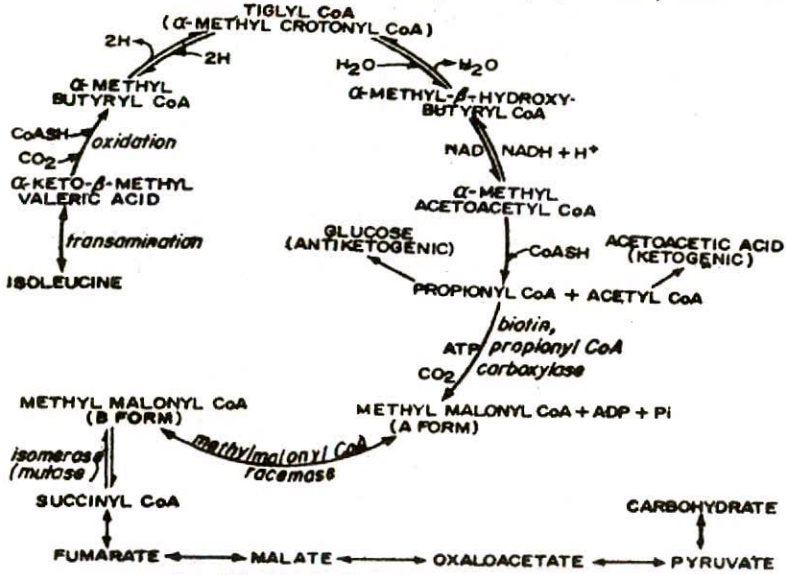
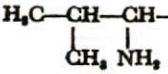


Fig. 420. Metabolic pathways of isoleucine.

VALINE. It is α-aminoisovaleric acid. It is essential and not synthesised in mammals. In the micro-organism, valine is synthesised from the pyruvic acid.



Valine

Synthesis of valine. Biochemical processes of the synthesis of valine in micro-organism have been presented in Fig. 421.

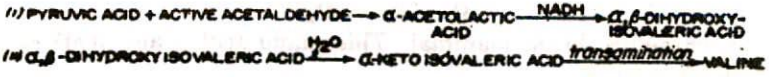


Fig. 421. Scheme shows biosynthesis of valine.

Pyruvic acid is decarboxylated with the formation of acetaldehyde which condenses with another molecule of pyruvic acid to form α-aceto-lactic acid. Thiamine pyrophosphate is required for such process of condensation.

Catabolism of valine. Metabolic degradation of valine in animals is the formation of pyruvic acid and glucose. It is antiketogenic and 3 molecules of carbon atoms of valine yield glucose in the diabetic animal. The pathways (FIG. 422) are as follows:

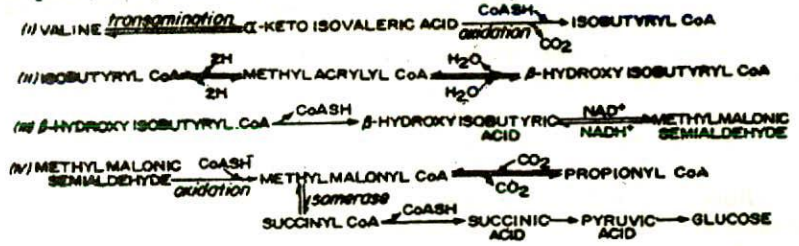
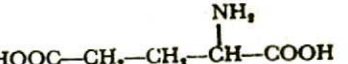


FIG. 422. Schematic representation of valine catabolism.

GLUTAMIC ACID. It is dicarboxylic amino acid and is readily synthesised in the body. Metabolically it is the most reactive amino acids. Chemically it is known as α-aminoglutaric acid.

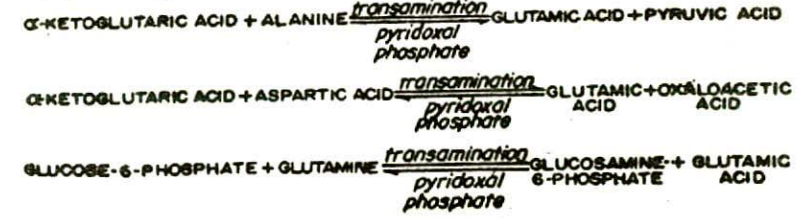


Glutamic acid

It is the active agent in the process of deamination and amination through the process of transamination reactions. It is glucogenic and antiketogenic. Metabolic pathways are shown below:

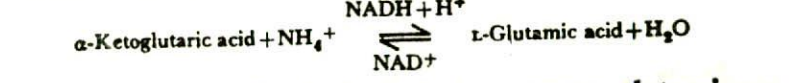
Synthesis of glutamic acid. It is synthesised in the body by the following processes:

(*a*) **through the process of transamination**

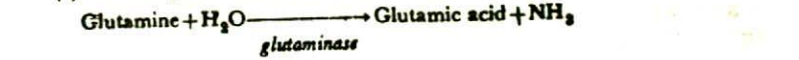


(*b*) **by the process of dehydrogenation**

Major portions of glutamic acid are provided by the action of liver enzyme glutamic dehydrogenase in presence of NAD or NADP.



(*c*) **from glutamine by hydrolysing enzyme, glutaminase**



Catabolism of glutamic acid. Metabolic degradations of glutamic acid are as follows:

1. **In the formation of α-ketoglutaric acid.** Glutamic acid is oxidised in the TCA cycle through the formation of α-ketoglutaric acid by transamination process with other keto acids like oxaloacetic acid and pyruvic acid (p. 599).

2. **In the formation of glutamine.** Glutamine provides a large proportion of the available free amino nitrogen of tissues and blood. It also constitutes metabolic nitrogen pool. It supplies ammonia for neutralisation of cations in the urine to conserve Na of blood.

Glutamine is synthesised from glutamic acid through the action of the enzyme *glutamine synthetase.*

$$\text{Glutamic acid} + \text{ATP} + NH_3 \xrightarrow[Mg^{++}]{\textit{glutamine synthetase}} \text{Glutamine} + \text{ADP} + \text{Pi}$$

Glutamine is formed in the liver, kidneys, brain, retina, etc. Major portions are synthesised in the liver. Glutamine has got immense role in the body. It serves as a *reservoir of amino nitrogen* in tissues.

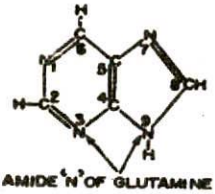
(*a*) It also takes part in transamination process. Glucosamine is formed from glucose-6-phosphate and glutamine by the process of transamination.

(*b*) Glutamine may be hydrolysed by the enzyme *glutaminase* with the formation of glutamic acid (*vide* above).

(*c*) Glutamine takes part in the process of detoxication in which phenylacetic acid, the toxic substance of the body is excreted with the formation of phenylacetyl glutamine.

$$\text{Phenylacetic acid} + \text{Glutamine} \longrightarrow \text{Phenylacetyl glutamine}$$

(*d*) **In the synthesis of purine.** Glutamine donates nitrogen at positions 3 and 9 of purine nucleus (Fig 423).



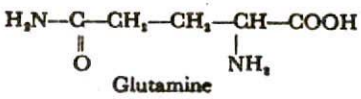


Fig. 423. Glutamic acid in the synthesis of purine.

(*e*) **In the synthesis of histidine.** Glutamine also takes part in the synthesis of histidine in micro-organism.

(*f*) **In the synthesis of NAD and NADP.** NAD and NADP, both contain adenine, so glutamine takes part in their synthesis.

(*g*) **In the synthesis of pyrimidine.** Glutamine also takes part in the synthesis of pyrimidine through initially the formation of carbamyl phosphate (p. 577).

3. **In the formation of γ-aminobutyric acid (GABA).** Glutamic acid content in the brain is high. An enzyme, glutamic decarboxylase is present and which decarboxylates the glutamic acid with the formation of γ-aminobutyric acid.

$$\text{Glutamic acid} \xrightarrow[\text{pyridoxal phosphate}]{\text{decarboxylase}} \gamma\text{-Aminobutyric acid (GABA)} + CO_2$$

4. **In the formation of glutathione.** Glutathione is tripeptide and contains glutamic acid, cysteine and glycine (p. 590).

5. **In the formation of alanine.** By the process of transamination, glutamic acid may take part in the synthesis of alanine from pyruvic acid.

$$\text{Glutamic acid} + \text{Pyruvic acid} \rightleftharpoons \alpha\text{-Ketoglutaric acid} + \text{Alanine}$$

6. **In the synthesis of proline.** Participation of glutamic acid in the synthesis of proline (FIG. 424) is as follows:

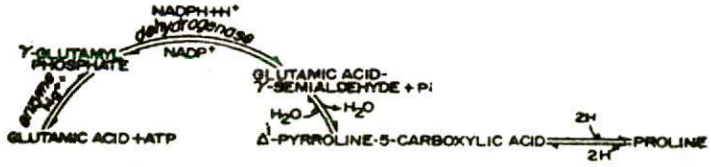


FIG. 424. Biochemical process of conversion of glutamic acid to proline.

7. **In the synthesis of ornithine.** Glutamic acid also takes part in the synthesis of ornithine (FIG. 425) through glutamic acid-γ-semialdehyde.



FIG. 425. Glutamic acid in ornithine synthesis.

Ornithine may be converted into arginine through Krebs-Henseleit urea cycle (p. 577).

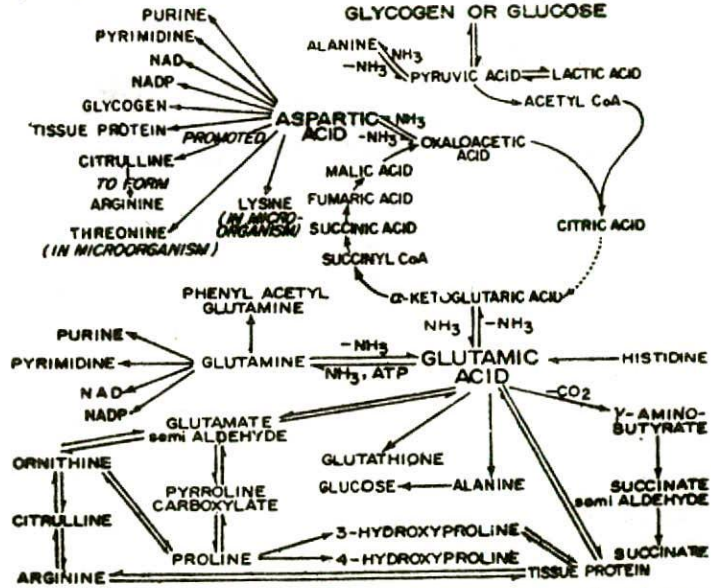


FIG. 426. Diagram showing the common pathways of glutamic and aspartic acids metabolism.

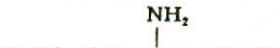
8. **In the synthesis of aspartic acid**

$$\text{Glutamic acid} + \text{Oxaloacetic acid} \xrightleftharpoons[\text{pyridoxal phosphate}]{\text{transamination}} \alpha\text{-Ketoglutaric acid} + \text{Aspartic acid}$$

9. **In the formation of glucose and glycogen.** Through the formation of α-ketoglutaric acid with the components of citric acid cycle, glutamic acid takes part in the synthesis of glucose and glycogen in the animal body.

Metabolic role of glutamic acid has been summarised in FIG. 426.

ASPARTIC ACID It is α-aminosuccinic acid and also dicarboxylic. It



Aspartic acid

is synthesised in the body and non-essential. Metabolism of aspartic acid is described below:

Synthesis of aspartic acid. The aspartic acid may be synthesised in the body by the process of transamination with the glutamic acid and oxaloacetic acid (*vide* above).

$$\text{Oxaloacetic acid} + \text{Glutamic acid} \xrightleftharpoons[\text{pyridoxal phosphate}]{\text{transamination}} \text{Aspartic acid} + \alpha\text{-Ketoglutaric acid}$$

Catabolism of aspartic acid. Metabolic degradations of aspartic acid are as follows:

1. **In the synthesis of purine.** Aspartic acid takes part in donating the nitrogen at position 1 of purine nucleus (FIG. 427).

2. **In the synthesis of pyrimidine.** For the formation of pyrimidine, initially aspartic acid reacts with carbamyl phosphate and ureidosuccinic acid is formed. From ureidosuccinic acid, uridine-5-phosphate is formed ultimately.

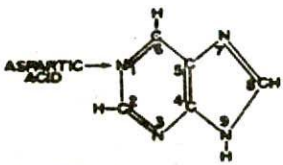


FIG. 427. Aspartic acid in purine biosynthesis.

3. **In the synthesis of NAD and NADP.** Through the synthesis of adenine, aspartic acid functions in the synthesis of NAD and NADP

4. **In the formation of arginine from citrulline.** Aspartate promotes the synthesis of arginine from citrulline.

(*a*) $\text{Citrulline} + \text{Aspartate} + \text{ATP} \longrightarrow \text{Arginosuccinate} + \text{AMP} + \text{PPi} + H_2O$

(*b*) $\text{Arginosuccinate} \rightleftharpoons \text{Arginine} + \text{Fumaric acid}$

5. **Oxidation in the TCA cycle and formation of glucose and glycogen.** Aspartic acid is oxidised in the TCA cycle through the formation of oxaloacetic acid, which may ultimately be converted into glucose and glycogen.

6. **In the synthesis of glutamic acid.** By the process of transamination, aspartic acid may take part in the synthesis of glutamic acid.

$$\text{Aspartic acid} + \alpha\text{-Ketoglutaric acid} \xrightleftharpoons[\text{pyridoxal phosphate}]{\text{transamination}} \text{Oxaloacetic acid} + \text{Glutamic acid}$$

7. **In the synthesis of threonine in micro-organism.** In the micro-organism, aspartic acid takes part in the synthesis of threonine (p. 594).

8. **In the synthesis of lysine in micro-organism.** In the micro-organism, aspartic acid also takes part in the synthesis of lysine (p. 595).

Metabolic pathways of aspartic acid have been presented in FIG. 426.

SULPHUR-CONTAINING AMINO ACIDS. Methionine, cysteine and cystine are the sulphur-containing amino acids of the body. Methionine is nutritionally essential amino acids, whereas the cysteine and cystine are not. But all of these are glycogenic.

Metabolism of METHIONINE. Methionine is α-amino-γ-methyl thiobutyric acid. Methionine is essential in the diet and may be substituted with its corresponding keto acids, α-keto-γ-methyl thiobutyric acid which on amination gives rise to methionine. The body cannot synthesise the corresponding keto acids. In micro-organism, the methionine can be synthesised by methylating the homocysteine.



Synthesis of methionine. In micro-organism, the mechanism of synthesis is as follows:

(*i*) Aspartic acid → β-Aspartyl phosphate → Aspartic semialdehyde → Homoserine

(*ii*) Homoserine + Cysteine → Cystathionine → Homocysteine + Serine

(*iii*) Homocysteine $\underset{-CH_3}{\overset{+CH_3}{\rightleftharpoons}}$ Methionine

Weissbach and his associates have shown that for the methylation process, though active methionine (adenosyl methionine) is required yet methyl-B_{12} coenzyme acts as methyl donor in transformation of homocysteine to methionine.

Catabolism of methionine. Methionine takes part in the following metabolic functions:

1. **As methyl donor.** Methionine as *active methionine* donates methyl group for the synthesis of creatine (p. 586) and choline (p. 592). Active methionine (FIG. 428) is formed as follows:

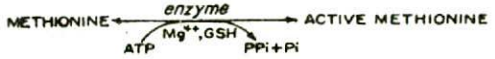


FIG. 428. Formation of active methionine from methionine.

2. **In the synthesis of cysteine and homoserine.** Main metabolic degradation of methionine is through cysteine and homoserine (FIG. 429), which are as follows:

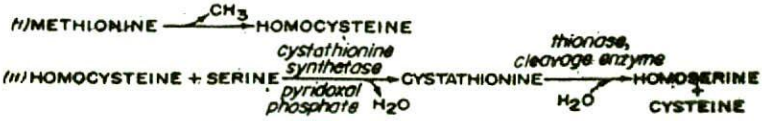


FIG. 429. Pathways of methionine breakdown to homoserine and cysteine.

This shows that methionine only donates S for the synthesis of cysteine.



Fig. 430. Pathways of homoserine breakdown.

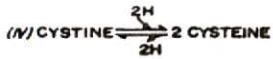


Fig. 431. Formation of cystine from cysteine and *vice versa.*

3. **In the formation of α-keto-γ-methyl thiobutyric acid.** Methionine may take part in the process of transamination with the formation of α-keto-γ-methyl thiobutyric acid.

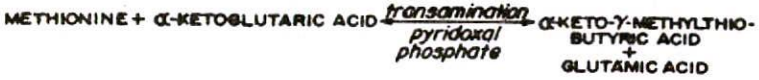


Fig. 432. Transamination process of methionine in the liver.

In liver disease, methylmercaptan is formed and is excreted in the urine. In such patients, foul odour in the breath is felt. It is due to activities of certain enzymes on the α-keto-γ-methyl thiobutyric acid with the formation of α-keto-γ-hydroxybutyric acid and methylmercaptan. The bad odour is due to the formation of this substance—methylmercaptan. α-Keto-γ-hydroxybutyric acid is ultimately oxidised in TCA cycle or may give rise to α-aminobutyric acid.

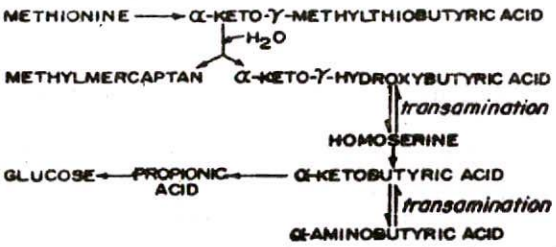


Fig. 433. Formation of methylmercaptan and pathways of methionine breakdown.

In **Fanchoni syndrome,** α-aminobutyric acid is excreted in the urine.

Homocysteine, the demethylated products of methionine, may be acted upon by the liver desulphydrase enzyme, *thionase* with the formation of α-ketobutyric acid, NH_3 and H_2S.

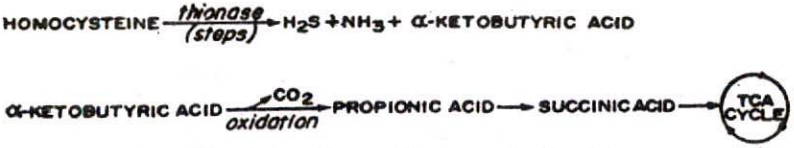
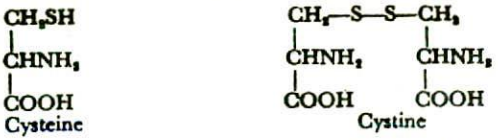


Fig. 434. Main pathways of homocysteine breakdown.

CYSTEINE AND CYSTINE metabolism. Cysteine is α-amino-β-mercaptopropionic acid and cystine is β, β-dithio-(α-aminopropionic acid).



Metabolism of cystine is through the degradation of cysteine. Main pathways of cysteine breakdown are as follows:

1. **In the formation of pyruvic acid, H_2S, NH_3**

$$\text{Cysteine} \xrightarrow[\text{desulphydrase}]{\text{pyridoxal phosphate}} H_2S + NH_3 + \text{Pyruvic acid}$$

2. Cysteine may transaminate with α-ketoglutaric acid with the formation of glutamic acid and β-mercaptopyruvic acid.

$$\text{Cysteine} + \alpha\text{-Ketoglutaric acid} \overset{\text{transaminase}}{\rightleftharpoons} \beta\text{-Mercaptopyruvic acid} + \text{Glutamic acid}$$

3. β-mercaptopyruvic acid is converted into pyruvic acid and 'S' which is converted into H_2S by reducing agents like cysteine and glutathione.

(*i*) β-Mercaptopyruvic acid ⟶ Pyruvic acid + S

(*ii*) 2 Cysteine + S ⇌ Cystine + H_2S

4. β-mercaptopyruvic acid after acting upon cyanide ion (CN^-) by the liver transulphurase enzyme may give rise to sulphocyanate (SCN^-) and pyruvic acid.

β-Mercaptopyruvic acid + CN^- ⟶ SCN^- + Pyruvic acid

5. β-mercaptopyruvic acid may react with sulphite (SO_3) with the formation of thiosulphate (S_2O_3) and pyruvic acid.

6. Another pathway of cysteine metabolism is through the formation of cysteinesulphinic acid, which may give rise to either pyruvic acid (FIG. 435) or taurine.

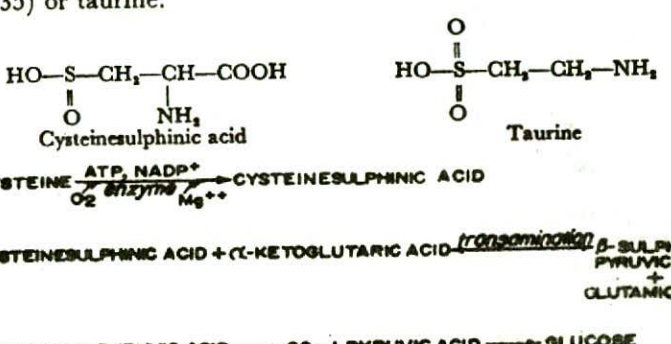


FIG. 435. Pathways of cysteine breakdown to sulphur dioxide (SO_2) and pyruvic acid.

Taurine is formed from cysteinesulphinic acid by two ways (Fig. 436).

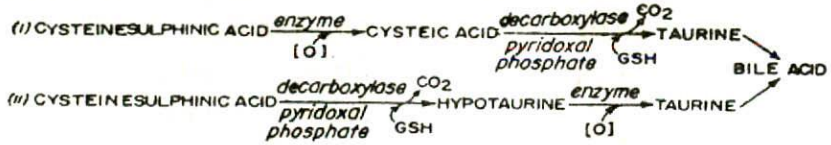


Fig. 436 Two pathways of bile acid formation.

Taurine reacts with cholyl CoA in the liver with the formation of taurocholic acid (bile acid).

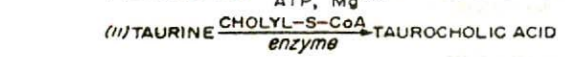


Fig. 437. Formation of taurocholic acid.

7. Cysteine takes part in the synthesis of glutathione (p. 590).

8. **In the process of detoxication.** Cysteine in its acetylation form, plays an important role in the detoxication of halogenated aromatic hydrocarbons.

Bromobenzene + Acetylated cysteine → *p*-Bromophenyl mercapturic acid

Metabolism of methionine, cysteine and cystine has been presented in Fig. 438.

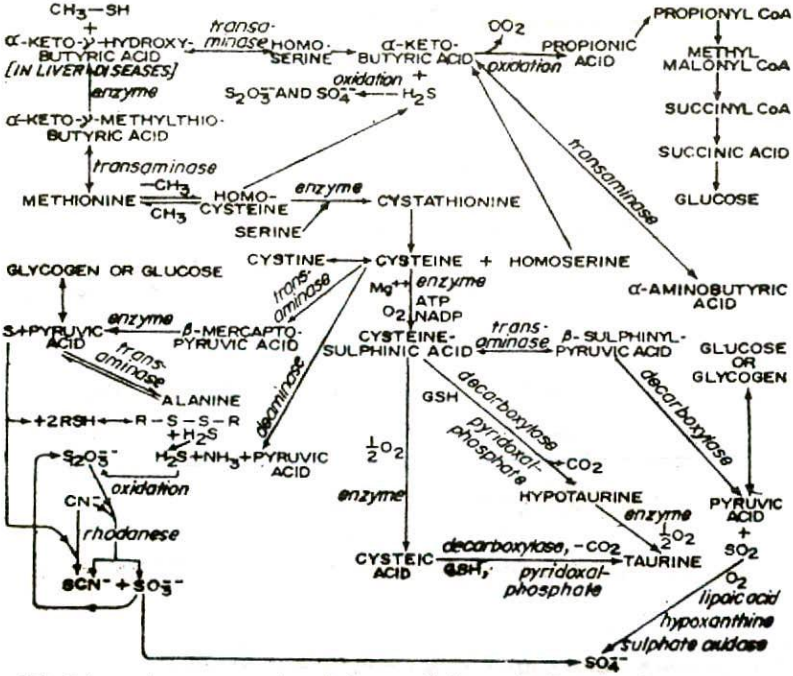


Fig. 438. Schematic representation of the metabolic path of methionine, cysteine and cystine where their interrelationship with other amino acids and carbohydrate are depicted.

Metabolic abnormalities of sulphur-containing amino acids. Cystinuria—It is an inherited metabolic disease where an increased amount of cystine (20–30 times the normal) is excreted in the urine. It is suggested that cystinuria is a manifestation of renal transport defect not only for cystine but also for lysine, arginine and ornithine. Milne (1961) claimed that cystinuria may be due to intestinal transport defect for all these four acids. But aetiology of this disease may require further revision. **Cystinosis**—It is different from cystinuria and is also known as *cystine-storage disease*. In this congenital disease, cystine crystals are stored in several tissues and organs (specially the R. E. system). This is generally accompanied by *aminoaciduria*. **Homocystinuria**—In this inborn metabolic error, plasma homocystine is excreted in the urine in large quantities with methionine. It is due to decreased activity of *hepatic cystathionine synthetase*. Subjects may also suffer, in addition, from mental retardation, bilateral posterior dislocation of lenses, palpable liver, etc. The cause of this defect is still debatable.

PHENYLALANINE AND TYROSINE. In animal, phenylalanine is an essential amino acid but tyrosine is not, because the latter is synthesised readily from the former. Tyrosine has got sparing action, because if tyrosine is fed then phenylalanine—need of the body is decreased. Tyrosine and phenylalanine have got immense metabolic role in the body. Both the amino acids are ketogenic and at the same time glycogenic (in fasting rats).

Metabolism. Both the amino acids are metabolised through the common pathways. Phenylalanine is initially but irreversibly hydroxylated to tyrosine. Tyrosine then undergoes series of metabolic changes leading to the formation of hormones (epinephrine, norepinephrine, thyroxine and tri-iodothyronine) and melanin. *Metabolic pathways are discussed below:*

Synthesis. *Phenylalanine is not synthesised in the body but, in the micro-organism, it is synthesised* where one molecule of D-erythrose-4-phosphate and two molecules of phospho-enolpyruvic acid are required.

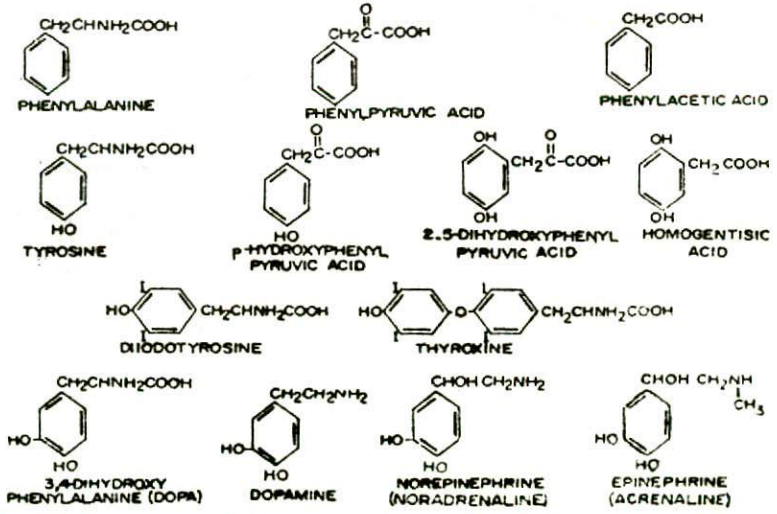


FIG. 439. Important metabolic products of metabolism of phenylalanine and tyrosine.

Tyrosine is synthesised in the body from phenylalanine by a complex enzymatic processes requiring hydroxylation of phenylalanine at the para (p-) position. The reaction requires enzyme phenylalanine hydroxylase and coenzyme NADP and folic acid coenzyme—*tetrahydropteridine.* The overall reaction is as follows:

$$\text{PHENYLALANINE} + O_2 \xrightarrow[\text{NADPH} + H^+ \text{ tetrahydropteridine}]{\text{hydroxylase}} \text{TYROSINE} + H_2O + \text{NADP}$$

Kaufman and Levenberg (1959) have described that hydroxylation of phenylalanine requires two steps:

(*i*) Phenylalanine + Tetrahydropteridine + O_2 ⟶ Dihydropteridine + Tyrosine + H_2O

(*ii*) Dihydropteridine + NADPH + H^+ ⟶ Tetrahydropteridine + NADP

Catabolism. Metabolic breakdowns of phenylalanine and tyrosine are as follows:

PHENYLALANINE. 1. **In the formation of tyrosine.** Formation of tyrosine has been described. Metabolic degradation of phenylalanine is mostly through tyrosine.

2. **In the formation of phenylacetic acid and phenyllactic acid.** Through the process of transamination phenylalanine may give rise to phenylpyruvic acid. Phenylpyruvic acid may further be reduced to phenyllactic acid. Phenylpyruvic acid, through the process of oxidative decarboxylation, gives rise to phenylacetic acid which is not reconverted into phenylalanine. It is toxic to the body.

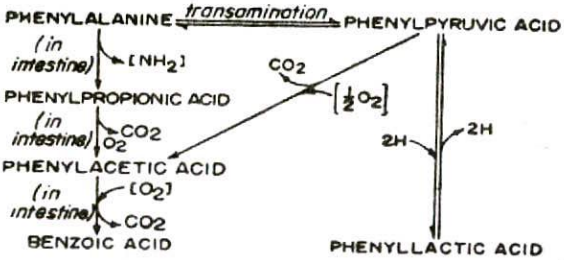


FIG. 440. Formation of phenylacetic and benzoic acids from phenylalanine.

Phenylalanine may undergo bacterial breakdown in the intestine by the bacterial enzyme with the formation of phenylacetic acid. This phenylacetic acid may be converted into benzoic acid (FIG. 440). These toxic substances are absorbed and detoxicated in the liver. Benzoic acid is detoxicated by glycine with the formation of excretable product—the **hippuric acid** and by glucuronic acid with the formation of excretable product—**benzoyl glucuronide.**

Phenylacetic acid is detoxicated by two processes, one with glycine and other with glutamine.

(*a*) Benzoic acid + Glycine ⟶ Hippuric acid
Benzoic acid + Glucuronic acid ⟶ Benzoyl glucuronic acid

(*b*) Phenylacetic acid + Glycine ⟶ Phenylaceturic acid
Phenylacetic acid + Glutamine ⟶ Phenylacetyl glutamine

3. **In the formation of tissue protein.** Like all other amino acids, phenylalanine also takes part in the synthesis of tissue protein.

TYROSINE. 1. **Oxidation in the TCA cycle.** Main pathways of this amino acid are the formation of fumaric acid and acetoacetic acid which are oxidised finally in the TCA cycle. Acetoacetic acid may also take part as usual in the formation of acetone, cholesterol and lipids. The detailed pathways are as follows:

First step of its oxidation is the process of transamination with α-ketoglutaric acid. By such process parahydroxyphenyl pyruvic acid is formed.

$$\text{Tyrosine} + \alpha\text{-Ketoglutaric acid} \underset{\text{pyridoxal phosphate}}{\overset{\text{transaminase}}{\rightleftharpoons}} p\text{-Hydroxyphenyl pyruvic acid} + \text{Glutamic acid}$$

p-Hydroxyphenyl pyruvic acid through steps gives rise to *homogentisic acid*. Homogentisic acid in presence of homogentisic oxidase is transformed into *maleylacetoacetic acid*. From maleylacetoacetic acid, *fumarylacetoacetic acid* is formed in presence of enzyme isomerase. Fumarylacetoacetic acid in presence of hydrolase splits into *fumaric acid* and *acetoacetic acid* (FIG. 441).

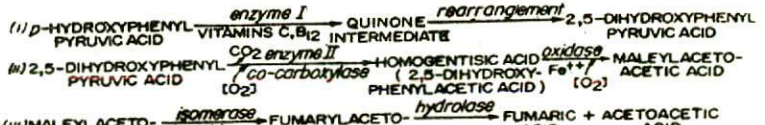


FIG. 441 Formation of fumaric and acetoacetic acids from *p*-hydroxyphenyl pyruvic acid.

Metabolic products (homogentisic acid and dihydroxyphenyl pyruvic acid) of tyrosine by the above reactions have got powerful inhibitory effects over the enzymes that take part in the oxidation process. But if vitamin C is present then this effect is removed.

2. **In the formation of phenol.** In the intestine, *paracresol* and *phenol* may be formed by bacterial enzymes (FIG. 442). These substances after being absorbed are detoxicated in the liver by sulphuric acid and glucuronic acid.

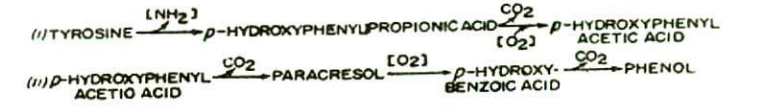
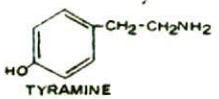


FIG. 442. Formation of phenol from tyrosine.

3. **In the formation of tyramine.** In the intestine, tyrosine may be decarboxylated by the bacterial enzymes with the formation of tyramine. Tyramine may be formed in the kidneys by O_2 deficiency. But in presence of sufficient O_2, tyrosine is instead deaminated by the tissue. Tyramine is a pressor substance.

$$\text{Tyrosine} \xrightarrow{-CO_2} \text{Tyramine}$$



4. **In the formation of epinephrine (adrenaline) and norepinephrine (noradrenaline).** For the formation of epinephrine, tyrosine is first oxidised to 3,4-dihydroxyphenylalanine (DOPA) by enzyme *tyrosinase*. DOPA is decarboxylated to hydroxytyramine (dopamine) by

DOPA decarboxylase. Hydroxytyramine on oxidation gives rise to norepinephrine (*arterenol*). After methylation of norepinephrine, epinephrine is formed. For the methylation of norepinephrine, adenosyl methionine is required (Fig. 444).

5. **In the formation of thyroid hormones.** Thyroid hormones—thyroxine and tri-iodothyronine are formed from tyrosine. It is claimed that di-iodotyrosine is first formed. Two molecules of di-iodotyrosine on condensation give rise to tetra-iodothyronine—the *thyroxine* where H_2O_2 and peroxidase enzyme are also required. Tri-iodothyronine is possibly formed after losing one atom of iodine from the thyroxine.

6. **In the formation of melanin.** Melanin is the dark pigment of the skin and is synthesised from tyrosine by complex enzymatic processes.

Until recently there was an idea that DOPA oxidase is required for the formation of melanin from DOPA. But recent observation claims that Cu-enzyme, *tyrosinase*, is responsible for the entire process.

(*i*) Tyrosine $\xrightarrow[tyrosinase]{+O_2}$ DOPA $\longrightarrow$ DOPA-quinone

(*ii*) DOPA-quinone $\longrightarrow$ 2-Carboxy-2,3-dihydro-5,6-dihydroxyindole $\longrightarrow$ 2-Carboxy-2,3-dihydro-indole-5,6-quinone $\longrightarrow$ 5,6-Dihydroxyindole

(*iii*) 5,6-Dihydroxyindole $\longrightarrow$ Indole-5,6-quinone $\longrightarrow$ Melanin

Fig. 443. Pathways of melanin formation from tyrosine.

In *albinism*, this melanin pigment is not formed due to inborn lacking of enzyme tyrosinase in the melanocyte cells. Melanin may be reversibly oxidised and reduced. The *oxidised* form of melanin is *black* and *reduced* form is *tan*.

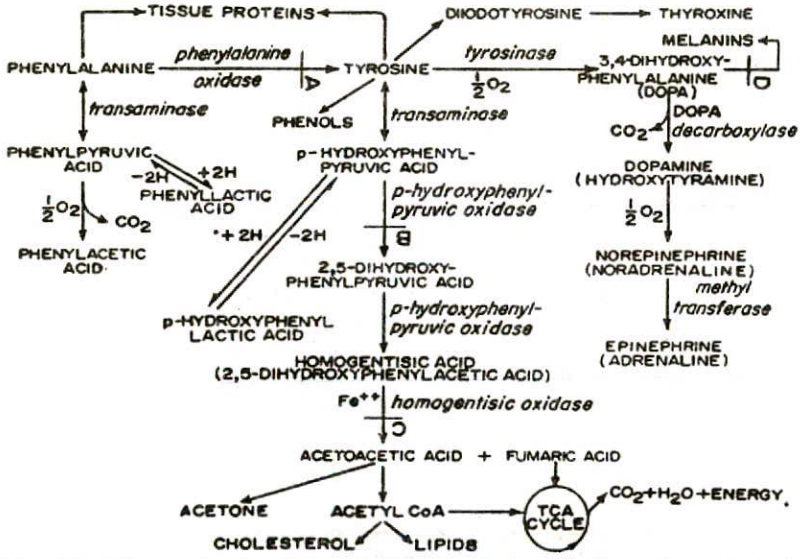


Fig. 444. Diagram showing the metabolic paths of phenylalanine and tyrosine and stages of inborn errors of metabolism shown by A = Phenylketonuria, B = Tyrosinosis, C = Alcaptonuria and D = Albinism.

Hereditary defects in phenylalanine and tyrosine metabolism. Four hereditary defective metabolic abnormalities of phenylalanine and tyrosine have been observed. They are known as *inborn errors of metabolism* and are as follows: (*a*) **Phenylketonuria**—In this condition, metabolism of phenylalanine is interfered with and as a result tyrosine cannot be formed from it in the normal way. The defect is due to absence of the enzyme phenylalanine oxidase responsible for this conversion. Children suffering from this disease have defective mental development and a high plasma phenylalanine and its increased excretion in urine. (*b*) **Tyrosinosis**—In this very rare case of metabolic abnormality, *p*-hydroxyphenyl pyruvic acid cannot be oxidised and so excreted in urine in considerable amounts. (*c*) **Alcaptonuria (Ochronosis)**—Due to deficiency of the enzyme homogentisic acid oxidase, persons suffering from this disease are unable to oxidise homogentisic acid which is excreted in urine. On standing, homogentisic acid (**alcapton**) present in urine becomes darker and finally black. (*d*) **Albinism**—The defect in this congenital disease is lack of enzyme tyrosinase in the melanocyte cells and so the pigment melanin are not formed. Localised failure of pigment formation might also occur, e.g., the eyes (FIG. 444).

TRYPTOPHAN. It is α-amino-β-indolepropionic acid. It is essential and should not be omitted in the diet. It is not synthesised in the animal but in micro-organism it may be synthesised. This amino acid is neither ketogenic nor glycogenic.

Metabolism of tryptophan. Though this amino acid is highly essential of the body yet it is inefficiently utilised by the body. A considerable amount of tryptophan is excreted through the urine.

Synthesis of tryptophan. It is not synthesised in the animal but in micro-organism, *E. coli*, it is synthesised.

Catabolism of tryptophan. Metabolic degradations of tryptophan are as follows:

1. Through the process of transamination, tryptophan is reversibly deaminated with the formation of β-3-indolepyruvic acid. β-3-indolepyruvic acid (FIG. 445) thus can substitute in the diet.

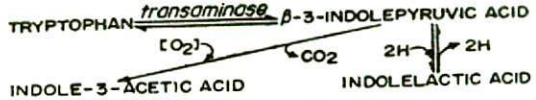


FIG. 445. Transamination process of tryptophan biosynthesis.

β-3-indolepyruvic acid may give rise to indole-3-acetic acid through oxidative *decarboxylation* and also indolelactic acid through reduction.

2. **Main pathways of tryptophan metabolism.** Main pathway of tryptophan metabolism is through the formation of formylkynurenine, kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid to α-ketoadipic acid, glutaryl CoA, acetyl CoA and ultimately with the oxidation in the TCA cycle. On the way of its breakdown *nicotinic acid* is also formed. Detailed pathways of the metabolic breakdown of tryptophan have been presented in FIG. 448.

3. **Formation of nicotinic acid and NAD.** Formation of nicotinic acid has been shown in FIG. 448. NAD (nicotinamide adenine dinucleotide)

is also formed. According to Hayaishi and others, **quinolinic acid,** the metabolic breakdown products of tryptophan, and in presence of phosphoribosylpyrophosphate (PRPPi) are acted upon by the mammalian liver

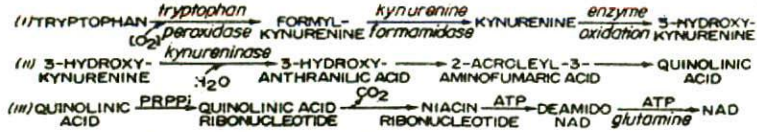


FIG. 446. Biosynthesis of nicotinic acid and NAD.

enzymes with the formation of quinolinic acid ribonucleotide. This quinolinic acid ribonucleotide on decarboxylation gives rise to niacin ribonucleotide. The detailed pathways have been summarised in FIG. 446.

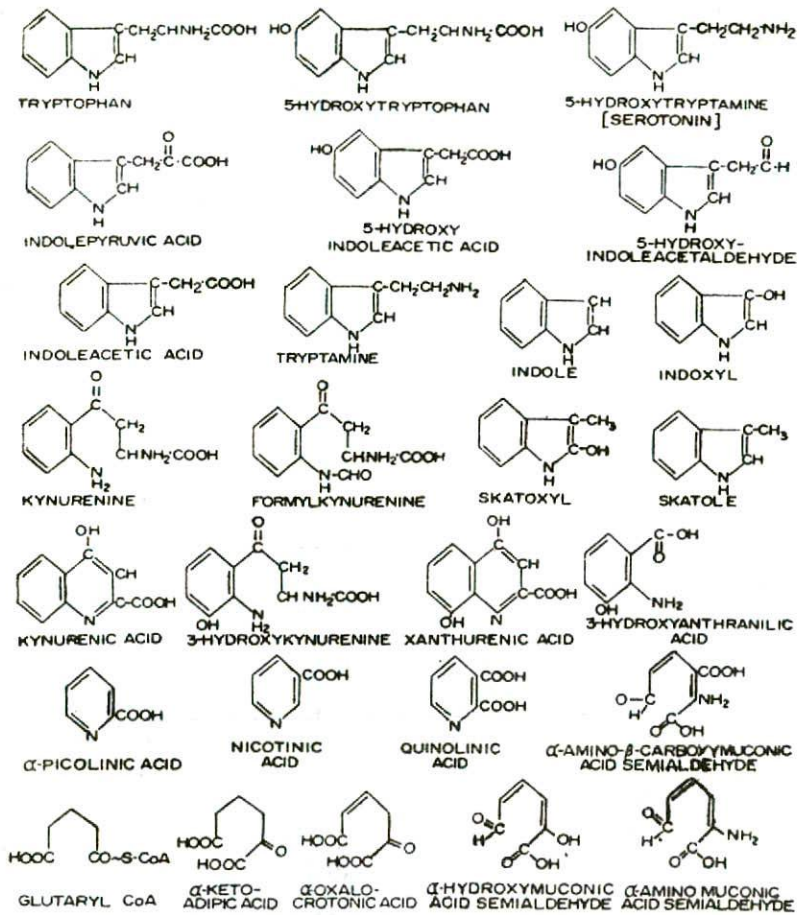


FIG. 447. Important metabolic products of tryptophan metabolism including nicotinic acid.

4. **Formation of serotonin.** Serotonin is 5-hydroxytryptamine. It is synthesised from tryptophan. Tryptophan on hydroxylation is converted into 5-hydroxytryptophan. 5-Hydroxytryptophan on decarboxylation gives rise to serotonin. Monoamine oxidase in the tissue destroys the

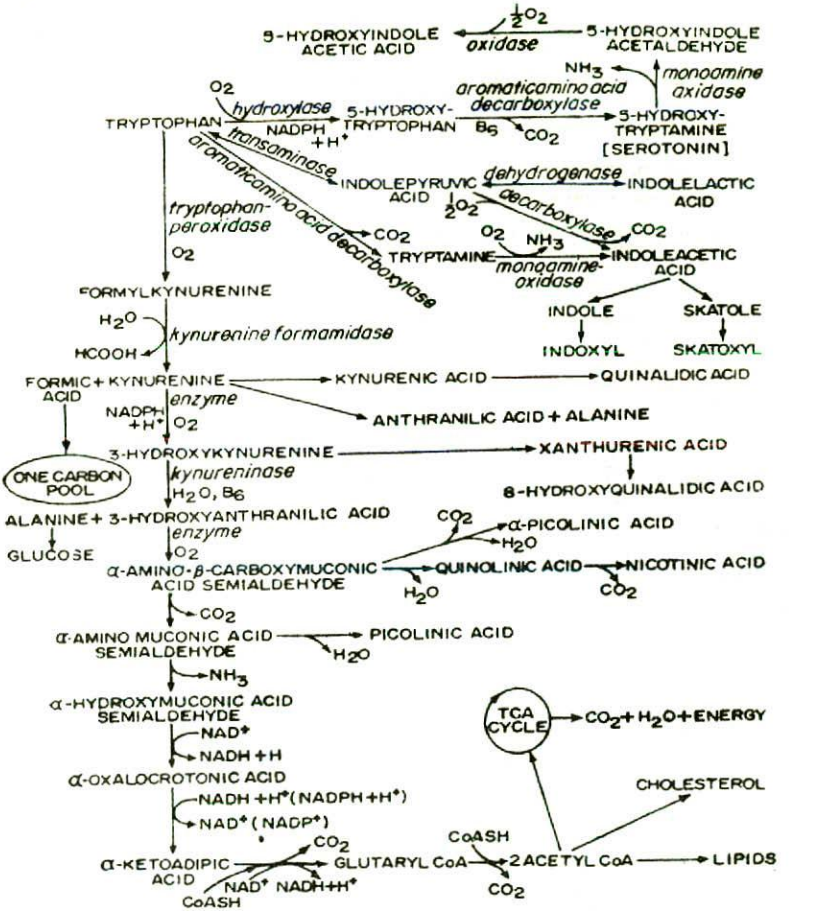
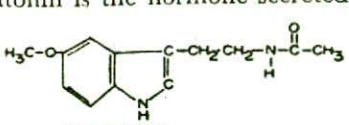


Fig. 448. Diagrammatic path of tryptophan metabolism.

serotonin through oxidative deamination and 5-hydroxyindoleacetic acid is formed. There are sufficient evidences that serotonin is related with important brain functions. It is found in the brain but is not uniformly distributed in the brain.

5. **Formation of melatonin.** Melatonin is the hormone secreted from pineal gland. This hormone may be synthesised from serotonin. Serotonin on acetylation by acetyl CoA gives rise to N-acetyl serotonin which, on methylation by s-adenosyl methionine, gives rise to



melatonin. Experiment shows that *major portion of melatonin is metabolised in the body* and a *small portion is bound and retained.* The melatonin is degraded by hydroxylation and followed by conjugation with sulphate and glucuronic acid.

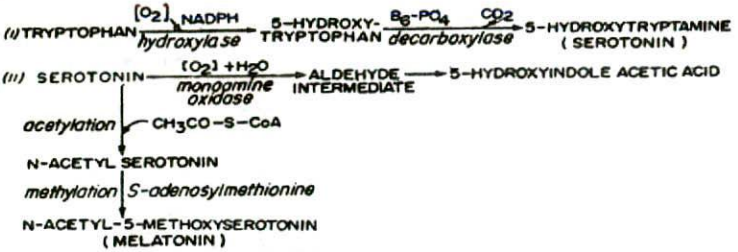


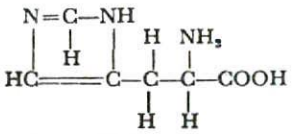
FIG. 449. Synthesis of serotonin and melatonin.

6. **Bacterial putrefaction in the intestine.** Tryptophan may undergo bacterial putrefaction in the intestine causing the formation of indoleacetic acid, indole, indoxyl, skatole, skatoxyl and also indoxyl sulphate. These are toxic substances and after being absorbed are detoxified in the liver by conjugation with sulphuric acid and glucuronic acid.

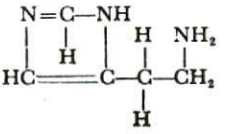
Metabolism of tryptophan has been presented in FIG. 448.

Abnormalities of tryptophan metabolism. Hartnup disease is a hereditary defective metabolic condition in which tryptophan is not normally metabolised and excreted as such along with indoleacetic acid. The symptoms are pellagra-like skin and mental abnormality.

HISTIDINE. It is α-amino-β-imidazolepropionic acid and appears to be essential for growth, but not for the maintenance of nitrogen equilibrium.



Histidine



Histamine

Metabolism of histidine. It is not synthesised in the animal body, but in the micro-organism it is synthesised.

1. Main pathway of histidine metabolism is the successive formation of urocanic acid, formimino-L-glutamic acid, glutamic acid, formic acid and ammonia.

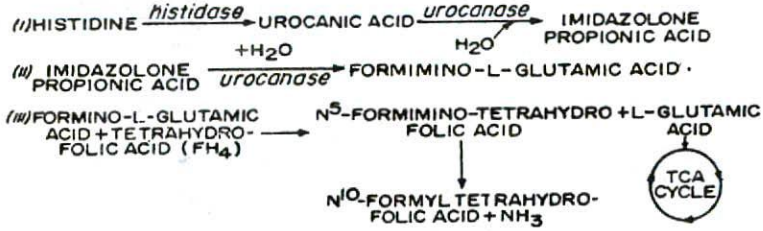


FIG. 450. Schematic representation of histidine catabolism.

2. **Formation of histamine.** This is formed in the intestine from histidine by bacterial decarboxylation. It is also normally formed in normal tissue and formed excessively in a state of allergic condition.

$$\text{Histidine} \xrightarrow{-CO_2} \text{Histamine}$$

Histamine is destroyed by *histaminase* present in the animal tissues, with the formation of β-imidazoleacetaldehyde. This β-imidazoleacetaldehyde is further oxidised in the liver by aldehyde oxidase into β-imidazoleacetic acid.

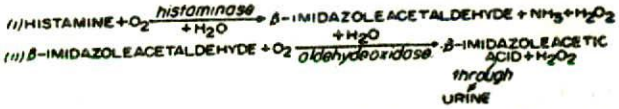


FIG. 451. Diagram shows the metabolic degradation of histamine.

β-imidazoleacetic acid is excreted through the urine.

Besides this, major portion of histamine is metabolised through the formation of 3-methylhistamine and 3-methylimidazole acetic acid, which are excreted in the urine.

3. **Histidine compounds.** Certain histidine compounds, viz., carnosine, anserine and ergothioneine, are found in the blood and tissues. *Carnosine* is β-alanylhistidine and *anserine* is β-alanyl-1-methylhistidine. These two are found in the muscle. *Ergothioneine* is found in the red blood corpuscles in large amount. It is not synthesised in the body and exclusively comes from dietary source.

Abnormalities of histidine metabolism. Two genetic disorders of this amino acid metabolism have been noted: (*i*) **Imidazole amino aciduria**—In this condition large amounts of carnosine and anserine as well as histidine and 1-methylhistidine have been observed to be excreted through urine together with a condition of *cerebromacular degeneration*. (*ii*) **Histidinemia**—The defect is due to hypoactivity of the enzyme histidase present in the liver, interfering with the normal degradation of histidine. Retardation of development of speech might take place in such disease.

Metabolic degradations of histidine have been presented in FIG. 452.

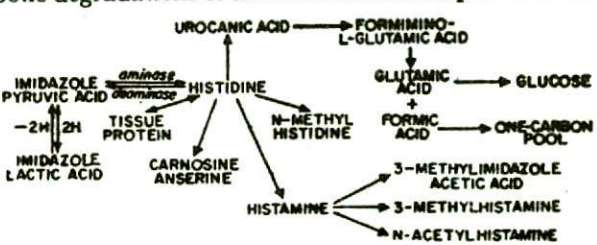


FIG. 452. Diagram showing metabolic pathway of histidine.

ARGININE, ORNITHINE AND CITRULLINE

Arginine is α-amino-δ-guanidovaleric acid and an essential amino acid. But this amino acid though synthesised in the body (page 578), yet the amount is so little which cannot maintain the adequate demand

of the body at the growing period. **Ornithine** and **citrulline** are formed in the body and are not essential.

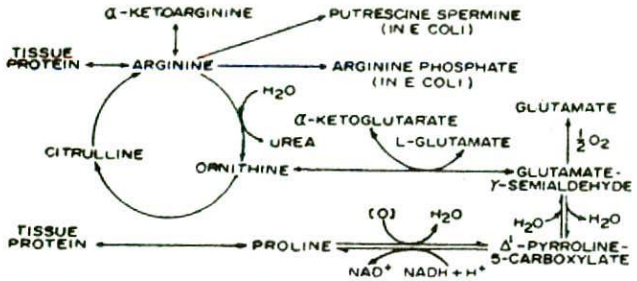


FIG. 453. Metabolic breakdown of arginine, ornithine and proline.

Metabolic role of these amino acids are mostly related in the formation of *urea* through Krebs-Henseleit cycle (pp. 579 & 584).

Metabolic roles of these three amino acids are discussed below:

Arginine

1. **In the formation of urea.** By enzyme *arginase*, the arginine is hydrolysed into ornithine and urea in the liver. Ornithine and citrulline also play an important role in the synthesis of urea. Ornithine practically acts as catalyst whereas citrulline donates one of its nitrogen for the synthesis of urea (p. 578).

2. **In the synthesis of creatine.** It is utilised in the synthesis of creatine. Creatine is guanidoacetic acid. Amidine group of arginine is transferred to glycine by arginine-glycine transamidinase with the formation of guanidoacetic acid.

3. **In the synthesis of ornithine.** Ornithine is formed under the following reactions:

(*a*) during the formation of creatine (p. 585),

(*b*) during the formation of urea.

4. **In the formation of γ-guanidobutyric acid.** Arginine also donates its amidine group to γ-aminobutyric acid for the formation of γ-guanidobutyric acid in the brain.

Ornithine

It is α-δ-diaminovaleric acid. It is synthesised from (*a*) arginine (*vide* below). It is closely related in the formation of urea. In the Krebs-Henseleit urea cycle, ornithine takes part in the synthesis of citrulline where ornithine reacts with carbamyl phosphate to form citrulline (p. 578).

Ornithine may take part in the synthesis of proline and glutamic acid. The pathways are as follows:

(*i*) Ornithine + Keto acid $\underset{\textit{transaminase}}{\rightleftharpoons}$ Glutamic acid-γ-semialdehyde + Amino acid

(*ii*) Glutamic acid-γ-semialdehyde

$\uparrow\downarrow$ *steps* $\qquad +H_2O \uparrow\downarrow -H_2O$

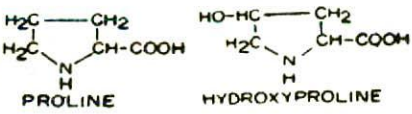
Glutamic acid $\qquad$ Δ′-Pyrolline-5-carboxylic acid $\underset{-2H}{\overset{+2H}{\rightleftharpoons}}$ Proline

FIG. 454. Pathways of proline and glutamic acid biosynthesis from ornithine.

Citrulline

Citrulline is α-amino-δ-ureidovaleric acid. It is formed from ornithine (p. 578) and takes part in the synthesis of urea. It donates one of its nitrogen for the synthesis of urea. Arginine is synthesised from citrulline in presence of aspartic acid, ATP and Mg^{++} (p. 578).

PROLINE AND HYDROXYPROLINE. Proline and hydroxyproline are among the dispensable amino acids in animals. **Proline** is pyrrolidine-α-carboxylic acid and **hydroxyproline** is γ-hydroxypyrrolidine-α-carboxylic acid. These are cyclic compounds and do not possess *free* amino group, but react like α-amino acid. They are non-essential and formed in the body from glutamic acid and ornithine.



Metabolism. From different studies, there is a close relation among the metabolism of proline, ornithine, arginine and glutamic acid. Hydroxyproline is the oxidation product of proline. There is no evidence that hydroxyproline is reconverted into proline. Both proline and hydroxyproline have been described to be glycogenic and this effect is manifested after being converted into glutamic acid.

Interrelationship of proline, hydroxyproline, arginine, ornithine and glutamic acid are summarised in FIG. 455.

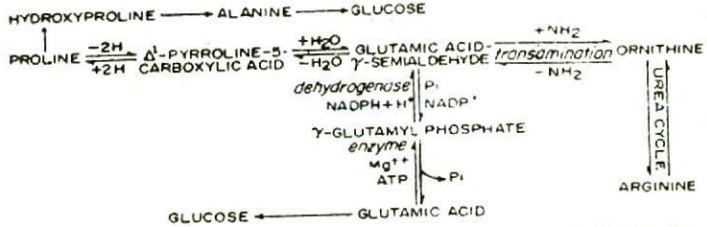


FIG. 455. Metabolic relationships between arginine, ornithine, proline, hydroxyproline and glutamic acid.

ENERGY TRANSFORMATION AND INTERRELATIONS BETWEEN PROTEIN, FAT AND CARBOHYDRATE METABOLISM

Energy transformation. In the analysis of energy transformation and biosynthetic processes in organisms, great advances have been made during the last three decades. The fundamental energy-liberating reactions which are essential for maintaining the diverse and sometimes complex cellular mechanisms are relatively simple. These reactions are examples of electron or hydrogen transport. To trigger biochemical reactions, energy-yielding reactions to energy-consuming steps might be simply coupled. The special form of chemical energy which is employed by biological systems for these coupling reactions is stored in the pyrophosphate bond of ATP (FIG. 456).

According to Krebs the fundamental step of energy transformation in living matter terminates in the synthesis of ATP at the liberation of free energy during metabolism. In these energy transformations oxidation, dehydration, reduction, hydration, acetylation, phosphorylation, amination, decarboxylation and transamination are a few basic processes. For protein, fat and carbohydrate metabolism, acetyl CoA, α-ketoglutaric acid

and oxaloacetic acid are key substances that are formed prior to any significant energy liberation. Acetyl CoA, α-ketoglutaric acid and oxaloacetic

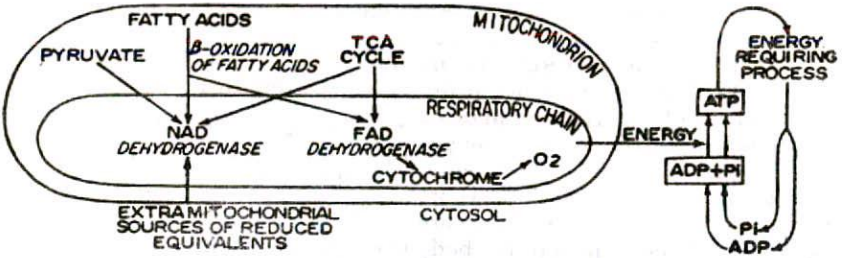


FIG. 456. Diagram explains the relationship of electron transport in the respiratory chain to the fatty acid oxidation and extra-mitochondrial sources of reducing equivalents and role of ADP in the respiratory control.

acid are broken down in the TCA cycle that liberates more than two-thirds of the cellular energy. The reactions of numerous food materials may be summarised in FIG. 457.

Interrelation. There are much informations about the chemical mechanism of metabolism in general. There are also certain common reversible reactions through which one type of food may be converted to another type and *vice versa*. The metabolic reactions of different foodstuffs produce intermediates which lead to final oxidation in a oxidative cycle known as TCA cycle. This cycle integrates carbohydrate, fat and protein metabolism and shows the chemical reactions of interrelationship of path formation of carbohydrate to protein and fat and *vice versa*. The reactions of this cycle provide the mechanism of oxidation of fatty acids through the oxidation of **acetyl CoA** and that of carbohydrate through the oxidation of pyruvic acid and acetyl CoA. The final stages of amino acids oxidation are also carried out through this cycle.

There are some amino acids, e.g., *leucine*, *tyrosine* and *phenylalanine* which go to form acetoacetic acid, and fumaric acid and acetyl CoA. These are oxidised through this cycle or acetyl CoA may be used for the synthesis of fatty acids, cholesterol and other acetylation reactions. There are other amino acids, e.g., *alanine*, *cystine*, *cysteine* and *serine* which go to form pyruvic acid, and finally they are either oxidised in this cycle or converted into glycogen or glucose through reversible glycolytic path or oxidised to acetyl CoA which go to form fatty acids, cholesterol, etc.

Some amino acids, e.g., aspartic and glutamic acids after transamination reaction, enter directly in the TCA cycle at oxaloacetate and α-ketoglutarate stages respectively from where they are either oxidised in the TCA cycle or go to form carbohydrate or fatty acids, etc., through this cycle.

Other amino acids, *arginine*, *histidine*, *ornithine*, *proline*, etc., also enter at the α-ketoglutaric acid stage through glutamic acid and serve the same purpose. These amino acids are also synthesised through this cycle, e.g., pyruvate to alanine, oxaloacetate to aspartate and α-ketoglutarate to glutamic acids respectively. Protein-sparing actions of carbohydrate indicate that protein may be formed from carbohydrate through this cycle.

Increased amount of ATP is formed due to intake of protein diet indicating large amount of carbohydrate being formed from protein and oxidised through TCA cycle.

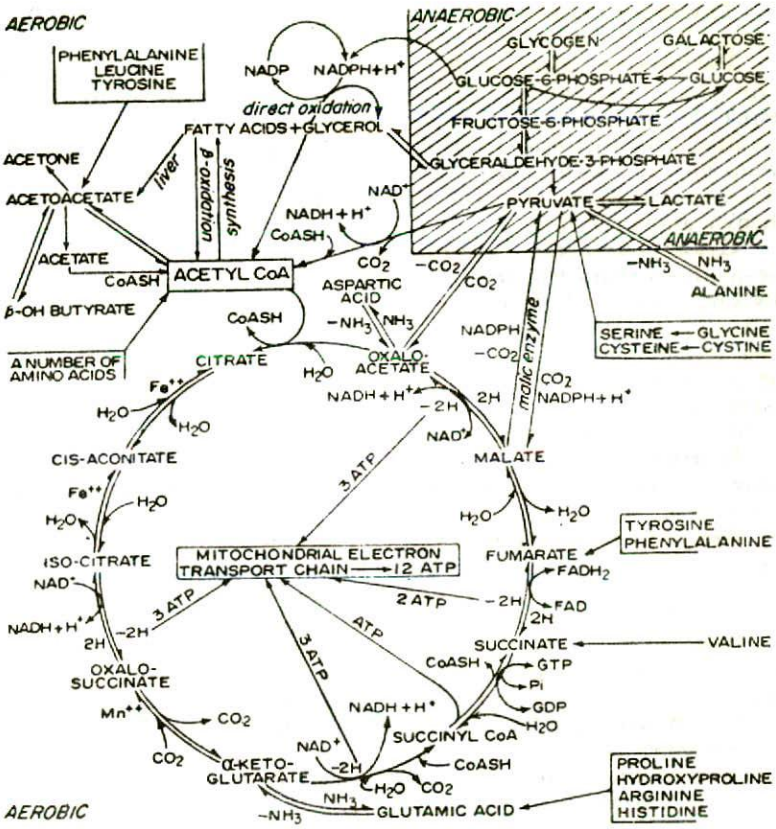


FIG. 457. Diagram showing TCA cycle where interrelation and integration of carbohydrate, protein and fat metabolisms are briefly depicted.

ROLE OF ENDOCRINES ON PROTEIN METABOLISM

1. **Anterior pituitary.** Growth or somatotrophic hormone (STH) is found to be protein anabolic, provided the insulin level is high. There is marked increase in the plasma glutamine as well as increased uptake of N_2 by the tissues when growth hormone is introduced.

2. **Insulin.** Insulin prevents neoglucogenesis. It stimulates protein synthesis and growth. Insulin and growth hormone of the anterior pituitary operate in close co-ordination since in the absence of either of the two, protein synthesis is retarded.

3. **Adrenal cortex.** Adrenal glucocorticoids are protein catabolic. Excess of this hormone causes retardation of growth, negative nitrogen balance, etc., due to increased neoglucogenesis.

4. **Thyroid.** Thyroxine stimulates protein synthesis. Excessive secretion of thyroxine promotes protein catabolism, wasting of the muscles, etc.

ROLE OF VITAMINS ON PROTEIN METABOLISM

Thiamine (vitamin B_1). Thiamine helps the enzyme system which is responsible for the synthesis of fats from proteins.

Pyridoxine (vitamin B_6). This vitamin takes part in the normal tryptophan metabolism, being a constituent of the enzyme *kynureninase*, responsible for conversion of kynurenine to anthranilic acid and 3-hydroxykynurenine to 3-hydroxyanthranilic acid. Pyridoxine acts as a prosthetic group for the enzyme *transaminase* and as a coenzyme for those enzymes which decarboxylate tyrosine, arginine, glutamic acid and 3,4-dihydroxyphenylalanine (DOPA).

Cyanocobalamin (vitamin B_{12}). This plays an important role in the synthesis of nucleic acid. Deficiency of it causes a disturbance of the DNA metabolism. This vitamin helps in connection with the formation of methyl groups and in the reactions of transmethylation as in the biosynthesis of methionine. It acts as coenzyme and has been found to catalise the enzymatic conversion of glutamate to β-methyl aspartate in bacterial system and to catalise the isomerase reaction in animal tissues where methylmalonyl CoA is converted to succinyl CoA.

Biotin (vitamin H). Biotin is linked up with riboflavin. It acts as a coenzyme and helps in decarboxylation. It also helps in the deamination of threonine, serine and aspartic acid.

Ascorbic acid (vitamin C). This vitamin helps in the development of protein matrix in the bones. Ascorbic acid takes part in the oxidation of *p*-hydroxyphenyl pyruvic acid to homogentisic acid which is the intermediate metabolic end product of tyrosine metabolism.

Folic acid. It is related with nucleoprotein metabolism. The reduced form of folic acid known as tetrahydrofolic acid acts as a coenzyme in the transfer of formyl and hydroxymethyl group in the biosynthesis of purines.

Riboflavin. Riboflavin is a constituent of FMN and FAD which act as coenzymes in enzyme system, e.g., L-amino acid oxidase, xanthine oxidase, and thus related to protein metabolism. [*Vide* also under the chapter of *Vitamins*.]

NUCLEOPROTEIN METABOLISM

Nucleoproteins. Nucleoproteins contain phosphoric acid and also other prosthetic group. Hence, they should be placed in a special class. Nucleoproteins are formed by the union of *nucleic acid with basic protein*. The nucleic acids, viz., deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are found both in animal and plant cells. In the animal cells DNA is the chief nucleic acid—specially found in chromosome, whereas RNA is found in smaller quantity in the nucleus. RNA is also found in ribosomes (*Claude's particles*) inside the cytoplasm where they help in the synthesis of proteins. The breakdown and synthesis of RNA are continuously going on within the cell with the help of intracellular enzymes. DNA is a stable compound and controls the hereditary characteristics of the cell. The nucleic acid is made up of many nucleotides (mononucleotide) making a double helical structure of two polynucleotide chains. Each mononucleotide is again composed of

nucleoside and phosphoric acid. Each nucleoside is composed of a pentose and a base as follows:

1. **Pentose.** The pentose, *ribose*, is present in ribonucleic acid (RNA), whereas *deoxyribose* is present in deoxyribonucleic acid (DNA).

2. **Bases.** The bases are of two types: (*a*) *Purine bases*—adenine and guanine (FIG. 458), are present in both the nucleic acids. (*b*) *Pyrimidine bases*—cytosine and uracil are present in RNA, whereas cytosine along

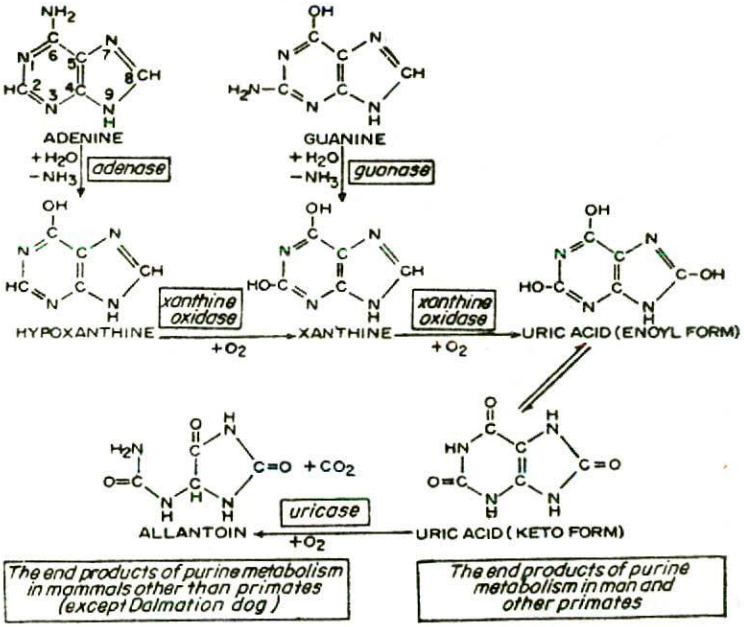


FIG. 458. Schematic representation of metabolic degradations of adenine and guanine.

with thymine is present in DNA. In addition to DNA and RNA, nucleosides are also component of a number of coenzymes, like NAD and NADP and metabolically important compounds, such as ATP, UDPG, etc.

Since nucleoprotein is a composite product, its complete metabolism will mean the life history of all the component parts in it. The protein parts of the nucleoprotein molecules contain a high proportion of *diamino acids* and undergo the same fate as the other proteins in the body. The phosphoric acid part and the pentose molecule are treated by the body in the same way as the phosphates and carbohydrates derived from other sources. *The special importance of the metabolism of nucleoproteins lies in the life history of its characteristic ingredients—the purine and the pyrimidine bases.*

Nucleoprotein metabolism may be of two types: *exogenous* and *endogenous*. The **exogenous nucleoprotein metabolism** is undergone by the end products of nucleoprotein digestion, after they are absorbed. These end products are *phosphoric acid*, *carbohydrate* (pentose), the *pyrimidine nucleosides* (which are not further digested), the *two purine bases* (*adenine* and *guanine*) and probably some nucleotide. The **endogenous nucleoprotein metabolism** starts in a different way (FIG. 459). *There are two kinds of tissue*

nucleotidases. The *phosphonucleotidase* splits off phosphoric acid and forms nucleosides. But the *purine nucleotidase* (more effective in slightly alkaline medium) takes away the purine bases, leaving phosphoric acid molecule combined with carbohydrate.

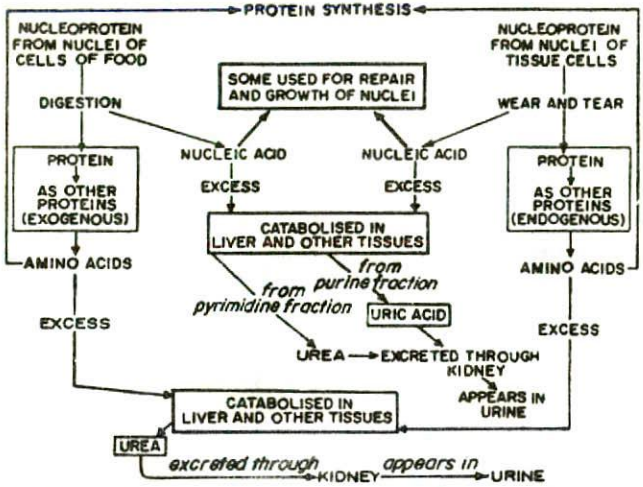


FIG. 459. Schematic representation of exogenous (dietary) and endogenous (tissue) nucleoprotein metabolism.

Taking everything together it will be seen that metabolism of purine may take place under two different conditions—*first*, when the purines remain combined as nucleosides and *secondly*, when they remain free as adenine and guanine.

The end product of purine metabolism is chiefly uric acid and slightly hypoxanthine and xanthine. But this is not all. All purine bases undergoing metabolic changes in the body, are not excreted in these forms. A good deal of it is lost in some unknown way. In dogs, excepting the *Dalmatian* variety, uric acid is further oxidised into *allantoin* and is excreted as such (FIGS. 458 & 460).

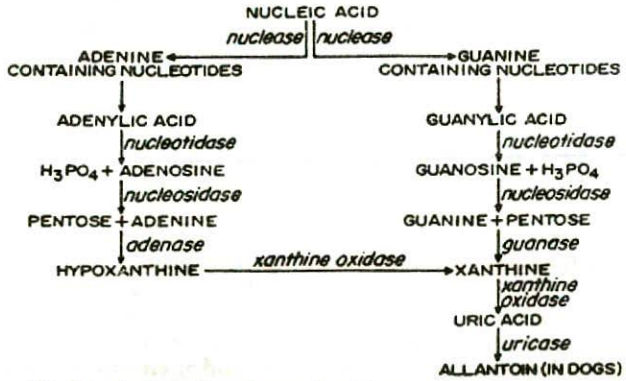


FIG. 460. Metabolism of purine nucleotides to uric acid and allantoin.

The intermediate metabolism of pyrimidine bases, e.g., *cytosine*, *uracil* and *thymine* is not clear. The catabolism of pyrimidines occurs mainly in the liver. Diets rich in thymine or DNA in rats produce increased excretion of β-aminoisobutyric acid. Based on fragmentary evidences obtained so far, the catabolic pathway of pyrimidines is shown in Fig. 461.

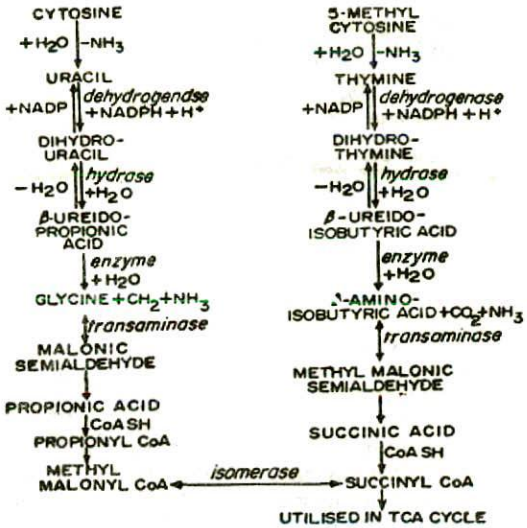


Fig. 461. Diagram showing the catabolic pathway of pyrimidine.

Feeding with pyrimidine bases increases the output of urea in the urine of dogs indicating the breakdown of the pyrimidine ring. Little is known about the fate of pentose. Probably they are oxidised.

The way by which purine bases undergo metabolic changes is briefly summarised in Fig. 460.

Functions of DNA and RNA. In recent years it has been definitely established that genetic information from one cell to the daughter one is carried by DNA and different forms of RNA that help in protein synthesis.

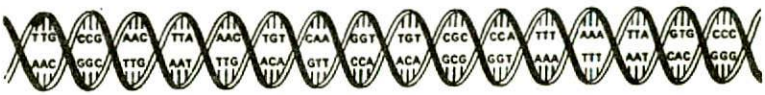


Fig. 462. Arrangement of amino acids in the helical form of RNA molecules.



Fig. 463. Arrangement of amino acids in mRNA.

Chromosomes of the cell nucleus is composed of DNA, which is responsible for the passage of genetic code from one cell to the other. Just before mitosis (page 21) the double helix of DNA separate from each other and with the help of an enzyme, **DNA polymerase,**



FIG. 464. Arrangement of mRNA in ribosomes.

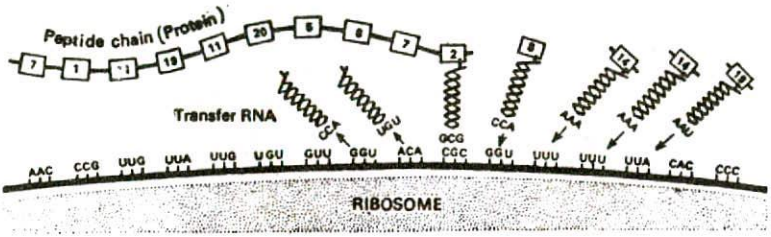


FIG. 465. Arrangement of tRNA in ribosomes.

a second chain exactly similar to the parent one is formed, thus giving it the original double helical structure. The two similar double helixes go to the two daughter cells formed as a result of cell division giving them the same amount of DNA as the parent cell. In the process of meiosis (page 23) such duplication of DNA in the parent cell does not take place and so the daughter cell contains half the number of original chromosomes.

Two types of RNA are also formed in the nucleus. One of them is single stranded messenger RNA (mRNA) which has a smaller molecule than DNA. Its synthesis is catalysed by the enzyme **RNA polymerase.** On being synthesised the mRNA comes out of the nucleus and becomes incorporated into the ribosomes of the cytoplasm. The

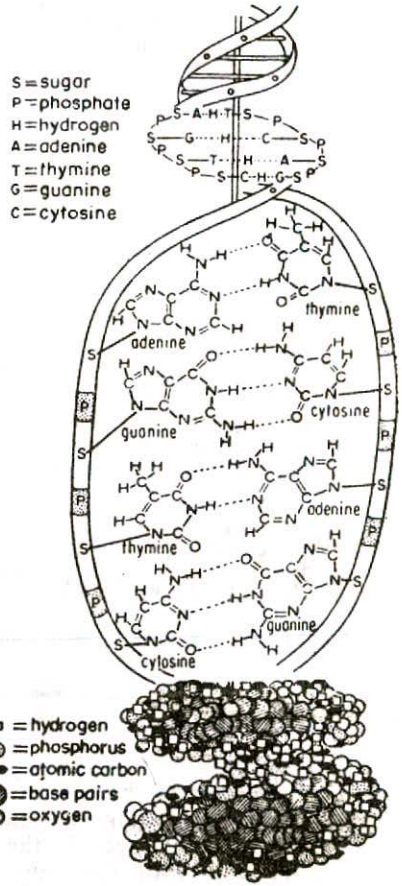


FIG. 466. Schematic representation of the helical model showing structural and molecular configuration. The top portion depicts the double helix with phosphate-sugar combination making up the outside spirals. The next part represents somewhat more details with the sequence of complementary nucleotides. The next portion shows molecular arrangements with connecting hydrogen bonds. The lower portion represents the detailed structures showing how the space is filled with atoms.

mRNA carries the genetic information to determine the sequence in which the amino acids are to be lined up in the polypeptide chain. In this process of synthesis, another type of RNA called **soluble RNA** (sRNA) or transfer RNA (tRNA) is also formed in the nucleus to participate in the process of synthesis. The tRNA attaches itself to the activated amino acids (**amino acyl AMP**) forming sRNA amino acid and carries them to the mRNA at the ribosomes. The sRNA finds out the definite code word or **codon** on the mRNA, attaches to the specific amino acids and is set itself free to be used again. The long mRNA thread has a number of ribosomal structures known as **polysomes (polyribosomes, ergosomes)** which are thus responsible for the synthesis of many amino acids forming a polypeptide. Finally the mRNA is set free from the polypeptide chain of the completed protein and renews its help in protein synthesis.

The RNA codes or codons for different amino acids are as follows:

AMINO ACIDS	RNA CODE WORDS			
1. Alanine (α-aminopropionic acid)	CCG			
2. Arginine (α-amino.δ-guanidovaleric acid)	CGC	AGA		
3. Asparagine (γ-amide of α-aminosuccinic acid or α-amino-γ-succinamide)	ACA	AUA		
4. Aspartic acid (α-aminosuccinic acid)	GUA			
5. Cysteine (α-amino-β-mercaptopropionic acid)	UUG			
6. Glutamic acid (α-aminoglutaric acid)	GAA			
7. Glutamine (δ-amide of α-aminoglutaric acid or α-amino-δ-glutaramide)	AAC	AGA		
8. Glycine (α-aminoacetic acid)	UGG	AGG	CGG	
9. Histidine (α-amino-β-imidazolepropionic acid)	ACC			
10. Isoleucine (α-amino-β-methylvaleric acid)	UAU	UAA		
11. Leucine (α-aminoisocaproic acid)	UUG	UUC	UCC	UUA
12. Lysine (α-ε-diaminocaproic acid)	AAA	AAU		
13. Methionine (α-amino-γ-methyl thiobutyric acid)	UGA			
14. Phenylalanine (α-amino-β-phenylpropionic acid)	UUU	CUU		
15. Proline (pyrrolidine-α-carboxylic acid)	CCC	CCU	CCA	
16. Serine (α-amino-β-hydroxy propionic acid)	UCU	UCC	ACG	
17. Threonine (α-amino-β-hydroxy-n-butyric acid)	CAC	CAA		
18. Tryptophan (α-amino-β-indolepropionic acid)	CGU			
19. Tyrosine (α-amino-β-(parahydroxyphenyl) propionic acid)	AUU			
20. Valine (α-aminoisovaleric acid)	UGU			

SYNTHESIS OF NUCLEOPROTEINS

Although in higher animals the power of protein synthesis is limited, yet nucleoproteins can certainly be synthesised in their bodies. A hen's egg before incubation contains very little purine bases but after hatching the chick contains large amounts of nucleoproteins. Obviously, this must have been synthesised from other substances present in the egg. In human infants the amount of nucleoprotein rapidly increases as growth advances, although, the chief food is milk which is almost free from nucleoproteins. From similar observations *it has been definitely proved that the nucleoproteins can be synthesised in the body. Liver is the most probable site of nucleoprotein synthesis.*

Nucleoproteins are compounds of simple basic proteins, protamines and histones with nucleic acid. Nucleic acids are composed of (*a*) **phosphoric acid,** (*b*) **a pentose**

and (*c*) a nitrogenous base—**purine** and **pyrimidine.** Both the nitrogenous bases are synthesised in the body not as such but as their corresponding nucleotides.

Purine nucleotides are **adenylic acid** and **guanylic acid** (guanosine-5′-phosphate). These nucleotides are not formed directly. At first the nucleotide **inosinic acid** (hypoxanthine-ribose-5′-phosphate) is synthesised from the metabolic products of carbohydrate and protein. Different compounds of the purine ring are derived from formic acid, CO_2, glutamine, aspartic acid, glycine and have been shown in Fig. 467. The inosinic acid nucleotide, after being formed, is then converted into adenylic acid and guanilic acid.

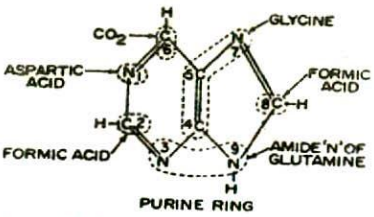


Fig. 467. Sources of the different components of purine nucleus.

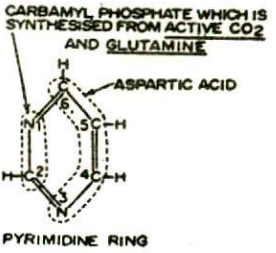


Fig. 468. Sources of the different components of pyrimidine nucleus.

Pyrimidine is also synthesised as nucleotides. Pyrimidine nucleotides are **cytidylic acid, thymidylic acid** and **uridylic acid.** The first step in the synthesis of pyrimidine nucleotide is the formation of **orotic acid.** It has been found by both *vitro* and *vivo* experiments that orotic acid is utilised for the synthesis of pyrimidine nucleotides. Carbamylphosphate and aspartic acid take the initial role in the synthesis of pyrimidine nucleotide. In the pyrimidine ring, nitrogen at position 1 and carbon at position 2 are donated by carbamyl phosphate. Carbons at positions, 4, 5, 6 and nitrogen at position 3 of the pyrimidine ring are donated by aspartic acid (Fig. 468).

URIC ACID METABOLISM

(Purine Metabolism)

Chemistry. Uric acid is 2 : 6 : 8 trihydroxypurine. It is derived as an end product of purine metabolism. [*Vide* pages 621 & 622.]

Sources. Uric acid comes from purines in case of man, other primates—Dalmatian dog and birds.

I. **From purines.** These bodies may be derived again from two sources: (*a*) **exogenous,** and (*b*) **endogenous.**

(*a*) **Exogenous purines.** (1) *Nucleoproteins of food.* (2) *Hypoxanthine* and *xanthine* present in meat extracts, meat soup, etc. (3) *Methyl purine*—theophylline, theobromine, caffeine, etc., present in tea, coffee and cocoa.

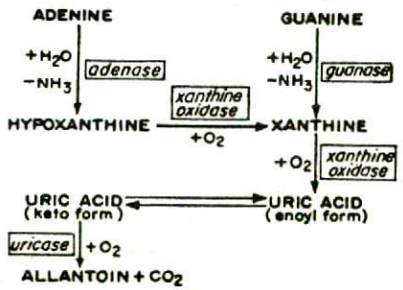


Fig. 469. Diagram showing the formation of uric acid.

(*b*) **Endogenous purines.** (1) *The nucleoproteins derived from tissue breakdown,* (2) *Several mononucleotides present in the body,* viz.,

(*i*) Adenosine triphosphate found in the muscles.

(*ii*) Inosinic acid, a hypoxanthine derivative, found in the muscle.

(*iii*) Guanylic acid, made up of guanine, pentose and phosphoric acid found in glands, viz., the pancreas.

II. **As a synthetic product.** Synthesis of uric acid and proteins is supposed to be a normal process. In birds urea is converted into uric acid in the liver. Moreover, it has been shown that in birds the greater part of uric acid is not derived from purines but by a process of synthesis from lactic acid and ammonia. Probably the same synthetic processes may take place in the mammals. The human infant which lives and grows on milk only, rapidly increases the nucleoprotein content of the body, although milk is poor in these products. Moreover, it is known that eggs are poor in purine bases but after hatching, the chick contains a large amount of purine compounds in the body. Histidine is a possible source of purine synthesis. Isotopic experiments with ^{15}N labelled glycine and ^{14}C labelled acetate show that uric acid is synthesised from them.

These evidences prove that synthesis of purine bases are normal phenomena in our body. Liver is probably the chief site for synthesis although other tissues take part in the synthesis as well.

Stages in the formation of uric acid from purine

It may take place in two ways: (*a*) from the free purine bases—adenine and guanine, and (*b*) from purine nucleosides (*vide* page 621).

Uric acid in blood. Blood contains 2–6 mgm uric acid per 100 ml (average 4 mgm).

The form in which uric acid is carried in blood is not yet fully known. *Probably, a part of it remains as free uric acid or urate. Another part remains in some colloidal form,* combined with the blood colloids. Benedict claims that in the red cells of ox and man major part of uric acid is present in combination with pentose and a small amount in the free form.

Fate of uric acid

1. **Destruction of uric acid.** Observations of Folin and his associates indicate that *nearly* 20% *of uric acid formed, is destroyed in the body. Liver is the probable site of uric acid destruction.* Injection of ^{15}N labelled uric acid definitely proves that some uricolysis occur in the normal human being and that the intestinal flora play very little part. But neither the details of the process nor the end products are known. Allantoin is certainly not the end product.

2. **Formation of allantoin (Uricolytic index).** In mammals, other than primates excepting the Dalmatian dog, uric acid is further oxidised to allantoin and is excreted as such. If we add together the amounts of allantoin and uric acid excreted and express the allantoin as a percentage of this sum, we get what is known as uricolytic index.

$$\textbf{Uricolytic index} = \frac{\text{Allantoin in urine}}{\text{Allantoin} + \text{Uric acid in urine}}.$$

It is a measure of the extent of conversion of uric acid into allantoin by the enzyme *uricase.* In chimpanzee it is 0; in man only 2; in dogs (excepting Dalmatian variety) it is 98. In man and higher apes, therefore, practically no allantoin is formed.

3. **Excretion of uric acid.** *Daily output of uric acid is* 0·75–1·0 *gm, of which nearly half is endogenous and the other half exogenous.* It is excreted either as free uric acid or as the urates of sodium, potassium, ammonium, etc. Reabsorption of the greater part of the uric acid occurs in the renal tubules, but there is good evidence for tubular secretion of uric acid. Uric acid is, therefore, cleared from the plasma both by glomerular filtration and by tubular secretion. The urinary excretion of uric acid is increased by the hormones of the adrenal cortex as well as by ACTH. Muscular exercise increases uric acid output.

Variations of uric acid excretion with different types of food

1. Protein food increases uric acid formation and output.*
2. Calorific value of food—If the calorific value of food be raised keeping the protein content constant, the uric acid output rises. It is interesting to note that this rise is only found if carbohydrates are added to raise the calorific value. With fats this is not seen.
3. High purine diet—This increases uric acid output.
4. Fats diminish uric acid output.

Thus proteins, purines, high carbohydrate diet and high calorific value of food raise the formation and output of uric acid, while fats diminish it. Depending upon this observation, it has been suggested that carbohydrates in some unknown way become converted into uric acid. *To diminish uric acid formation, therefore, the diet should be of low calorific value, poor in proteins, purine and carbohydrates but rich in fats.* But one should remember that these foodstuffs exert an opposite action on blood uric acid. For instance, proteins (even purine-free substances like egg) and carbohydrates increase uric acid excretion and lower the blood uric acid level. While fats diminish its output and raise its blood level. These facts should be carefully kept in mind while prescribing diet for gout cases.

Gout

In gout there is excess of uric acid in the blood and urine. There is also deposition of urate in the articular surfaces of joints and sometimes in periarticular structures. The joint of the big toe is first affected and in acute attack there appears sudden *swelling* of joints, which are extremely painful. The blood uric acid (which in the normal subject is about 4 mgm per 100 ml) rises to about 30 to 60 mgm per 100 ml. The urate deposits which occur in the joints, fingers, in the helix of the ear, etc., are known as **tophi.** Gout may arise from the following conditions:

(*a*) Increased synthesis of uric acid.
(*b*) Excessive nucleic acid breakdown, e.g., starvation.
(*c*) Overproduction as occurs in glycogen storage disease.
(*d*) Increased nucleic acid turnover, e.g., polycythemia, granulocytic leukaemia.
(*e*) Decreased excretion of uric acid which might arise from interference with secretion of it from kidney tubules.

Significance of variation of blood uric acid. Its value in blood is increased in gout where uric acid in larger amounts as monosodium urate may be deposited in the joints and tissues. In leukaemia and pneumonia the blood uric acid is elevated due to increased purine catabolism accompanying with increased destruction of leukocytes and tissue autolysis. It also increases often in arteriosclerosis with hypertension and in cardiac decompensation in which it may be some time due to renal insufficiency. In some diabetes or in acute infection of children this may increase in blood.

SULPHUR METABOLISM

Source. The various forms in which sulphur is taken are as follows:

ORGANIC

1. **Sulphur-containing amino acids,** viz., cystine, methionine, etc.

* **Muscles, liver, etc., contain enough nucleoproteins and increase uric acid formation and output.**

2. **Sulphur-containing proteins,** viz., mucin (mucoitin-sulphuric acid), cartilage proteins (chondroitin-sulphuric acid), etc.
3. **Sulpholipids** or **sulphatids** present in the nervous tissue.
4. **Thiamine** and **biotin** (members of vitamin B complex).

B. INORGANIC

Sulphates of sodium, potassium, magnesium, etc.

Distribution. (1) It is found as *keratin* in the hard appendages of the body, i.e., hair, nails, hoofs, horns, feathers, etc. (2) In the cartilage. (3) In various glycoproteins, such as mucin. (4) In nervous tissue as sulpholipids. (5) In traces in almost all cells in the form of *glutathione.* (6) The enzyme rennin, lipase, phosphatase, etc., contain sulphur. (7) The hormone insulin is a sulphur compound. (8) Heparin contains sulphur. (9) β-mercapto-ethylamine, a constituent of coenzyme A, is derived from sulphur-containing amino acids.

Functions. *Functions of sulphur should be regarded to be the same as the functions of sulphur-containing compounds.* From the distribution and forms in which it remains in the body, functions of sulphur may be summarised as follows:

1. **It is related to the maintenance of hardness with certain amount of elasticity in various tissues of the body,** for instance, hair, hoofs, horns, cartilages, nails, etc.
2. **It is a constituent of essential amino acid methionine.**
3. **Relation with bile salt.** Taurocholic acid of bile is derived from taurine, which is synthesised from sulphur-containing amino acid *cystine.*
4. **It is related to O_2 carriage and the oxidative processes of the body.** For instance, (*a*) Glutathione is a sulphur-containing tripeptide. It takes an important part in tissue oxidation. (*b*) It is an ingredient of the hormone insulin which is concerned with the metabolism of carbohydrate mainly, and partly protein and fat. (*c*) As a constituent of thiamine and biotin, it takes part in tissue oxidation.
5. **As the active radicle of some enzymes.** The enzymes lipase and phosphatase, etc., contain 'SH' (*sulphydryl*) radicle, upon which their characteristic action depends.
6. **Relation with milk clotting.** The milk-coagulating enzyme 'rennin' contains sulphur.
7. **Relation with blood clotting.** Heparin, which is believed to prevent intravascular clotting normally, contains sulphur.
8. **Detoxication.** The sulphur-containing amino acid cystine is used in the detoxication of toxic substances, such as bromobenzene, which is excreted as mercapturic acid. Exogenous sulphur is oxidised into sulphuric acid. This sulphuric acid is partly used to detoxicate substances like indoxyl and forms ethereal sulphate. A part of it forms inorganic sulphate by neutralising alkalies and thus helping to maintain blood reaction constant.

Brief life history of sulphur

Sulphur metabolism may be divided into two parts: **exogenous,** and **endogenous.**

Exogenous. The inorganic sulphates of food cannot be utilised for building tissue proteins. They are excreted as such: The **sulphur-containing amino acids** of food serve two broad functions. *First,* they are utilised by the body to build up tissue proteins and for the synthesis of various sulphur compounds mentioned above. *Secondly,* these amino acids break

down and the sulphur liberated from them is mostly oxidised in the liver into sulphuric acid. This acid is partly excreted as inorganic sulphates and partly as the ethereal sulphates in the urine.

Endogenous. Sulphur derived from the breakdown of the endogenous proteins undergo a different fate. It escapes further oxidation and is excreted as neutral sulphur compound which is a true index of endogenous protein metabolism.

Excretion of sulphur

Sulphur is excreted in the following ways:

I. **In the saliva**—as thiocyanates and inorganic sulphates (traces). The former is specially found in smoker's saliva. It gives a deep brown colour with ferric chloride.

II. **In the stool**—as mucin, H_2S gas and altered bile salts.

III. **In the urine.** The total sulphur of urine is equivalent to about 3·5 gm of H_2SO_4 per day. It is present in three forms: (1) inorganic sulphates 80%, (2) ethereal sulphates 5%, and (3) neutral sulphur (organic sulphide sulphur), 15–20%.

1. **Inorganic sulphates**—such as those of sodium, potassium, magnesium, calcium, ammonium, etc. It constitutes about 85–90% of total urinary sulphur. It is an index of exogenous protein metabolism. The excretion of sulphates and urea run closely parallel, showing that both of them are of exogenous origin.

2. **Ethereal sulphates.** It constitutes about 5% of total urinary sulphur. It is formed in the following ways: (*a*) Tyrosine or phenylalanine undergoes bacterial putrefaction in the intestine and is converted into phenol and cresol. These are absorbed, carried to liver where they are conjugated with sulphuric acid and form one group of ethereal sulphates. Recently it has been shown that in the process of esterification *active* sulphate is first formed by the combination of ATP and sulphate. The active sulphate (adenosine-3′-phosphate-5′-phosphosulphate) in the presence of an enzyme *sulphate transferase* transfers its sulphate group to phenols, alcohols, amines, etc. They are excreted by the kidneys as *ethereal sulphates*. (*b*) Tryptophan also undergoes bacterial putrefaction and is converted into indole and skatole. These are absorbed and are toxic substances. They are detoxicated in the liver. During detoxication they are at first oxidised into indoxyl and skatoxyl sulphuric acids and then they are conjugated with sulphuric acid to form indoxyl and skatoxyl sulphates. They are excreted in the urine as K salts and they form another class of ethereal sulphates. **Indican,** which is chemically potassium indoxyl sulphate, is an example of ethereal sulphate. The presence of ethereal sulphate, in the urine indicates, bacterial putrefaction of proteins in the intestine. The common clinical condition is *constipation.*

3. **Neutral sulphur.** It constitutes about 4–6% of total urinary sulphur. **Chemistry and forms**—Here, the sulphur remains in unoxidised form. The examples are—sulphur-containing amino acids (viz., cystine), urochrome, thiosulphates, mercaptans, thiocyanates and traces of taurocholic acid, oxyproteic acid, etc. **Amount in blood**—2·2–4·5 mgm per 100 ml of whole blood and 1·7–3·5 mgm per 100 ml of serum. This proves that *one quarter remains in the corpuscle and three quarters in the plasma.* **Significance**—It increases in that rare metabolic disease, in which

cystine is excreted in a large amount in urine (*cystinuria*). It also rises in cases of melanotic sarcomas when an abnormal sulphur-containing pigment appears in the urine.*

NITROGEN EQUILIBRIUM

Definition. *It is a state of the body in which the intake and output of nitrogen are same.* This is found in the adults where growth has ceased and no more protein can be stored. **Minimum protein requirement**—*It is the minimum amount of protein that must be supplied in the diet to maintain nitrogen equilibrium.* According to Chittenden it is equivalent to 7 gm of nitrogen, i.e., roughly about 44 gm (i.e., $7 \times 6{\cdot}25$) of protein. But other observations show that it may be still lower. In the normal diet much higher quantities are given (*vide* 'Normal Diet').

Determination. It can be determined by dietetic experiments. At first a diet of adequate calorific value containing proportionally high protein, is given. So that, the nitrogen intake and output will be same. Then the amount of protein in the diet is gradually reduced and is replaced by equivalent amounts of carbohydrates and fats to maintain the calorific value of the diet constant. With such a procedure it will be seen that if enough carbohydrate and fats be given, the amount of protein required to establish nitrogen equilibrium may be considerably reduced. Due to this property, fats and carbohydrates are called **protein sparers.** Thus normal daily nitrogen excretion of 16 gm with an ordinary mixed diet can be brought down to about 6 gm by supplying enough carbohydrate and reducing protein consumption proportionally. But there is a minimum limit below which proteins can no further be reduced. This is called the **irreducible protein minimum.**

The minimum protein requirement (or the 'irreducible protein minimum') *represents the essential protein requirement of the body, which is needed to replace the loss sustained by the endogenous protein metabolism. Hence, it is a direct measure of endogenous protein metabolism.*

From these considerations it will be evident that the minimum protein requirement will depend upon (1) the amount of fats and carbohydrates in the diet, (2) the amount of essential amino acids contained in the food protein (because it is not possible to maintain nitrogen equilibrium with 'second class' proteins or proteins of 'low biological value' even if large quantities may be taken), (3) the adequacy of the calorific value of the diet. If the diet be insufficient in quantity the subject will undergo partial starvation, more tissue protein will burn and therefore, output of nitrogen will be more than the intake.

Value of determining nitrogen equilibrium. Established nitrogen equilibrium in any subject shows the following facts: (*a*) That the subject is no more growing and therefore not storing any protein. (*b*) That the protein in his diet is sufficient in quality and quantity. (*c*) That his diet is adequate in calorific value. (*d*) That he is not suffering from any wasting disease.

Positive and negative balance. Nitrogen balance is defined as *the difference between the nitrogen taken in the food and that excreted in the faeces and urine.* The intake may be more than the loss. This is called **positive nitrogen balance.** It indicates that protein is being stored. This is found in growing children, pregnant women, convalescent patients, athletes whose muscles are developing. The loss may be more than the intake.

* **S/N ratio.** On a diet of meat (muscles) or in prolonged starvation the ratio of sulphur to nitrogen in urine is about 1 : 14. The same ratio is found to exist in the muscles. This constant ratio shows that sulphur and nitrogen are derived from the same source—the muscles. So that for every gram of sulphur there will be 14 gm of nitrogen.

This is called **negative nitrogen balance.** This is found in wasting diseases, in starvation and where the protein supply is inadequate in quality and quantity (FIG. 470). Nitrogen equilibrium is disturbed under the

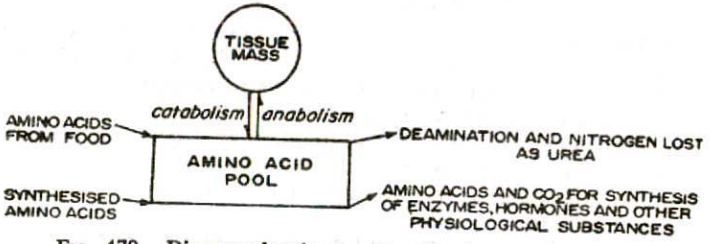


FIG. 470. Diagram showing reaction for nitrogen equilibrium.

above two conditions: positive nitrogen balance and negative nitrogen balance.

NITROGEN EQUILIBRIUM OF PURE PROTEIN DIET

As a rule, in the normal human subjects, who are habituated to a mixed diet, it is not possible to establish nitrogen equilibrium with pure protein food for two reasons. *First,* unusually large amounts of proteins have to be taken which will upset digestion, and *secondly,* the high specific dynamic action of proteins will increase the combustion of more tissue proteins, so that nitrogen output will be higher than the intake whatever the amount of proteins might be given. But in those subjects who are habituated to a very high protein diet (viz., Eskimos who consume about 3·5 Kg of meat per day) nitrogen equilibrium can be established with protein alone. In carnivorous animals the same thing is seen.

SPECIFIC DYNAMIC ACTION (S. D. A.)

All foodstuffs, after being ingested, increase the rate of heat production though the subject is in a basal state. This increased heat production due to ingestion of foods above the basal metabolic rate (B.M.R.) is known as **S.D.A.** or **calorigenic action of foods.** It can also be expressed as the **cost of metabolism** of the various food substances. *The highest S. D. A. is shown by protein and is about* 30%, whereas others, e.g., *carbohydrate* 6%, *fat about* 4%. By this it is meant that, if a quantity of protein, carbohydrate or fat, each having energy value of 100 calories, be given separately to an animal (whose basal metabolism is 100 cal) the actual heat production will be 130, 106 and 104 calories respectively. The extra heat comes from the combustion of the tissue substances causing loss of body weight. To prevent this loss of weight, the subject must be supplemented extra calorific allowance for the S.D.A. of the food itself. The following facts about S. D. A. are noteworthy. (1) *S. D. A. starts within an hour of taking food, becomes maximum in about the third hour and is maintained at this level for several hours.* (2) *Liver is the site of S. D. A. of proteins,* because proteins fail to exert S.D.A. after removal of liver. But it is not the seat of S. D. A. of carbohydrates and fats. Because the removal of liver does not affect it. (3) In fasting and undernutrition the S. D. A. of all foodstuffs increases. (4) During positive nitrogen balance, S. D. A. of protein does not seen to occur. (5) In order to exhibit S.D.A. the animal must have a temperature above 33°C. Below this temperature the S.D.A. of foodstuffs becomes less and less apparent. (6) *The S.D.A. of protein cannot be utilised in any other way. It is a waste heat*

and lost. But the case is different with fats and carbohydrates. Their S.D.A. can be utilised in the performance of work. Hence, when exercise is done with a food containing these substances, the extra heat due to S.D.A. is not seen, because it becomes used up in the energy exchanges underlying muscular action. For this reason high protein diet is not suitable for heavy muscular work. ***S.D.A. of protein is useful in maintaining heat balance in cold climates.***

Cause of specific dynamic action

S.D.A. of proteins. The following observations are significant: (1) That S.D.A. is not due to the synthesis of body proteins from the amino acids of food. Because proteins do not exert any S.D.A. upon growing infants or upon those subjects where nitrogen balance is positive. (2) That S.D.A. is not due to any specific-stimulating action of some amino acids. Although it is seen that S.D.A. of proteins depends upon the following amino acids only—glycine, alanine, glutamic acid, tyrosine and phenylalanine, yet it is not due to the effects of these amino acids. Because they exert no S.D.A. in infants. (3) S.D.A. of proteins is exerted only when proteins are breaking down. Breakdown of amino acids includes three processes—*first*, deamination, *secondly*, the changes undergone by the non-nitrogenous part, and *thirdly*, the changes undergone by the nitrogenous part. It has been shown that the first two processes are not responsible for S.D.A. S.D.A. of proteins is shown during the change undergone by the nitrogenous part. S.D.A. is due to largely associated with the nature of deamination, i.e., oxidative deamination (transformation into urea). ATP also helps in the urea formation. For the synthesis of ATP energy is required which leads to *increased metabolism.*

S.D.A. of carbohydrates. It is suggested that it represents the energy liberated in excess of that required for the conversion of glucose into glycogen. After a fast, when glycogen store is depleted, ingested glucose (which is mostly converted into glycogen), exhibits pronounced S.D.A. Glucose with thiamine exerts more S.D.A., since thiamine promotes and accelerates fat synthesis from carbohydrate.

S.D.A. of fats. According to Lusk (Plethora theory), it is due to more rapid oxidation of fats in the cells owing to their increased concentration in the tissue fluids (plethora) after absorption due to rate of digestive product reaching the tissue exceeds the rate of utilisation.

Three applications may be made of these facts: (1) The high S.D.A. of proteins should be made use of in cold climates by giving a protein-rich food. (2) While prescribing adequate diet, proper allowance should be made for the S.D.A. of proteins, otherwise tissue substances will burn and the subject will lose weight. (3) B.M.R. of a subject should be determined avoiding the period during which S.D.A. develops (*vide* page 525).

MINERAL METABOLISM

Introduction. The importance of the inorganic salts will be understood from the fact that salt starvation causes death much earlier than food starvation. *Although the salts give no energy yet they are essential for our life.*

To get a better conception as to why the salts should be of so much importance to us, one has to take a very long view and remember the old story that life was first born in the sea-water. If that is a fact, then it is only natural to expect that the sea salts will be intimately concerned with the structure and function of cells.

In course of evolution, although the human beings have departed a long way from their marine ancestors, yet the primitive aquatic life has left a permanent impression on our body. Each salt present in the sea is also found in the human body. Blood plasma is a typical example where the various inorganic salts are present almost in the same proportions as they are in the sea-water. Each one of these salts serves some essential functions. Even the minute quantity of iodine found in the sea-water is utilised for the synthesis of different thyroid hormones. Elements such as iron, copper, cobalt, manganese, iodine, selenium, etc., are required by plants and animals in very small quantities and are termed *trace elements.*

From these considerations and from various other experimental facts it is seen that these inorganic salts and elements—individually and collectively—are so intimately blended with our life processes that life becomes impossible without them.

If, thus, we remember our marine genesis and the fact that the inorganic elements are still carrying on the same intimate relationship with the life of a cell, then the fundamental principles of mineral metabolism in higher animals will not be very difficult to grasp.

IRON

[*Vide* pages 160–64.]

COPPER

Sources. It is widely distributed in the sea-water, plants, vegetables, milk, brain tissue, liver, blood, etc.

Daily requirement. In the adult about 2 mgm. Normal mixed diet is quite adequate.

Storage. Liver is the main store. Minute amount (0·1–0·5 mgm) is present in blood. In normal serum, a copper-containing globulin *ceruloplasmin*, has been found to occur. Copper content of the brain and liver of foetus and infant is much greater than that of adult. This high storage is useful to prevent deficiency in the suckling period as found in the case of iron. Total amount in adult body is 0·1 gm.

Functions. (*a*) Normochromic microcytic anaemia occurs during copper deficiency which is due to failure in proper absorption of iron. The anaemia can be cured by parenteral administration of iron. It is claimed that the therapeutic effect of iron in the cure of hypochromic anaemia is enhanced in presence of traces of copper. It is held that best effect is obtained if Cu and Fe are administered together in the ratio of 1 : 100. Most iron salts do not contain more than 0·02 mgm of copper per gram. Since, this is less than the optimum daily requirement traces of copper should be added for best therapeutic effect. (*b*) In certain marine animals the blood contains a copper compound—haemocyanin (with 0·38% of Cu)—instead of haemoglobin. There it serves the same function as haemoglobin. (*c*) Copper is present in a number of enzymes like tyrosinase, cytochrome oxidase, uricase, etc. In copper deficiency the marrow of red bones markedly decreased in cytochrome oxidase. In human red blood corpuscles, a copper-containing protein *erythrocuprein* is present, the function of which is not known as yet. (*d*) Cu in some way is related to development of bone. (*e*) In female rats, copper deficiency causes continued anestrus.

Wilson's disease is due to disorder of copper metabolism. There is a fall in plasma cerul[illegible]min and increase in copper content of brain and liver leading to neurological distu[illegible]ces and hepatic damage.

MANGANESE

Daily requirement. Traces. Ordinary mixed diet contains about 2 mgm, which is enough. Widely distributed in the body specially in the intestinal tract, liver and reproductive organs.

Absorption and excretion. Follows the same principles as iron. [*Vide* page 162.]

Functions. (*a*) It exerts a similar but less pronounced effect like copper in the synthesis of haemoglobin. Some workers believe that hypochromic anaemia is best treated by a combination of Fe, Cu and Mn. In its deficiency growth is poor, bone formation abnormal. (*b*) It has some role in normal reproductive physiology. Experimental deficiency of manganese in rats disturbs oestrous cycle and lactation in females and degeneration of germinal epithelium in males. (*c*) In the lamellibranch (Pinna squamosa) the blood pigment, instead of haemoglobin, is a manganese compound, called Pinna globulin. It serves the same functions as haemoglobin. (*d*) Mn ions activate many enzymes, viz., *arginase, phosphoglucomutase, muscle adenosine triphosphatase, choline esterase, hexokinase, isocitric dehydrogenase pyrophosphatase* and *various decarboxylases.*

Transmanganin is β_1- globulin which is different from transferrin and responsible for keeping Mn bound.

CALCIUM

[*Vide* under the chapter on *Endocrines.*]

PHOSPHORUS

[*Vide* under the chapter on *Endocrines.*]

SODIUM

Sources. Taken as common salt. Present in milk, water and all ordinary articles of diet.

Daily requirement. As NaCl—minimum 5–10 gm. The average intake is much above this, 8–10 gm and even more, hence natural sodium deficiency is rare.

Distribution. Total—0·1% of body weight. **In blood** (as NaCl)—whole blood contains 350–550 mgm per 100 ml. **Plasma** contains 560–630 mgm per 100 ml. As Na, plasma contains 340 mgm%, corpuscle 65 mgm%, whole blood 200 mgm%. **In the cerebrospinal fluid**—as NaCl—650–750 mgm%, as Na—330 mgm%, slightly higher than in plasma.

It is to be noted that the *distribution of Na and K in the cells and extracellular fluids are in reverse order. Na is chiefly found in the extracellular fluids and very little inside the cells, whereas, K is chiefly found inside the cells and very little in the extracellular fluids.*

Metabolism. Controlled by adrenal cortex. [*Vide* Adrenal Cortex.]

Excretion. Daily excretion is generally same as daily intake. Excreted chiefly through urine and partly through sweat and stool. The average daily excretion in urine as chlorides is about 10–15 gm (i.e., 1%).

Functions. The sodium in plasma provides over 90% of the total base of the body. Sodium works in the body in two capacities. Either (*a*) **as sodium ions** or (*b*) **as sodium compounds.**

I. **As sodium ions.** (1) **Initiates and maintains contraction of heart.** (2) **Essential for the normal functions of cells.** (3) **Essential for the contraction of voluntary and involuntary muscles.** (4) **Excites nerves**—Na ions are neuro-excitatory as opposed to calcium ions.

II. **As sodium compounds.** Such compounds are *bicarbonates, phosphates, chlorides, proteinates*, etc. Their functions are as follows: (1) **Maintains blood reaction**—This is done in many ways. For instance (*a*) *sodium bicarbonate* is the chief buffer of blood and other body fluids. (*b*) *The acid and alkaline phosphates* also constitute an important buffer system. (*c*) Sodium which remains combined with plasma proteins (*sodium proteinates*) can also act as a buffer. (*d*) Sodium of NaCl can also fix acids with the help of the phenomenon known as *chloride shift* (*vide* page 392). (2) **Controls reaction of urine**—Kidneys regulate the urine reaction by altering the proportion of acid and alkaline phosphates in the urine. (3) The **reaction of pancreatic juice and bile** is due to the presence of sodium carbonate. (4) **Maintains osmotic pressure**—NaCl is the chief regulator of the osmotic pressure of the body fluids. (5) **Helps in the formation of HCl of gastric juice**—NaCl takes part in the series of reactions as a result of which HCl is manufactured by the stomach. (6) **Fat absorption**—In adrenalectomised animals administration of Na salts rectifies defective fat absorption. (7) Maintains water balance.

Effects of deficiency. Na deficiency shows reduction in fat deposit, atrophy of muscle and testis, lung infection, retarded bone growth, reduction in osteoid tissue, and a definite eye changes in animals.

POTASSIUM

Sources. In all foodstuffs, specially in the animal cell.

Daily requirement. 4 gm approximately.

Distribution. Total—0·1% of body weight, i.e., same as sodium. It is chiefly intracellular as opposed to sodium. **In blood**—whole blood, 200 mgm, plasma, 15–20 mgm, cells, 180 mgm per 100 ml. **Cerebrospinal fluid**—12–17 mgm per 100 ml.

It is to be noted that when whole blood is stored at 2–4°C., as for the purposes of blood transfusion, potassium from corpuscles migrates into plasma in which the potassium concentration rises. Such a sample of blood, if administered, may produce serious effects.

Metabolism. Controlled probably by adrenal cortex, either directly or indirectly by regulating sodium metabolism.

Excretion. 2–3 gm excreted through urine per day which varies with the amount of intake. In potassium deficiency kidneys reabsorb more. With high meat diet and in the later stages of fasting, when tissues are breaking down, potassium excretion rises.

Functions. (1) **Maintains intracellular osmotic pressure as KCl.** (2) **Maintains intracellular reaction**—as *bicarbonates, phosphates*, and *proteinates*—potassium constitutes a very important buffer system in the cell. (3) **CO_2 carriage**—potassium of KHb in the red cells helps CO_2 transport. (4) **Potassium ions inhibit cardiac contraction and prolong** relaxation. (5) **Potassium ions inhibit muscular contraction in general.** (6) **Potassium exerts important effects upon the function of nervous system.** Some facts are given below: (*a*) *Synaptic transmission*—potassium is intimately

related to the formation and action of acetylcholine at the synapses upon which the transmission of nerve impulses depends. (*b*) *Potassium is intimately related to the excitability of nerve cells.* (*d*) *It is intimately concerned with the development of potential difference* with the help of which an impulse is conducted through a nerve. (7) **Hastens ciliary movement.**

Taking everything together, *the action of potassium is generally opposed to that of calcium.* In adrenal deficiency serum potassium rises, due to less excretion by the kidneys and more leakage from the tissue cells. In intestinal obstruction serum potassium rises as a terminal event. In shock, the same picture is seen.

Effects of deficiency. The heart rate is slow. There are scarring of heart muscle, hypertrophy of kidneys, paralysis of muscle and retarded bone growth. Bones become fragile excessively. Both sexes may become sterile.

MAGNESIUM

Sources. Green vegetables, meat, bread, etc.

Daily requirement. Not definitely known. Its presence in normal diet is generally enough for its requirement in the body. Recent recommendation is 350 mgm per day for adult males and 300 mgm per day for adult females.

Absorption. From the upper part of the small intestine. Mg ions are absorbed with great difficulty. It forms insoluble phosphates like calcium. Hence the principles, that apply for Ca absorption, are also applicable for Mg. [*Vide* Calcium absorption under the chapter on *Endocrines*.]

Distribution. (1) **Blood**—whole blood 3 mgm, plasma 2·5 mgm, corpuscles 3·5 mgm per 100 ml. Distribution of Ca and Mg in blood is generally opposite. Calcium remains almost wholly in plasma and very little in the cells, while Mg is mostly found in cells and less in plasma. (2) **Bone**—70% of the total Mg of the body is present in the bones as phosphates. Bones contain 2% of the $Mg_3(PO_4)_2$. (3) **Voluntary muscles**—about 0·02%. (Ca—0·007%). (4) **Chlorophyll**—it is the green pigment of plants. It is a porphyrin derivative of magnesium.

Excretion. In urine, 0·1–0·2 gm in 24 hours. It is generally not excreted through the large gut. Mg content of faeces largely represents unabsorbed Mg of diet. *Metabolism* of Mg is probably controlled by adrenal cortex directly or indirectly.

Functions. (1) **Forms bone and tooth.** (2) **Activator of enzymes**—Mg ions activate the enzyme phosphatase. It accelerates the action of bone phosphatase. (3) **Takes part in the chemistry of muscular contraction**—Mg is a member of a complex coenzyme system which takes part in the chemical changes underlying muscular contraction. It may be that here also it helps the action of muscle phosphatase. (4) **Antagonistic to calcium ion**—The distribution and function of Mg ion is generally antagonistic to calcium ion.

Effects of deficiency. In man vascular disturbances, hyperexcitability, convulsions and ultimately death. Something like tetany develops and is known as **Mg-tetany.** In rats Mg-tetany is fatal in about 18 days. In this case blood magnesium falls, blood cholesterol rises and the calcium content in the soft tissues, specially in the muscles and the kidneys, becomes very high. If Ca intake is increased the effect of Mg deficiency becomes very severe.

CHLORINE

Sources. Present as sodium chloride in milk, water, other articles of diet, etc.

Daily requirement. 10–12 gm per day (about 4 gm of sodium). **Excretion**—(1) **Mainly in the urine,** about 15 gm in 24 hours. In starvation or in deprivation of salts, chlorides are retained in the body and less than 1 gm per day is excreted. Under all such conditions blood chlorides remain unchanged. This low excretion is due to more reabsorption by the renal tubules. Chlorides are high threshold substances. (2) **In the sweat**—usually the chloride content of sweat is very low—0·15%, but in profuse sweating, a man may lose 10 gm or more in the course of three hours only.

Distribution. Mostly present in the form of inorganic chlorides both inside the cells as well as in the extracellular fluids. Whole blood contains about 0·25%; plasma, 0·36%; corpuscles, 0·19%. CO_2 tension of blood determines the proportion of chlorine between cell and plasma. [*Vide* page 392.] They are readily absorbed from the intestine. They cannot be replaced by other halides.

Storage. Large amounts of chlorides can remain stored in the skin and subcutaneous tissue.

Control of metabolism. (1) **Adrenal cortex**—due to its influence on sodium

metabolism. (2) Posterior pituitary controls urine output and indirectly controls chloride balance.

Functions. (1) Chlorine provides for about 2/3rds of the anions of plasma and is the chief anion of the body. Consequently, it is a main factor for regulating body reaction. (2) Chlorides, such as NaCl, KCl, etc., are important agents in the regulation of osmotic pressure in the body. (3) HCl of the gastric juice is ultimately derived from the blood chlorides. (4) Chlorine ions are essential for the action of ptyalin and pancreatic amylase. (5) Chloride shift is an important phenomenon by which chlorine ion shifts alternately from plasma to cell and helps CO_2 carriage and regulation of blood reaction.

IODINE

Sources. (1) In traces as iodides in water, food, common salts, etc. (2) Sea-water—0·2 mgm per litre. Sea weeds and spongy shells are very rich in iodine.

Daily requirement. 0·05 mgm. In children more.

Absorption. Readily absorbed from the small intestine.

Distribution. Total 25 mgm in the body, half of it remains in the thyroid. Iodine remains in the blood as (*a*) Inorganic iodide in the plasma and corpuscles and varies from 0·5 μ gm to 1 μ gm per 100 ml. (*b*) Bound iodine—Thyroxine and tri-iodothyronine in the plasma in combination with α-globulin. It is called protein-bound iodine or PBI. The normal value of PBI varies from 5·0 μ gm to 8·0 μ gm per 100 ml.

Iodine metabolism. It is controlled by thyroid. Deficiency leads to simple **goitre.** Iodine is excreted chiefly in the urine. [*Vide Thyroxine* under the chapter on *Endocrines.*]

Functions. (1) Iodine is an essential constituent of thyroid hormones and therefore essential for normal life.

BROMINE

Sources. In foodstuff, specially in the animal tissues.

Distribution. Distributed throughout the tissues in traces, and remains mixed with chlorides. In blood its concentration is higher than iodine.

Absorption and excretion. Readily absorbed from the small intestine.

Functions. Bromine ions (like chlorine ions) activate ptyalin and pancreatic amylase.

FLUORINE

Sources. Traces in food and water. Drinking small water should not contain more than 1–2 parts per million.

Distribution. Distributed throughout the body in traces. Enamel of teeth is particularly rich in it.

Functions. In bones it remains as CaF_2 but is neither essential nor constant. Very small amount possibly helps in development of tooth, but excessive intake causes mottling of teeth. Fluoride is a poison for some enzymes of glycolytic pathways, not clear if it is an essential element.

ZINC

Sources. Traces in ordinary articles of diet.

A vital enzyme, carbonic anhydrase, contains zinc. It is also essential for the activity of some enzymes and hence essential for the life of the organism. Other enzymes, viz., several dehydrogenases (alcohol, glutamic, certain pyridine nucleotide) and pancreatic carboxypeptidase contain zinc.

Daily requirement. About 12 mgm.

Distribution. Traces in all tissues.

Excretion. Chiefly in urine.

Functions. (1) Although active zinc free insulin preparations are available, yet zinc is an integral and essential element of insulin molecule. (2) Insulin crystallises readily as a zinc compound and zinc prolongs insulin action. (3) Possibly helps storage of insulin in the pancreas; because pancreas is rich in Zn.

Effects of deficiency. Reduced growth, alopaecia, skin lesions, etc., are observed.

ALUMINIUM

This is another trace element distributed widely in the body. Its **function** in the body is not properly known. Use of aluminium utensils does not produce any aluminium poisoning.

COBALT

Traces are essential for cattle life. Being a component of vitamin B_{12} (4·5%) it is also essential for human haemopoiesis. Only minute amounts in daily diet are sufficient. [*Vide* 'Vitamin B_{12}'.]

Administration of excess cobalt causes degeneration of α-cells of islets of Langerhans and abnormal increase in number of circulating R.B.C. (*cobalt polycythemia*) possibly due to stimulation of **erythropoietin,** a hormone which accelerates erythropoiesis.

SELENIUM

Excess selenium has some toxic effects in rats. This element is a constituent of **Factor 3** which prevents liver necrosis, muscular dystrophy, white muscle disease, etc., in animals.

SULPHUR

[*Vide Sulphur Metabolism*—pages 628–31.]

DELETERIOUS EFFECTS PRODUCED BY DEFICIENCY OF INORGANIC SALTS

It has been already noted that the salts are more important for our life than food. Salt starvation will bring about an earlier death than food starvation. In animals natural salt deficiency never takes place, enough being present in food. In man also total salt deficiency is never seen under natural conditions but partial deficiency or deficiency of individual elements or salts may take place in cases of increased excretion, such as profuse sweating, diarrhoea, haemorrhage, etc., or by taking a faulty diet or in certain diseases of the endocrine glands, for instance, parathyroid, adrenal cortex, etc. The effects of complete salt starvation as experimentally seen in animals and the effects of deprivation of individual salts on human beings, have been studied in detail. The following is a brief summary.

1. **IN ANIMALS.** With complete deprivation of salts, the function of the entire body becomes upset. The animal becomes weak, loses appetite—food is retained in the stomach for a long time in an undigested form (showing absence of gastric secretion and motility) and is finally vomited out. The animal gradually fails to move about, shows signs of paralysis, loses consciousness and ultimately dies.

Salt loss in the urine becomes extremely low, almost all being reabsorbed by the tubules as a measure of salt economy. If under such conditions food (without salts) is given, the salt excretion is much lower than when food is withheld. *This indicates that inorganic salts remain in some sort of chemical combination in the cell.* During food starvation the animal has to live by burning its own body tissues; the salts, thus freed, diffuse out of the cells and appear in the urine. Thus food starvation increases the salt loss and enhances the deficiency effects. Since potassium remains mostly in the cells, food starvation which leads to the breakdown of the cells, will increase the potassium content of the urine. In other words, *potassium excretion is a good guide as to the degree of disintegration of cells going on in the body.*

2. **IN MAN.** (*a*) **With a salt-free diet,** the NaCl almost disappears from urine due to maximum tubular reabsorption. Caffeine under this condition increases the salt loss by depressing the reabsorption of salts and water by tubules. So that caffeine will increase the salt deficiency and also cause dehydration. (*b*) **If salt deficiency be induced** by producing excessive sweating (giving water drinks without salts, only to replace the loss of water) the plasma non-protein nitrogen (N.P.N.) rises, the urea may rise to 80 mgm per 100 ml, *the man may be sick showing signs of early uraemia.* This is probably, at least partly, due to the depression of kidney functions. The symptoms quickly clear up by giving saline drinks. In those conditions where the man or the animal has to undergo a lot of sweating, he should always be given saline drinks. Otherwise, ill-effects may be produced. (*c*) **Heat cramps**—Deficiency of salts, particularly of NaCl, occurs in men doing heavy muscular work in hot and moist atmosphere. Such people are found to suffer from temporary painful contraction of skeletal muscles. This effect is due to salt loss as well as dehydration. The blood becomes thick, haemoglobin concentration rises, blood NaCl falls. 1% salt solution in water if taken by such subjects will lead to a speedy cure. (*d*) **A sense of fatigue, weakness and even temporary loss of consciousness** have been reported in man doing even sedentary work in hot humid climates. The cause has been suggested to be, at least partly, sweating and salt loss. Saline drinks will bring abont a speedy cure.

In addition to above, deficiency of individual elements may lead to specific harmful effects. Some of them are summarised below.

1.	**Deficiency of Ca**	.. *rickets, tetany*, etc.
2.	„ „ **P**	.. *phosphorus rickets.*
3.	„ „ **Mg**	.. *tetany, vascular disturbances, convulsions and death.*
4.	„ „ **Fe, Cu, Mn**	.. *secondary anaemia.*
5.	„ „ **I**	.. *simple goitre.*
6.	„ „ **K**	.. *checks growth and is ultimately fatal in animals.*
7.	„ „ **NaCl**	.. *heat cramp, fatigue, unconsciousness, loss of kidney functions*, etc.

BALANCE OF INORGANIC IONS

From the facts accumulated so far as to the functions of the inorganic salts in the body, there is ample evidence to conclude that the inorganic ions remain in the body *in a state of functional balance.* It is found that almost everywhere and in every function *they can be grouped into two classes.* One group having *complementary* effects, the other group being *antagonistic.* It may also be found that the interrelationship may vary according to the field of reaction. *At some places two elements may have antagonistic effects, whereas at others they may help each other.* The following is a brief picture of the interrelationship amongst the more important ions.

1. **Calcium and phosphorus.** Ca and PO_4 ions are generally complementary in nature. They help each other. One without the other can neither be retained in the body nor can function adequately. In the blood the relation between Ca and inorganic phosphorus is 2Ca : 1P. But in the diet an opposite ratio, i.e., 2P : 1Ca, is found optimum for absorption. Raised blood calcium reduces phosphorus and *vice versa.*

2. **Ca, Na, Mg and K.** Although in detail their effects vary, yet generally speaking Ca is antagonistic in many respects to Na, K, and Mg. Ca is neurosedative, whereas Na and K are neuroexcitatory. In heart, Na, K, and Ca act in a co-ordinated manner and keep up the normal function of heart.

3. **Fe, Cu, Mn.** Their functions is complementary, at least as far as haemoglobin synthesis is concerned.

4. **Cu, Mo and SO_4.** The action of copper against the other two elements are antagonistic. Copper intake remaining normal, low intake of Mo and SO_4 produces Cu poisoning whereas excessive intake of the two leads to copper deficiency.

It has been mentioned that at some fields of action two elements may be complementary, but at other fields they may be antagonistic. For instance, Mg is generally antagonistic to Ca. But in bone formation Mg probably helps the phosphatase mechanism by means of which Ca salts are deposited.

CONTROL OF MINERAL METABOLISM

The control of mineral metabolism is a very complicated phenomenon. In the unicellular animals the mechanism is relatively simple and is probably carried out by adjusting the permeability of the cell membrane. But in the higher animals and human beings, although this primitive system persists, yet a lot of other factors have stepped in, as a result of which, a very intricate system of control has evolved. The supply, metabolism, balance and excretion are controlled by the following factors: (1) **Salt hunger**—It has been mentioned that under natural conditions, salt deficiency does not take place. In any condition where partial deficiency occurs, it is at once replenished by the intake of more salts. This salt hunger is felt in such conditions of deficiency. In cattle establishments huge blocks of rock salts are kept, and the animals are often found to lick those blocks eagerly. (2) **Absorption**—It is known that absorption of inorganic salts is controlled by a number of factors, but above all, the need of the body for a particular salt, chiefly determines the degree of its absorption. As for instance, anaemic subjects absorb more iron than normal people. (3) **Storage**—There is ample evidence that almost all the essential

inorganic elements can be stored in the body to some extent. As for instance, iron is stored in the liver, spleen, bone-marrow, etc., Ca, P, Mg in the bones, NaCl in the skin and subcutaneous tissue, etc. In times of need body can derive these elements from the store. It is found that, with regard to the inorganic salts, the body follows a principle of utmost economy. (4) **Specific characteristic of the cells**—On comparing the distribution of the various inorganic elements in the body it is seen that there is a remarkable difference between their distribution inside and outside the cells—For instance, Ca is almost solely present in the plasma and is practically absent from the corpuscles. Na also follows the same principle. But the distribution of Mg and K is just the reverse. Moreover, the cell membrane of certain cells exhibits certain peculiarities of permeability. They allow certain types of ions to pass, not the others. This is best seen in the case of red blood corpuscles, the membrane of which allows the anions to pass, not the cations. With the help of this characteristic quality of the membrane, the cells control the distribution and metabolism of the inorganic elements in themselves. (5) **Endocrines**—The hormones are probably the chief controllers of mineral metabolism in human beings. Although the informations collected uptil now are fragmentary, yet there is ample evidence to show that number of hormones exert enormous influence upon mineral metabolism. A brief summary is given below: (*a*) **Adrenal cortex** secretes *mineralocorticoids*, e.g., deoxycorticosterone, aldosterone, etc., which increase reabsorption of Na, chloride and bicarbonates and depress that of potassium and phosphates by renal tubules. Thus by controlling the excretion of these salts, the mineralocorticoids keep an adequate balance of inorganic ions in blood, other body fluids and tissue cells. Aldosterone is most effective in controlling the electrolytic balance. (*b*) **Parathyroid** controls Ca and P metabolism. (*c*) **Thyroid** controls metabolism of iodine. It has also got some effect on Ca metabolism through *Thyrocalcitonin*. (*d*) **Posterior pituitary,** with the help of antidiuretic hormone, controls the excretion of water and therefore the excretion of salts which remain dissolved in it. (*e*) **Anterior pituitary,** by its thyroid-stimulating hormone or thyrotropic hormone (TSH) and adrenocorticotrophic hormone (ACTH), regulates the secretion of thyroid and adrenal cortex respectively, and through these glands controls the mineral metabolism. (6) **Kidneys**—One of the normal functions of kidney is believed to be to maintain an adequate concentration of salts and ions in the blood and tissue fluids. If excess salt is taken, the extra amount is readily excreted by the kidneys. In conditions of salt deprivation, the kidney tubules reabsorb these salts to the maximum degree so that almost nothing is excreted. In this way, kidneys act as one of the most important organs in maintaining the salt balance of the body. (7) **Nervous system**—Since nervous system controls the activity of all systems including the endocrine glands, it is reasonable to believe that it takes an important role in the control of mineral metabolism. A number of inorganic elements, for instance Na, K, Ca, Mg, etc., take an enormous role in maintaining the functions of nervous system. After stimulation of the vagus, potassium is found to increase in the perfusion fluid. The hypothalamus sends a bundle of fibre—the supra-opticohypophyseal tract to the posterior pituitary, and controls the secretion of antidiuretic hormone. From similar facts it is reasonable to believe that the nervous system,

particularly the vegetative nervous system, exerts some influence in regulating mineral metabolism.

PIGMENT METABOLISM

A number of pigments are found in the human body, some of which serve important physiological functions, while others represent simple waste products or that their functions are yet obscure. It is difficult to classify pigments on a single scientific basis. They may be **classified** in many ways, for instance: (1) **According to the body substance in which they are present,** such as, **blood pigments, bile pigments, urine pigments, skin pigments, intracellular pigments,** etc. (2) **According to their functions.** (3) **According to their chemical structure.** The following classification has been attempted with all the three views taken together.

I. PYRROLE DERIVATIVES

In these pigments four pyrrole rings remain joined together through a **methinyl** (≡**CH**) or **methylene** (—CH_2—) group to form either a *chain compound* or a *ring compound*. The following is a brief list:

Chain Compounds	Ring Compounds
1. **Bilirubin.**	1. **Haemoglobin, haematin, haem, haemochromogens** and similar *compounds*.
2. **Biliverdin** and other bile pigments.	2. **Cytochrome**—it is an iron-containing intracellular pigment required for tissue oxidation.
3. **Choleglobin.**	3. **Porphyrins,** e.g., protoporphyrin, coproporphyrin, uroporphyrin, etc.
4. **Urobilinogen.**	
5. **Urobilin**—present in urine.	
6. **Stercobilin**—found in the stool.	

Bilirubin, biliverdin and the other bile pigments are derivatives of haemoglobin. Urobilinogen and urobilin are derived from bile pigments. Stercobilin is believed to be identical with urobilin. For their life history *vide* pages 451–52.

Pyrrole compounds are widely distributed throughout animal and plant life. The porphyrins have the property of combining with proteins and various metals producing a number of coloured compounds known as *metalloporphyrins. These compounds, wherever found, are responsible for transport of oxygen or actual tissue oxidation.* For instance **chlorophyll,** the green-colouring matter of plants, is a metalloporphyrin containing Mg. **Haemoglobin** is an iron porphyrin which is the red pigment of blood. **Myoglobin** is an iron-containing red pigment found in the red muscles. **Haemocyanin** is a blue-coloured copper porphyrin present in the blood of molluscs and crustacea, and replaces haemoglobin in them. In certain pathological conditions—known as *porphyrinuria,* large quantities of porphyrins are passed out in the urine. Such people are highly sensitive to sunlight. *Porphyrins are believed to be highly photosensitive and are related to the production of light sensitiveness of these patients.*

II. TYROSINE COMPOUNDS

1. **Melanin.** This is the chief pigment of the body and is derived from tyrosine or phenylalanine. **Distribution.** It is very widely distributed in the body, but peculiarly enough, *it is limited only to those structures which have got an ectodermal origin,* for instance, skin, hair, choroid coat of retina, substantia nigra, etc. **Function.** In the skin and hair it is probable that melanin serves a protective action against the harmful effects of sunlight. Prolonged exposure to sunlight stimulates melanin formation and causes the so-called *sun-tanning.* The function of melanin in the choroid coat is mainly to convert the eyeball into a perfect dark chamber. Since, nervous tissue is derived from ectoderm, the melanin in the substantia nigra may represent the vestigial remnants of the melanin forming properties. **Synthesis.** Melanin, whenever it is found, is formed in the local cells by the enzyme—*tyrosinase* or *melanase.* The mother substance, upon which the enzyme acts, is a tyrosine derivative (DOPA) believed to be formed in the adrenals. The broad steps of melanin synthesis from the oxidation of phenylalanine or tyrosine are as follows:

Phenylalanine→tyrosine→Dihydroxyphenylalanine (DOPA)→**Melanin.**

Melanin formation in both human and amphibian skin is augmented by the hormone known as intermedin or melanocyte-stimulating hormone (MSH) secreted by the pars intermedia of the pituitary gland. Adrenocorticotrophic hormone (ACTH) secreted by anterior pituitary has melanocyte-stimulating activity similar to MSH although to a much lower degree. In Addison's disease, ACTH is secreted in a large amount and there is brownish-black pigmentation of the exposed parts of the skin, e.g., hands, feet, etc., and mucous membrane.

Melatonin, extract from bovine pineal gland, causes concentration of melanin near the nuclei of melanocyte in frog, and as a result of this the skin becomes paler. Its role in human is not known.

In melanotic sarcoma, melanin may be found in the urine.

2. **Homogentisic acid.** It is a metabolic product of tyrosine and is normally oxidised further to acetoacetic acid and fumaric acid. In one of the 'inborn errors of metabolism' known as *alcaptonuria*, homogentisic acid accumulates as it cannot be oxidised. The urine if made slightly alkaline produces a dark coloured melanin-like product.

III. **LIPOCHROMES** (Carotenoids). These are fat-soluble pigments having different shades of colour, ranging from light orange to yellow. **Chemically,** they are unsaturated hydrocarbons containing 40 carbon atoms. They may be of different types, such as the **carotenes,** present in the carrots and plant leaves, the **xanthophylls** and **lycopenes** in the tomatoes, etc. The lipochromes of the human body present in the red nucleus of the brain are probably derived from vegetable lipochromes. The most rich sources are carrots, vegetables and plant leaves. 25 gm of carotene is obtained from 1,000 Kg of carrot. The carotenes are of immense physiological importance, because they act as provitamin A, being converted into vitamin A in the liver.

LUTEIN. It, produced in the corpus luteum, is also a xanthophyll compound serving an essential role in the normal sex life of females. Carotenes and xanthophylls have been isolated from the body fat, milk fat, nervous tissue, etc. *The yellow colour of fat and butter (from cow's milk) is due to these lipochromes. The grey colour of the nerve cells and the red colour of the so-called red nucleus are believed to be due to lipochromes.* Lycopene has also been demonstrated in the human fat.

The reddish colour, sometimes seen in human hair, the bright red colour on the scales of the gold fish and the bright pigments of Canary's feather are all due to carotenoid compounds.

IV. **FLAVINS** (Lyochromes). These are water-soluble, nitrogenous pigments widely distributed in nature having a yellow fluorescence. As a rule the flavins remain combined with phosphoric acid, one specific protein radicle and a pentose molecule. The enzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) present in different cells, lactoflavin of milk, ovoflavin of eggs, etc., are the examples. Riboflavin is a component of vitamin B group. All these flavins take an important part in the oxidative processes inside the cells. Although very widely distributed, yet they are found in very low concentrations. From the whites of 10,000 eggs only 180 mgm of ovoflavins are obtained. 5,400 litres of whey yield only 1 mgm of lactoflavin.

V. **VISUAL PIGMENT.** Several visual pigments have been isolated which are as follows: (1) **Visual purple** or rhodopsin is a chromoprotein and is present in the rods of the retina. In presence of light, the rhodopsin is bleached into retinene$_1$ and opsin. Retinene is the derivative of vitamin A. In the dark, the rhodopsin is efficiently resynthesised. For details *vide* under Eye. (2) **Porphyropsin.** Like rhodopsin, there is also another pigment present in the fresh water vertebrates which is known as porphyropsin. In the light, it is bleached into retinene$_2$ and scotopsin. Retinene$_2$ is the derivative of vitamin A_2 (retinol$_2$). In the dark, the porphyropsin is resynthesised. (3) **Iodopsin.** It is a visual pigment of the cone of the retina. It has been isolated from the cone of the chicken. In presence of light, it is also bleached into retinene$_1$ and photopsin. Retinene$_1$ is the derivative of vitamin A_1 (retinol$_1$). In the dark, the iodopsin is resynthesised. (4) **Cyanopsin.** This pigment has been synthesised in the laboratory and has not been isolated in the retina. It is also bleached in the light into retinene$_2$ and cone opsin. Retinene$_2$ is the derivative of vitamin A_2 and is also resynthesised in the dark.

VI. **URINE PIGMENTS.** (1) **Urobilin** (*vide* pages 451–52). (2) **Urochrome**—It is a sulphur-containing yellow pigment, being the chief colouring matter of the urine. Its amount does not depend upon food and is constant under normal conditions. It depends upon endogenous metabolism and increases in all conditions where tissue damage is going on. It should be considered as an end product of endogenous protein metabolism (neutral sulphur). (3) **Uroerythrin**—reddish colour, nature unknown. (4) **Coproporphyrin**—derivative of haemoglobin.

VII. **FAECAL PIGMENTS. Stercobilin**—same as urobilin. Various other pigments may be found in the stool derived directly from diet, drug and probably bacterial products.

METABOLISM IN STARVATION

Total starvation includes complete deprivation of water, salts and food. It results death of the animal in the shortest possible time. *Deprivation*

of water only follows death of the animal in about **an week,** *that of salts i.* **two weeks,** *whereas food starvation in about* **3–4 weeks** or even longer. The length of time, a man can survive, depends upon the fat stores. The longest period of survival never exceeds 9 to 10 weeks. Here, the effects of food starvation only will be discussed. Starvation induces a number of metabolic changes, some occurring within a few days. There is a progressive fall in B.M.R. body temperature, pulse rate and blood pressure. Ketosis develops and some retention of salt and water occurs.

Experimental observations. The effects of food starvation have been mostly observed in animals. But some direct observations on human beings have been carried out upon professional fasting men. The following effects are seen:

General condition. During the first two days there is a craving for food, particularly at meal times. But later on this craving subsides, provided water and salts are freely allowed. Gradually the desire for food vanishes; weakness increases and a strong dislike to undertake any physical or mental effort develops. At about this time the subject falls into a state of semiconsciousness. The pulse rate and the body temperature remain almost normal till before death. The sleep increases and respiration becomes slower. Temperature falls before death. The amount of urine as well as its urea content falls. Stool is formed and may be passed during or at the close of the starvation period.

Body weight. The body weight is steadily lost. The daily loss in man during the first ten days, amounts to between 1–1·5% of the original body weight. At the onset of the fast the fat depots and subcutaneous tissue bear the brunt. The extracellular fluid in large quantity is also lost. Dissolution of the muscular tissue and protoplasmic structure occurs later. The muscle fibres are much reduced in size and many of the fibres are degenerated. The muscles lose about 35% of its weight. The brain and heart lose about 3% of their weight. Kidney loses about 20%, the liver loses about 55%, and the spleen loses about 70% of their weight. Thyroid, adrenal and gonads lose about 2–6% of their weight. The organs and the tissues of the body are not affected alike. The more vital organs lose the least weight, whereas the less vital ones lose the most.

Metabolism of carbohydrate, fat and protein. During starvation, the body has to depend upon its own tissue materials. Of the three foodstuffs—glycogen, fat and protein—the liver glycogen store is first mobilised. The protein, mobilised from stored protein in the tissue, is transformed into glucose (neoglucogenesis). This *initial stage will last for not more than two days.* In the next stage, 80–90% of the energy requirement will be derived from fats and the remainder (10–20%) from the proteins. Since adipose tissue represents the largest amount of stored food, *the second stage will last for the longest period*—over two weeks. *In the third stage,* when the fat-store is almost exhausted, energy requirements is derived from the breakdown of the body proteins. The cell substance will break up with a consequent dislocation of cell metabolism and cell life. This state of affairs, if continued, it will lead to death. *The third stage lasts for less than one week.*

Thus from the metabolic point of view, the whole period of inanition can be divided into three stages—the **first stage** is the stage of carbohydrate depletion, the **second stage** is that of fat depletion and the **third stage** is that of breakdown of body proteins. The determination of total

R.Q. and of non-protein R.Q. will indicate the extent to which these three foodstuffs are burning at the three stages. The R.Q. is highest at the first stage and diminishes later on. A brief description of the different metabolic changes is given below.

Carbohydrate metabolism. Carbohydrate store becomes almost depleted in the first two days. The blood sugar is maintained at a steady level even up to the end. This steady level is believed to be due to neoglucogenesis in the liver.

Fat metabolism. Fat which remains in adipose tissue (element variable) is utilised first. It goes into the liver where it is completely oxidised and an increased amount of acetyl coenzyme A is produced, resulting formation of ketone bodies whereas due to absence of carbohydrates, fat oxidation usually remains incomplete producing ketosis and acidosis. Thus alkali reserve is diminished and various ill-effects of ketosis are produced. **Ketones** from the liver pass into the blood and ketone bodies appear in the urine. The *acidosis* is compensated by bicarbonate of blood, increased pulmonary ventilation and increased elimination of CO_2 from the alveoli, and increased ammonia formation in the kidney and excretion of ammonium salts in the urine.

Protein metabolism. Tissue protein is broken down and amino acids formed after hydrolysis constitute the **amino acid pool.** The amino acids from this pool is utilised for the maintenance of the structural and functional efficiency of the vital organs. The amino acids also undergo deamination in the liver and the non-nitrogenous part helps in the maintenance of the blood sugar level. The amount of nitrogen excretion during the first few days is directly proportional to the amount of protein intake before starvation. The average daily excretion in the first week is about 10 gm. During the second and third weeks the values are very low. But just before death (when proteins are rapidly breaking down) the urinary nitrogen rises (premortal rise). The end products of endogenous protein metabolism, i.e., creatinine, creatine, neutral sulphur compounds and uric acid are the main nitrogenous products. Creatine excretion gradually falls as the weight of muscles diminishes. On the fifth day of starvation a man excretes 11·4 gm of nitrogen and the energy output is about 2,000 calories. 6·25 gm of protein when broken down eliminates 1 gm of nitrogen. The urinary nitrogen indicates $6{\cdot}25 \times 11{\cdot}4 = 71{\cdot}5$ gm of protein catabolism. Thus the energy liberated at that amount of protein catabolism is $71{\cdot}5 \times 4{\cdot}1$ Cal = 300 calories. The rest of the calories are derived from fat, i.e., 190 gm of fat.

Mineral metabolism. Phosphorus and sulphur excretion in urine at first rises and then falls. The excretion of calcium is elevated and that of chloride, sodium, potassium and magnesium is reduced during starvation.

Changes in blood and urine during starvation

Blood changes. (1) Acidosis with diminished alkali reserve. (2) Low blood sugar. (3) Increased blood fats. (4) Presence of ketone bodies. (5) Raised potassium (Showing breakdown of tissue cells).

Urine changes. (1) Volume becomes less. (2) Nitrogen content steadily falls. (3) Presence of abnormal constituents like creatine, ketones, etc. (4) Ammonia excretion increases as also the ammonia coefficient. (5) Rise of acidity. (6) Fall of potassium that causes chlorine retention (Index of breakdown of tissues).

Premortal changes. The following changes indicate impending death: (1) 50% *loss of body weight.* (2) *Rise of nitrogen excretion.* (3) *Fall of body temperature.*

WATER METABOLISM

Of the three factors, water, salts and food, water is the most important. If we remember that life evolved first in an aquatic medium, there will be nothing to be astonished that water is the most essential substance for life. Deprivation of water will kill a subject much earlier than deprivation of salt or food. With water starvation death takes place in the shortest possible time (in about one week), when only 20% of the total body weight is lost.

Chemistry of water

Our former idea about the chemical constitution of water, having the formula H_2O with a molecular weight 18, is fast changing and is probably no more tenable now. By studying the various properties of water, modern chemistry suggests that water is actually a polymer of H_2O. Critical temperature of water is 365°C. Boiling water is $(H_2O)_3$, ice is $(H_2O)_4$ and ordinary water is a mixture of the two. Experiments with heavy water (D_2O) have clarified many aspects of water metabolism.

I. **Distribution of water in the body.** *Total water content is* 60% to 70% of the adult body weight, i.e., 45–49 litres, females having somewhat lower values than males. Accumulated evidences suggest that using body weight as a parameter of reference, body water content is inversely related to the adiposity of the organism. Thus increase of fatty tissue in the body will automatically result in a reciprocal decrease in total water content when expressed in terms of per cent. body weight. Behnke, to minimise the errors in the concept of total body water, introduced the term **lean body mass** which is made up of functional tissue, containing only essential fat. The total body water calculated on a fat-free basis, according to the concept of lean body mass, on a large number of animals, e.g., rat, guinea-pig, cat, dog, monkeys, etc., averaged 73·2% range being 70–76%. This measurement is applicable only to normal adults. In very young, and in those having other abnormalities, deviations are to be expected.

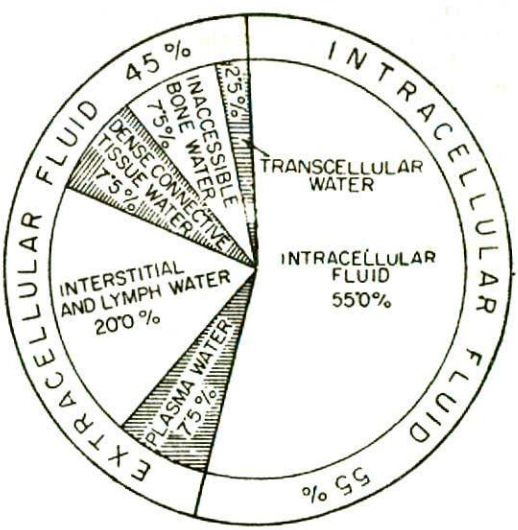


FIG. 471. Diagram showing the relative percentage of water content in different compartments of the body.

The total body water is distributed throughout two main compartments: (1) **intracellular,** approximately 50% of the body weight (i.e.,

39 litres) and (2) **extracellular**—20% of the body weight, i.e., 14 litres, of which 3 litres in plasma and 11 litres in interstitial fluid and lymph.

Recent investigations indicate that although the concept of a single intracellular water component is still useful, the extracellular component is more heterogeneous and is subdivided into four subcomponents:

1. Blood plasma (4·5% body water).
2. Interstitial fluid and lymph (8%).
3. Dense connective tissue, cartilage, bone (6%).
4. Transcellular fluids (1·5%), such as aqueous and vitreous humour, cerebrospinal fluid, endolymph, perilymph, etc.

A man weighing 11 stones (70 Kg) contains about 47 litres of water in the body. Of this, 20 litres (about half) are in the muscles and 10 litres (about one-fifth) in the skin. Blood contains about one-fourteenth part of the total body water. In young animals and in very active tissues, the water content is much higher. A baby contains water much over 70% of its body weight. Water content is maximum in the foetus and diminishes with age.

II. **Water content in various tissues.** The percentage of water in various tissues are as follows: **skin,** 20%; **muscles,** 75–80%; **blood,** 76%; **plasma,** 92%; **connective tissues,** 60%; **corpuscles,** 60%; (total amount of water in blood is 4–5 litres); **nervous tissue—grey matter,** 85% (more than in blood, yet it is solid); **white matter,** 70%; **adipose tissue,** 20%; **dentine,** 10% (least and therefore hardest); **bones** (without marrow), 25%; **cerebrospinal fluid,** 99%.

These figures are approximate and average. The water content of the tissues and organs varies from time to time according to the loss and supply of water and the degree of activity. It must be remembered that the water content of the body is derived from two sources—(*a*) **from food and drink,** (*b*) **as end product of metabolism.** It is better to call the former as **exogenous water** and the latter as **endogenous water.** The body water remains in two states: (*a*) *In the free state,* i.e., not combined with anything. Most of the body water remains in this form. Various substances can remain dissolved in this water and be removed by ultrafiltration. (*b*) *Bound water.* This is a very small quantity. In this form water remains combined with the colloids and other substances.

METABOLIC WATER (ENDOGENOUS WATER)

This water comes as an end product of metabolism. Almost the whole of H of solid food is converted into water, only about 5 gm of H being excreted in the form of ammonia, urea, etc. Different foodstuffs yield different quantities of water. Approximate figures are given below:

100	gm	of fat	gives	107	gm	of	water
,,	,,	,, starch	,,	55	,,	,,	,,
,,	,,	,, protein	,,	41	,,	,,	,,
,,	,,	,, alcohol	,,	117	,,	,,	,,

FUNCTIONS OF WATER

Some of the important physiological functions of water are summarised below:

1. **It is an essential constituent of living cell.** No living thing can resist drying.
2. By its **solvent action** it forms a great number of crystalloidal and colloidal solutions and thus serves as a universal medium in which the

intracellular and extracellular chemical reactions take place. Probably no chemical reaction inside the body can take place without water.

3. **It acts as a medium for various physical processes,** such as osmosis, diffusion, filtration, etc.

4. **Hydrolysis.** It is an important chemical process involved in digestion and metabolism. In this process the H and OH ions of water are introduced into bigger molecules and the latter are broken down into smaller units.

5. **Dehydration and condensation.** In these processes water molecule is removed. This takes place in certain synthetic processes in which bigger particles are formed by the union of smaller ones. For instance, glycogen from glucose. This action is the reverse of hydrolysis.

6. **Ionising medium.** Water is a very good ionising medium. The *dielectric constant* of water being very high, oppositely charged ions can coexist in water without much interference.

7. **It acts as a vehicle for various physiological processes,** such as (*a*) for absorption of food material from the intestine; (*b*) for reabsorption from kidney tubules; (*c*) for the transport of the various foodstuffs from place to place; (*d*) for the drainage and excretion of the end products of metabolism; (*e*) for the manufacture of various secretions, such as, digestive juices, etc.; (*f*) for carrying the hormones to their places of activity, etc.

The physical and chemical properties of water permit chemical reactions requiring large amounts of heat to take place at a low body temperature.

8. **Heat regulation.** Body temperature is regulated by water in the following ways: (*a*) **Heat absorption**—Due to high specific heat of water more heat is required to raise the temperature of 1 gm of water through 1°C. than most of known solids and liquids. By virtue of this property water can mop up large quantity of heat. (*b*) **Heat conduction and distribution**—Heat-conducting power of water being very high it acts as a very good agent in carrying away heat from the site of production and distributing it throughout the body. By the two above properties, water acts as an important heat-buffer. (*c*) **Heat loss**—Water helps heat loss through urine and stool and by evaporation from skin, lungs, etc. Water has got the highest latent heat of evaporation.

By the help of these properties water takes an important part in regulating body heat.

9. **Lubricant action.** Water acts as a lubricant to prevent friction and drying. In joints, pleura, peritoneum, conjunctiva, etc., the aqueous solution is practically free from fats and acts as a lubricant against rubbing and drying.

10. **Refractive medium.** The *aqueous humour* helps to keep up the shape and tension of the eye-ball and acts as a refractive medium for light.

11. **Mechanical action.** The cerebrospinal fluid which contains nearly 99% water acts as a great mechanical buffer preventing injury to the nervous system.

12. **Respiratory function.** Although CO_2 and O_2 are only slightly soluble in water, yet this little solubility is of immense importance for the gaseous exchange in the tissues and lungs. The fish derive oxygen almost exclusively from dissolved O_2 in water.

WATER BALANCE

Water is continuously being supplied and lost from the body. But still the total water content of the body is kept more or less constant, by maintaining a balance between supply and loss. This indicates that there must be an efficient machinery for maintaining water balance.

WATER REQUIREMENT

The total water requirement of an adult, under ordinary conditions, is about 2,500–3,000 ml, i.e., *about* 1 *ml per calorie of food intake.* Half of this quantity (i.e., about 1,500 ml or half ml per calorie) should be taken as free drinks.

Balance sheet

Supply of Water		Loss of Water	
1. Drink	1,450 ml	1. Kidney	1,500 ml
2. Solid Food (All solid foods contain some water as free H_2O)	800 ml	2. Skin (visible and invisible perspiration) ..	600 ml
3. Oxidation	350 ml	3. Lungs	400 ml
		4. Faeces	100 ml
Total ..	2,600 ml	Total ..	2,600 ml

The above figures are average and approximate. Water loss by any one of the channels may rise or fall under various conditions. Loss through skin varies according to the temperature and humidity of the atmosphere and also upon the amount of muscular exercise done. In hot climates and with exercise, excretion through skin may vary from 3–10 litres per day. Higher atmospheric humidity reduces water loss through the skin. Water excretion, by lungs also increases in hot dry weather. In diarrhoea, dysentery, cholera, etc., more water is lost in the faeces, while in conditions of diuresis more is passed out by the kidneys. The water secreted in the digestive juices is not lost water. Because it is almost completely reabsorbed and about 5–7 litres of water circulate in this way per day. The loss in saliva and lachrymal secretion is negligible under normal conditions.

Positive and negative water balance. *Water balance is said to be positive* (intake exceeds loss) in growing infants and children, in convalescents, athletes and pregnant women who are storing water and building their body tissues. Each gram of protein is laid down with about 3 gm of

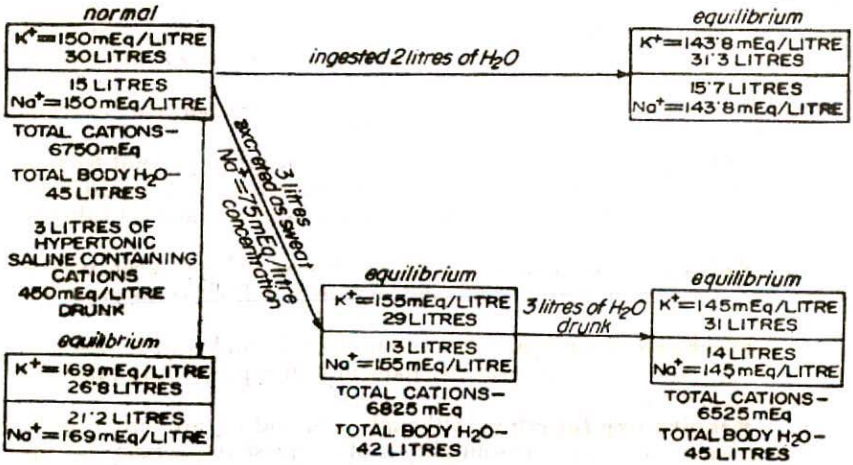


FIG. 472. Diagram showing the exchange of water between intracellular fluid (ICF) and extracellular fluid (ECF) when they are disturbed in different ways.

water. Fat and glycogen are deposited with less amount of water. When diet is changed from high fat to high carbohydrate, water retention takes place and the balance becomes positive.

Water balance is negative (loss exceeds intake) under the following conditions: (1) When the subject is thirsty. (2) When a pre-existing oedema is clearing up due to diuresis. (3) When diet is changed from high carbohydrate to high fat.

In any condition of increased water loss, the relative proportion of Na and K content of the fluid excretion will indicate whether the water is coming chiefly from the extracellular or intracellular sources. High Na content will indicate extracellular source, whereas high K content will indicate intracellular source, provided intake remains constant.

REGULATION OF WATER BALANCE

In spite of large amount of water is constantly appearing in and disappearing from the body, a fairly accurate balance is maintained between its gain and loss, which indicates that there must be a strong regulating machinery. The mechanism which regulates water balance is very intricate and is not yet fully known. The following factors are closely involved in it: (1) **Endocrine,** (2) **Autonomic nervous system**—hypothalamus and the vasomotor system, (3) **Renal regulation,** (4) **Respiration** and **skin,** (5) **Phenomenon of thirst.** The role of these factors are discussed below.

I. Endocrines

A number of endocrines takes part in water regulation. They are as follows:

1. **Posterior pituitary.** It manufactures **two hormones,** e.g., **antidiuretic hormones** or **vasopressin** and **oxytocin,** of which *antidiuretic hormone* has got influence upon water balance (FIG. 473). The **antidiuretic hormone**—This increases the reabsorption of water from the distal renal tubules and thus reduces urine volume. It is very interesting to note that *the secretion of this hormone is controlled by the water content of the body.* Excess of water depresses, while dehydration stimulates the secretion of this hormone. In the thirsting animals the presence of an antidiuretic substance has been demonstrated in the urine.

2. **Adrenal cortex.** Adrenal cortex secretes aldosterone which plays an important part in maintenance of water balance. The secretion of aldosterone is controlled by the angiotensin II and also by high serum K^+ and low serum Na^+. The aldosterone regulates the water balance through ADH release from posterior pituitary, causing retention of water and thus increase of blood volume (FIG. 473). In adrenal cortical insufficiency there is decreased reabsorption of Na^+ and as a result more Na^+ is lost in the urine. There is increased reabsorption of K^+. The reabsorption of Cl^- is also depressed. There is consequential changes in the body fluids. The intracellular crystalloid osmotic pressure exceeds that of the extracellular crystalloid osmotic pressure, and water flows from the extracellular fluid to the intracellular fluid. Plasma volume decreases, and there is anhydraemia and haemoconcentration.

3. **Adrenal medulla.** Injection of adrenaline reduces renal circulation by causing constriction of renal vessels and thus decreases the volume of urine.

4. **Thyroid.** Thyroxine increases urine volume along with increased elimination of salt, probably not by a direct effect on the kidney but by raising the general metabolism and thus increasing nitrogenous end products which acted as **diuretics.** In myxoedema there is increased fluid retention in the extracellular tissue.

II. The autonomic nervous system

1. **Hypothalamus.** The hypothalamus controls the secretion of antidiuretic hormone of the posterior pituitary through the supra-optico-hypophyseal tract. Lesion of this tract or the corresponding region of

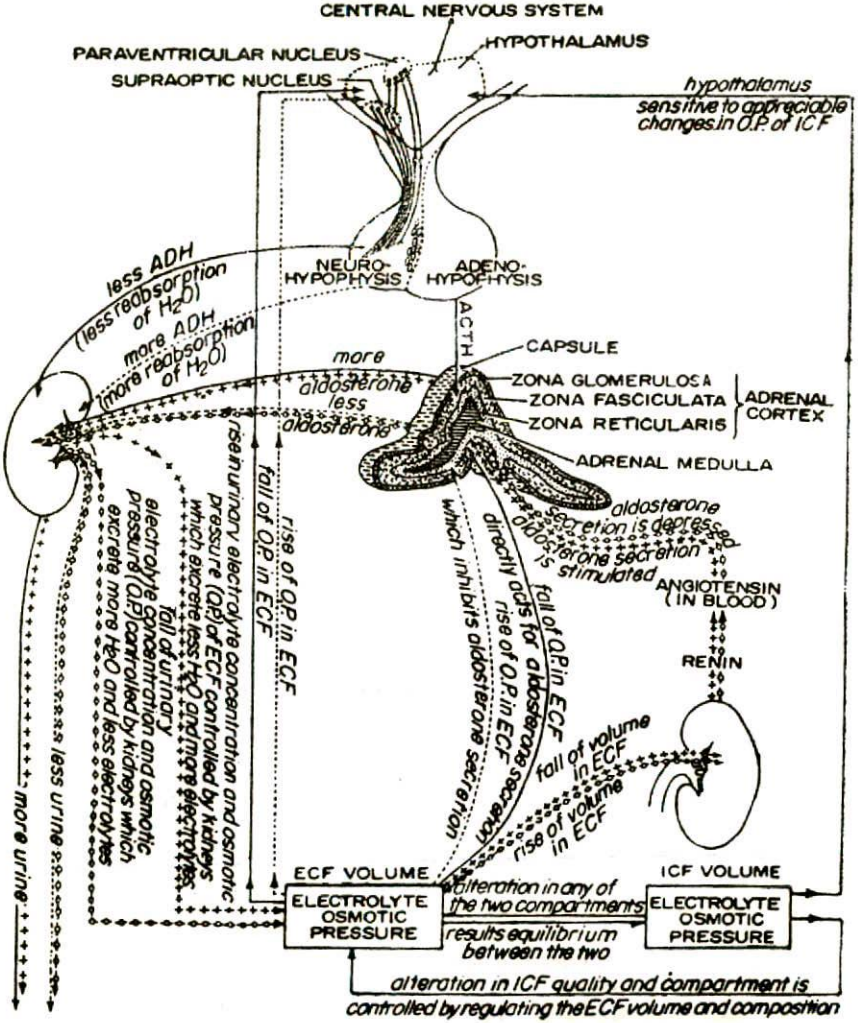


FIG. 473. Diagram showing the control of body fluids by posterior pituitary hormone (ADH) and mineralocorticoids of adrenal cortex.

hypothalamus or disease of the posterior pituitary causes intense polyuria known as **diabetes insipidus.** This function of hypothalamus may be controlled in the following way: the **water content of the body**—Excess water dilutes blood and reduces osmotic pressure as a result of which the hypothalamus is depressed leading to less secretion of antidiuretic hormone and consequently diuresis is produced. When body water is reduced, the osmotic pressure of blood increases, hypothalamus is stimulated—more antidiuretic hormone is secreted and consequently urine volume is reduced. [*Vide* under the chapter on *Excretion.*]

2. **Vasomotor system.** The vasoconstrictor and vasodilator nerves also play an important role in the regulation of renal circulation and general blood pressure.

III. Renal regulation

When water content of the body rises, such as by excess water intake, or saline injections, etc., kidneys excrete more water. This effect may be ascribed to: (1) Increased blood volume and consequent rise of blood pressure and thereby increased filtration pressure. (2) Dilution of plasma proteins, reducing colloidal osmotic pressure and consequently increasing the available filtration pressure. (3) Increasing the number of active glomeruli. (4) Depressing the degree of water reabsorption by the renal tubules. It has been shown that the first two effects are negligible. In man no increase of glomerular filtration takes place until the urine volume exceeds 900 ml per hour. Regarding the third factor it has been proved that in certain species of animals, there is some increase in the number of active glomeruli under such conditions. It is doubtful whether in man this change at all takes place. But even if it is assumed that it does take place, yet this cannot explain the huge increase of urine volume which may be as much as twenty times its normal value. (5) It has been observed that the increase of central blood volume enhances the urine output through the inhibition of secretion of ADH. It is suggested that the inhibition of ADH secretion takes place reflexly through the stimulation of stretch receptors present in the left atrial wall. (6) Besides these, angiotensin II which is formed by the kidney-*renin*, takes an important part in regulation of water balance through the secretion of aldosterone (FIG. 473).

The fourth factor is, therefore, the chief agent in regulating water excretion by the kidneys under physiological conditions. It has been already explained how the water content controls the secretion of antidiuretic hormone from the posterior pituitary upon which the degree of renal reabsorption depends.

IV. Lungs and skin

These channels also take considerable part in regulation of water-balance by excreting variable amounts of water.

V. Phenomena of thirst

When more fluid is lost, such as, in diarrhoea, vomiting, diuresis, sweating, haemorrhage, etc., the subject feels thirsty, and takes water. **Thirst** may be defined as the specific **'hunger for water'.** In this way the amount of lost water is replenished. In hibernating animals metabolism is so low that the water produced by the oxidation of foodstuffs is enough to equalise the water loss. Hence, under such condition no thirst is felt. **Drinking** is stimulated by three types of stimuli:

1. A *rise in vascular tonicity* even without any change in blood volume.
2. A *fall in blood volume* even when unaccompanied by a rise in osmolarity.
3. A *third factor* which operates in some animals is a rise in temperature which can stimulate drinking even before there is any obvious change in body water content.

Little is known about the receptors which mediate the sensation of thirst. Presumably the initial sensation of thirst depends on blood volume and osmolarity changes—when appropriate amount of water has been drunk, the sensation vanishes because of the activity of oral and gastric receptors. The thirst centre is situated in the midhypothalamic region near the paraventricular nucleus (caudal to the osmoreceptors).

Thus when the water content of the body increases, the water balance is maintained in two ways: (1) **By reducing water intake**—The subject does not feel thirsty and takes no water. (2) **By increasing water loss**—This is done by reducing secretion of antidiuretic hormone through hypothalamus and thus causing diuresis.

When the water content of the body is reduced (by loss, etc.)—exactly opposite processes take place and the balance is thus maintained.

LIVER

Introduction. The liver is the largest organ in the body and acts as a well-equipped biochemical laboratory where practically metabolism of all the nutritional substances, viz., carbohydrates, proteins, lipids, vitamins and minerals, takes place as well as heat is produced. So it has been considered appropriate to discuss the functions of liver at the end of the chapter dealt with metabolism of these substances.

Anatomy and histology. The liver is both a secretory and an excretory gland and is situated on the upper and right side of the abdominal cavity. This gland is relatively much larger in the foetus than in the adult. It is almost a solid organ consisting of several lobes. Each lobe is made up of numerous lobules. Under the microscope, each lobule is found to be composed of rows of *polygonal cells* radiating from the centre—like the spokes of a wheel (FIG. 474), the periphery of the lobule being delineated by the presence of portal triads. The centre is occupied by the *central* (*intralobular*) *vein.* The portal vein and hepatic artery enter into, and bile duct and hepatic vein emerge out of the liver through a connective tissue sheath **(Glisson's capsule).** After entering, they branch repeatedly. It has been discussed earlier (*vide* page 339) that there is frequent hepatoportal anastomosis in between the hepatic artery and portal vein in the *interlobular region.* Recent investigations show that the liver cells are arranged in the form of plates, having a thickness of single-cell diameter, which provide a honeycomb or sponge-like structure. Throughout this structure, the cell plates are tunnelled by a communicating system of cavities or **lacunae.** These lacunae contain hepatic sinusoids which are formed by endothelial cells and phagocytic cells of the R. E. system called **Kupffer cells.** Electron microscope reveals that Kupffer cells may contain phagocytosed substances and they are elongated structures having an irregular outline, crenated nucleus, few mitochondria and varying number of lysosomes. The narrow space separating the sinusoidal wall from the liver cell plates is known as **Disse's space.** The flow through the

sinusoids is guarded by *inlet and outlet sphincters* and the intermittent flow is mostly due to the presence of these sphincters. In the liver lobule the sinusoids are drained in the central vein. The interlobular branches of the hepatic artery also end in the sinusoids directly. The central vein while passing through the long axis of the lobule, constantly receives sinusoids from all sides and ultimately leaves the lobule at its base. Here it joins with the central vein of the neighbouring lobules thus forming the *sublobular vein* which ultimately ends in the large hepatic vein. The hepatic veins

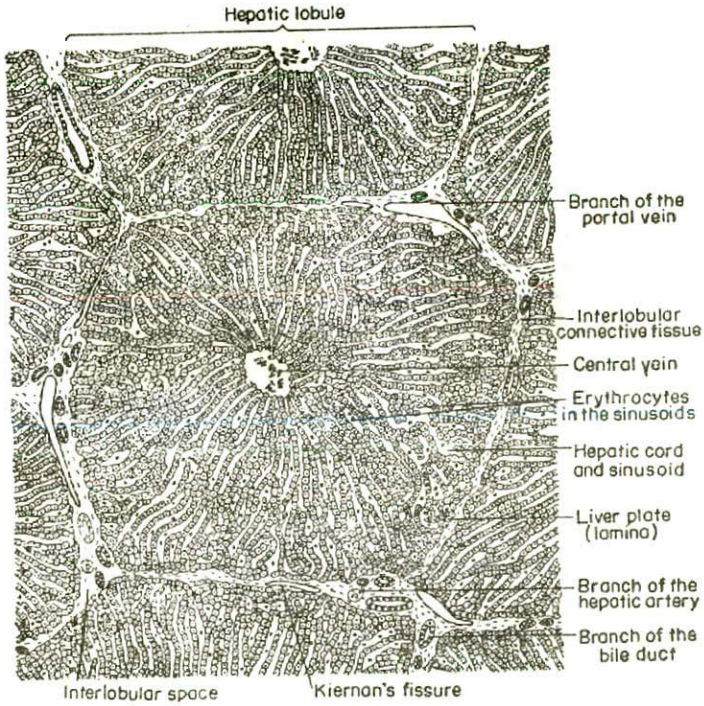


FIG. 474. Histological structure of a liver lobule.

drain the blood from the liver into the inferior vena cava. *Each radiating column of hepatic cells has, on one side, a vascular capillary going to join the central vein and, on the other side, a bile capillary going out of the lobule* to join bigger bile channels. The intercellular canaliculi radiate outward from the central axis of the lobule to join with the intralobular canaliculi which then end in the interlobular ducts—the *terminal bile duct*. Many such interlobular ducts join to form the longer duct. The interlobular bile ducts while proceeding through the connective tissue septa, always run in accompaniment with the branches of the portal vein and hepatic artery (FIGS. 475 & 476). The liver capillaries contain numerous *mast cells*. Bile is formed in minute vacuoles in the true hepatic cells which are discharged through fine *intercellular canaliculi* into the bile capillaries. Hepatic cells are polygonal

in shape and on the average 25 μ in size. 85% of hepatic cells being parenchymal type has a clear cell membrane and often binucleated, and mitosis is rare normally. The cytoplasm contains stored glycogen and fat, also basophilic material. Mitochondria, Golgi apparatus, granular and agranular endoplasmic reticula and lysosomes are present. The excretory

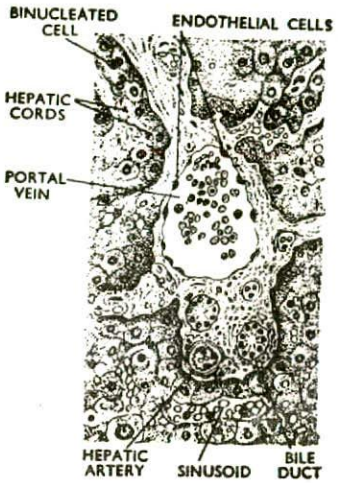


FIG. 475. Diagram showing the section of human liver where hepatic artery, portal vein and bile duct pass side by side.

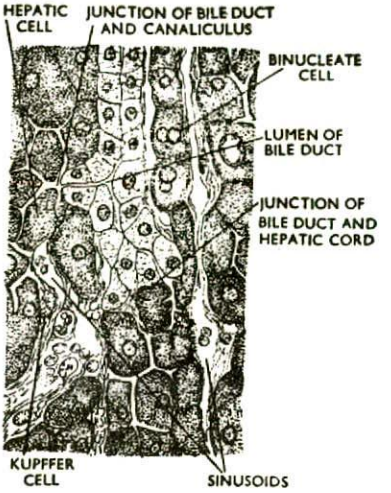


FIG. 476. Diagrammatic representation of section of human liver showing the course of bile canaliculi leading to bile duct.

system of the liver starts with bile canaliculi which are lined by single membrane and the membrane is separated by two or more liver cells. The membrane is protruded into canaliculus in the form of microvilli which increase the area for interchange. Partial removal of liver in man and animals results rapid regeneration by cell replication. Some hormonal regulations may be present in the mechanism of rapid regeneration.

Under electron microscopy, the border of liver cell is straight except for a few desmosomes from where microvilli project into the lumen of bile canaliculi and they also project with irregular size and space along the sinusoidal margin into perisinusoidal tissue space. Due to their such structure, active secretion or absorption of fluid, etc., takes place.

Liver-cell nuclei contain chromatin and DNA as revealed by the histologist and the biochemist respectively. Human liver-cell nuclei become tetraploid after puberty, octoploid at the age of about 20 years and have a double contour with pores. These pores allow interchange with surrounding hyaloplasm. In the chromatin network there are one or more embedded nucleoli.

The inner invaginations of the mitochondria form cristae where most of the oxidative phosphorylation takes place. Mitochondria contain mostly cytochrome oxidase, succinoxidase and phospholipids in large amounts.

They can oxidise numerous substrates including fatty acids and intermediates of TCA cycle. Mitochondria transport energy releasing in the form of ATP.

The rough-surfaced endoplasmic reticula contain granules, ribosomes with RNA and are the seat of protein synthesis. Under light microscopy, these granules are responsible for basophilia. The smooth-surfaced endoplasmic reticula are the sites of detoxification of drugs, conjugation of bilirubin, synthesis of steroid hormones and enzymes.

The liver-cell lysosomes, adjacent to bile canaliculi, are the sites of deposition of ferritin, lipofuscin, bile pigment and copper, and contain many hydrolytic enzymes. Pericanalicular dense bodies (microbodies) and pinocytic vacuoles are also seen.

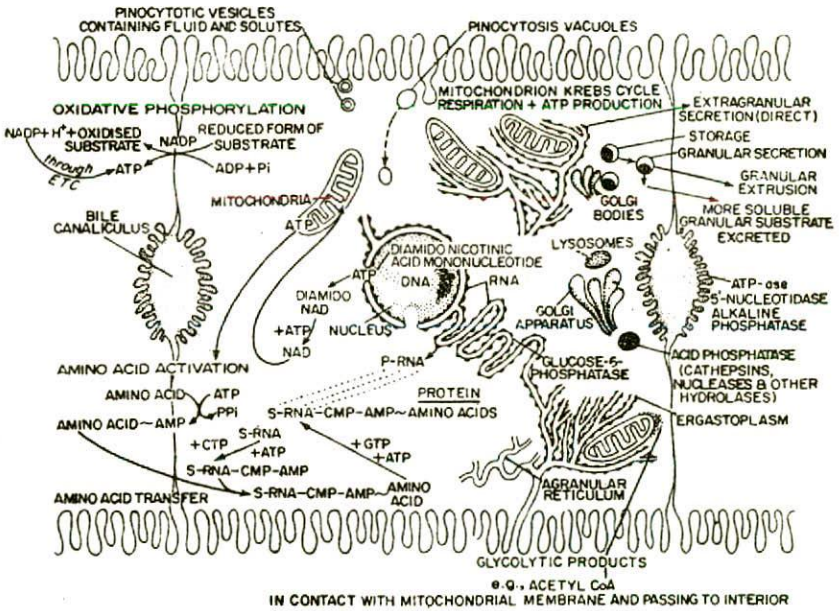


FIG. 477. Diagram showing the intracellular metabolism in a liver cell (after *Novikoff*).

Golgi apparatus or body acts as a *packaging* site for excretion of bile. This apparatus comprises a system of particles and vesicles lying near the canaliculus. Lysosome, Golgi apparatus and microbodies sequester any ingested materials, and secrete, excrete and store them for metabolic purposes of the hyaloplasm.

The intervening hyaloplasm contains granules of lipid, glycogen and fibrils. Bile ductules are surrounded by collagen fibres.

FUNCTIONS OF LIVER

Liver is an essential organ of the body. Its functions are numerous which are briefly summarised below:

I. **In connection with blood and circulation:**

(*i*) R. B. C. formation—in foetal life.

(*ii*) R. B. C. destruction—in adult life.
(*iii*) Storehouse of blood and regulates blood volume.
(*iv*) Relation with blood clotting—(*a*) Manufactures prothrombin (with the help of vitamin K) and fibrinogen and thus essential for clotting. (*b*) Mast cells form heparin and prevent intravascular clotting.
(*v*) Related to activity of its R. E. system in immune mechanism.
(*vi*) It transfers blood from portal to systemic circulation.
(*vii*) Manufactures all plasma proteins.

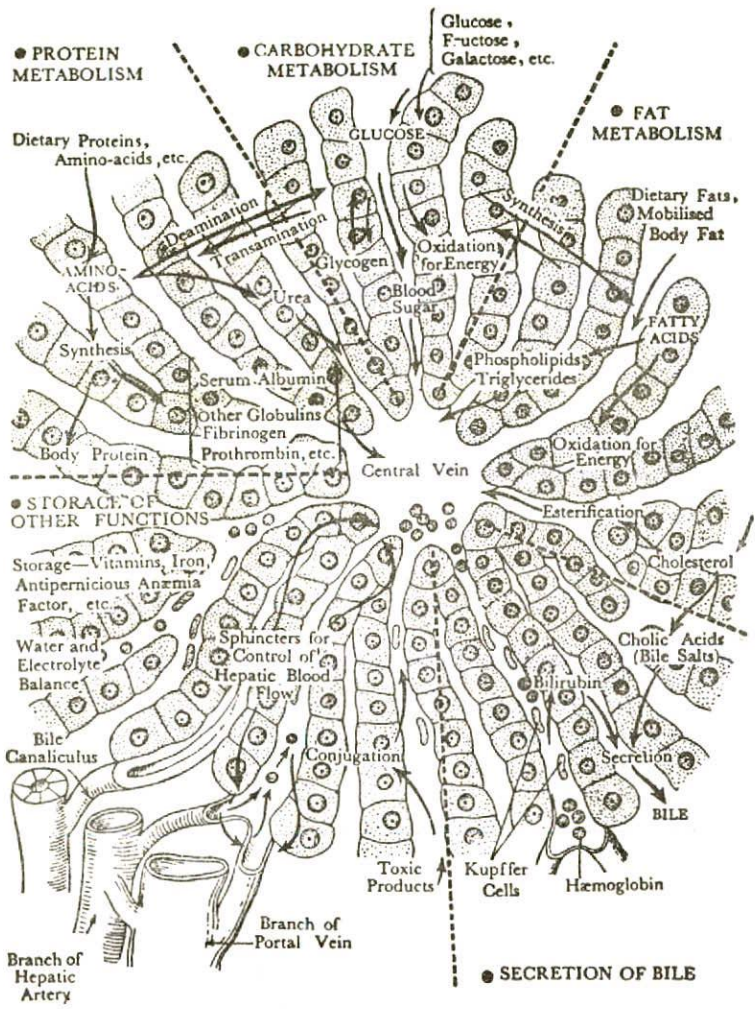


FIG. 478. Diagrammatic representation of functions of a liver lobule. Rows of radially arranged liver cells; each row has a bile capillary on one side and a blood sinus on the other. Stellate cells of van Kupffer are shown in the walls of the sinus.

(*viii*) Stores iron, haematinic factor also known as vitamin B_{12} and copper, and thus helps in the formation of red cells and haemoglobin.

(*ix*) **Hepatic and portal circulation.** The total flow of blood in the liver through both hepatic artery and portal vein is about 1·5 litre/minute. 20–30% of which is carried by the hepatic artery and 100% saturated with O_2, whereas the remaining is carried by portal vein which is 80–90% saturated with O_2. Portal venous blood is derived mainly (60%) from superior mesenteric vein and remaining from splenic vein. The bloods of two streams are well mixed in man before they are supplied to the cell and thereby every cell receives the same blood. The pressure in the portal system is normally between 5 and 13 mm of Hg. Due to high pressure in arterial blood of hepatic sinusoids, the portal blood mixes with it freely. The hepatic vein formed from the central vein in which hepatic venous pressure is about 6 mm of Hg and normally 60% is saturated with O_2.

In vascular components of the liver there are sphincters which regulate local contributions of hepatic artery, portal vein, total liver blood flow and the capacity of total venous bed. Thus the liver can accommodate blood up to one-third of the total blood volume.

The liver produces 75 ml of lymph per minute and its protein content is 90% of that of plasma. Through hepatic lymphatics, 40% of the total plasma protein is returned to the blood in each 24-hours.

II. **Manufactures bile.**

Bile is secreted continuously from the liver cells and stored in the gall bladder. Bile contains water (11·14%), total solid (5·18%), mucin and pigments (3·42%), neutral fat (0·37%), fatty acids (0·97%), phospholipids (0·2%), cholesterol (0·63%) and inorganic ions (0·85%). Cholesterol is synthesised in the liver from active acetate. Cholesterol is also excreted from the liver. Bile acids—cholic acid, deoxycholic acid and lithocholic acid have been considered to be the derivatives of cholanic acid and formed in the liver. The bile acids in conjugation with glycine and taurine, form the compounds—glycocholic acid and taurocholic acid

$C_{23}H_{39}O_3.CO.NH.CH_2.CH_2.SO_3H$ — Taurocholic acid

$C_{23}H_{39}O_3.CO.NH.CH_2.COOH$ — Glycocholic acid

respectively. Bile salts are the Na-salts of taurocholic acid and glycocholic acid. Bile salts have got important functions in absorption of fats and also for the emulsification of fats with the concurrent production of a great surface area to enable lipase and other enzymes to act more efficiently.

Bile pigments are the biliverdin and bilirubin. These are the excretory products of haemoglobin of broken down R.B.C. and are formed in the R. E. system in the various parts of the body. Bone-marrow, liver and spleen have been considered to be the site of formation of the bile pigments.

III. **Relation with carbohydrate metabolism** (Figs. 479, 480, 481, 482 & 484):

(*i*) Converts non-glucose monosaccharides into glucose (and glycogen).

(*ii*) Converts lactic acid, pyruvic acid and glycerol into glucose and also glycogen.

(*iii*) Stores carbohydrate in the form of glycogen (*glycogenesis*) and when blood sugar tends to be low, it mobilises glycogen (*glycogenolysis*).

(*iv*) Takes an important part in blood sugar regulation.

(*v*) It is the seat of *neoglucogenesis*.

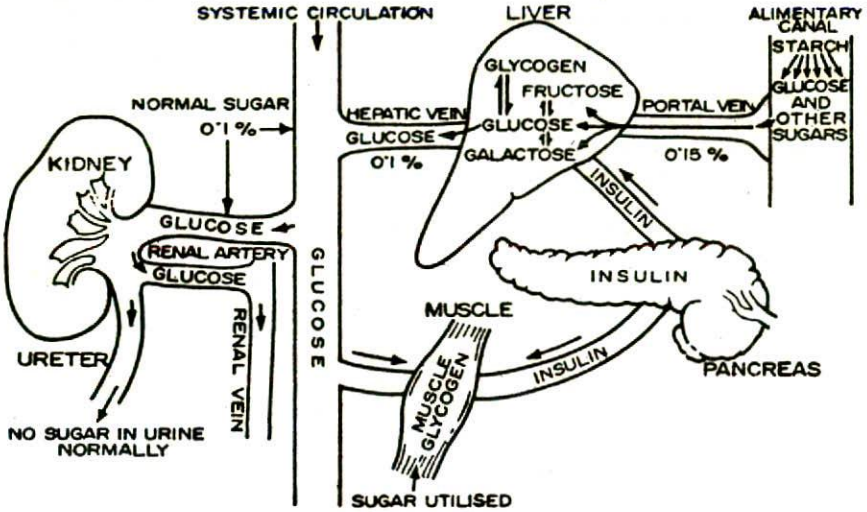


FIG. 479. Diagram showing glucose utilisation in a normal individual. Excess glucose present in blood stream stored as glycogen in the liver and additional glucose in muscle.

(*vi*) Manufactures fats from carbohydrates, etc.

(*vii*) Glucose is metabolised here through TCA cycle and other alternative pathway (HMP shunt).

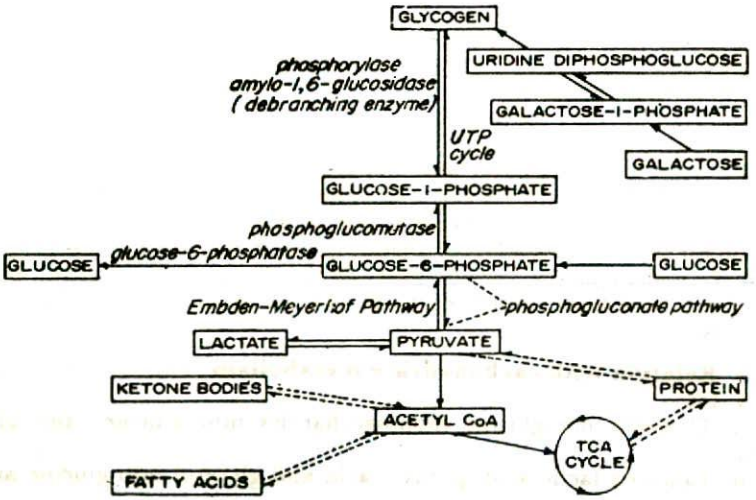


FIG. 480. Diagram showing the metabolic pathway in glycogen breakdown.

(*viii*) Glucuronic acid is formed from uridine diphosphate glucose (UDPG) which plays an important role in the conjugation of bilirubin and other substances (Fig. 481).

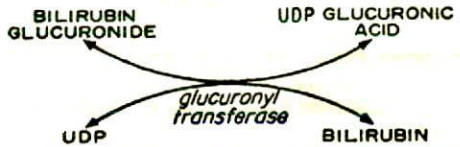


Fig. 481. Diagram showing the conjugation of bilirubin by liver as glucuronide.

(*ix*) **Alcohol metabolism** (Fig. 482). The liver is the main seat of alcohol metabolism. A zinc-containing enzyme, *alcohol dehydrogenase* catabolises acetaldehyde. A coenzyme *acetaldehyde dehydrogenase* converts acetaldehyde to acetyl CoA. Acetaldehyde can be further catabolised to acetate. Acetate may be oxidised to CO_2 and H_2O or converted to other biochemical compounds including fatty acids through TCA cycle.

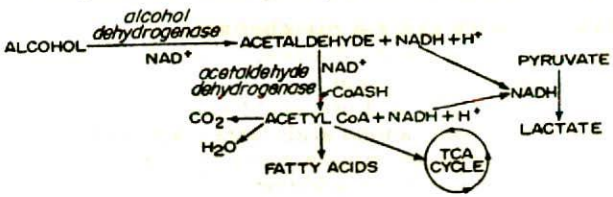


Fig. 482. Metabolism of alcohol (ethanol) in the liver.

When alcohol is converted to acetaldehyde and then to acetyl CoA, NAD acts as a cofactor, i.e., hydrogen acceptor. ATP, generated by the oxidation of NADH through electron transport chain (ETC) of oxidation, provides energy for the synthesis of fatty acids. The reduction of activity of TCA cycle substrates is due to decreased fatty acid oxidation. On the other hand, NADH+H+, being hydrogen carrier, is utilised for the conversion of pyruvate to lactate and for this reason alcohol raises blood lactate level. It is postulated that this mechanism explains post-alcoholic hypoglycaemia.

Hepatic microsomes help in the esterification of fatty acids to triglycerides rather than phospholipids. The direct effect of alcohol may be *alcoholic fatty liver* which is the cause of increased hepatic fatty acid synthesis as well as decreased hepatic fatty acid oxidation.

IV. **Relation with fat metabolism** (Figs. 483 & 484):

(*i*) It stores fats. Liver contains about 3% of fat (with mixed diet).
(*ii*) It helps in the oxidation of fat, releasing energy in the form of ATP.
(*iii*) Site of synthesis of cholesterol from acetate.
(*iv*) Synthesises phospholipids.
(*v*) Synthesises fats from carbohydrates and proteins.
(*vi*) It is the seat of ketone body formation.
(*vii*) Unused free fatty acid (FFA) released from fat depot, is converted to triglycerides and other lipids to meet energy requirement.
(*viii*) Glycerol is oxidised here *via* the pathway of carbohydrate metabolism.

(*ix*) In a carbohydrate deficiency, the fat metabolism in the liver is increased and fat is partially converted to glucose or glycogen.

(*x*) Fat-soluble vitamins, e.g., A, D, E, K, are stored here.

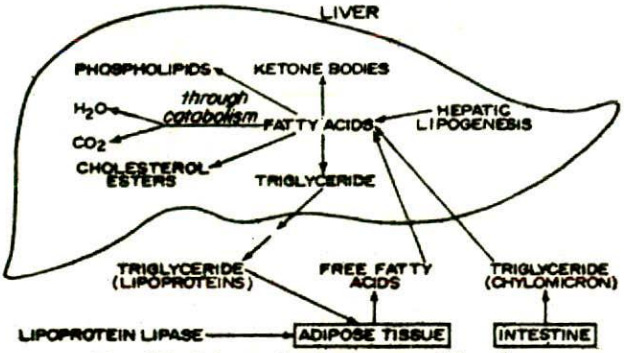


FIG. 483. Scheme showing sources of liver fat.

V. **Relation with protein metabolism:**

(*i*) It is the seat of specific dynamic action of protein.

(*ii*) Chief seat of deamination.

(*iii*) Main seat of urea and uric acid formation.

(*iv*) Synthesis of some amino acids takes place here.

(*v*) Plasma proteins are manufactured here except immune globulin.

(*vi*) Coagulation factors, in addition to fibrinogen and prothrombin, are manufactured here.

(*vii*) It is the seat of nitrogen metabolism as evident from deamination process, urea synthesis, etc.

VI. **Hormone metabolism:**

(*i*) Reduces the circulating adrenal cortical and sex hormones by degradation and conjugation.

Steroid hormones like oestrogen, cortisol, testosterone, etc. are insoluble and solubility is increased and made excretable after being conjugated with glucuronic acid and sulphuric acid in the liver. Normally the liver inactivates adrenocortical hormones by reduction of the 4–3-ketonic group with the formation of tetrahydroderivatives. The tetrahydroderivatives, are then either oxidised to the 17-ketosteroids or the C-20 ketone. In hepato-cellular disease there is defect at the stage of reduction of the 4–3 ketone group and not in its conjugation.

The liver normally extracts about 97% of aldosterone delivered and is converted into both acid hydrolysable conjugate (AHC) and to tetrahydroaldosterone. Extra-hepatic tissue also metabolises a certain amount into AHC. In the liver disease the utilisation by extra-hepatic tissue is increased.

Oestrogens are inactivated by the liver. The enzyme 16-α-hydroxylase is concerned in conversion of oestrone to 16-hydroxyoestrone, the precursor of oestriol. In patients with severe liver disease, the activity of this enzyme is impaired and oestradiol excretion is increased in association with decreased excretion of oestriol.

The liver cells can clear testosterone from the blood rapidly through conjugation. In liver disease, though the testosterone is removed from the blood but excretion of 17-ketosteroids in the urine is reduced.

(*ii*) Inactivation of insulin, glucagon, antidiuretic hormone (ADH), and anterior pituitary trophic hormones, etc., occur here.

VII. Relation with vitamins:

(*i*) Manufactures prothrombin with the help of vitamin K.

(*ii*) It forms vitamin A from carotene and stores vitamins A and D.

(*iii*) Chronic liver disease is always associated with *folic acid* deficiency. It is known that the liver converts *folate* to its active form—tetrahydrofolate. Tetrahydrofolate is the storage form of folic acid. In liver disease, this enzymatic transformation of tetrahydrofolate becomes impossible and thus folic acid is excreted in the urine.

(*v*) The liver is the principal storage organ for vitamin B_{12} and in condition of hepato-cellular disease, the uptake of vitamin B_{12} by the liver tissue is decreased.

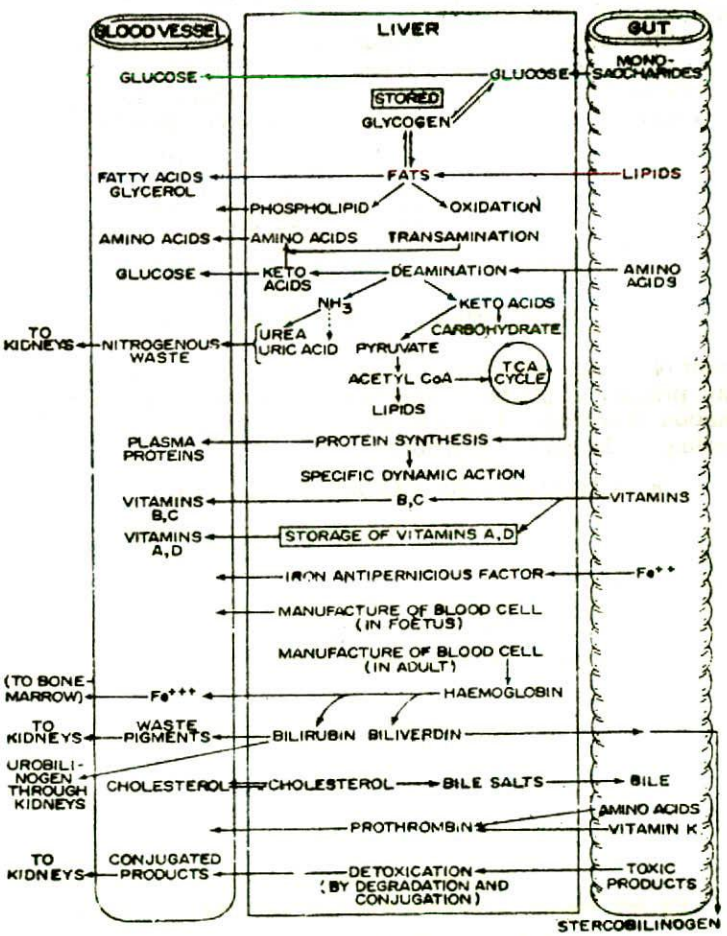


FIG. 484. Schematic representation of different metabolic functions of hepatic cell in brief.

VIII. Excretory functions:

(*i*) Certain heavy metals are temporarily fixed by the liver cells which are then excreted in the bile.

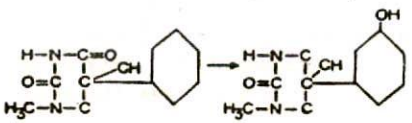
(*ii*) Various toxins, bacteria and drugs are excreted through bile.

(*iii*) Cholesterol and bile pigments are excreted in the bile.

IX. Detoxicating and protective functions:

The liver is the site of detoxication of different toxic substances either produced in the body or taken along with food. Detoxication is the process by which toxic substances are rapidly made excretable through different biochemical changes. This is done by the process of *oxidation*, *hydrolysis*, *reduction* and *conjugation*. Drugs are also made excretable by the liver enzymes. The enzymes for the above processes are bound to hepatic microsomes. Detoxication processes of the drugs and other toxic substances are as follows:

(*i*) **Oxidation.** Drugs *phenylbutazone* and *hexobarbital* are detoxified mainly in the liver cells. It requires NADPH, O_2 and microsomal enzymes. Phenylbutazone is oxidised to oxiphenbutazone and the hexobarbital is oxidised to an inactive substance (Fig. 485). Besides these, a large



Hexobarbital Inactive substance

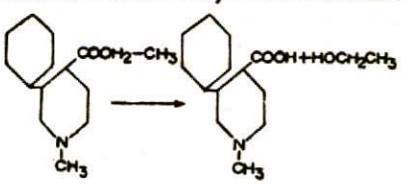
Fig. 485. Hepatic detoxication of drug by *oxidation*

amount of foreign substances are metabolised in the body by oxidation. Many primary aliphatic amines are detoxified in the liver with the formation of corresponding acid and the nitrogen is converted into urea according to the general reaction

$$R—CH_2NH_2 \longrightarrow R—COOH + NH_2CONH_2$$

The sulphur of a good number of organic sulphur compounds is oxidised to sulphate to varying degrees and the sulphate is excreted in organic or inorganic form.

(*ii*) **Hydrolysis.** Pethidine is hydrolysed by microsomal enzymes to inactive metabolite (Fig. 486). The drug procaine is also rapidly hydrolysed into *para*-aminobenzoic acid and diethylamino ethanol.

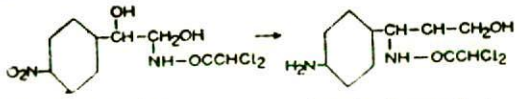


Pethidine Inactive substance

Fig. 486. Hepatic detoxication of drug by hydrolysis (de-esterification).

(*iii*) **Reduction.** This process requires NADH or NADPH as hydrogen

donor and also flavoprotein enzyme. Chloramphenicol or chloromycetin is detoxified by this process into its inactive by-product (FIG. 487).



Chloramphenicol Inactive by-product

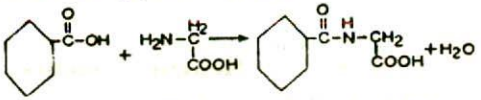
FIG. 487. Hepatic detoxication of durg by the process of reduction.

Many nitrocompounds are also detoxified by this process. Picric acid is reduced into picramic acid. But both the acids are excreted into the urine. Chloral hydrate is partly reduced into trichloroethanol. This reduction process is also employed for the conversion of

(*a*) —S—S— linkages to — SH groups,

(*b*) azo compounds to amines, e.g., *para*-dimethylaminoazobenzene (butter yellow), and *para*-aminophenol.

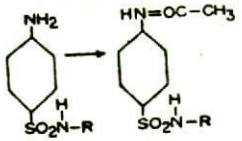
(*iv*) **Conjugation.** By this process a good number of toxic substances are detoxified and eliminated from the system. These are (*a*) bilirubin as glucuronide, (*b*) phenol as glucuronide or phenyl sulphuric acid, (*c*) benzoic acid in conjugation with glycine as hippuric acid (FIG. 488), and also



Benzoic acid Glycine Hippuric acid (Benzoyl glycine)

FIG. 488. Hepatic detoxication of drug by conjugation.

with glucuronic acid as glucuronide, (*d*) phenylacetic acid in conjugation with glycine as phenylaceturic acid and in conjugation with glutamine as phenylacetyl glutamine, (*e*) bromobenzene in conjugation with acetylated cysteine as *para*-bromophenyl mercapturic acid, (*f*) sulphanilamide in conjugation with acetyl CoA (through acetylation) as *para*-N-acetyl benzene sulphonamide (FIG. 489).



Sulphanilamide *p*-N-acetyl benzene sulphonamide

FIG. 489. Hepatic detoxication of drug by acetylation.

X. **Takes part in heat regulation:**

The liver produces a large amount of heat and takes part in heat regulation.

XI. It is also responsible for the liberation of a **depressor principle.**

XII. It is the storehouse of Fe, fat-soluble vitamins, glycogen, labile protein, fat, etc.

Different metabolic functions of the liver have been presented in FIG. 484.

Fatty liver: Lipotropic agents. Fatty, yellow-looking enlarged liver, observed in certain pathological condition is called fatty liver. This can be

produced experimentally in animals on a low carbohydrate and high fatty diet. Substances like lecithin, choline, methionine, etc., prevent or cure fatty liver and are called lipotropic agents.

Effects of portal obstruction (clinical)

(*i*) Congestion at the lower end of oesophagus and in the mucous membrane of the anus, etc., and occasional haemorrhages from the dilated venules of these sites.

(*ii*) Dilated venules over the anterior abdominal wall.

(*iii*) Rise of portal venous pressure.

(*iv*) Accumulation of fluid in the peritoneal cavity—ascites.

Clinically portal obstruction is noticed in *cirrhosis of liver.*

Jaundice

Jaundice is the condition when excessive bile pigment is formed and escapes into the tissues, causing it yellow. It may be due to the production of bile pigment more than the liver can excrete it. In diseases of the liver, bilirubin excretion may be decreased and accumulated in the blood. Sometimes, obstruction in the transport system of the bile may cause jaundice. Thus the cause of jaundice may be subdivided into three main groups which are as follows: (*a*) **Haemolytic jaundice** in which excessive bile pigment is formed due to increased destruction of the erythrocytes. In such condition, bilirubin concentration in the serum is raised and accumulated in the tissues. (*b*) **Hepatic jaundice** occurs due to various liver poisons, toxins, viral infection, cirrhosis, etc. In such condition, the normal liver function is lost due to damage of the parenchymal cells, (*c*) **Obstructive jaundice** is the condition when hepatic or common bile duct may be obstructed and failing to be excreted by the bile capillaries, it is absorbed into the hepatic veins and lymphatics.

EFFICIENCY TESTS FOR LIVER

From the above facts, it will be evident that liver functions are so varied that any one test may not be competent in investigating all of its functions. Hence, many tests have been devised. The following is a brief summary:

I. INVESTIGATION OF THE SECRETORY AND EXCRETORY FUNCTIONS OF LIVER

1. **Van den Bergh reaction.** Van den Bergh reaction is based on the application of Ehrlich's test for bilirubin present in the serum. Ehrlich's reaction is based on the coupling of diazotised sulphanilic acid and bilirubin for the production of reddish-purple azo compound. In the original procedure of Ehrlich test alcohol was used as a solvent for both bilirubin and the diazo reagent. But Van den Bergh (1916) observed the normal development of colour without the addition of alcohol. The bilirubin which gives normal colour reaction of diazo reagent without the addition of alcohol, has been termed the 'direct reacting' bilirubin. However, another form of bilirubin, still present in the serum which requires alcohol for colour reaction with the diazo reagent. This form of bilirubin is termed as 'indirect reacting' bilirubin. It is now claimed that 'direct reacting' bilirubins of Van den Bergh are conjugated bilirubin. These bilirubins after being formed in reticulo-endothelial system pass through the liver, where these are conjugated with glucuronic acid, forming the bilirubin glucuronide. 'Direct reacting' bilirubin is found in obstructive jaundice. 'Indirect reacting' bilirubin is free (unconjugated) bilirubin which remains as such without being conjugated in the liver. As this form of bilirubin is insoluble, it requires alcohol for extraction as well as reaction with diazo reagent. This form of bilirubin is found in haemolytic jaundice. In obstructive jaundice both types (direct and indirect) are detected.

2. **Tests for bile pigments and their derivatives in the faeces.** Such as, for unaltered bile pigments, hydrobilirubin (*Schmidt's test*), etc. Positive reaction indicates that bile is being secreted by liver and enters into the intestine.

3. **Bilirubin excretion test.** This indicates the excretory power of liver. A standard amount of bilirubin is given intravenously and its concentration is determined in plasma from time to time. Normally not more than 5 to 6% of the pigment should be retained after 4 hours.

4. **Bromosulphalein excretion test.** A standard amount of the dye is given intravenously and its concentration in blood is estimated from time to time. If any dye is retained in blood after 30 minutes—the liver is damaged.

5. **Rose Bengal dye test.** Ten ml of 1% Rose Bengal dye are injected intravenously. In normal individual, 50% of the dye is excreted within 8 minutes.

6. **Test for urobilinogen** (and urobilin) in the urine. Positive reaction shows two things: (*a*) that bile is entering into the intestine and (*b*) that liver cannot excrete the urobilinogen—which is reabsorbed from the intestine. On the whole, liver function is deficient.

II. INVESTIGATION OF THE METABOLIC FUNCTIONS OF LIVER

TESTS BASED ON METABOLIC FUNCTIONS

Carbohydrate metabolism

1. **Galactose tolerance test.** 40 gm of galactose along with 400 ml of water is taken by the fasting subject. Blood is taken every half an hour interval after the intake up to 2 hrs. and the blood galactose level is determined. Normally the blood galactose rises slightly within an hour and falls within two hours. The sum of the four galactose values is called *galactose index*. In case of liver damage blood galactose is found to be high in all the four samples of blood and the galactose index rises.

2. **Laevulose tolerance test.** 40 gm of laevulose along with 250 ml of water is taken by the fasting subject. Blood is taken every half an hour interval after the intake up to two hours and the blood sugar level is determined in all the four samples. The maximum value reaches within one hour and falls up to the fasting level within two hours. In case of liver damage blood sugar is found to be high in all the samples and does not come down to fasting level within two hours.

3. **Glucose tolerance test.** 100 gms of glucose in 300 ml of water is given by mouth to a fasting subject and samples of venous blood are drawn at half-hourly intervals for two hours, a fasting sample having been collected before giving glucose. In absence of other abnormalities, the glucose tolerance test gives an indication of liver function.

4. **Glycogen storage.** This test is based on the ability of epinephrine to raise blood sugar above fasting level through the process of hepatic glycogenolysis. In this test the subject is allowed to take high carbohydrate for three days and on the fourth day, overnight fasting blood sugar is estimated and noted. Thus 10 μ gm of epinephrine per Kg body weight is injected and blood sugar is estimated after an hour. Normally blood sugar above the fasting level exceeds 40 mgm per 100 ml. This is decreased in hepatic disease.

Lipid metabolism

1. **Cholesterol and cholesterol ester ratio.** This ratio gives a good indication of liver function because liver takes an important part in synthesis, esterification, oxidation and excretion of cholesterol. In normal individual total blood cholesterol ranges about 150–250 mgm% and about 60–70% of it is esterified.

2. **Phenol turbidity test.** Total lipid in the serum gives turbidimetric reaction with a phenol reagent. A measure of the total lipid in the serum gives an indication of the functional status of the liver.

Protein metabolism

1. **Hypoalbuminaemia.** Liver is the site where the albumin is synthesised. In liver disease, the serum albumin concentration is decreased and there is always a tendency of hypoproteinaemia.

2. **Prothrombin time and response to vitamin K.** In decreased liver function prothrombin time is increased. Measurement of prothrombin time and also its response to vitamin K are the useful indication of liver function. Normal prothrombin time is approximately 15 seconds but in condition of liver disease it is increased to 22 to as much as 150 seconds. In such decreased state of the liver, if vitamin K is parenterally administered then slight increase of prothrombin time indicates severe liver disease.

3. **Tests for altered production of different protein fractions.** There are certain tests for the altered synthesis of different protein fractions. These are as follows:

(*a*) **Cephalin-Cholesterol flocculation test.** This test is done by mixing 4 ml

of saline with 1 ml of cephalin–cholesterol emulsion and also with a mixture of 0·2 ml of serum. It is kept in the dark for 24–48 hrs.

In normal liver there is no flocculation but in diseased state, varying degrees of flocculation and precipitation occur. This test is indicated by positive (+) signs or negative (—) signs. Positive signs indicate flocculation and precipitation. It gives an indication of an acute pathological process in hepatitis.

(*b*) **Thymol turbidity test.** About 3 ml of the thymol reagent are added to 0·05 ml of abnormal serum. Turbidity that appears is due to precipitation of globulin.

Sera having high β- and γ-globulin fractions give positive test.

(*c*) **Zinc turbidity test.** Serum with abnormally high γ-globulin if diluted with zinc sulphate then turbidity occurs in proportion to the γ-globulin present.

(*d*) **Serum colloidal gold test.** Positive reaction indicates liver damage. This is due to faulty globulin formation by liver.

4. **Amino acid tolerance test.** This test is an important metabolic function of liver. If amino acids are administered intravenously then it is removed from the blood by the liver within a certain period. In liver disease the rate of removal of the amino acids are appreciably retarded.

Other tests

1. **Taka and Ara reaction.** Mercuric chloride, acid fuchsin and sodium carbonate when added to the abnormal serum, mercuric chloride is precipitated. Positive reaction indicates parenchymatous liver damage.
2. **Serum iron.** Increased in hepatitis, decreased in cirrhosis and remains normal in biliary obstruction.

Enzyme test

Estimation of blood enzymes. A number of enzymes present in blood changes in liver diseases: (*a*) increase in **serum alkaline phosphatase** in obstructive liver diseases, (*b*) elevated **lactic dehydrogenase** and also **aldolase** in acute hepatitis, (*c*) increased **leucine aminopeptidase** resulting from interference with biliary excretion. GPT (glutamic-pyruvic transaminase) activity is relatively high in the liver than that of GOT (glutamic-oxaloacetic transaminase) activity. Measurement of GPT may give an indication of liver function.

III. INVESTIGATION OF THE DETOXICATING POWER OF LIVER

1. **Oral hippuric acid excretion test.** This is not commonly done now, because the next one gives better results.
2. **Intravenous hippuric acid test.** A standard amount of sodium benzoate is given intravenously and after one hour hippuric acid is estimated in urine. Liver can conjugate benzoic acid with glycine forming hippuric acid. [This function is also present in the kidney.] Normally 0·7 gm to 0·95 gm is excreted as hippuric acid in the first hour.