Metabolism of Carbohydrate, Lipids, Amino acids and Proteins

Metabolism is a term meant to denote all the biochemical processes that occur in animals, resulting in the production of energy, and waste products, rendering the latter harmless. Intermediate (intermediary) metabolism refers to metabolic processes that are not immediately concerned with energy release, but refers to the events that occur between the assimilated energy source and the release of energy. The fundamental processes are cellular. Metabolism comprises all of the chemical reactions that occur in a living organism. It includes anabolism (reactions that result in the formation of more complex molecules and are, therefore, energy-requiring) and catabolism (reactions from which simpler molecules result and energy is released). Anabolic reactions include, for example, the formation of structural proteins or enzymes from amino acids, and the formation from simple sugars of polysaccharides that serve as an energy store. Many catabolic reactions have evolved for the specific purpose of producing the large quantities of energy required by the organism for performance of work. Metabolic processes show variations in their rate depending on the stage (larva, adult) and activities. External factors like temperature also influence the rate. Metabolic control has been attributed to hormonal and neural mechanisms. These cellular phenomena occur in living cells on a general pattern and details of these will be available in books on cell physiology or comparative physiology.

The constituents of food which will be available to the insect may be characterised as carbohydrates, lipids, and proteins. Carbohydrates are broken up into a simple sugar, usually glucose. Three pathways are common for energy release from glucose *viz.*, Embden-Meyerhoff pathway, the pentose pathway (hexose monophosphate shunt), and the cycle of Krebs (citric acid cycle or monocarboxylic acid cycle.). The latter is aerobic md completes the degradation of sugars, besides proteins and lipids. The three pathways of breakdown and the enzymes responsible for the activities indicated in Figs 1, 2 and 3.

Studies have shown that all pathways studied in mammals occur in insects also. Within the cell the mitochondria form the organelles playing a key role in these phenomena. All the enzymes of the Krebs cycle occur in the mitochondria of insects and the complete oxidation to pyruvate has been established.

Sites of Metabolism

Chemical reactions are carried out by all living cells, though they are usually limited in number and, of course, are related to the specific function of the cell in which they occur. For example, in midgut epithelial cells, metabolism is directed largely toward synthesis of specific proteins, the enzymes used in digestion. Metabolism in muscle cells is specifically concerned with production of large amounts of energy, in the form of ATP, for the contraction process. In epidermal cells reactions leading to the production of chitin and certain proteins, the components of cuticle, are predominant. Certain tissues, however, are not so specialized and in them a multitude of biochemical reactions, involving the three major raw materials (sugars, amino acids, and lipids), are carried out. In vertebrates the liver performs these multiple functions. The analogous tissue in insects is the fat body.

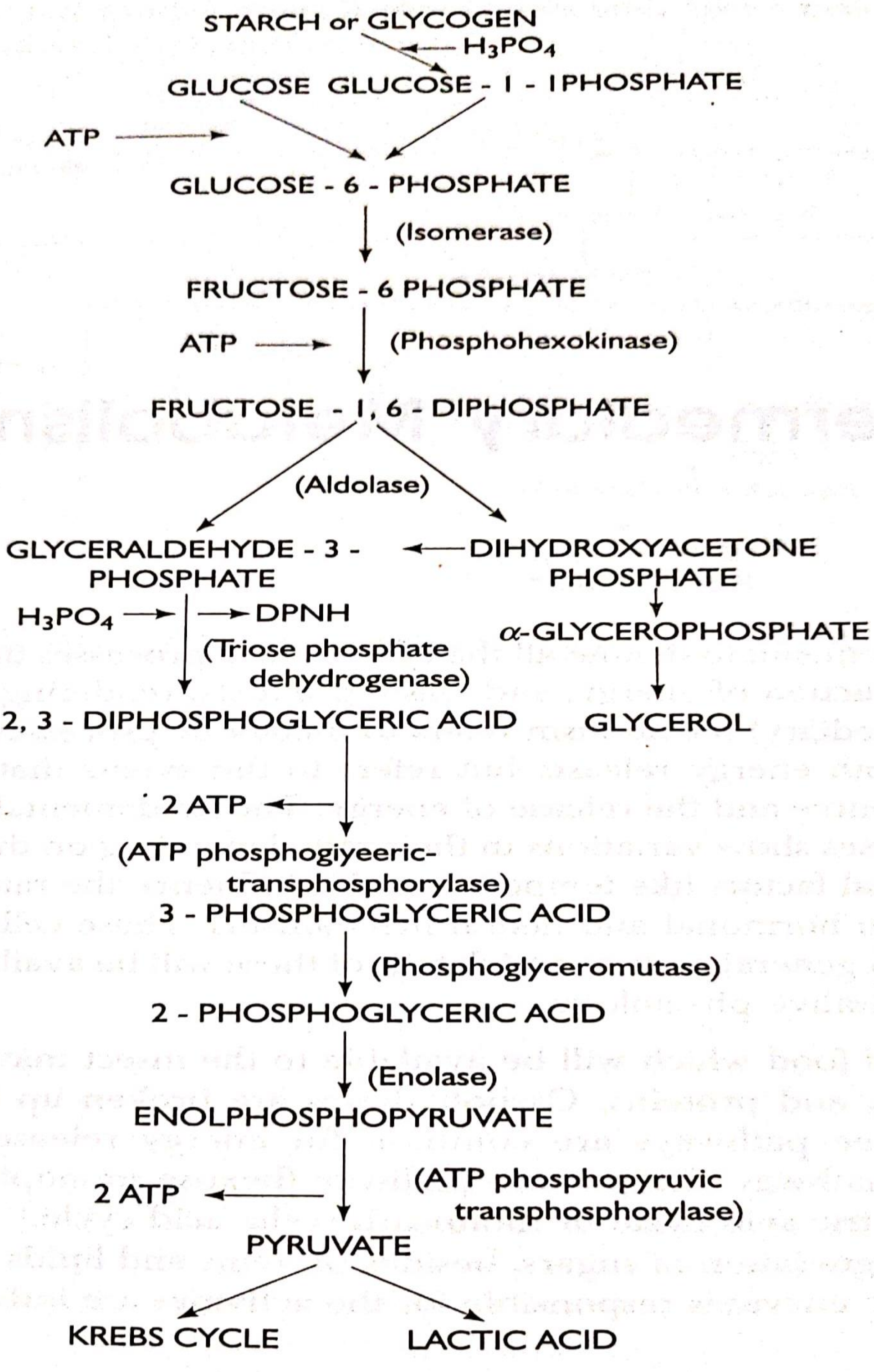

Fig. 1: Embden-Meyerhoff pathway

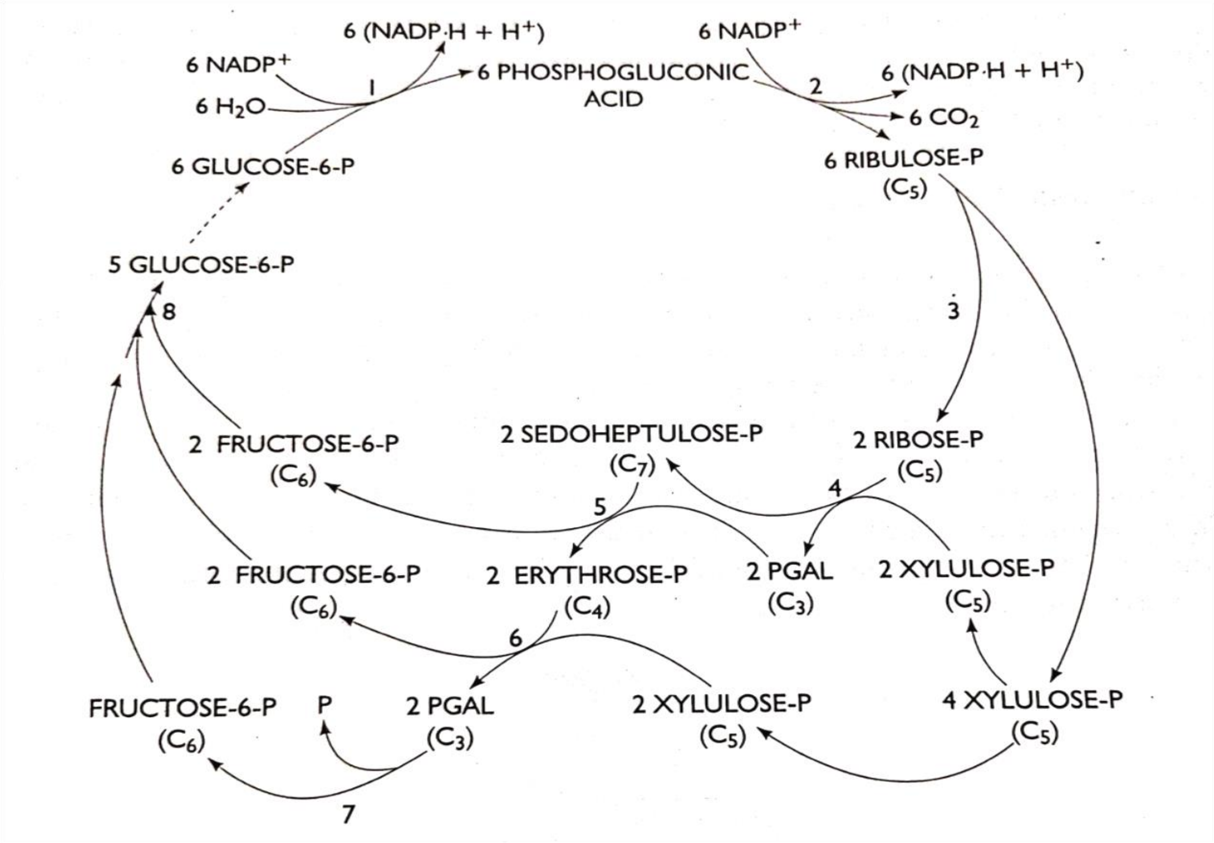

Fig. 2: Hexose monophosphate shunt

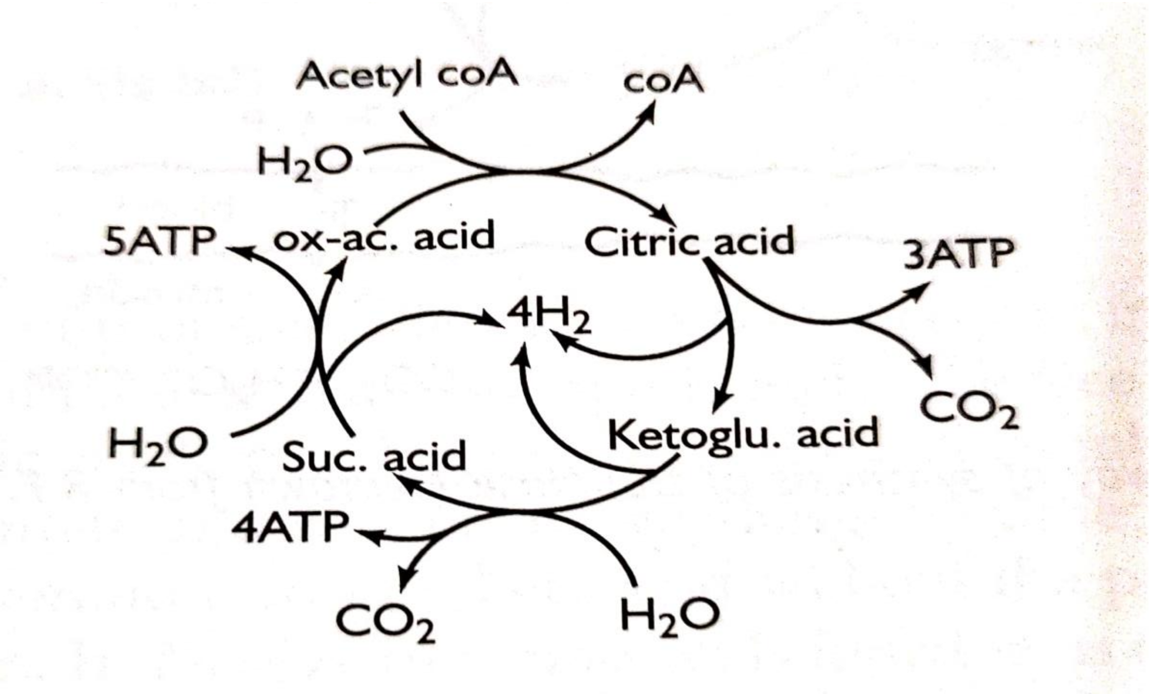

Fig. 3: Krebs cycle

Carbohydrate Metabolism

The carbohydrates are involved in energy storage while their conjugate forms (glycolipids and glycoproteins) participate in various physiological processes.

The storage carbohydrates

In insects glycogen exists in cells and tissues and trehalose, a disaccharide, occurs in the haemolymph. Both these are the sources of glucose. Trehalose consists of two glucose molecules joined through an α1, 1-linkage. Trehalose supplies energy in flight, during starvation, and metamorphosis. The metabolic pathways are shown in Fig. 4. Trehalose is hydrolysed by the enzyme trehalose to yield glucose and the enzyme occurs in blood, gut, and fat bodies. Ordinarily fat bodies contain stores of trehalose. The concentration of trehalose varies from 88ng$^{1:1}$ in silver fish, *Lepsima* to more than 70 ng$^{1:1}$ in aphids, *Megoura*.

Glycogen is stored in tissues like fat body, muscles, midgut cells, etc. Glycogen yields energy after transformation into the circulating blood sugar, trehalose and trehalose after conversion into glucose. In forms like the larva of *Aedes*, amino acids have been seen to yield glycogen. The glycogen is stored as 117 mole g1 wet weight in the fat body of *Locusta migratoria* and 275 mole g fat body of Phormia regina, The flight muscles contain 7, 91 and 150 g'1 wet weight in *Schistocerca*, *Periplaneta* and *Phormia,* respectively.

Chitin, a chief cuticular constituent is formed from carbohydrate reserves, of which trehalose is important. It is the helical polymer consisting of 1500 N-acetyl glucose amine residues. There are three polymorphs of chitin: α, ß andγ. Most of the cuticular material is reabsorbed after digestion by the ecdysial fluid.

The mixed carbohydrates are represented by glycoproteins and are distributed in various tissues and secretions. The silk-proteins and yolk-proteins are the best examples of glycoproteins in insects. Some glycoproteins are attached to the cell membrane.

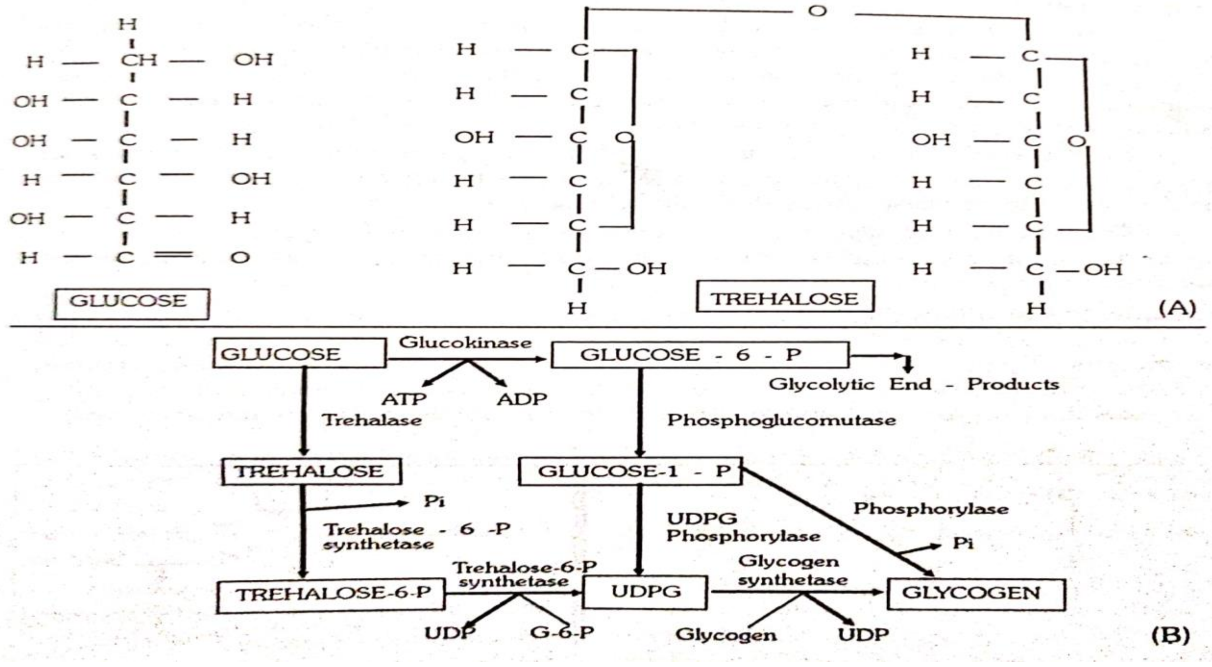

Fig. 4: Carbohydrate metabolism, (A) Haemolymph sugars, (B) Inter-conversion of sugars, Abbreviations: UDPG- Uridine diphosphoglucose, UDP- Uridine diphosphate, P- Phosphate, Pi- Inorganic Phosphate

Catabolism

The glycogen phosphorylase is responsible for the primary cleavage of glycogen in *Schistocerca* flight muscle. Glycogen concentration decreases by 64% within 10 seconds after the inception of flight and in the fat body by 75% into 2 hours of flight as a result of phosphorylase activity.

The trehalose is broken by the enzyme trehalase. The enzyme is specific for the α, α form and occurs in various polymorphs differing in pH optima, Km-Trehalose and molecular weight. The enzyme is demonstrated from the midgut, hindgut, haemolymph, flight muscles, etc.

The chitin is degraded by the moulting fluid containing the enzymes-chitinase degrading the chitin molecules to chitobiose units and ß-N-acetyl-D glucose aminidase hydrolysing terminal non-reducing N-acetyl glucose amine residues in chitobiose and, oligosaccharides.

The glycolysis is a long reaction occurring in various tissues and catalized by specific enzymes-hexolinase, phospho-fructokinase, trios-phosphale-isomerase, glycerol aldehyde phosphate dehydrogenase, pyruvate kinase, etc. The pentosephosphate pathway is facilitated by two primary enzymes- glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

Gluconeogenesis, i.e., the synthesis of glucose from amino acids or glycerol takes place by the enzymatic reactions. The enzymes involved in gluconeogenesis are pyruvate carboxylase; fructose-di-phosphatase, etc.

The synthesis of glycogen from glucose is catalized by glycogen synthetase. Similarly, synthesis of trehalose takes place by trehalose phosphate synthetase and trehalose phosphatase.

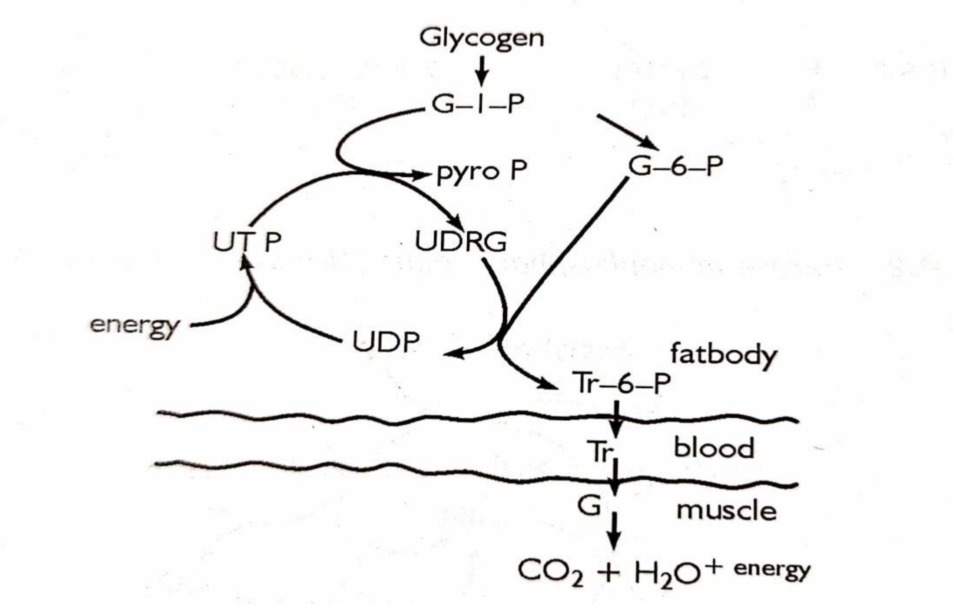

Fig. : Pathway of synthesis of trehalose

Lipids

These include neutral fats (triglycerides), phosphatides, cerebrosides, fat soluble vitamins, and steroids. They comprise the major food reserves of insects. The fatty acids are synthesised in the fat bodies and in other tissues from amino acids, sugars, and simpler fatty acids. The enzyme lipase is highly active in the fat body; glycerol is produced and then phosphorylated to glycerophosphate. These are moved into the flight muscles. The cuticular water-proofing is afforded by a thin layer of wax. Shellac produced by *Kerria*, plates of wax of scale insects, and comb material of bees are all constructed of wax. Composition of these waxes varies. For example in beeswax, 12% is paraffin, 72°/o esters, and 13% free long chain acids. Cells of fat body and oenocytes help in its synthesis in honeybees, getting extruded as flakes from abdominal wax glands. It is also known that oenocytes play a part in the formation of the cuticle. Steroids are fat-like compounds which do not form esters or saponify. They have a characteristic structure, the rings always having at least one OH group. Cholesterol occurs in insects, and sterols are all converted into cholesterol. For normal life an insect requires steroids as a dietary material. Insect moulting hormones are steroids. Various lipid derivatives are also known to carry out specific functions. To this category belong pheromones, royal jelly of honeybees, etc.

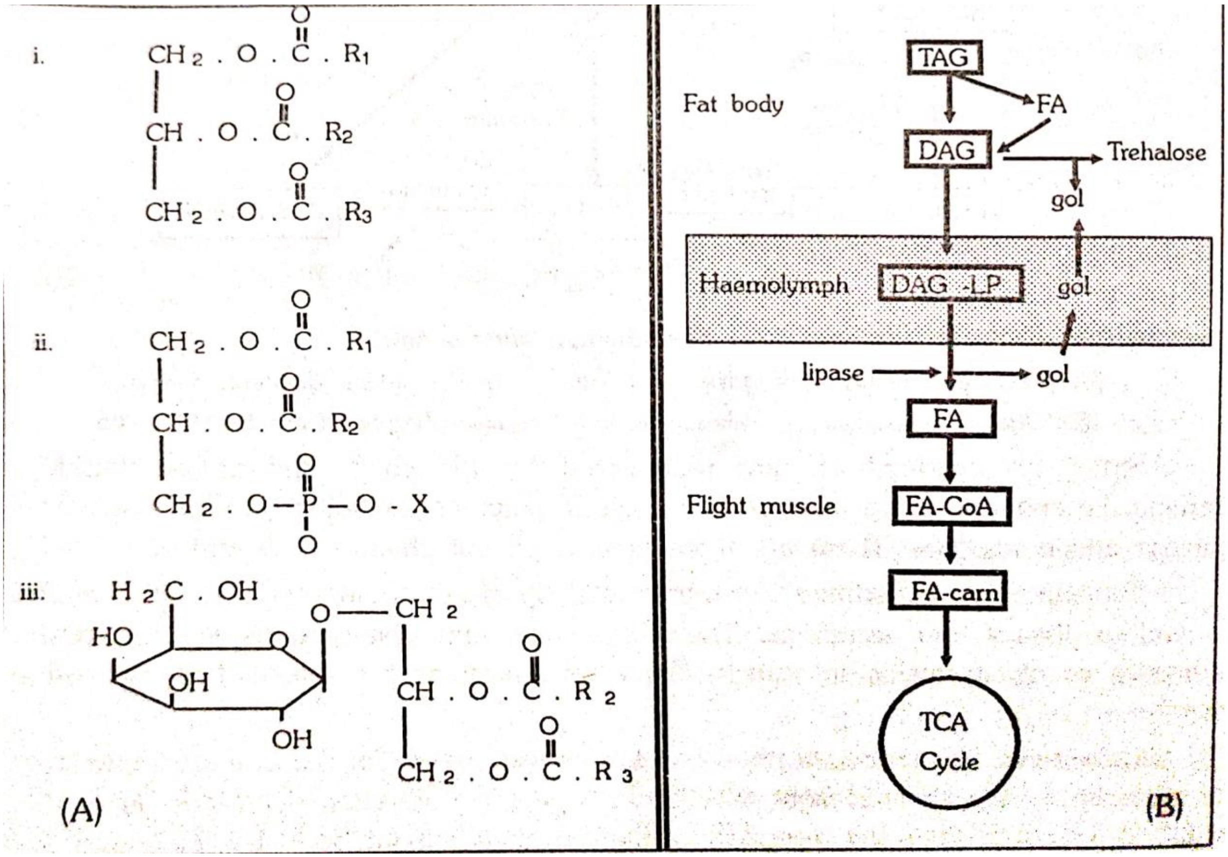

Fig.5: Lipid metabolism- (A) Common dietary lipids, i- triglycerides, ii- glycerophospholipids, iii- monogalactosyl diglycerides and (B) metabolism
Abbreviations: TAG- Triacylglycerol, DAG-Diacylglycerol, FA- Non-esterified-fatty acid, LP-Lipoprotein, Carn- Carnitine, TCA-Tricarboxylic acid cycle.

Storage lipids

The storage lipids are acylglycerols particularly triacyl-glycerol which is the major source of metabolic energy in insects. The distribution of triglycerols varies according to demand of the insect, developmental stage and also varying between tissues. The major lipid storage tissue is the fat body in which more than 50% of the wet weight and 75% of the dry weight is represented by Triacyl-glycerol. Large quantity of triacyl-glycerol is also accumulated in the developing oocytes and eggs. Triacyl-glycerol may also be present in the haemolymph although in most species the dominant haemolymph lipid is diacylglycerol which is bound to the carrier protein, lipophorine. Transport of lipid in the form of diacyl-glycerol is the specialized feature of insect biochemistry. 90% of the haemolymph diacyl-glycerol is present as the 1, 2-isomer.

The fatty acids, both esterified fatty acids and non-esterified fatty acids accumulate in all tissues and constitute a major metabolic reserve. There are various types of fatty acids in the insects, these are:

(i) Saturated fatty acids such as myristic acid, palmitic acid and stearic acid,
(ii) The mono unsaturated fatty acids such as myristoleic acid, palmitoleic acid and oleic acid and

(iii) The poly unsaturated fatty acids such as lingleic acid and linolenic acid.

Structural lipids

Phospholipids are the major structural lipids and are essential component of cell membranes and distributed throughout all tissues of the insect body. The phosphatidylcholine and phosphatidyl-elhanolamine are the major phospholipids in insects amounting for over 70% of the total phospholipid contents. In Hymenoptera, Lepidoptera and Orthoptera the first type of phospholipid while in Diptera the second type predominates.

Sterols

The insect tissues are incapable to synthesize sterols and need a dietary requirement for sterols. They are important structural component of biological membranes.

Cuticular Lipids

They form a thin lipid layer on the outer integumentary surface as a part of epicuticle.

Lipogenesis

The fatty acid biosynthesis pathway is carried out by two enzyme systems: Acetyl-CoA carboxilase and fatty acid synthatase. The first enzyme catalyzes carboxylation of acetyl-CoA to the 3-C compound malonyl-CoA. The condensation of malonyl-CoA with acetyl-CoA is catalyzed by the fatty acid synthetase to produce a 4C-Butyrvl intermediate. The reaction is continued until the acyl chain of appropriate length is formed. A capacity for mitochondrial synthesis of fatty acid has been reported in *Drosophila* by reversal of ß-oxidation pathway.

The acylglycerol formation in insects takes place through the biosynthetic pathway described for other animal groups. The enzymes involved m acylglycerol formation in insects are the *α*-glycerophosphatase and diacylglycerol transferase of the α-glycerophosphate pathway. Similarly phosphatidate phosphohydroiase activity has also been found in fat body and flight muscle of *Hyalophora cecropia.*

The phospholipid biosynthesis occurs through the cytidine pathway involving phosphorylation by the enzyme, phosphokinase. The phosphorylated base is then activated and transferred to a 1, 2 diacylglycerol to form the appropriate phosphoacyl 1-glycerol. The major site of the phosphoglycerol synthesis is the fat body, but may occur in the nervous tissue, flight muscle and gut.

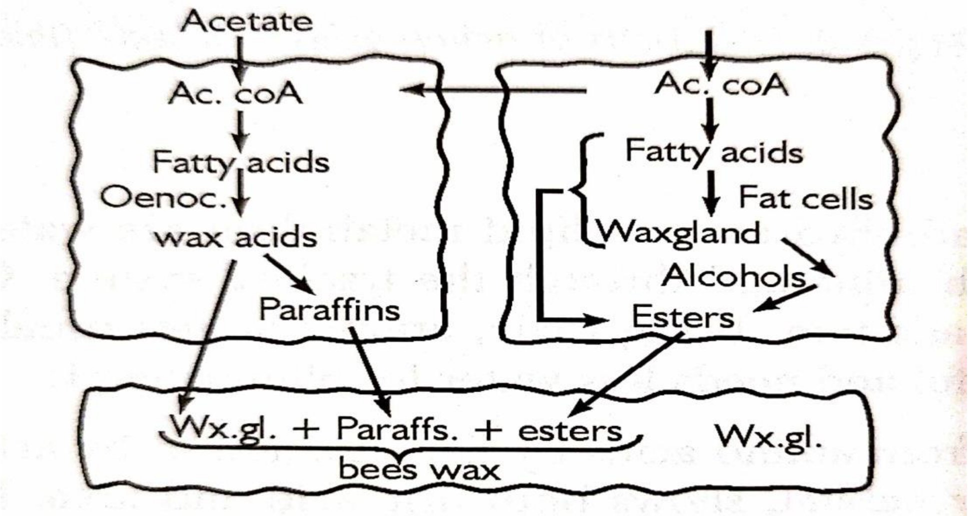

Fig.7 : Pathway of synthesis of wax

Amino acid and Protein Metabolism

The simpler substances of proteins called amino acids occur as free amino acids in blood and the concentration of these in haemolymph is high.

Amino acid

Insect haemolymph is characterized by high concentration of free amino acid, i.e., 100 to 300 times higher than that in the human blood. The significance might be related to repeated moulting where protein synthesis is cyclical, yolk synthesis and other active substances are involved in reproduction, osmoregulation, neural co-ordination, detoxication, phospholipid synthesis and energy production, etc. The composition of free amino acids is species specific in insects, cysteine, hydroxyproline, methionine sulfoxide; ornithine and amino butrate are of much less common occurrence than other components. The free amino acid pattern in insects is tissue and sex specific. The free amino acids give rise to compounds like butyric acid, ornithine and taurine. A number of amino acids like glutamate, proline, aspartate and alanine are synthesised in the fat body. The transfer of amino groups from an amino acid to a keto acid without the production of ammonia is known as transamination. The glutamate content is ten times higher in tissues than that in the haemolymph in *Drosophila* while the adult female *Culex* contains five times more methionine sulfoxide than the male and ß-alanine in reverse proportion.

In *Bombyx* 19 amino acids function as donors in transamination, Malpighian tubules show the greatest transaminase activity. Glutamate plays a major role - the transfer of nitrogen from one compound to another. This central role aids in incorporating and distributing nitrogen. Body proteins get synthesised from amino acids. RNA acts as a template for synthesis. Functionally proteins form essential components of cells, secretions, proteins, materials like silk, etc.

The amino acids are rapidly decreasing during starvation while feeding elevates the amino acid concentration. In the developing eggs qualitative and quantitative in the amino acid content occurs at the initial phase and reaches at the maximum level at the advance stage, mostly due to break down of yolk reserve to yield amino acids for the synthesis of tissue proteins. Total quantity of free amino acids increases rapidly after the oviposition. It varies greatly during the post-embryonic development

At metamorphosis, the total free amino acids exhibit initial rise followed by a fall during the late stage of development resulting due to breakdown of tissues of the preceding instars and formation of succeeding instar tissues. In the adult, both male and female, the concentration of free amino acids is increasing before copulation while decreasing thereafter, as a result of their involvement in yolk protein synthesis and accessory sex gland secretion.

Amino transferase activity occurs in the insects. Molecular weight of some of the amino transferase enzymes has been determined. The most important physiological significant one is the L-alanine-amino-transferase which maintains the amino acid pool at a proper level for protein synthesis as well as supplies metabolites for energy metabolism and catalyses interactions between protein and carbohydrate metabolism.

Tyrosine and ß-alanine are involved in the process of sclerotization and therefore occur in higher concentration before each moult during the post embryonic development. Besides tyrosine, proline, and tryptophan occur in large quantity in the insect haemolymph which serve as a source of metabolic energy and ommochrome precursor, respectively.

Proline participates in flight muscle metabolism and provides intermediates of the citric acid cycle and acts as a primary energy substrate. Adipokinetic hormone increases the rate of proline synthesis in the fat body suggesting hormonal regulation of proline synthesis in insects.

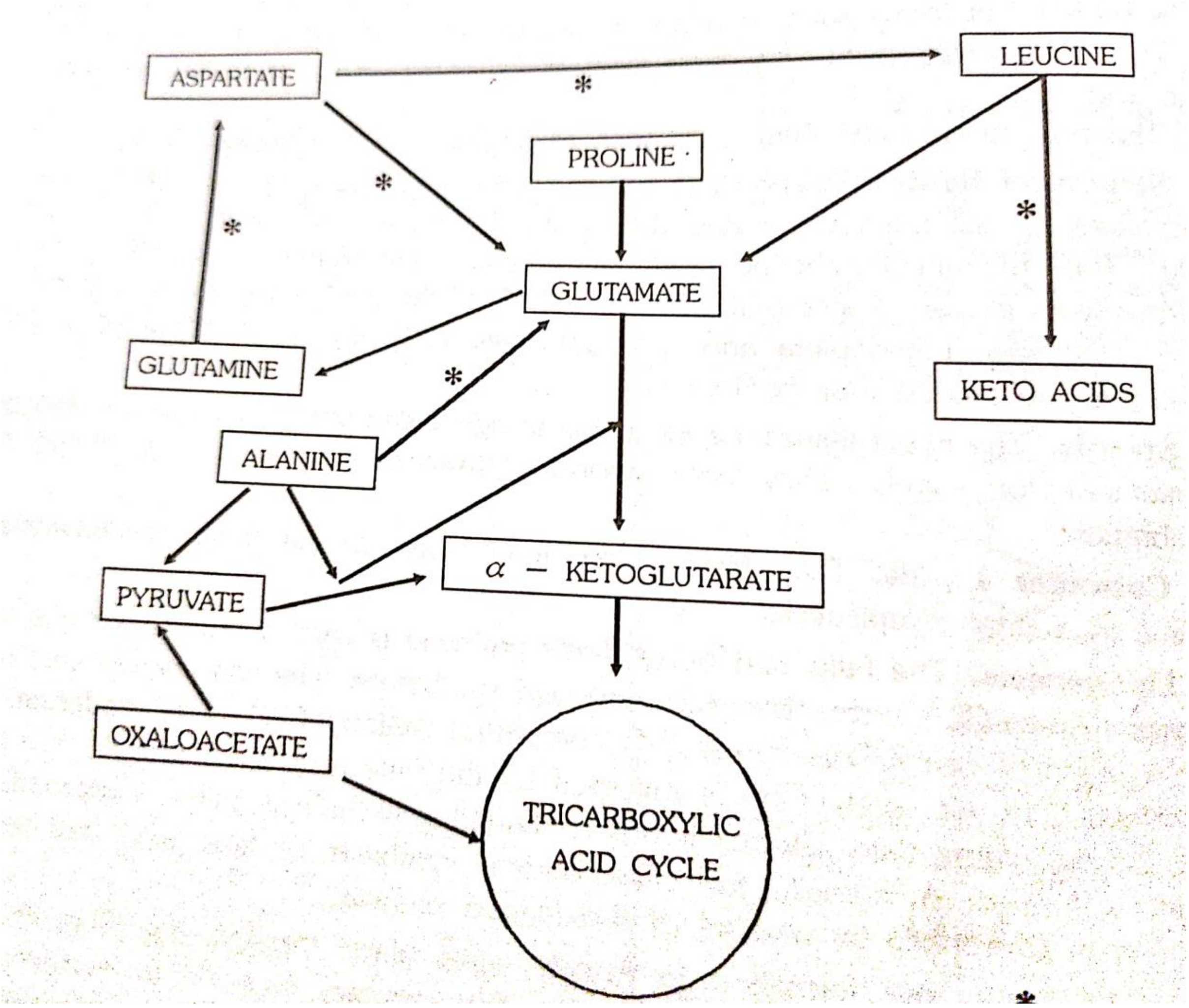

Fig.8: Amino acids metabolism in insects

Proteins

Storage proteins are synthesized in the fat body and are released into haemolymph and their concentration increases in the last larval instar. The larval proteins are often undetected in the adult stage. They are of two types:

Larval haemolymph proteins (LHP)

All storage proteins are of a range of 5, 00,000 molecular weight and are composed of six sub-units. All these proteins are highly aromatic containing a high proportion of tyrosine, and phenyl alanine residues. They are synthesized predominantly in the fat body and their concentration increases enormously in later larval instars. They did not occur in the adult stage. Cailiphorin, Manducin, Lucilin, Phormin, Sarcophagin, Bombyxin and Bombycin are some of the storage proteins detected in *Calliphora, Manduca, Lucilia, Phormia, Sarcophaga* and *Bombyx mori* respectively. The larval haemolymph proteins are also denoted simply as the LHP 1, 2, 3, etc. In these proteins aspartic acid is most abundant amino acid residue and cystine is absent. The LHP are also rich in phenyl alanine, glutamic acid and tyrosine residues in some insects.

Heat shock proteins (HSP)

Heat shock proteins are present in some of the insects particularly, *Drosophila melanogoster*. These are a specific group of proteins and the synthesis of which is induced by heat and some chemicals or environmental stimuli.

The catabolism of protein produces ammonia in addition. Being toxic, ammonia gets usually converted into uric add which is less harmful and needs less water for eliminating it

Urea arises directly from amino acids by deamination or by action of urease on urea. *Tenebrio*, living in a dry habitat, shows both uric acid and urea. Fluid feeders like *Apis* produce comparatively more ammonia. Urea is the end product of mammalian catabolism and arises through the ornithine cycle, a process poorly understood in insects. Blood feeders like *Rhodnius* derive urea directly from the food.

Uric acid is formed from utilisation of glycine, glutamine, etc. in the fat body, and also from the catabolism of nucleic acids. Adenine and guanine released from the breakdown of nucleic acids give rise to uric acid.

Adenine→Hypoxanthine→Xanthine→Uric acid→Guanine→Xanthine→Uric acid

Allantoin is excreted by aquatic insects and in Diptera, Coleoptera, and Orthopteti allantoin arises from uric acid by the action of uricase. By action of allantoinase, allantoic acid is produced, forming nearly 0.5% of wet weight of faeces of Lepidoptera. Allantoic acid constitutes 25% of the waste product of pupal metabolism forming the meconium.

Detoxication

Metabolic events have been studied in toxicology of insecticides. Several insects have been known to possess the detoxication mechanisms.

The detoxication mechanism renders the organic toxic substance into harmless prod¬ucts which gets excrected. In insects this has been brought about by several methods. In Orthoptera, Hemiptera, Coleoptera and Lepidoptera, the phenols are conjugated to glucose to form p-glucosides. 2-4 Dinitro-o-cresol and phenothiazine are tackled this way. In the housefly naphthol is converted into etheral sulphate. Cockroaches acetylate amino groups; houseflies metabolise hexa chloro cyclohexane (HCH) by conjugating it with glutathione or cysteine. Oxidations occur as a detoxicating mechanism in cyclodiene poisoning. Rarely reduction of organic compounds is met with. These examples illustrate that poisonous substances get converted or linked up with substances, making them harmless to the insect several detoxication mechanisms are indeed poorly understood, though some of the major pathways involved have been elucidated in toxicological studies.