PRACTICAL MANUAL

INSECT ANATOMY AND PHYSIOLOGY

ENT- 502 3(2+1)

M.Sc. (Ag.) Entomology

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SYLLABUS: INSECT ANATOMY AND PHYSIOLOGY

Practical: Latest analytical techniques for analysis of free amino acids of haemolymph; determination of chitin in insect cuticle; examination and count of insect haemocytes; preparation and evaluation of various diets; consumption, utilization and digestion of natural and artificial diets.

Name of Student .....

Roll No. .....

Session .....

Semester .....

Course Name: .....

Course No. : .....

Credit .....

CERTIFICATE

This is to certify that Mr./Miss/Mrs. .....ID No..... has completed the practical of course.....course No. ..... as per the syllabus of M.Sc. (Ag.)..... Semester in the year.....in the respective lab/field of College.

Date Course Teacher

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Practical No. 1

Objective: To study the comparative anatomical details of digestive system in Grasshopper

Activity: Collection of Grasshopper, dissection and observe the parts of digestive system

Materials required: .....

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Procedure:.....

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Observation:.....

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Practical No. 2

Objective: To study the comparative anatomical details of nervous system in Grasshopper

Activity: Collection of Grasshopper, dissection and observe the parts of nervous system

Materials required: ……

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Procedure: .....

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Practical No. 3

Objective: To study the comparative anatomical details of the male reproductive system in Grasshopper

Activity: Collection of Grasshopper, dissection and observe the parts of the male reproductive system

Materials required: .....

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Procedure: .....

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Observation: .....

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Practical No. 4

Objective: To study the comparative anatomical details of the female reproductive system in Grasshopper

Activity: Collection of Grasshopper, dissection and observe the parts of the female reproductive system

Materials required: .....

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Procedure: .....

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Observation:.....

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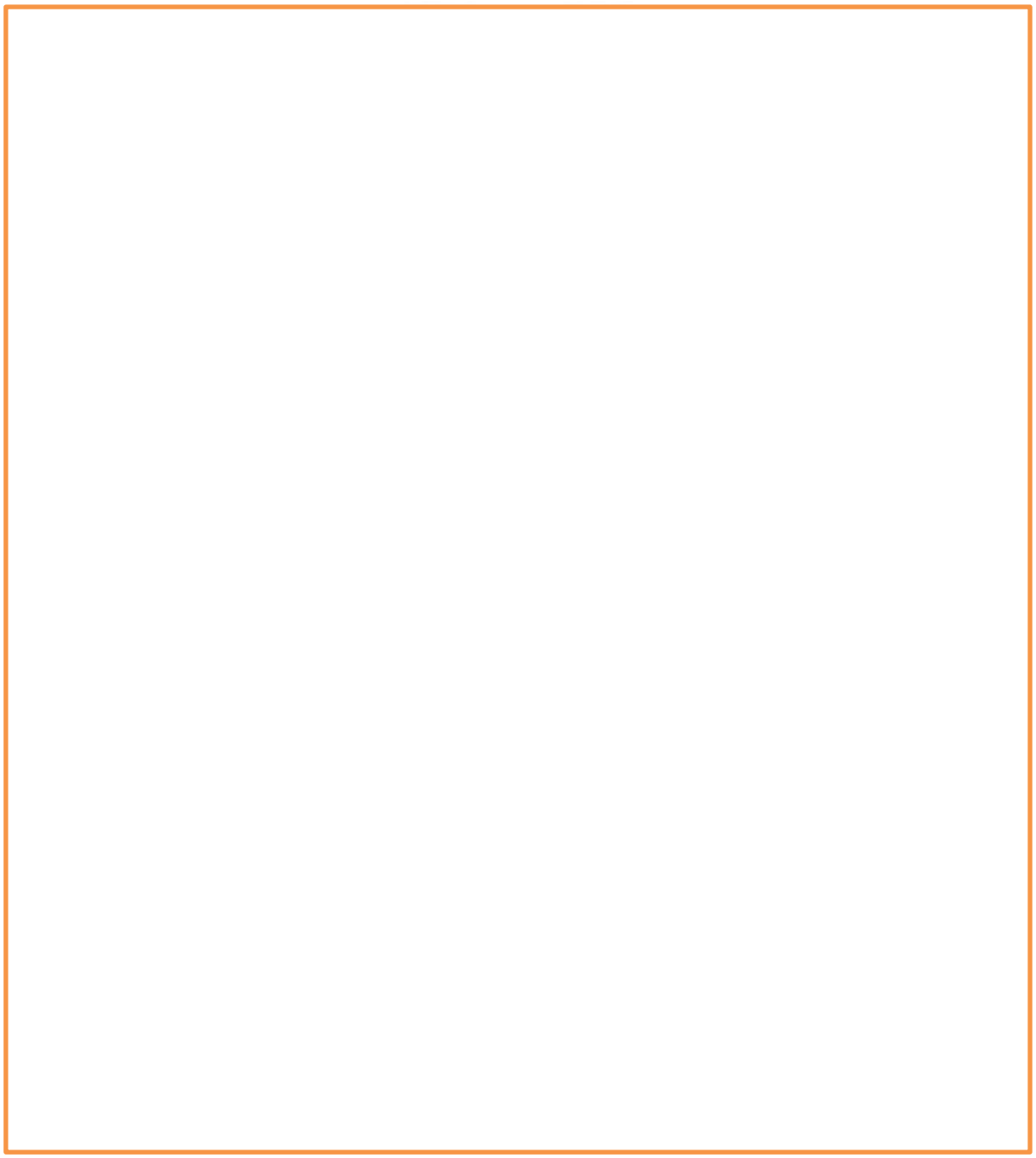

Practical No. 5

Objective: To study the different types of artificial diet for insect rearing

Activity: Discuss about the different types of artificial diet for rearing

Materials required: .....

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Procedure: .....

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Observation:.....

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Practical No. 6

Objective: The preparation of the artificial diet for *Helicoverpa armigera* (Hub.)

Activity: To prepare the artificial diet for rearing of *H.armigera*

Materials required: .....

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Procedure: .....

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Observation:.....

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Practical No.7

Objective: To study the anatomical details of the tracheal system in a given insect

Activity: The details of the tracheal system in insect

Materials required: .....

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Procedure: .....

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Observation:.....

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Practical No.8

Objective: To study about the detection of waxy components in insect cuticle

Activity: The detection of wax in insect cuticle

Materials required: .....

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Procedure: .....

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Observation and Results.....

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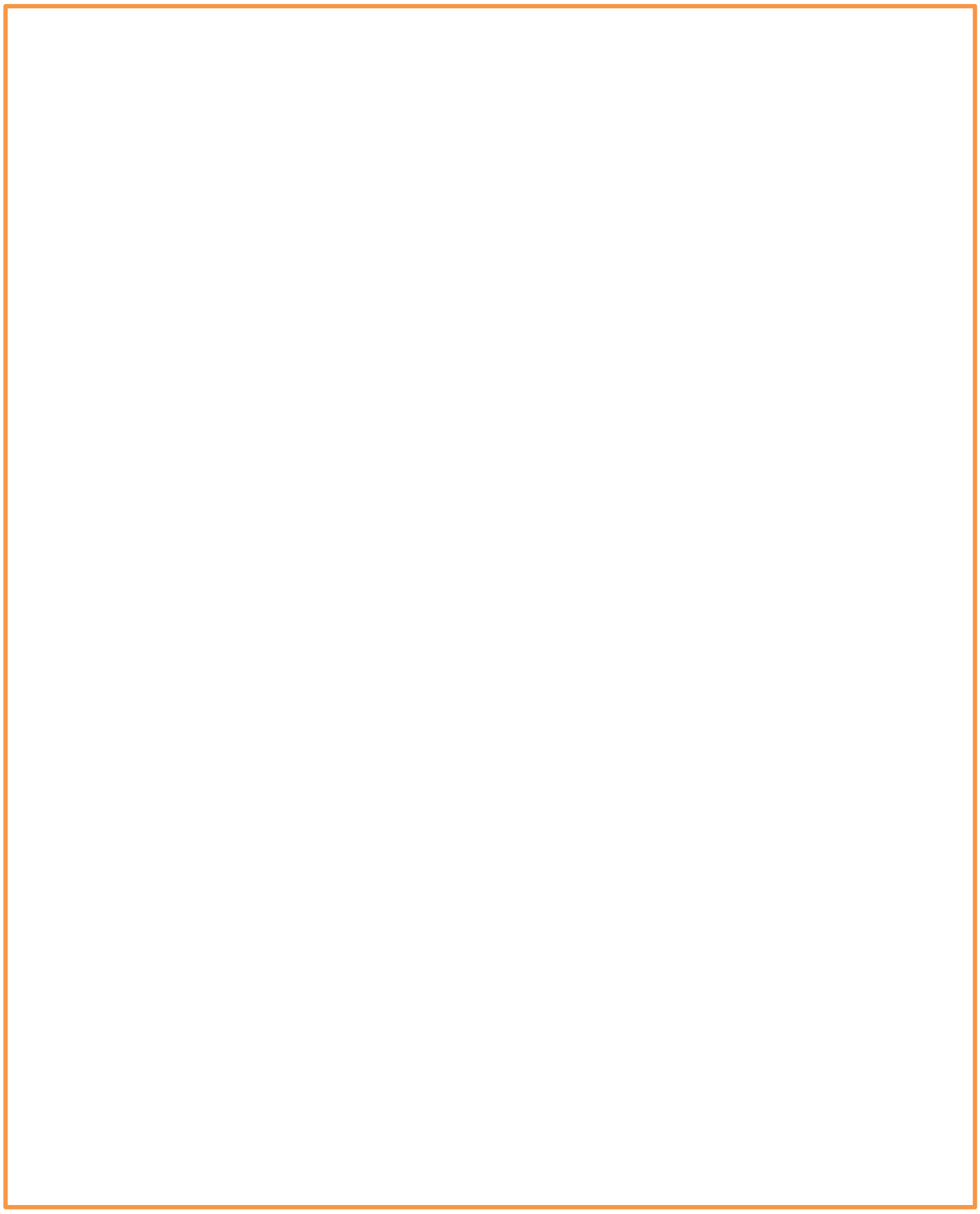

Practical No. 9

Objective: The detection of uric acid in a given insects

Activity: Procedure for the detection of uric acid in a given insect

Materials required: .....

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Procedure: .....

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Observation and Result.....

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Practical No. 10

Objective: To study about the different excretory organs in a given insect

Activity: Observe the different excretory organs in various specimens such as Grasshopper/Cockroach

Materials required: .....

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Procedure: .....

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Observation:.....

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Practical No. 11

Objective: To study the anatomical details of Malpighian tubule in Grasshopper

Activity: Observe the Malpighian tubules and identification of different parts

Materials required: .....

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Procedure: .....

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Observation:.....

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Practical No. 12

Objective: To demonstrate the of uptake of dye by Malpighian tubules in a given insect

Activity: Observe the functions and process of the excretion by Malpighian tubules

Materials required: .....

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Procedure: .....

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Observation:.....

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Practical No. 13

Objective: The determination of Chitin Content in insect cuticle

Activity: The determination of chitin content in insect cuticle

Materials required: .....

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Procedure: .....

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Observation:.....

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Practical No. 14

Objective: To study about the histological preparation of haemocytes in a given insect

Activity: Observation of haemocytes from haemolymph of a given insect *viz*. cockroach/ grasshopper

Materials required: .....

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Procedure: .....

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Practical No. 15

Objective: To learn about the counting of haemocytes in a given insect

Activity: The procedure for qualitative counting of haemocytes

Materials required: .....

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Procedure: .....

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Observation & Results: .....

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Practical No. 16

Objective: To study the Quantitative count of haemocytes in a given insect

Activity: The Quantitative count of haemocytes using haemocytometer in a given insect

Materials required: .....

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Procedure: .....

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Observation & Results:.....

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Practical No. 17

Objective: To determine the amino acids in haemolymph from the chromatographic separation method

Activity: The procedure for the separation of amino acid from haemolymph

Materials required: .....

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Procedure: .....

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Observation & Results:.....

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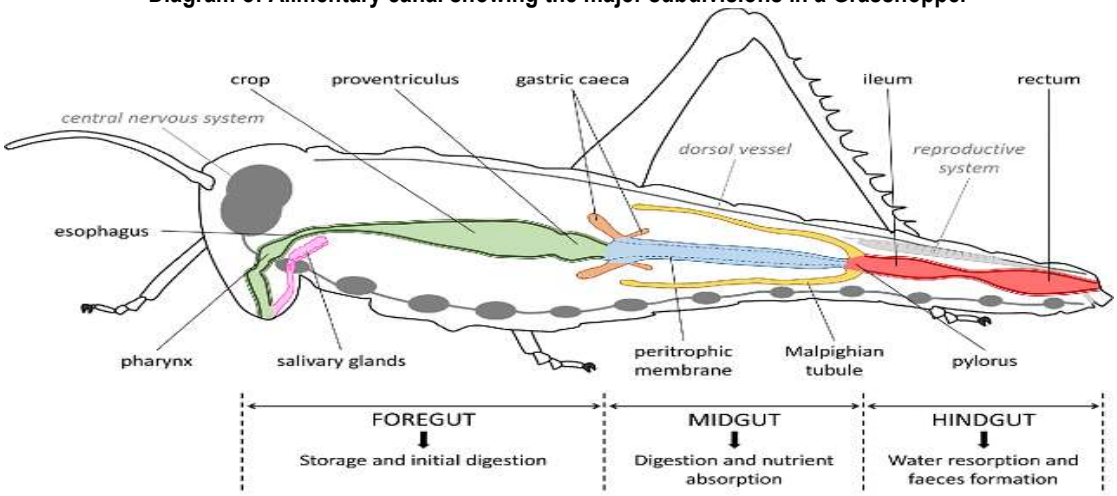
The anatomical details of digestive system in Grasshopper

The best learning situation requires one specimen and set of tools per two students for studies. Students working in pairs have ample opportunity to fully participate in the dissection and to carefully examine the specimen. They are also able to share and discuss their observations during and after the dissection.

Materials required for dissection

Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle, Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes, Dissection tray, Dissecting pins, Rigid metal ruler, And case Camel hair brush etc.

Diagram of Alimentary canal showing the major subdivisions in a Grasshopper



Digestive system: It includes the organs of ingestion (alimentary canal and its associated glands) and the physiology of digestion. The organs of ingestion are located in the head and are meant for the intake of food. The preoral cavity is enclosed by the mouth parts and is divided into two parts by the hypopharynx, the anterior region in which the alimentary canal opens is termed as cibarium and in which the salivary duct opens is known as salivarium. In the sucking Insects the cibarium is modified into a sucking pump while salivarium serves as the salivary syringe.

Alimentary canal: The alimentary canal of grasshopper/cockroach is a simple, hollow and tubular in structure which runs from the buccal cavity to anus. It is distinctly divided into the following three primary regions

Foregut or Stomodaeum: It constitutes the anterior region of the alimentary canal which is primarily an organ of ingestion and shows as a site for storing food. It consists of the following paris

Pre-oral food cavity-It has been described previously and indeed it is not a part of alimentary canal.

Pharynx-It is situated in between the pie-oral cavity and the oesophagous and is provided by the dilateral muscles. These muscles are highly developed in those insects which pharynx helps in forming the suking pump.

Oesophagous-It is simple straight tube which runs from the posterior region of the head to thorax and joins with the crop.

Crop- It is simple bag like structure and serves as a storage reservoir for the food. Apparently it is a dilated portion of the oesophagous but differs histologically by the presence of sclerotized ridges which are arranged transversely in the crop. Since it serves as a reservoir for food hence its walls are thin and the muscles are poorly developed**.**

Gizzard-It is situated in the posterior region of the crop which cannot be apparently distinguished from crop but differs internally by having the longitudinal folds into the lumen in which cuticular teeth are attached. Its posterior part is concentric in the internal layer of six 'V' shaped processes are attached which form the cardiac valve with the folds of gizzard. Its major function is to regulate the passage of food into the mid gut. Histologically, the following layers may be distinguished in the walls of the stomodaeum.

1. **Intima** - The inner most layer of chitin found in continuation of body cuticle.
2. **Epithelial layer**-It is a thin layer secreting the intima.
3. **Basement membrane**- Bounding the outer most surface of the epithelium.
4. **Longitudinal muscles**-These muscles are less developed than circulatory muscles.
5. **Circulatory muscles** - These are well developed.
6. **Peritoneal membrane** - It is often difficult to detect and consists of apparently structure less connective tissue.

Midgut or Mesenteron: It is relatively a short tube or elongated sac with uniform diameter extends from hepatic caecae or cardiac valve to Malpighian tubes or pyloric valve. Histologically, the inner wall of mesenteron or stomach is not made up of chitin, but consists of following layers

i. Peritrophic membrane
ii. Enteric epithelium
iii. Basement membrane
iv. Circular muscles
v. Longitudinal muscles
vi. Peritoneal membrane

The enteric epithelium is made up of three types of cells:

i. The columnar cells which secret the enzymes and absorb the digested food,
ii. The regenerative cells which renew the destroyed and dead epithelial cells through secretion or in the process of degeneration**.**
iii. The goblet cells which are of uncertain functions.

Thus, there are following five major function of enteric epithelium:

- to make digestive enzymes
- to absorb the digested food
- to produce new cells
- to absorb the water
- to excrete the waste material outside the body.

The inner surface of midgut is sometime lined by a thin membrane known as peritrophic membrane which protects the epithelial cells from the direct contact of food particles. This membrane is absent in Lepidopterans and Hemipterans.

Hindgut or Proctodeum: It extends from the posterior end of midgut to the anus and is also an invagination of the body wall. The hind gut consists of the some layers as the fore gut except that the circular muscles are developed both inside and outside the layer of longitudinal muscles. The hind gut is externally marked by the insertion of the Malpighian tubes and internally by the pyloric valve. It may be divided into three distinct regions

Ileum- It is a small tube which has many folds in its inner wall.

Colon- It is situated on the 5th and 6th segments of the abdomen and is a slender tube which, cannot be easily distinguished from the **ileum**. In some insects it is just like 'S' in structure.

Rectum- Both the ends of the rectum are comparatively slender while the middle portion is thick and large which consists of six rectal papillae internally and six ridges of longitudinal muscles externally. The rectum opens to exterior through the anus which is situated at the caudal end of the abdomen.

Salivary Glands - The labial glands which are associated with the gnathal appendages are the salivary glands. A pair of salivary glands is found in the grasshopper which generally lie in the thorax and are convoluted tubes often branched and racemose. Both the ducts of salivary glands unite together beneath the oesophagous to form a common salivary duct which opens into the salivarium.

MALE REPRODUCTIVE SYSTEM IN GRASSHOPPER

Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle, Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes, Dissection tray, Dissecting pins, Rigid metal ruler and case Camel hair brush etc.

Male reproductive organs consist of the followings- (i) A pair of testes (ii) A pair of vasa deferentia (iii) Seminal vesicles (iv) Ejaculatory duct (v) Penis or Aedeagus (vi) Accessory glands (vii) Male genitalatrium The Testes-They are located above the midgut and held in position by the surrounding fat bodies and tracheae. Each testis is a more or less ovoid body partly or completely divided into a variable number of follicles or lobes which are cylindrical in shape. Each follicle is connected with vas deferens by a relatively well developed slender tube known as vasa deferentia. The peritoneal investment of the follicle is developed to the extent of enveloping the testis as a whole in a common coat known as scrotum.

The presence of the sex cells in different stages of development.

These zones are as follows

The germarium - It is the region having primordial genii cells or spermatogonia which undergo multiplication.

The zone of growth – In this zone the spermatogonia increase in size and undergo repeated mitotic division and develop in to spermatocytes.

The zone of division and reduction-Here the spermatocytes undergo meiosis and produce spermatids.

The zone of transformation - The spermatids are transformed into spermatozoa. The masses of spermatozoa are generally enclosed in the testicular cyst cells from which they are released in the vas deferens. In addition, the testes contain large elements known verson's cells or apical cells.

Vas deferens- These are the paired canals leading from the testes which are partly or wholly mesodermal in origin.

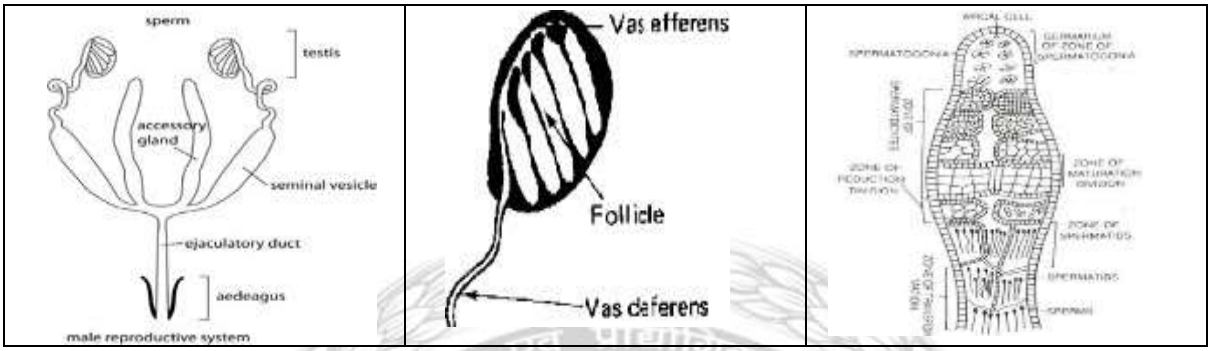
Seminal Vesicles- The Vas deferens vary greatly in length in the majority of insects. Each Vasa deferens becomes enlarged along its course to form a sac known as seminal vesicle in which spermatic fluid is collected.

Ejaculatory duct -Posteriorly, the vasa deferentia unite to form a short common canal which is continuous with a median

ectodermal tube known as ejaculatory duct. The terminal end of ejaculatory duct opens in the male genital atrium.

Aedeagus- The terminal end of the ejaculatory duct is enclosed in a finger-like evagination of the ventral body wall which forms the male intermittent organ known as aedeagus. It is situated on 9th abdominal sternum of the grasshopper on the conjunctival membrane of the posterior margin.

Accessory glands- These are one to three pairs in number and usually present in relation with the genital ducts opening into seminal vesicle. These are tubular or sac-like in structure. In most of the cases their secretions mix with spermatozoa and in some insects glands are directly concerned with the formation of the spermatophores.

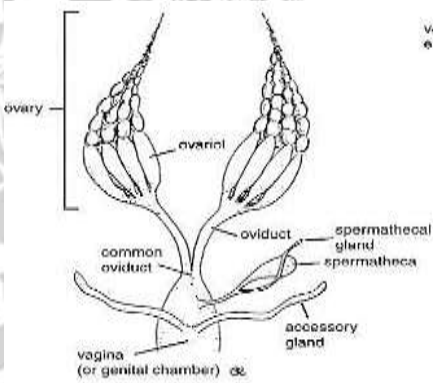


FEMALE REPRODUCTIVE SYSTEM IN GRASSHOPPER

Materials required for dissection: Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle, Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes, Dissection tray, Dissecting pins, Rigid metal ruler, And case Camel hair brush etc.

Reproductive organs: The female reproductive system consists of the following organs- (i) A pair of ovaries (ii) A pair of lateral oviducts (iii) Spermatheca (iv) Vagina and genital chamber (v) Accessory glands (Collaterial glands)

The ovaries- These are typically more or less compact bodies lying in the body cavity of the abdomen on either side of the alimentary canal. Each ovary is about 2 cm long and composed of a variable number of ovarioles and open into the oviduct. A typical ovariole is an elongated tube in which the developing eggs are disposed one after the other in a single chain. The oldest oocyte is situated nearer the union with the oviduct. The wall of an ovariole is made of follicular epithelium whose cells rest upon a basement membrane known as tunica propria. Each ovariole may be differentiated into three zones:



Terminal filament- It is the slender thread like apical prolongation of the peritoneal layer. The filaments of the ovary combine to form a common thread termed as terminal filament. The terminal filament of one ovary units with the filament of the other ovary to form a median ligament. It aids in maintaining the ovaries in the position and is attached to the dorsal diaphragm.

The germarium- It is situated below the terminal filament and forms the apex of an ovariole. It consists of a mass of cells which are differentiated from the primordial germ cells.

The region of growth- It is also called as vitellarium which constitutes the major portion of an ovariole. The vitellarium contains the developing eggs (oocytes). The epithelial layer of the wall of vitellarium grows inwards to enclose each oocyte in a definite sac known as follicle. The cells of the follicle secrete the chorion of the egg and in some cases serve to nourish the oocytes.Three types of ovarioles may be recognized on the basis of presence or absence of nutritive cells.

Panoistic type– Nutritive cells are absent e.g., grasshopper and other insects of Orthoptera and Isoptera.

Polytrophic type- Nutritive cells are present and arranged in alternate with the oocytes e.g., Hymenoptera.

Acrotrophic type- Nutritive cells are present and situated at the apices of the ovarioles e.g., Hemiptera. The oviducts– The lateral oviducts are paired canals leading from the ovaries and are formed from the mesoderm. These lateral oviducts form the common oviduct which opens into the vagina. Each oviduct is an enlarged pouch which stores eggs. The vagina is greatly enlarged to form a chamber, known as uterus, for the reception of developing eggs.

The Spermatheca– This is a pouch or sac for the reception and storage of the spermatozoa (seminal fluid) and is also known as receptaculum seminis. It generally opens by a duct into the dorsal wall of the vagina, which is known as sperm duct. In many insects pairing takes place only once and since the maturation of eggs may extend after the union of the sexes, the provision of spermatheca allows for their fertilization from time to time. A special sperermathecal gland opens into

the duct of spermatheca and secretes a fluid which length of the life of sperms.

Genital chamber– The vagina opens into the genital chamber on 9th sternum and this chamber is called bursa copulatrix which helps in copulation.

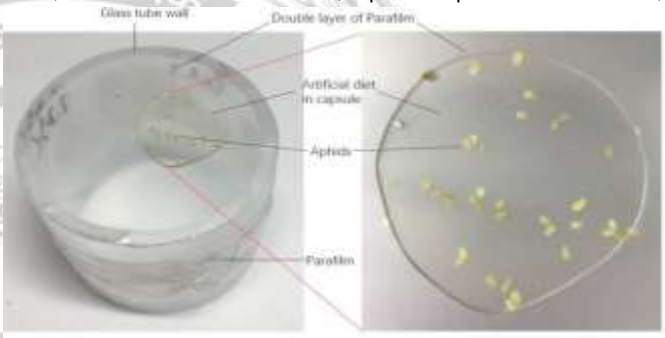
Accessory glands– These are paired structures opening into the distal portion of the vagina. These glands provide material for the formation of egg pod or ootheca.

Fertilization– After copulation; the spermatic fluid is received in the spermatheca. The egg comes down from the oviduct to the vagina which has an opening (micropyle) into its shell for the entrance of male germ cell (spermatzoa). One or two spermatozoa enter the egg through micropyle and only one succeeds in fertilizing the egg. After fertilization the accessory glands secrete a fluid around the egg which hardens it.

ARTIFICIAL DIET FOR INSECT REARING

Artificial diets- The term artificial diet, when applied to insects is one that has been defined as any diet that is not the natural food of the insect. It includes all the various terms such as synthetic, chemically defined, holidic, meridic, and oligic.

A nutritionally complete diet for most insects in axenic culture must contain all or most of the proteins or amino acids including ten essential ones, carbohydrates, fatty acids, cholesterol, choline, inositol, pantothenic acid, nicotinamide, thiamin, riboflavin, folic acid, pyridoxine, biotin, vitamin B12, beta carotene or vitamin A, alpha tocopherol ascorbic acid, several minerals and water.



Uses of artificial diets

- Diets are used for studying insect nutrition
- Used for studying biochemistry, behavior and other biological process.
- Testing of compound for physiological effects
- Maintaining colonies
- Mass production of insects for various purposes

Insect artificial diets are classified into 3 types

Holidic diet: Diets in which the ingredients can be represented by chemical formulae are known as chemically defined diets or holidic diets. These diets are used primarily for nutritional studies. Most nutritional studies have been conducted with diets containing one or more of the following ingredients such as agar, protein (casein) vegetable oil, starch, and cellulose. With appropriate descriptions, these diets could be designated as defined diets.

Meridic diet: Diets which contain one or more unrefined substances from plant, animals or microorganisms such as plant tissue, liver extract, and yeast products. The main characteristic of these diets is that most of the nutrients are provided as pure or refined substances also. The large number of diets for the laboratory rearing of insects is included in this group.

Oligic diet: These diets are made up of crude material. They are designed to imitate the natural food and are assumed to have all the required nutrients with undigestible inert material. These diets are economical and are used of mass rearing of insect

ARTIFICIAL DIET FOR *Helicoverpa armigera* (Hub.)

Materials and Methods: At the start, 1050 larvae (different instars) of *H. armigera* were collected from chickpea fields and reared on artificial diet. The rearing room temperature ranges from 20 to 29°C, relative humidity varied from 60 to 80 percent, and a 15.9 light and dark cycle was maintained.

Ingredients for the preparation of diet: The ingredients and quantities used in preparation of a 4.251 batch of the diet are as follows:

Ingredients Quantity

Agar - 25.0 g
Bean powder (*Vigna unguiculata*) - 600 g
Ascorbic acid - 7.0 g
Sorbic acid - 3.0 g
Vitamin E - 0.2 g
Dried active yeast - 20.0 g
Mehtyle-p- Hydroxyhenzoate - 10.0 g
Vitamin mixture - 5.0 ml

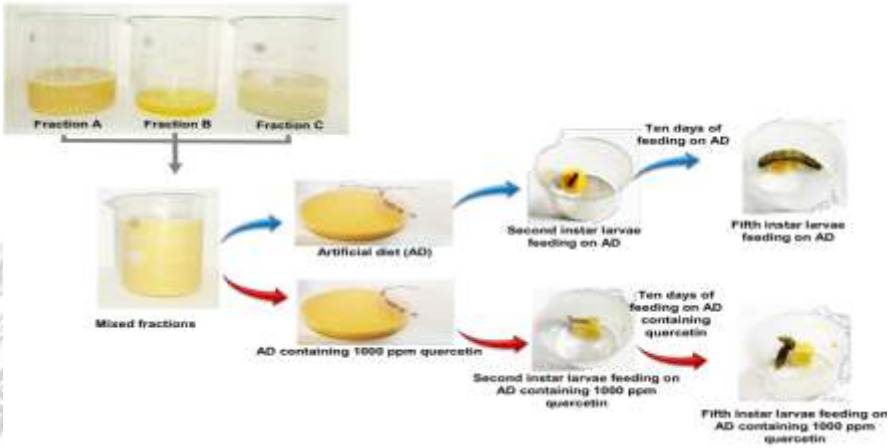
Composition of *H. armigera* larval diet (1 litre)	
Chickpea	100 gm
Yeast extract	30 gm
Sucrose	25 gm
Wesson's salt mixture	5 gm
Methyl para hydroxy benzoate	5 gm
Ascorbic acid	10 gm
Sorbic acid	6 gm
Streptomycin sulphate	100 mg
Choline chloride (5%)	10 ml
Formaldehyde	2 ml
Multivitamin capsules	03
Protein-X	2 gm
Autoclaved double distilled water	1000 ml

Formaldehyde (10%) - 6.0 ml
Tap water - 3500.0 ml

Vitamin mixture preparation in 200 ml sterile distilled water:

Calcium pantothante - 4.8 g
Nicotine acidamide 2- 4 g
Riboflavin - 1.2 g
Folic acid - 1.2 g
Thiamine Hydrochloride Pyridoxine - 0.6 g
Biotin - 0.048 g
Vitamin B_{12} - 0.0024 g

Procedure: The dry ingredients of the diet were weighed carefully and kept in separate containers. The wet ingredients were measured and also kept in separate containers. The entire quantity of agar was suspended in a 5 lit. capacity container and brought to a boil. The total quantity of bean powder (*Vigna unguiculata*) was added to the boiled agar and mixed, which resulted in cooling of the mixture to nearly 80°C. Then all the dry and wet ingredients were added to this mixture and the entire mass was mixed. The vitamin mixture, including vitamin E, was added last, and the mass was again mixed thoroughly. The prepared diet was the poured into the desired number of sterilized glass capsule vials (5-6 ml diet/capsule vial) with the help of a squeeze bottle and allowed to cool and harden.



NERVOUS SYSTEM OF GRASSHOPPER

Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle, Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes, Dissection tray, Dissecting pins, Rigid metal ruler and case Camel hair brush, binocular microscope etc.

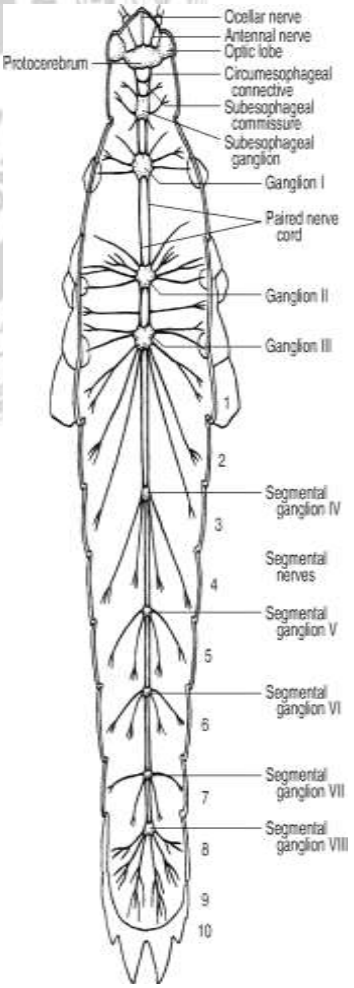
Central Nervous System of Cockroach: The central nervous system consists of the supra-oesophageal ganglion or brain, sub- oesophageal ganglion and the nerve cord.

The supra-oesophageal ganglion or cerebral ganglion is a bilobed structure situated in the head in front of oesophagus, above the tentorium and almost between the bases of the antennae. It is formed by the fusion of three pairs of ganglia. It represents the brain and is concerned chiefly with sensory function. From the supra- oesophageal ganglia arise two circumoesophageal connectives which encircle round the oesophagus and meet below it with the sub-oesophageal ganglion. The sub-oesophageal ganglion is also situated in the head and formed by the fusion of 3 pairs of ganglia. Thus, the supra-oesophageal ganglion, circumoesophageal connectives and sub- oesophageal ganglion together constitute the nerve ring round the oesophagus in the head capsule. The sub-oesophageal ganglion is the principal motor centre and controls the movements of muscles, mouth parts, wings and legs.

From the sub-oesophageal ganglion arises a double nerve cord which travels through the thorax and abdomen below the alimentary canal on the ventral side up to the posterior end of the body. The nerve-cord has three large ganglia in the thorax, one each for pro-, meso-and metathoracic segments, therefore, they are called prothoracic, mesothoracic and metathoracic ganglia.

Further the nerve cord has six ganglia in the abdomen which lie in the 1st, 2nd, 3rd, 4th, 5th and 7th segments.

Each ganglion of the nerve cord is formed by the fusion of two ganglia except the ganglion in the 7th segment. The ganglion in the 7th abdominal segment is the largest of all the abdominal ganglia and probably formed by the fusion of 3 pairs of ganglia. Both the nerve cords run parallel and very close to each other but unlike earthworm they are not enclosed in a common sheath.



But they are fused only at the place of the presence of ganglion and they are solid.

Peripheral Nervous System of Grasshopper: The nerves originating from the nerve ring and ventral nerve cord to innervate different parts of the body constitute the peripheral nervous system.

Three pairs of nerves originate from the supra-oesophageal ganglion—optic, antennary and labora frontal nerves. The first two innervate the eyes and antennae but the third one divides into labral nerve supplying to the labrum and the frontal nerve which runs forwards to join the sympathetic nervous system.

Similarly, three pairs of nerves originate from the sub-oesophageal ganglion—mandibular, maxillary and labial to innervate the mandibles, maxillae and labium respectively. Several pairs of nerves arise from each thoracic ganglion to supply the different parts of their own segment.

A pair of nerves, however, from metathoracic ganglion innervates the 1st abdominal segment. The nerves originating from first five abdominal ganglia innervate the 2nd, 3rd, 4th, 5th and 6th abdominal segments. From the last abdominal ganglion three pairs of nerves are given off to supply the 7th, 8th, and 9th segments. It also gives a branch to innervate the cercus and other associated structures.

Sympathetic Nervous System: The autonomic or stomogastric or sympathetic or visceral nervous system of cockroach consists of some ganglia and their connectives. It includes the frontal, occipital, visceral and pre-ventricular ganglia. The nerves from these ganglia are connected with the supra-oesophageal ganglion.

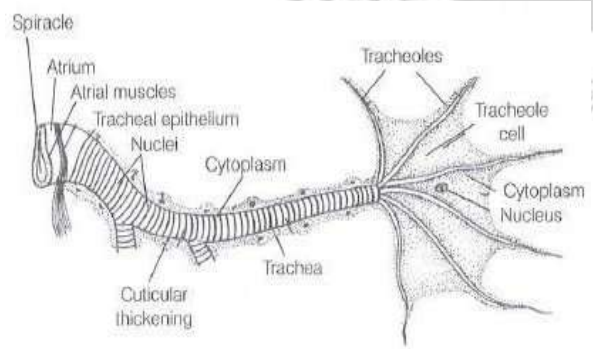
The frontal ganglion is a small ganglion situated on the oesophagus in front of the supra-oesophageal ganglion. A pair of frontal connectives from the frontal ganglion is connected with the supra-oesophageal ganglion, a median recurrent nerve passes backward from it and connects the occipital or hypo cerebral ganglion behind the supra-oesophageal ganglion.

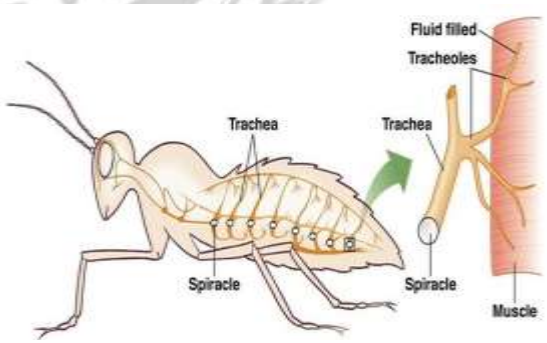
Three nerves, two lateral and one median originate from the occipital ganglion; the lateral nerves are connected with the corpora cardiaca and corpora allata, which are endocrine glands, while the median nerve runs backwards over the oesophagus and joins the visceral ganglion situated on the crop. From the visceral ganglion a pair supply the alimentary canal, one of them is connected with the pro-ventricular ganglion situated on the gizzard

RESPIRATORY SYSTEM

Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle, Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes, Dissection tray, Dissecting pins, Rigid metal ruler and case Camel hair brush, binocular microscope etc.

Tracheal system: The trachea are ectodermal in origin and are tubular invagination of the body wall. The trachea present a ringed appearance due to thickening of intima known are taenidia. The taenidia_prevent the collapse of trachea if the pressure within is reduced. The trachea divide and re divide into smaller branches known as tracheoles. The tracheoles are less than 2 microns and are lined by taenidia. The base of each cluster of tracheoles has a web like cell known as end cell or tracheole cell which lie within the tissue cells of the body. The trachea from neighbouring spiracles join to form longitudinal trunks running along the length of the body. There are lateral trunks in addition to the dorsal and ventral longitudinal trunks. The longitudinal trachea are connected to each other by transverse commissures. The trachea has direct contact with air and with the tissues through a number of openings known as spiracles.



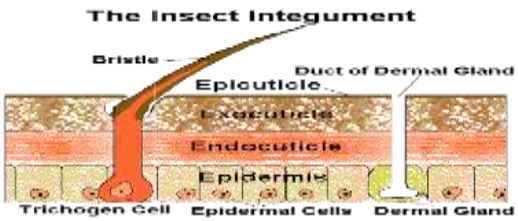


DETECTION OF WAXY COMPONENTS IN INSECT CUTICLE

Experimental Insect: Living black ants (*Formica* sp.) (Order: Hymenoptera).

Apparatus: Compound microscope, cavity blocks (2), Camel hair brush, etc.

Chemicals: Liquid paraffin, absolute alcohol (methylated spirit), xylene, *etc*.



A large waxy layer is present in the epicuticle of the insects. The waxy component may have different characteristics in different insects. It is soft greasy in cockroaches and waxy, pale yellow, non-crystalline white in the larvae of sawfly *Athalia proxima*, *Nematus* sp. and *Pieris brassicae* (Cabbage caterpillar) while, in stored grain pests like *Tenebrio* sp. it is hard and crystalline.

If the insect is treated with xylene, the waxy component of cuticulin is removed and the waxy layer presents a flow of water from the insect body. Water bubbles are different from air bubbles by the characteristics that the air bubbles explode immediately when they come to the air surface. The water has great affinity towards alcohol therefore, it comes out on large scale and attracted toward the alcohol layer. Wax is a mixture of araffins of the probable order C-C, and esters of n-alcohols and acids. The waxy thickness from 0.1 u to 0.4 µ.

Procedure: Take a cavity block (1) containing few drops of xylene. Then submerge a live black ant *Formica* sp. in it for 15 minutes. The xylene will react on the waxy component of ant cuticle/epicuticle. Then arrange one more cavity block (II) containing a mixture of methylated spirit and liquid paraffin. These two chemicals doesn't mix with each other. Now submerge xylene treated ant into this cavity block (II) containing mixture of LP and MS/absolute alcohol. After few minutes observe the cavity block under microscope.

It will be observed that- Bubbles emerge out of the cuticle. Number of bubbles increase with respect to increase in time. By rising temperature small bubbles becomes large bubbles.

Results: Water bubbles coming from the cuticle proves the presence of waxy layer in the insect cuticle which dissolves on treating with xylene.

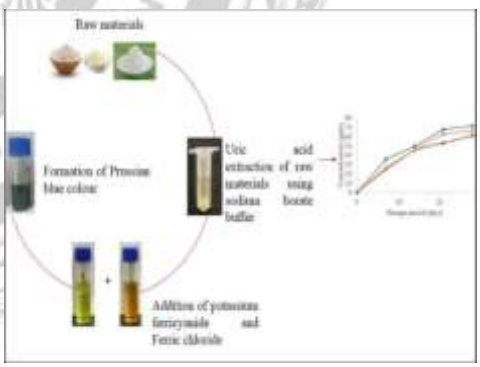
DETECTION OF URIC ACID IN INSECTS

Experimental Insect: Blister beetle (Coleoptera: Meloidae).

Source: Malpighian tubules and Rectum.

Requirements Apparatus: Dissecting box, mortar and piston, filter papers, spirit lamp, test tubes, slides, coverslips, etc.

Chemicals: Uric acid, $AgNO_3$ solution, conc. HNO_3, dil NH_4OH, Na_2CO_3, NaOH solution, distilled water etc.



Importance and Theory: Uric acid is the most important nitrogenous component of the urine. It contains less hydrogen than other nitrogenous compounds excreted and thus useful in water conservation. As a free acid or as Ammonium salt it is highly insoluble but, eliminated with a very little water. It helps refilling the water supply. It helps the insect in egg and and pupal development where there is no source for refilling the water supply. Nitrogen is excreted as uric acid with 85.8% in silkworm. In dried excreta of *Tineola* it is 28% while in *Tenebrio* it is 50% and in *Antheraea perneyi* 26.2%. It is in solution when sufficient water is available but in scarce, it is in a crystalline spheres. The crystalline sphere may have the range of diameter from lu to 60µ or more. The uric acid also have other nitrogenous constituents.

Procedure - I

- Dissect out Blister beetle and remove the malpighian tubules present at junction of hindgut and midgut of the alimentary canal.
- The malpighian tubules are cleared by removing tracheae, fat bodies and other elements.
- The malpighian tubules are then taken in a cavity block containing little quantity of distilled water.
- Prepare a homogenous paste of M.T.
- Add to this a little quantity of Na_2CO_2 solution and stir a little while.
- Take few drops of $AgNO_3$ on the filter paper, then add a drop of homogenized extract on it.
- Yellowish brownish or black coloured ppt is developed.

This indicates the presence of uric acid in the extract. As due to the reduction of $AgNO_3$ ppt is developed.

Result: Yellowish or brownish color confirms the presence of uric acid in the extract.

Procedure – II

- Dissect out live Blister beetle.
- Take out rectal content from rectum of blister beetle and place on clean slide.
- Prepare a smear of the extract on slide. 4. Dry the slide.
- A few drops of conc. HNO3, are added and evaporated it to dry.
- Heat the slide.
- Content will turn brown due to the formation of alloxanthine. 5/12
- Add a drop of NH4OH - the reduced turns purple in colour.
- 9 Dry the slide with NH4 OH. 10. Examine the slide under microscope.
- Blue spots confirms the presence of uric acid in the extract. Reaction
- Uric acid + Conc. HNO3 →Alloxanthine (Purple) Alloxanthine + NH4OH →Murexide (Blue).

Result: Uric acid is present in the rectum of insect (Blister beetle)

EXCRETORY ORGANS IN INSECT

Experimental insect: Test insect

Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle,Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes,Dissection tray, Dissecting pins, Rigid metal ruler, and case Camel hair brush etc.

Excretory organs:

Malpighian tubules: Thin, blind-ending tubules, originating near the junction of mid and hindgut predominantly involved in regulation of salt, water and nitrogenous waste excretion. This structure was discovered by Marcello Malpighi.

Nephrocytes: Cells that sieve the haemolmph for products that they metabolize (pericardial cells).

Fat bodies: A loose or compact aggregation of cells, mostly trophocytes suspended in the haemocoel responsible for storage and excretion.

Oenocytes: The cells of haemocoel, epidermis or fat body with many functions.

Integument: The outer covering of the living tissues of an insect.

Tracheal system: The insect gas exchange system, comprising trachea and tracheoles.

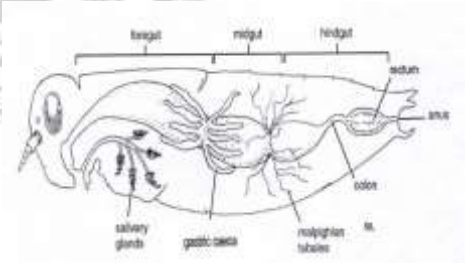
Rectum: The posterior part of hind gut. Among the above organs, malpighian tubules are the major organ of excretion.

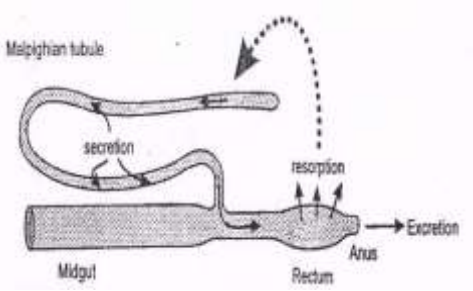
ANATOMICAL DETAILS OF MALPIGHIAN TUBULE IN GRASSHOPPER

Experimental insect: Grasshopper Materials required for dissection

Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle, Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes, Dissection tray, Dissecting pins, Rigid metal ruler, and case Camel hair brush etc.

Malpighian tubules: The main organ of excretion and osmoregulation in insects are the malpighian tubules, acting in association with rectum or ileum. Malpighian tubules are outgrowths of the alimentary canal and consist of long thin tubles formed of a single layer of cells surrounding a blind-ending lumen, generally they are free, waving around in the haemolymph where they filter out solutes. Each tubule is externally covered by peritoneal coat and supplied with muscle fibres and tracheloes. Functional differentiation of the tubules was seen, with the distal secretory region and proximal absorptive region. The malpighian tubules produce a filtrate (the primary urine) which is osmotic but ionically dissimilar to the haemolyph and selective reabsorbs water and certain solutes, but eliminates others. The malpighian tubules produces an osmotic filtrate which is high in K+ and low in Na+ with Cl as major anion. The active transport of ion especially K+ into the tubule lumen generates an osmotic pressure gradiant for the passive flow of water. Sugars and most amino acids are also passively filtered from the haemolymph via junctions between the tubule cells, whereas amino acids and non-metabolizables and toxic organic compounds are actively transported into the tubule lumen. Sugars are reabsorbed from the lumen and returned to the haemolymph. The continuous secretory





activity of each Malpighian tubule leads to a flow of primary urine from its lumen towards and into the gut. In the rectum, the urine is modified by removal of solutes and water to maintain fluid and ionic homeostasis of the body

UPTAKE OF DYE BY MALPHIGIAN TUBULES IN INSECT

Experimental Insects: Cockroach, *Periplanata americana* (Blattaria: Blattidae).Grasshopper, *Heiroglyphus banian* (Orthoptera: Acrididae) or Blister beetle (Coleoptera: Meloidae).

Requirements Apparatus: Shallow dish bee wax tray, dissection box, dissecting binocular/microscope with good lighting, *etc.*

Chemicals: Indigo-carmine/0.01% Neutral red.

M.T. Number and Species Diversity: Grasshoppers, and nymphal cockroaches contain 60 or more malpighian tubules, lepidopterous caterpillars contain 6 tubules while, Dipterous larvae (Drosophila) contain only 4 tubules, the pairs unite together and open into the rectum by single pore in Drosophila.

Importance and Theory: Malpighian tubules are elongated, tubular bodies which extends from the junction of mid and hind gut and are found in insects. These vary greatly in number, from a pair to over 100 in different species and also vary with the forms. Nitrogenous waste, uric acid, are passed into the tubules in solution. Excess water is resorbed in the tubules or in the rectum, making the urine and fecal material in a more or less dry form. Thus, malpighian tubules have excretory function.

There are several types of dyes which enter into the lumen of malpighian tubules. In cockroach indigo carmine is excreted by only some part of malpighian tubules. In certain insects like *Rhodnius,* the above dye is excreted only by the upper segment. Similarly, neutral red enters the lumen by the same route. However, later, it is taken up by the lower segment, deposited and stored in the cells. Many dye enters into the lumen through cells. Thus, sometimes, they store some part of dye in their bodies. The stored dyes may be later, absorbed by the lumen in many cases.

Procedure: Select any of the above given insect for experiment. Anaesthetize the insect with CO2 or ether or chloroform before dissection. Pin the insect in a shallow dish bee wax tray and dissect away the dorsal or ventral abdomenal wall and carefully lift or tease away structures obscuring the M.T. Tease out a malpighian tubule into a few drops of saline. Add a small amount of neutral red solution and observe the uptake of dye by the tubule cells. Also observe its appearance in the M.T. lumen. An evidence of red dye will be observed by the presence of crystals in M.T.

Results: Crystals are seen in malphigian tubules

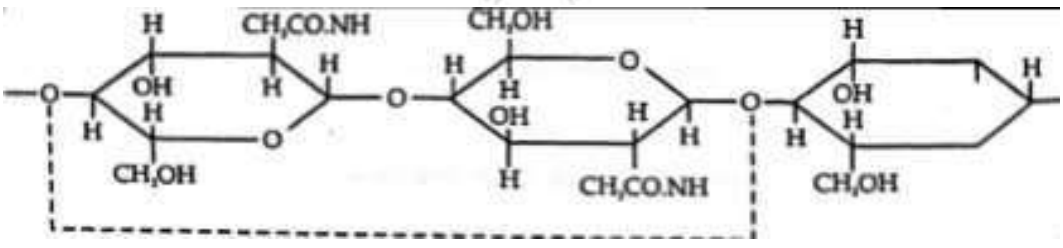
DETERMINATION OF CHITIN CONTENT IN INSECTS CUTICLE

Insect required: Living Grasshopper (*Hiroglyphus banina*)

Apparatus: 500 ml Beaker, Petridis, glass rod, cavity block, watch-glass, thermometer -200° C, glycerine bath, spirit lamp, test tubes, etc.

Chemicals: Glycerine, dist. water, alcohol grades 90 -30%, saturated KOH solution, 5% CH3COOH, 2% Iodine, 1% H_2SO_4

Importance and Theory: Chitin is nitrogenous polysaccharide polymer and major part of the insect body wall (cuticle). According to Wigglesworth (1968) chitin is protein complex of cuticle with empirical formula (C6H13O5H)x and high molecular wt. The chitin molecules are composed of N-acetyl glycosamine and β-glucosine linkage. Single chitin molecule contains hundreds of N-acetylamine units. "Kitobiose" is a single unit of two adjacent of n- acetyl glucose amines. The units can join together in a linear fashion forming branched long chain. Endocuticle contain main constituent of chitin and vary with the species in per cent.



Properties: Chitin is soluble in dilute acids and concentrated alkalies. It is also soluble in water, ethers, alcohol, all solvents including acetic acid and H_2S0_4. It forms iodate and chitoson sulphate when treated with H_2S0_4. Chitin is soluble in conc. mineral acids and it is hydrolyzed to lower saccharides when reacted with alkali at high temperature.

Procedure

- The procedure includes following steps:

- Take out a living cockroach and remove few pieces of tergites or sternites from it, clean the pieces.
- Remove all other parts such as tracheae, muscles, etc. from these pieces so as to clean the matter.
- The pieces are then taken into test tube containing saturated KOH solution.
- Uniform and indirect heating of pieces on glycerine bath containing thermometer 200°C.
- The test tubes containing pieces and KOH solution then heated continuously till the temperature rises to 140°C.
- At 140°C the colour of pieces becomes faint slowly.
- Heat the test tubes continuously up to 155°C, at this temperature cuticle becomes decolourised completely.
- Continue the heating of pieces till the temperature goes to 160°C.
- KOH solution starts boiling at 160°C. Heating is stopped when material becomes decolourised completely. Arrange temperature 160°C constant for continuous heating and this process is repeated for about15 minutes.
- In decolourised material chitoson is left. Chitoson is colourless and transparent.
- After proper heating the test tube is taken out from glycerine bath and cooled at room temperature.
- Take out chitoson (Pieces) in clean watchglass. Some pieces are washed in cavity block with degraded alcohol, from absolute to 30 percent for 2 -5 minutes each.
- Take out chitoson pieces on at clean slide/watch glass and treat with 1% H2SO4. Material will change to brown.
- The same matter is treated with 0.2% iodine solution for observing violet colour. The violet-colour to the matter will indicate the presence of chitin.
- Confirmatory Test of Chitin
- Treat the chitoson pieces on slide with 3% acetic acid, the matter will be dissolved immediately adding 1 %. H_2SO_4 to this matter will develop white ppt. This will confirm the presence of chitin.

Special Precautions

- Heating the material beyond 160 °C should be avoided, residue may not be left in the test tube if heated beyond 160°C
- For hoping positive results, acetyl bond, must be broken completely by heating.
- Direct heating is avoided, heating should be uniform and indirect.
- Adopt gradual cooling process.

Results: The intense violet colouration confirm the presence of chitin in the matter.

HISTOLOGICAL PREPARATION OF HAEMOCYTES IN INSECT

Experimental Insect: Cockroach, *Periplanata americana*. (Blattaria: Blattidae)

Requirements Apparatus: Slides, coverslips, microscope, water bath, beaker 500 ml, thermometer, lamp etc.

Chemicals: Geimsa's stain or Leishman's stain, 2% ringer solution, 2% Versene, alcohol grades, xylene, DPX, etc.

Importance and Theory: Haemocytes are an important constituent of the blood of insect and are loose cells present in the blood in body cavity of insect. These cells are generally located on the surface of various organs and also found circulating freely in the haemolymph or blood. The haemocytes are present in lumen of heart certain insects such as *Periplanata americana, Pieris brassicae, Apis indica, etc* but not in certain insects such as Bed bugs, etc. In most insect species, the haemocytes are found either independently in blood or in aggregations. They change their shape as per the need. The number of circulating cells vary with the species from 15,000 to 2,75 ,000 per cubic mm, a cockroach averaged 30,000 per cubic mm.

Sites of haemocytes: Tissues, surface of various organs, blood, along out surface of heart, wing vessel, wing veins, heart lumen, etc. In certain cases haemocytes are sedentary, e.g. *Corethra* larva.The haemocytes perform the functions such as the ingestion of small solid particles (phagocytosis), resistance to micro- organisms, blood coagulation, connective tissue formation, resistance to metazoan parasites, haemocytes and intermediary metabolism, immunity, giant cell formation, etc.

Procedure: A cockroach is immersed for about 2 -3 minutes in hot water bath (Temp. 53 -54°C but, not more than 60°C) for removing haemocytes from their aggregation sides. Cut the tip of antenna and press the cockroach gently for a drop of haemolymph from the cut point. A monolayer prepared degreased slide should be kept ready with a drop of 2% versene ringer solution as haemocyte fixative. Then take a drop of haemolymph in fixative. The slide is then air dried and then stained with either Leishman's stain or Giemsa's stain for 10 minutes. Then remove the slide and wash it with running tap water and allow to dry at room temperature. Make the dehydration of the matter by brief bath of acetone. The stained slides are cleared in xylene and then finally mounted in DPX.

Steps Involved in Technique

1. Dip the insect in hot water (Temp. 53°C -54°C) for about 2-3 minutes
2. Take out blood
3. Smear on slide
4. Add dilution fixative (2% versene)
5. Dry it with lamp (bulb)
6. Stain it
7. Dehydration
8. Prepare slide
9. Mount under microscope

10. Observe the characters of haemocytes in slide
11. Identify the haemocytes
12. Haemolymph dilution Fluid
13. 2% versene-ringer solution+ traces of methelene blue
14. Adherent medium
15. One part egg albumin+ 4 parts 2% versene-ringer solution
16. Chemical fixative
17. Disodium EDTA (Versene) should be used as chemical fixative.

It is prepared by dissolving 2g of disodium EDTA crystalline powder in 100 ml solution of 2% versene. Ringer solution. (Insect ringer).

Preparation of insect ringer's solution

Dissolve following in distilled water to make the volume 1000 ml (pH -7 to 7.2)

- NaCl- 9.8 g
- KCl- 0.77 g
- $CaCl_2$- 0.5 g
- Na_2HCO_3- 0.18 g
- NaH_2P0_4- 0.01 g
- Dextrose -1.0 g

Working solution - (0.1 ml in 0.9% distilled water).

- Leishman's stain Leishman's powder -0.5 g
- Methyl alcohol-50 ml
- Giemasa's stain
- Giemsa powder - 0.5 g
- Methyl alcohol - 50 ml
- Glycerine -50 ml
- Acetone -10 ml.
- Glycerine -50 ml
- Acetone - 10 ml

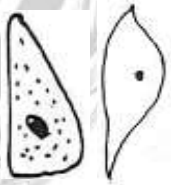
COUNTING OF HAEMOCYTES IN INSECT

Experimental Insects: Cockroach, *Periplanata americana* (Blattaria: Blattidae) or Gram pod borer, *Helicoverp aarmigera* (Lepidoptera: Noctuidae)

Requirements Apparatus: Microscope, slides, cover slips, needle, water bath, beaker 500 ml, thermometer, *etc*.

Chemicals: Leishman's stain, Giemsa's stain, xylene, DPX, alcohol grades *etc*.

Procedure: For the fixation of haemocytes, the insects are emmersed for 1-2 minutes in a hot water bath of temperature 55 to 60°C. A grease free slide is kept ready for taking haemolymph drop emerging from tip of antenna. With the help of scissor a cut is taken to the terminal portion of antenna. Immediately haemolymph will start emerging as a drop from cut point of antenna. It is taken on slide and a thin/monolayer is prepared and then dipped into either Giemsa's stain or Leishman's stain for 10 minutes. Then the slide is washed with tap water and dehydrated. After giving a wash of xylene, the slide is mounted with DPX and observed under microscope. Following haemocytes are observed under microscope. For *H. armigera* blood is taken from cutting prolegs of the larvae.



Proleucocytes: These cells are small rounded or oval in shape with deeply stained cytoplasm. These are abundant in blood smear especially in ribonucleic acid and nucleus. A huge bulk of basophilic granules are found in cytoplasm. They are abundant in young Lepidopteran larvae like Gram pod borer. They are found undergoing mitosis in all insects and have referred as young growing individuals.

Cystocyte Plasmatocyte (spindle shaped)

Plasmatocyes: These are polymorphic, rounded, spindle shaped or pear shaped haemocytes when they are free in the haemolymph but, found flattened or elongated when attached to different organs in the insect. Their number is quite large in Lepidopterus insects. These cells are characterized by rounded and oval nucleus, granular eosinophillic, and hyaline non vacuolated cytoplasm, stained faintly bluish.

Granular cells: These cells contain numerous reddish granules. However, their shape and structure is more or less similar to plasmocytes.

Spherule cells: The size of nucleus vary with the cells. Spherules are rarely present, particularly in the blood of cockroach. But, common in many lepidopterous insects. Cytoplasm resolution is poor.

Plasmocytes: These are bigger sized cells with oval or ovoid shape and distinct cell membrane. These are most common and most active cells found in the haemolymph. These cells are further characterized by having prominent rounded nucleus deeply stained, granular eosinophilic and 9.12 µ in diameter. Cytoplasm is a huge bulk of basophilic granules.

Results: Following types of haemocytes were present in the blood of cockroach:

- Plasmocytes
- Spherules
- Spindle shaped plasmocytes
- Proleucocytes
- Granular cells
- In *H. armigera* oenocytes were present as additional to cockroach

QUANTITATIVE COUNTING OF HAEMOCYTES IN INSECT

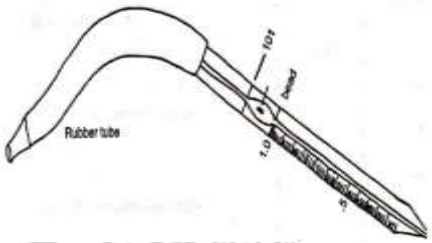
Experimental Insect: Cockroach *Periplanata americana* (Blattaria: Blattidae)

Requirement Apparatus: Haemocytometer (1.5 WBC) (Fig), counting slide (Fig) with special coverslip, pipette, cotton needle, hot water bath, beaker 500 ml, thermometer, microscope.

Chemicals: Diluting fluid, (Versene), distilled water.

N.B: Haemocyte number vary with the different stages of the insects and with the different species.

Procedure: Take the cockroach and dip it into hot water bath (temperature 55 to 60°) for about 1 to 2 minutes and then take a cut to the terminal point of antenna. Then collect the blood from antenna as a drop by sucking it through a clear pipette. The blood is sucked up to the mark one and immediately it is diluted in physiological saline – versene up to 101 ml. Then shake the solution for about 20 minutes so that the haemocytes will be distributed uniformly. Then take clear counting slide and a drop of diluted haemolymph solution and immediately covered with the special type of coverslip. Now observe the slide under microscope. The picture will show that haemocytes are uniformly dispersed on squares of the counting slide. Now count the number of cells present in each big square and calculate the average, volume of each square and then 1 mm^3 is calculated.



Calculations

Formula: No. of haemocytes per mm^3 = 25,000 x n n = is average no. of cells present in 1 sq3.

Volume of square = length x breadth x depth = 1/5 × 1/5 × 1/10 = 1/250

Thus each square having volume 1/250 mm3 when the average no. of cells present in each square is = n. Then i.e. 100 times dilution contains 250n cells/cubic mm

That is if no dilution then 250 x 100 = 25,000 n

Hence, the formula is = 25,000 n

When n is the number of cells present in one square and dilution is 100 times. Therefore, average number of haemocytes present in one sq is = Total no. of haemocytes present in all counting square/No. of all sq3 counted n = 36/16= 2.25 = 2.25 x 25,000 =56,250/mm^3

Result: The number of haemocytes present in one cubic mm area is 56,250. Thus, 56,250/mm^2/haemocytes are present in a given insect haemolymph

Precautions:

- The count should be made immediately.
- Dilute solution should be kept in convenient container and place for immediate use.
- Greatest care should be taken for preparing dilutions.
- The chamber of coverslip should be dry and clear.
- The counting chamber should be washed with distilled water and dried with soft fabrication.

Observation

No. of corner	No. of cell sq counted	No. of leucocytes present in each sq	Total squares counted= 16
I	1 2 3 4	1 2 3 2	No. of leucocytes present in sq = 8
II	1 2 3 4	2 3 2	No. of cell present in sq = 9
III	1 2 3 4	3 2 3 2	Total cells present = 10 in sq
IV	1 2 3 4	3 2 3 1	Total cells present = 9 in sq.

Total no. of sq counted = x = 16
I, II, III, IV Total no. of cells counted = y = 36 (8 + 9 + 10 + 9 = 36)
Average no of cells present in each sq = y/x=36/16 = 2.25
Hence the total no of cells = 2.25 x 25,000 = 56,250/mm³

CHROMATOGRAPHIC SEPARATION OF AMINO ACIDS FROM HAEMOLYMPH IN INSECT

Experimental Insect: Cockroach *Periplanata americana* (Blattaria: Blattidae)

Requirements apparatus: Circular chromatographic paper (Fig) micropipette, Petridish pair, beaker 500 ml, table lamp etc.

Chemicals: Standard amino acids, solvent system, Ninhydrin, N-butanol = acetic acid = distilled water (4: 1:1).

Importance and Theory: Twelt (1908), first introduced the chromatography. In paper chromatography, Paper acts as carrier of aqueous phase of the two phases solvent system. The chromatography depends on method of absorption, partition and ion exchange. By absorption method, different components of solutes are separated. Partition part is carried out on paper and ion exchange depends on exchange of either cations or anions in chromatography method. The solute is separated into two different components moving at various rate along the paper depending upon alternate partition coefficient, solvent system, direction of flow and type of paper used. The rate offlow is ratio of linear movement of solute to linear rate of movement of solvent system.

Rate of flow (R.F.) = Distance travelled by solute / Distance travelled by solvent

Amino acids are identified with the help of paper chromatography using solvent system containing nynhydrin, N-butanol, acetic acid and distilled water in the ratio 4: 1:1. With the help of R.F. values, the amino acids can be identified as after separation.

Procedure: Take a circular chromatographic paper and draw circle at centre. Then mark various points for loading known amino acids. Likely, an unknown points also marked for loading haemolymph of nymph and adult cockroach. Loading at different points now load the various amino acids and unknown samples of haemolymph. During loading drying is also essential. Drying is done with the help of table lamp. Minimum 10 drops should be loaded at each known point and simultaneous drying is done on table lamp and finally unknown points are loaded and dried as suggested above.

Spreading: After loading and drying make a hole at centre through which a paper strip is inserted and dipped into solvent in petridish which is in sufficient amount. Later, make it air tight by applying grease along the end of the chamber.

Developing: After sufficient running take out paper mark solvent front and dry it over lamp. Then pour ninhydrin 0.1% on that paper and again dry it. Coloured bands of amino acids will be developed on paper. Mark it and calculate the Rate of Flow (R.F) and finally compare with standard amino acids and confirm the amino acids present in unknown samples. The above procedure is repeated for comparing larva. Pupa and adult of the insect.

Observation:

Spot	Standard amino acid	Distance travelled by solute	Distance travelled by solvent	R.F. value
A	cystine	0.5	5.0	0.10
B	valine	3.0	5.0	0.60
C	serine	1.5	5.0	0.30
D	leucine	3.7	5.0	0.74
E	Alanine	2.0	5.0	0.40
F	Histidine	0.33	5.0	0.06
G	Unknown	0.5	5.0	0.10
	Larva	1.5	5.0	0.30
		2.0	5.0	0.40
		3.7	5.0	0.74
H	Unknown	0.5	5.0	0.10
	Adult	1.5	5.0	0.30
		3.0	5.0	0.60
		3.7	5.0	0.74