

**METAMORPHOSIS OF SOME OF THE ORGAN
SYSTEMS IN THE PULSE-BEETLES,
Callosobruchus analis FABRICIUS
AND *C. chinensis* LINNAEUS
(BRUCHIDAE : COLEOPTERA)**

A dissertation submitted to the University of Dhaka
for the degree of

DOCTOR OF PHILOSOPHY

by

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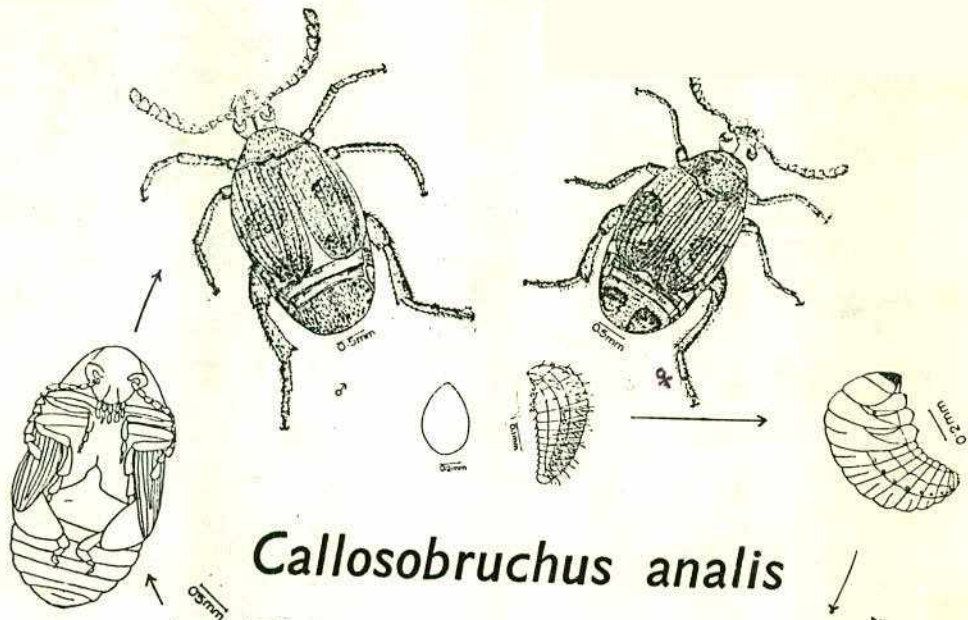


I do hereby declare that this dissertation submitted to the University of Dhaka for the degree of Doctor of Philosophy is based on my own investigation, carried out under the supervision of Dr. Mahmud-ul Ameen, Professor, Department of Zoology, University of Dhaka and that this or any part of this work has not been submitted for any other degree anywhere.

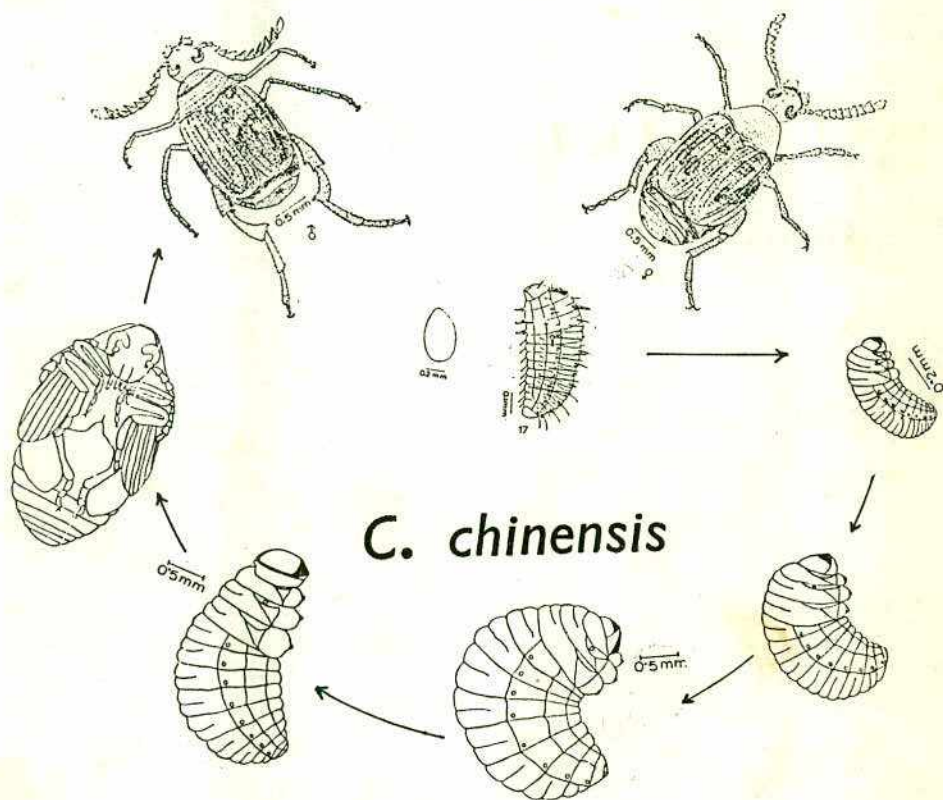
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Callosobruchus analis



C. chinensis

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SYNOPSIS

The anatomy and histology of the alimentary canal and Malpighian tubules of the larva, pupa and adult of two species of Callosobruchus are described separately. The metamorphic changes (anatomical and histological) of the above two organ systems have also been described in detail. Descriptions have been elaborated for Callosobruchus analis and made briefly for C. chinensis because they are very similar to C. analis.

I. INTRODUCTION

Callosobruchus analis Fabricius and C. chinensis Linnaeus (Bruchidae : Coleoptera) are serious pests of stored pulse grains. Their postembryonic developmental stages are completed within the pulse grains causing total damage of the kernels.

These beetles lay eggs on the surface of the seed. After hatching, the 1st instar bores into the cotyledon of the seed with the help of its strong mandibles. The larva lives on the kernel and it grows enormously with successive larval instars. In all there are four instars, of which the 3rd and 4th eat away the kernel voraciously. The larva stops feeding before the last larval moult, when its appearance changes a little. It is then called the "prepupa". Major anatomical and histological changes of the internal organs begin from this stage.

The hollowed out grain appears like a capsule with the pupa inside it. Only a thin lining of the kernel remains within the seed-coat. The early adult does not leave its pabulum immediately after the pupal-adult moult, but it remains for sometime inside the seed till the metamorphic changes of the internal organs are complete. The adult insect eats the remaining

part of the kernel before leaving the seed. It leaves behind excreta and exuviae within the seed-coat. Thus the infested pulse grains become absolutely denuded of any food substance.

A review of literature shows that many works were done on the biology and control of these two beetles, (e.g. Rehman et al., 1942; Arora & Pajni, 1957; Srisan, 1966; Rahman, 1971; Begum & Rahman, 1974; Rahman et al. 1975; Huda & Rahman, 1982; Rezaur et al. 1984), but very little has been done on the organ systems, specially the alimentary canal and Malpighian tubules of these beetles. Only Vats (1976) made brief observations on the larval alimentary canal of C. analis while comparing them with other bruchid larvae. Saini (1964) referred to the cryptonephridial system of adult C. analis and C. chinensis. But no information is available on the metamorphic changes of the various organ systems of these two pulse beetles.

Metamorphosis of the organ systems of insect had been a subject of great interest among entomologists for a long time. Extensive works have been done on the anatomy, histology, and metamorphosis of the alimentary canal and Malpighian tubules of insects by many workers. From their findings it is obvious that there exists a lot of controversy regarding various metamorphic

procedures in different insects. It varies greatly from one insect order to the other, and even among the different members of the same family. For instance, in the Bruchidae, the metamorphosis of the midgut of Acanthoscelides obtectus (Say) (Bushnell, 1936) differs much from that of Callosobruchus maculatus F. (Pajni, 1967).

The present study has been taken up with a view to describe in detail the anatomical and histological changes of the two important organ systems, viz., the alimentary canal and the Malpighian tubules of two bruchid beetles, during metamorphosis. In doing so, the larval, pupal and adult alimentary canal and Malpighian tubules are described separately. Then their metamorphic changes have been described precisely. Descriptions have been elaborated in C. analis. Whereas the descriptions are very brief for C. chinensis, to avoid repeatation.

More emphasis has been given to the histological changes as the metamorphic processes differ much from one individual to the other and consequently there arise a lot of controversy. To overcome such confusion, the histological changes in the alimentary canal and Malpighian tubules have been precisely described, discussed and compared with other beetles, and a few insects of other orders.

Two species of a single genus were selected for the present investigation with a view to find out the following :

Firstly, if there exists any peculiar difference in the anatomy and histology of the alimentary canal and Malpighian tubules of these two closely allied species living in the same medium. And secondly, to obtain a comparative picture of the metamorphic changes in these two organ systems if any, among two closely allied bruchid beetles.

II. MATERIALS AND METHODS

Rearing

Adults of Callosobruchus analis and C. chinensis were collected from a ready stock culture maintained at 30.0°C and 70-75% R.H. in the Institute of Food and Radiation Biology (IFRB), AERE, Savar, Dhaka which was previously known as Radiation Biology Division at Dhaka and IPCORI at Tongi. A few adult beetles were collected from that stock culture and reared at room temperature (29.0°C $\pm$ 3.5°C) in the Department of Zoology, University of Dhaka for constant and ready supply of the various developmental stages of these insects.

In the departmental laboratory, the stock culture was maintained in petridishes or glass jars. Grains of Moong pulse (Phaseolus radiatus) were used as the media. They were disinfested in an incubator at a constant temperature of 70°C for about three hours. A little amount of the pulse was kept in a covered petridish or a glass jar, the mouth of which was covered with a fine piece of cloth. To start a colony, a few female adults were allowed to lay eggs on these seeds for about an hour. Then either the insects were separated from the medium or the egg-containing pulses were transferred to another similar container.

The egg-containing pulses were then examined from time to time. From each medium a few seeds were soaked

in water for about five minutes to make them tender and softer. Then the seed coat was removed, and the specimen was taken out of their grooves with the help of fine forceps and needles under a stereoscopic binocular microscope. Their developmental stages were determined by counting the number of exuvae present inside the kernels, by their morphological characters, and their relative body lengths, etc. Normally, four larval instars, prepupa, pupa, and adults were examined six-hourly.

Dissections

The dissections were made on a microscopic slide or a petridish under a stereoscopic binocular microscope. Dissections were performed in the following saline solution :

10% Sodium Chloride	= 9.0 ml
10% Aqueous Calcium chloride (anhydrous)	= 0.3 ml
Distilled water	= 90.0 ml

A few drops of saline solution was poured over it before dissection. During dissection, the required organ system was cleaned by washing away the unwanted tissues with the saline solution. A dark background was used which made it easier to see the desired organs of the insect during dissection. Then the dissected out organs were placed either on a grooved slide for anatomical study or transferred to a second container for histological preparation.

Histology

Either the dissected organs or their parts, or entire animal were fixed for embedding.

(i) As the dissected organs and their parts were too small and difficult to see, they were first stained with aquatic eosin for a couple of minutes before placing them in the fixative solution.

Several fixatives were tried but aqueous or alcoholic Bouin's fluid gave the best result. After fixation the tissues were dehydrated through grades of ethyl alcohol, passed through xylol and then embedded in paraffin wax (melting point 58°C).

(ii) When the entire larva or pupa was fixed, the body was perforated at several places with a sharp needle allowing the fixative to enter into the body. Alcoholic Bouin's fluid was used as the only fixative. The insect body was dehydrated through grades of ethyl alcohol. Embedding was through a modified Peterfi's Celloidin-Paraffin method (Pantin, 1960). However, Parlodion(R), a non-explosive cellulose nitrate (of Mallinckrodt Chemical works, New York & Montreal) was used instead of Celloidin.

A Cambridge Rocking Microtome was used for sectioning. Serial longitudinal and transverse sections

were cut at 4-6 μ m. Sections were stained with Heidenhein's or Delafield's haematoxylin. Usually, 2.5% iron-alum was used as a differentiator. Often, the tissues were counterstained with eosin. Finally, the sections were mounted in Canada balsam after dehydration through alcohol and xylol.

Drawings were made with the help of a Camera lucida or a "drawing prism". Measurements were taken with the help of a micrometer. Photomicrographs were taken from the photographic section of BANSDOC, Dhaka, and Bangladesh Diabetic Association, Dhaka.

III. ALIMENTARY CANAL

A. ANATOMY

III.A. 1(a). ANATOMY OF THE LARVAL GUT OF C. ANALIS

The alimentary canal of Callosobruchus analis is a very spacious organ which occupies a major part of the body cavity of the larva. It commences as a tube of variable diameter from the buccal cavity and ends at the anus. The anterior and the posterior portions of the alimentary canal are almost straight whereas the middle portion is coiled and convoluted in a twisted manner. As in all insects, the larval alimentary canal is clearly distinguishable into three primary divisions - the foregut or stomodaeum, the midgut or mesenteron, and the hindgut or proctodaeum. In gross appearance the divisions are easily recognised, the midgut being larger and wider in the middle, whereas the foregut and hindgut at the anterior and posterior ends are narrow and slender (Figs. 1a, 1b, 2, 3, & 4). Moreover, in the late larva the foregut-midgut junction is made distinct by the antero-lateral folds of the midgut wall which forms a slight bulge (Figs. 2, 3, & 4). The midgut-hindgut junction is made prominent by the origin of the Malpighian tubules.

The length of the alimentary canal and its proportion to the body length of the larva in various instars of C. analis varies considerably (Table 1). The larval alimentary canal is about two times longer than

the body length in the 1st instar, two and a half times in the 2nd instar, three times in the 3rd instar, and more than three and a half times in the 4th instar or full grown larva of C. analis.

Table 1: Measurements of the body and the gut lengths in different larval instars of C. analis.

Instars	Body (mm)	Gut (mm)	Proportion Body : Gut
Ist	0.85	1.82	1 : 2.14
2nd	1.15	3.05	1 : 2.64
3rd	1.45	4.50	1 : 3.11
4th	3.35	12.38	1 : 3.70

Little information is available regarding the alimentary canal of bruchid larvae. Vats (1976) gave an account of the alimentary canal in eight bruchid larvae including Callosobruchus maculatus (F.), C. analis (F.), Bruchidius saundersi (Jek.), B. albizzaiae Arora, Bruchus pisorum L., Zabrotes subfasciatus (Boh.), Caryedon serratus (Ol.) and C. languidus Gyll. Vats observed only the last instar larvae of the above bruchids. The alimentary canal of the 4th instar larva of the present material is quite similar to that of the last instar larva of C. analis.

described by Vats (1976). Description of the alimentary canal of another bruchid larva, Bruchus quadrimaculatus Fabr. was given by Mukerjii (1939). He described in detail the larval mouth parts which are similar to the mouth parts of C. analis larva. Mukerjii's (1939) description of the larval alimentary canal of B. quadrimaculatus is similar to the early instar larvae of C. analis. The larval alimentary canal of Tribolium castaneum (Herbst) (Tenebrionidae) was described by Ameen & Rahman (1973), of three species of Catopidae by Strambi (1976), of several scarabaeid larvae by Areekul (1957), of Phyllophaga anxia (LeConte) (Scarabaeidae) by Berberet & Helms (1972). Of those the alimentary canal of tenebrionid and catopid larvae is more or less similar to that observed for C. analis during this study except for a few minor differences. The alimentary canal of the scarabaeid larva described by Berberet & Helms (1972) do not agree with the larval structures in C. analis. On the contrary, the alimentary canal of their adults show similarity in their morphological appearance and disposition. However, the crypts formed by the midgut wall in the adult scarabaeid do not occur in C. analis larva.

Foregut

The foregut begins from the mouth which is situated at the base of the buccal cavity. The buccal cavity helps to engulf food materials in large quantity and then release them through the mouth into the pharynx. The mouth parts of C. analis larva is of a typical biting and chewing type which include a semi-circular and well muscularised labrum, a pair of large and well cuticularised mandibles with inward denticular processes, a pair of maxillae with various sensory bristles and hairs, and a labium with an attached sclerotized hypopharynx.

The foregut of the larva extends posteriorly as a short and slender uniform tube within the head region and joins with the midgut at the posterior end of the metathorax. It is introduced into the anterior tip of the swollen midgut like the stalk of an apple (Fig. 2).

The foregut of an early larva consists of two parts, the pharynx and the oesophagus (Figs. 1a, 1b, & 2). There is no morphological distinction between the pharynx and the oesophagus except the presence of dilator muscles in the former. But, the foregut of the full grown larva consists of the pharynx, oesophagus, and the proventriculus (Figs. 3 & 4). The proventriculus is the most dilated part of the foregut.

The length of the foregut with its divisions in relation to the body length of the larva in various instars of C. analis is shown in Table 2.

Table 2. Measurements of the body and foregut lengths in different instars of C. analis.

Instars	Body (mm)	Foregut (mm)		Total
		Pharynx	Oesophagus + proventriculus	
Ist	0.85	0.06	0.07	0.13
2nd	1.15	0.08	0.09	0.17
3rd	1.45	0.18	0.25	0.43
4th	3.35	0.43	0.57	1.00

Pharynx

Pharynx is the anterior part of the foregut situated within the head capsule. It extends from near the frontal ganglion as a narrow tube passing through the cerebral nerve ring. It ends just behind the sub-oesophageal ganglion. The pharynx is always provided with a few bands of dilator muscles. The arrangement of the dilator muscles observed in the larval pharynx include a tuft of antero-dorsal dilator muscles inserted on the dorsal surface of the pharynx near the frontal ganglion, two ventro-lateral sets of dilator muscles lying on the two ventrolateral sides of the pharynx, and a tuft of postero-dorsal dilator muscles inserted on the dorsal part of the pharynx just behind the cerebral ring (Fig. 4). All these dilator

muscles of the pharynx originate from the head capsule. A few prominent dilator muscles were also observed on the dorsal and lateral surface of the buccal cavity. But the exact nature and position of the dilator muscles on the buccal cavity were not determined. However, these should be regarded as the buccal and cibarial dilator muscles. The arrangement of the dilator muscles on the pharynx of C. analis larva is similar in many respects to the larval pharynx of Coccinella septempunctata Linn. (Coccinellidae) described by Pradhan (1939).

According to Snodgrass (1935), pharynx is the part of stomodaeum from which dorsal dilator muscles make their insertion. He further pointed out that the pre-cerebral and post-cerebral pharyngeal regions are distinguished as the "anterior pharynx" and "posterior pharynx" respectively. Ameen (1969) in Ptychoptera albimana F. (Ptychopteridae : Diptera) and Khattar (1972) in Schizodactylus monstrosus (Drury) (Schizodactylidae: Orthoptera) also found similar differentiation of the pharyngeal region. The same condition was also observed in the foregut of C. analis larva. The pre-oral portion of the larva of C. analis is represented by a very simple cibarial part, a food pocket which is an extrabuccal structure situated between the base of the hypopharynx and the clypeus.

Oesophagus and proventriculus

The oesophagus is morphologically similar to the pharynx except that it has no dilator muscles. It continues from behind the sub-oesophageal ganglion as a straight narrow tube upto the apple-headed anterior end of the midgut. it

The oesophagus is almost uniform in diameter throughout its length in the 1st, 2nd and 3rd larval instars. But it gradually becomes conical or bulbous towards the posterior end during the 4th (last) instar. This dilated portion of the oesophagus near the midgut end is a simple proventriculus. In fresh dissections this proventricular portion showed rapid and continuous contractile movements, more distinct than the other parts of the alimentary canal. This type of rhythmic movement probably exert a squeezing pressure over the epithelial folds along with the intimal articulation. This helps in grinding and triturating the food materials entering into the region. Thus, the food materials are broken down into finer form which then gradually pass through the oesophageal invagination into the anterior midgut. h

It was also observed that the last instar (4th instar) larva becomes a vigorously voracious eater before pupal preparation. As a result, the larva readily takes a large amount of food materials at that time which are passed towards the posterior part of the foregut. The

continuous rapid transport of the food materials through the pharynx and anterior side of the oesophagus is slowed down by the oesophageal invagination. This may cause the temporary blockage at the hindermost part of the foregut. As a consequence, a large amount of food remain temporarily blocked in the area and as a result the adjacent oesophageal wall becomes dilated. Concomitantly the muscles and intimal articulations in the area also become more developed. All these lead to the formation of a simple proventriculus in the last larval instar of C. analis (Figs. 3 & 4).

Oesophageal or proventricular invagination

The oesophageal invagination is a part of the foregut directly introduced into the midgut. In most insects it strictly ~~restries~~ regurgitation of food materials from the midgut to the foregut. The invaginated oesophageal part has been described by many recent workers under the name of "cardiac valve" (Bahadur & Kathuria, 1971, Kathuria, 1972, Berberet & Helms, 1972, and many others); "Valvule stomodiale" - (Strambi, 1976), and "Valvula esofagiana" - (Goncalves, 1979); and "oesophageal invagination" - (Ameen, 1969; Ameen & Rahman, 1973; Ameen & Imam, 1976, and many others). In the present investigation the term "oesophageal invagination" and "proventricular invagination" have been used in the early instars and late instars respectively.

The oesophageal invagination is not clearly distinguishable morphologically in C. analis larva. The anteriormost part of the midgut which is slightly wider than the rest of the midgut can be roughly considered as the oesophageal invagination.

Midgut

The midgut of C. analis larva is a large capacious part which is very prominent in comparison to the other two regions of the alimentary canal. It is divided into two distinct regions, the anterior and the posterior midgut. The anterior midgut is more prominent, i.e., broader and longer than the posterior midgut. This regional differentiation of the midgut continue into the adult stage. Similar regional differentiation of the midgut has been described by Pradhan (1936) and Ameen (1970) in Epilachna indica and E. vigintioctopunctata respectively. They described the anterior midgut as "straight proximal lobe" and the posterior midgut as "narrow coiled distal loop". These above terms cannot be applied to C. analis since the anatomical disposition of the midgut is not similar and identical in all the stages of development. Potts (1927) also observed such regional differentiation of the larval midgut in the mexican beam beetle, Epilachna corrupta; such regional differentiation was not observed in the tenebrionid beetle,

T. confusum (Sinha, 1958a) and T. castaneum (Ameen & Rahman, 1973). Regional differentiation of the midgut has been observed in many beetles, e.g. by Woods (1918) in Altica bimarginata Say, Potts (1927) in Epilachna corrupta, Swingle (1930) in Popilla, Bigham (1931) in Asaphes memnonius Herbst., Schaefer (1931) in Sphaeroderus nitidicollis, Burgess (1932) in Epilachna corrupta Muls. Sundman & King (1964) in Anthonomus grandis, Shukla & Kumar (1969) in Odoiporus longicollis, Shukla & Upadhyay (1980) in Catharsius, and many others. Shukla & Upadhyay (1980) used the term "anterior part or pre-midgut" and "posterior part or post-midgut". According to Vats (1976), the midgut measures more than 8 mm in the mature larva of C. analis, which confirmed the present finding, shown in the Table 3.

Table 3. Measurements of the body and the midgut lengths in different instars of C. analis.

Instars	Body (mm)	Midgut (mm)			Proportion	
		Anterior	Posterior	Total	Ant.Mg. : Post.Mg.	
1st	0.85	0.58	0.42	1.00	1	: 1.38
2nd	1.15	1.38	0.57	1.95	1	: 2.42
3rd	1.45	2.18	0.76	2.94	1	: 2.87
4th	3.35	6.84	1.86	8.70	1	:: 3.68

Anterior midgut

The anterior midgut in the early larval condition (1st and 2nd instars) is a saccular tube which apparently looks like a simple slightly tortuous tube (Fig. 1b). Its anterior and posterior ends are narrower whereas the mid-dorsal part is dilated irregularly to form a dorsal convexity when the ventral surface remains more or less flat or slightly curved along with the ventral body wall. In the 3rd instar, the anterior midgut becomes a broad tube, slightly broader towards the anterior end and gradually narrowing towards the posterior end (Fig. 2). In this condition, there is a bend at the anterior end of the midgut. In the full grown larva (late 4th instar), the anterior midgut appears as a broad tube almost uniform in diameter throughout its whole length. In this condition the "anterior bend" portion of the 3rd instar larva becomes straightend to form a tube of uniform diameter (Figs. 3 & 4).

In all the above cases, the anterior midgut touches the mid-dorsal roof of the abdominal cavity.

Posterior midgut

The posterior midgut is a tubular part which is shorter and narrower than the anterior midgut. It appears almost like a posterior tail-like prolongation of the anterior midgut. It is about one-third in diameter than the anterior midgut. It is coiled round the posterior end of the anterior midgut in the form of a single loop (Figs. 2 & 3).

In the young larva (1st instar), the posterior midgut loop is not prominent. It is just a bend or curved portion along with the anterior saccular midgut (Figs. 1a & 1b). In the 2nd and 3rd instar, the posterior midgut coils round the broader anterior midgut in the manner of a single coil of watch spring (Fig. 2). In full grown larva, the coiling of the posterior midgut is loosely placed on the posterior end of the anterior midgut (Fig. 3). The proportion in length between the anterior and the posterior midgut varies increasingly with the development of the larva which is least in the 1st instar and highest in the last instar larva, as shown in the table 3.

Hindgut

The hindgut of C. analis larva is a long tube which is convoluted in the form of a twisted "S" at the ileo-colon region. The rest of the hindgut including the hind portion of the colon and rectum together is more or less straight and spindle-shaped i.e. broader in the middle and narrower towards the two ends. The dilation of the colon is due to the external embracement or association of the distal parts of the Malpighian tubules in a convoluted manner. The part of the Malpighian tubules associated with the colon is generally known as "cryptonephridia" and thus

the system involved is known as the "cryptonephridial system". The cryptonephridial system is an important aspect of coleopteran hindgut.

The hindgut of the larva is clearly divisible into the pylorus, the ileo-colon and the rectum. The length of the hindgut with its divisions in relation to the body length of the larva in various instars is shown below.

Table 4. Measurements of the body and the hindgut lengths in different instars of C. analis.

Instars	Body (mm)	Hindgut (mm)				
		Pylorus	Ileum	Colon	Rectum	Total
Ist	0.85	0.10	0.17	0.25	0.17	0.69
2nd	1.15	0.12	0.23	0.35	0.35	0.93
3rd	1.45	0.13	0.34	0.42	0.24	1.13
4th	3.35	0.29	0.85	1.00	0.54	2.68

Pylorus

The pylorus is a short knob-like or funnel-shaped structure of the hindgut at the transition of the midgut and hindgut. Its broad end remains attached to the posterior midgut whereas the narrow end remains with the ileum. From the anterior tip of the pylorus a whorl of

six free Malpighian tubules originate. Two very short Malpighian tubules directly run towards the posterior side to meet with the colon. Due to the direct contact of these two Malpighian tubules between the pylorus and the colon, the intermediate portion of the ileo-colon remain in a single looped condition. The other four Malpighian tubules extend forward along the midgut in the body cavity then reflex on themselves and extend posteriorly upto the colon region.

The pylorus of C. analis larva is similar to the pylorus of T. castaneum larva (Ameen & Rahman, 1973). Ampular attachment of the Malpighian tubules with the pylorus has been observed by Haridas & Anathakrishnan (1981) in sixteen reduviids. The present investigation reveals that there is no such ampular arrangement of the Malpighian tubules with the pylorus. Each of the six Malpighian tubules originates independently from the apex of the pylorus. The basal part of the pylorus is provided with musculature which may act as a sphincter of the pylorus. Probably, strong musculature of the pyloric region helps in regulating the entrance as well as movement of the gut content including the fecal matters or excretory waste products in their respective pathway towards the exterior. Reinecke et al. (1973, 1977 & 1978) worked on the musculature of the hindgut of lepidopteran larvae. Later on they proposed

a hypothesis, according to which, "Muscles of the pylorus contract rhythmically to press small quantities of ingesta and Malpighian waste into the ileum".

It was observed in fresh dissections that the alimentary canal of the larva is in constant rhythmic contractile movement which has been referred to as "peristaltic movement" of the alimentary canal in T. castaneum by Ameen & Rahman (1973). Due to this activity of the alimentary canal food materials gradually pass towards the rectum via ileo-colon. This type of contractile movement was found to continue even more than half an hour after dissection. Probably, due to this activity, the hindgut was found irregularly dilated in the fixed specimen.

Ileo-colon

There is no clearcut external demarcation between the ileum and the colon of the larva. The ileum begins from the narrow end of the pylorus, extends backwards parallel to the posterior midgut, then turns towards the posterior side of abdominal cavity, thus making an angle between its two arms. The first portion of the ileo-colon near the pyloric region is the "ascending portion" or simply the ileal part. The second arm which extends backwardly is the colon portion and may be called as the "descending portion". The general disposition of the

ileo-colon region of young C. analis larva is very similar to be larval ileo-colon of Apis mellifera L. (Dobrovsky, 1951). they

The ileal portion is slightly narrower than the colon part. The ileum is uniform in diameter throughout its whole length. The colon becomes gradually dilated towards the posterior end due to the insertion, innervation and accommodation of the cryptonephridia on the surface of the colon wall. The insertion of the Malpighian tubules to the hindgut wall marks the beginning of the cryptonephridial system.

In the mature larva, there is no distinct ascending and descending arms of the ileo-colon. In this condition, the ileo-colon region twist in a manner to form a "S"-shaped structure.

Rectum

The rectum is a very short tubular structure. It becomes narrower gradually towards the anus. The anterior broader end of the rectum remain adherent to the broad end of the cryptonephridial part of the colon. A simple, almost invisible constriction represents the adjoining line of union of the colon and rectum. When viewed carefully with a compound microscope, the demarcating line of the two portions, the surface view of the convoluted cryptonephridia on the colon and the muscular striations on the wall of the rectum is slightly visible, if it is stained perfectly.

III.A.1 (b). ANATOMY OF THE PUPAL GUT OF C. analis

The alimentary canal of the pupa of C. analis (Figs. 7 & 8) is a long and **almost** straight tube of variable diameter except the ileo-colon region where it is slightly bent upon itself. It is simpler than those of the larval and adult alimentary canals. It closely resembles the adult alimentary canal in its general morphology and anatomical disposition.

The most striking feature of the pupal alimentary canal is the cylindrical form of the midgut. The surface of the midgut is smooth and the gut wall is thin and more or less transparent, which is due to the thinness of the metamorphosing epithelium. In this case, a large amount of "yellow bodies" which accumulated in the midgut lumen is indistinctly visible through the gut wall.

Of the three principal divisions of the alimentary canal, the foregut is the shortest and the midgut is the longest part. In one case, the pupal alimentary canal was found to be 5.25 mm in length when the body of the pupa was 3.5 mm long, i.e. the pupal gut is one and a half times longer than the body length. In this specimen, the foregut measured 0.6 mm, midgut 2.65 mm, and the hindgut 2.0 mm in length. Thus, the foregut covered about 11.5%, midgut 50.5%, and the hindgut 38.0% of the total length of the pupal alimentary canal.

Foregut

The foregut is a tube of almost uniform diameter except the posterior end which is slightly dilated. Anteriorly it is bent downwards according to the facial disposition of the head region. It consists of three regions, viz., the pharynx, oesophagus and the proventriculus. The proventriculus of the pupa is simpler than that of the larva, i.e., the normal shape and size has been changed in the pupal stage due to the metamorphic changes in this region described later.

The foregut measures 0.6 mm long, where the pharynx, oesophagus and the proventriculus measure 0.2 mm, 0.3 mm, and 0.1 mm respectively. In this case, the diameter of the pharynx and the oesophagus varies between 0.05 mm and 0.08 mm; whereas in the proventriculus it varies between 0.08 and 0.18 mm.

Pharynx

The pharynx is a narrow and short tube which extends towards the anterior side upto the buccal region. It continues from near the frontal ganglion, passes through the cerebral ring upto a little behind the sub-oesophageal ganglion. Anteriorly, the pharynx along with the buccal region is bent downwards, whereas posteriorly it is straight and continuous with the

oesophagus. The pharynx is provided externally with a few sets of dilator muscles, as was observed in the larval pharynx.

Oesophagus

The oesophagus is a straight and uniform tube behind the pharynx. The posterior end of the oesophagus is continuous with the narrow neck of the proventriculus. There is no external musculature like the dilator muscles of the pharynx in the oesophagus.

Proventriculus

In gross appearance, the proventriculus of the pupa is not a prominent structure as in the full grown larva or the adult. In this case, it is just a broad end of the oesophagus which is a continuous and gradual dilation of the posterior end of the foregut. It resembles neither a bulbous structure as in the larva nor it is flask-shaped as in the adult C. analis. It appears somewhat squeezed compared to the larval proventriculus. The squeezed type of simple proventriculus in the pupa may be due to the remodelling of the inner intimal part and the muscular re-arrangement during metamorphic changes. The anterior end of the proventriculus is narrower and continuous with the oesophagus whereas the posterior end is broader and introduced into the anterior end of the midgut. There is

no clear external demarcation between the proventriculus and the midgut except that the midgut end is more dilated than the proventriculus.

There is no morphological differentiation of the proventricular invagination, though it plays an important role during metamorphosis. However, structure of the proventricular invagination is quite distinct and illustrative in the histological preparations of the present material.

Midgut

The midgut of the pupa is almost a straight tube. It is cylindrical, thin-walled and more or less glassy and transparent in appearance. It is clearly distinguishable into an anterior midgut and a posterior midgut as in the larva and the adult. The two regions of the midgut is easily separated from each other by their relative width. The anterior midgut is about two times longer and two to three times wider than the posterior midgut. The pupal midgut closely resembles the adult midgut except that there are no "crypsts" or "mesenteric diverticula" on the surface of the pupal midgut as found in the adult condition.

The midgut forms about 50% of the total length of the pupal alimentary canal. The midgut measured 2.65 mm in length when the anterior and the posterior midguts

were 1.75 mm and 0.9 mm long. In this case, the diameter of the anterior midgut varied between 1.5 mm and 3.0 mm, and the posterior midgut 1.0 mm and 1.5 mm.

Anterior midgut

The wider part of the midgut, i.e., the anterior midgut is also the broadest part of the alimentary canal. Its middle part is slightly broader than the two ends. The external surface of the gut is smooth and plain. The gut wall is very thin, appears like a thin transparent outer limiting membrane. The lumen is filled up with histolysed epithelial contents in the form of "yellow bodies". These can be clearly seen through the gut wall if stained.

Posterior midgut

The posterior midgut is a tubular tail-like continuation of the anterior midgut. It is a straight, narrow and almost uniformly dilated part which includes the posterior one-third of the midgut. Its surface is smooth and the wall is thin and almost transparent like the anterior midgut. Similar to the anterior midgut, the "yellow bodies" formed in this region are also easily recognizable inside the gut lumen. Posteriorly it is continuous with the pylorus of the hindgut which is more dilated than the posterior midgut. Again, the posterior

midgut is marked off from the hindgut by the origin of the six Malpighian tubules.

Hindgut

The hindgut of the pupa is a long tube of almost uniform diameter except the posterior part of the colon which is slightly dilated due to the accommodation of the distal parts of the Malpighian tubules which form the cryptonephridial system. This system which was previously found in the larval colon still persists and continue to persist around the adult colon. The ileum with a little portion of the colon region is bent upon itself in a looped manner. On the otherhand, the cryptonephridial part of the colon along with the rectum is straight which ends into the anus.

The hindgut consists of the same regions as in the larva, viz., the pylorus, ileum, colon and the rectum. It is 2.0 mm long, of which the pylorus, ileum, colon and the rectum are 0.1 mm, 0.6 mm, 0.8 mm, and 0.5 mm respectively. The diameter of these successive regions varies from 0.08-0.1 mm, 0.05-0.08 mm, 0.08-0.15 mm, and 0.05-0.09 mm, respectively.

Pylorus

The pylorus of the pupal alimentary canal is a knob-like structure situated at the anterior end of the hindgut. Its anterior end is broader than the posterior end.

From the top of the pylorus, just surrounding the base of the posterior midgut, six Malpighian tubules originate freely and regularly. The posterior narrow end of the pylorus continues with the ileum. Certain sphincter muscles associated with the basal sides of the pylorus are indistinctly visible in well stained materials.

Ileum

The ileum is a coiled part of the pupal alimentary canal. It bends upon itself twice in a looped manner forming a convoluted part. The convolutions of the pupal ileum is less prominent than those of the adult ileum, although both are more or less similar to each other. The width of the ileum is apparently similar to that of the posterior midgut. Due to this minor difference in diameter between the two regions, both of them, i.e., the posterior midgut and the ileum are often confused in the freshly dissected materials. But the presence of Malpighian tubules at the end of the pylorus distinguishes the posterior midgut from the pylorus. Besides these, two very short Malpighian tubules which join directly with the cryptonephridial chamber, run along sides the ileum.

Colon

The colon is almost a straight tube which becomes gradually dilated towards the posterior end. The dilation

of the colon region is due to the association of the Malpighian tubules around the colon wall in a separate chamber, as observed in the larva and adult. The cryptonephridial system of the larva is continued into the adult insect through the pupal stage. The pupal cryptonephridial system helps in redifferentiating the cryptonephridia from larva to the adult form. However, the reconstructive process of the cryptonephridial system during the pupal stage has been observed clearly in the histological preparations of the present material.

Rectum

The rectum is the short terminal part of the hindgut which begins from the posterior broad end of the colon. It becomes slightly narrow towards its extreme posterior end and ultimately ends in the anus. There is a slight constriction between the colon and the rectum which demarcates the two adjacent regions(Figs. 7 & 8).

III.A. 1(c). ANATOMY OF THE ADULT GUT OF C. ANALIS

The alimentary canal of the adult C. analis (Figs. 9, 10 & 11) is dissimilar to that of the larva in many respects. It resembles those of other beetles, viz., Criocer~~us~~us asparagi Linn. (Davidson, 1931), Sphaeroderus nitidicollis (Schaefer, 1931), Bolitotherus cornutus (Auten, 1933), Penthe pimelia Fabr. (Brubaker, 1934), Anthonomus grandis Boheman (Sundman & King, 1964), and Lytta nuttalli Say (Morgan et al. 1970), and many others. In gross appearance, it is almost a straight tube of variable diameter except that its ileo-colon region is slightly bent downwards in front and the ileo-colon region is looped upon itself in the form of a twisted "S". The alimentary canal is clearly distinguishable into the three major primary divisions. The foregut is the shortest part which is anteriorly bent downwards. The hindgut is the longest part, marked off from the midgut by the origin of the Malpighian tubules. The midgut is divisible into two distinct regions, as in the larval midgut, but quite dissimilar in appearance. The anterior midgut is about two times longer and two to three times wider than the posterior midgut. The whole surface of the midgut is provided with numerous minute finger-like projections, known as "mesenteric diverticula" or "crypts", which are absent in the larval midgut.

The alimentary canal is about one and a half times longer than the body length of the adult insect. For example, in one case, the alimentary canal measured 4.9 mm in length when the adult insect was 3.25 mm long. Thus the proportion between the body length and the gut length is about 1 : 1.5. Similar relative lengths were also observed in the beetles, Phyllophaga gracilis Brum (Fletcher, 1930), Sphaeroderus nitidicollis Chev. (Schaefer, 1931), Penthe pimelia Fabr. (Brubaker, 1934), and Pheropsophus occipitalis (Shukla & Upadhyay, 1980).

In many beetles the gut length has been reported to be longer than the body length. Thus, the gut was reported to be one and a one-fourth times longer than the body length in Crioceris asparagi Linn. (Davidson, 1931) and Coccinella septempunctata (Pradhan, 1938); almost double the body length in Popilla (Swingle, 1930), Aulacophora foveicollis (Lucas) (Saksena, 1951), Odoiporus longicollis (Shukla & Kumar, 1969), Prionus laticollis (Benham, 1970); three times longer than body length in Passalus cornutus (Lewis, 1926), Epilachna corrupta (Potts, 1927), Bolitotherus cornutus (Auten, 1933), and Epilachna indica (Pradhan, 1936). In some cases, the gut length has been reported to be four times longer than the body length, e.g., in Dactylosternum hydrophiliodes (Macleay) (Shukla & Upadhyay, 1976), and even eight to ten times, e.g., Phanaeus vindex MacL. (Becton, 1930), Onthophagus

Catta Fabr. and Aphodius moestus Fabr. (Verma, 1969),
Canthon laevis (Miller, 1961).

Foregut

The foregut begins from the mouth which is situated at the base of the buccal cavity. It extends backward through the head capsule upto the thorax as a narrow tube of almost uniform diameter, the end part of which dilates to meet with the midgut. In gross appearance, the foregut is bent and narrow towards its anterior end, straight and uniform at the middle and flask-shaped towards the posterior end. It is clearly distinguishable into a pharynx, oesophagus and a proventriculus (Fig. 10).

The foregut is the shortest part of the alimentary canal which forms only about 12.5% of the total gut length. For example, in one case, the foregut measured 0.61 mm when the alimentary canal was 4.9 mm long. In this case, the pharynx was 0.20 mm, oesophagus 0.25 mm, and proventriculus 0.16 mm in length; whereas the diameter of the pharynx and oesophagus varied between 0.05 to 0.08 mm, and that of the proventriculus between 0.08 to 1.5 mm.

Pharynx

Pharynx is the anteriormost part of the alimentary canal, which is anteriorly bent downwards due to the facial disposition of the head (Fig. 9). It is a tube of

almost uniform diameter, situated within the head capsule in between the brain and the sub-oesophageal ganglion. It passes from below the frontal ganglion and extends through the cerebral ring upto a little behind the sub-oesophageal ganglion. It is well provided with a few sets of dilator muscles characteristics of this region. Four sets of pharyngeal dilator muscles can be seen in the adult which are very similar to those in the larva. The pharyngeal dilator muscles are antero-dorsal, postero-dorsal and ventro-lateral in position (Fig. 10). These dilator muscles remain confined within the head capsule. Disposition of the stout dilator muscles in the buccal region and its surrounding were also observed. But their exact ramifications were not determined. These dilator muscles in the buccal and pre-oral regions may be regarded as the "dilatores buccales" and "dilatores cibarii", described by Snodgrass (1935).

Oesophagus

The oesophagus is the straight middle part of the foregut. It is narrow and tubular and almost uniform in diameter as in the pharynx. But it lacks any external dilator muscles as found in the pharynx. The posterior end of the oesophagus is connected with the neck of the proventriculus. There is no clear external demarcating line or constriction mark between the pharynx and the

oesophagus, and between the oesophagus and the proventriculus. However, the pharyngeal region can be recognized by the presence of the dilator muscles. The posterior dilated part marks the proventriculus of the foregut. The part lying between the two is the oesophagus.

Proventriculus

The most prominent part of the foregut of the adult is the proventriculus. It is a flask-or bulb-shaped structure, the narrow end or the neck region is continuous with the oesophagus and the posterior broad end is introduced into the anterior end of the midgut as a proventricular invagination. But from external appearance it seems that the broad end of the bulb is fixed on the midgut end. The exact contact of the proventriculus with the midgut is clearly understood only from its histological study.

Presence of proventriculus in the alimentary canal in the beetles and its various functions have been described by many previous workers, which are similar to the proventriculus of the adult C. analis in many respects. Balfour-Browne (1934) in Dytiscidae, Dennell (1942) in Calandra granaria L., Gupta (1965) in Meloidae, Shukla & Awasthi (1969) in Mylabris pustulata Thumb., Shukla & Kumar (1969) in Odoiporus longicollis, Strambi (1970) in Catopidae, Singh & Mukharji (1971) in Tribolium

castaneum, Mukharji & Singh (1971) in Sitophilus oryzae Linn., Shukla & Upadhyay (1980) in Pheropsophus occipitalis (MacL.), Macgown & Sikopowski (1981) in Anthonomus grandis grandis Boheman are some of the examples.

There is no clear external differentiation of the adult proventricular invagination like that of the pupa, though it bears certain special internal structures.

Midgut

The midgut is a prominent part of the adult alimentary canal. Like that of the larva, the midgut of the adult is also divisible into two distinct regions, the anterior and the posterior midgut. Morphologically, the whole midgut is quite dissimilar to the midgut of the larval stages. The entire surface of the midgut is provided with numerous evaginations of the gut wall, known as the "mesenteric diverticula" or the "crypts". They are very short and blunt. They appear like densely arranged minute finger-like projections, almost similar in structural appearance and more or less uniform in distribution over the whole surface of the midgut (Figs. 9 & 10). Ameen & Rahman (1973) in Tribolium castaneum (Tenebrionidae) described similar crypts which are uniformly distributed all over the midgut surface but they become gradually longer from the anterior to the posterior end of the midgut. Strambi

(1970) described the crypts in Troglodromus bucheti gaveti (Catopidae) which are more elongated and almost finger-like projections, but their distribution is similar to the present material. Similar crypts have been observed by Shukla & Upadhyay (1980) in Catharsius molossus (Scarabaeidae); but there the crypts are more dense anteriorly than in the posterior region.

Anterior midgut

The anterior midgut of the adult is a irregularly dilated saccular part. It is about two times longer and two to three times wider than the posterior midgut. In a freshly emerged adult, the anterior midgut appears like an elongated sac with a few regular and alternate dilations and constrictions (Fig. 10). This is probably due to the "peristaltic movement" of the gut wall during digestion of food materials in that region.

The dilation and constriction of the anterior midgut is more or less regular and feeble in the freshly emerged adults whereas the dilations are irregular and the constrictions deeper in the older adults. From this observation it may be presumed that the midguts of older larvae do not take active part in digestion any more. Whereas the newly hatched adults which live still within its pabulum of pulse seeds and whose gut contains a considerable amount of ingested food remains active in

its digestive function, which decreases gradually and ultimately stops as no new food is ingested by the older adults.

Posterior midgut

The posterior midgut is a short, narrow and almost uniform tube. It is slightly bent upon the posterior end of the anterior midgut. Its broader end remain united with the anterior midgut and the narrower end remain attached with the anterior end of the hindgut.

The midgut includes about 40.5% of the total length of the alimentary canal in the adult insect, of whose about two-third constitutes the anterior midgut and one-third the posterior midgut. As for example, one midgut measured 2.0 mm in length when the total gut length was 4.9 mm. In this case, the anterior and the posterior midgut measured 1.3 mm and 0.7 mm respectively. In diameter, the anterior midgut varied from 0.2 to 0.5 mm whereas the posterior midgut varied between 0.1 and 0.2 mm.

Hindgut

The hindgut is the longest part of the adult alimentary canal (Fig. 11); it forms 45% to 47% of the total gut length. It is a tube of variable diameter of which the anterior half is uniform in diameter, convoluted,

i.e., coiled around itself on the left side of the main axis of the gut in the form of a twisted "S", whereas the posterior half is more or less straight and gradually broaden towards the posterior side terminating in a narrow prolongation which ends in the anus (Fig. 9). The hindgut consists of four regions, viz., the pylorus, ileum, colon and the rectum. The colon is characterised by the presence of cryptonephridial system which is an enveloped condition of the Malpighian tubules around the colon wall. The association of the Malpighian tubules in this manner was also observed in the larval stage of this insect.

The hindgut of the adult measured 2.3 mm in length when the total gut length was 4.9 mm. In this case, the pylorus, ileum, colon and the rectum were 0.1 mm, 0.8 mm, 0.9 mm, and 0.5 mm, respectively.

Pylorus

The pylorus is a short funnel-shaped structure situated at the anterior end of the hindgut (Figs. 10 & 11). The narrower end of the pylorus is continuous with the ileum whereas the broader end remain attached with the posterior midgut. From the broad end of the pylorus i.e. from the meeting point of the midgut and the hindgut, a whorl of six Malpighian tubules originate independently, which then extend forward in the abdominal cavity. The pylorus is provided with strong musculature which may act as a sphincter.

Ileum

The ileum begins from behind the pylorus, extends as a narrow and convoluted tube of almost uniform diameter. The posterior end bends and meet the colon. At first the ileum continues backwards as a straight tube, then it makes a loop and then extends in a forwardly direction along with its anterior straight part upto the junction of the midgut and hindgut where it again bends to make another loop and joins the colon. In this condition, the looped ileo-colon appears like a twisted "S" (Figs. 9 & 11). The middle point of the second loop is the beginning of the colon, which can be marked off from the ileum by a feebly developed constriction in between the two adjacent regions of the hindgut (Fig. 9). The convoluted part of the hindgut, i.e., the entire looped section of the ileum and a very short anterior portion of the colon remain together towards the left of the long axis of the hindgut.

Colon

The colon is a more or less straight and dilated part of the hindgut. It begins from the last loop of the ileum as a slightly bent end, continues towards the posterior extremity dilating gradually. It appears like a inverted fusiform structure (Figs. 10 & 11). The narrow end of this portion is connected with the ileum whereas the broad end joins the conical rectum. The dilation of

the colon is due to the association of the distal parts of the Malpighian tubules which form the cryptonephridial system, as in the larva. In this case six Malpighian tubules originating from the pylorus travel freely and independently in the body cavity. Then their terminal ends come to lie outside the colon wall and become invested by a membrane, thereby forming the cryptonephridia. The binding membrane forms a separate chamber encircling the colon, which is called the "fascial chamber" or "cryptonephridial chamber". The distal tips of the Malpighian tubules lie within this chamber and are known as the "cryptonephridia ". The cryptonephridia within the cryptonephridial chamber remain in a regular convoluted manner which are more prominent in the broader part of the cryptonephridial chamber (Fig. 9). This arrangement and disposition can be seen easily in stained dissections under a compound microscope, provided the staining is good. It should, however, be noted that the cryptonephridial chamber does not encircle the entire colon, it starts from a little behind the bent portion separating the colon from the ileum.

Rectum

The rectum is an elongated more or less conical structure; the broad end of which remains with the posterior end of the cryptonephridial part of the colon (Figs. 9, 10

& 11). It gradually narrows down towards the exterior to end into the anus. There is a slight constriction mark between the cryptonephridial part and the broad end of the rectum which marks the junction of the colon and the rectum. The presence of the transverse striations of the rectal sphincter muscles on the rectal wall, is also roughly visible when observed carefully.

III. A. 1 (d) METAMORPHIC CHANGES (ANATOMICAL) IN THE
ALIMENTARY CANAL OF C. ANALIS

Post-embryonic, i.e., metamorphic changes of the alimentary canal of C. analis takes place throughout the larval and pupal stages and continues even during the newly attained adult condition of the insect. The insect has four larval instars and, therefore it moults thrice during its larval life and changes to the pupal stage after the 4th moult. Finally it moults for the 5th time to become the adult. Of these moults, the first three may be regarded as "growth moults", because they are mainly concerned with the growth of the organs, which remain more or less similar before and after the moults. But during the last two moults drastic changes occur in their structure. Hence, they may be called "transformation moults". These anatomical changes are described below in three stages, viz.,

- a) **changes during larva to larva moults,**
- b) **changes during larva to pupa moult, and**
- c) **changes during pupa to adult moult.**

a) Changes during larva to larva moults

The 1st instar larva of C. analis after hatching out of the egg actively bores into the cotyledon of the pulse where it eats away the cotyledon and makes room

for its growth. In this stage it does not feed voraciously. It is very small in size (less than 0.5 mm) and very difficult to dissect. However, in cleared specimen the alimentary canal could be seen easily. In this stage the alimentary canal is rather simple with a short narrow foregut, a straight dilated midgut, and a narrow, slightly coiled hindgut (Fig. 1a).

The foregut remains as a simple, very short and uniform tube upto the 2nd moult (Fig. 1b & 2). After that, a simple pear-shaped dilation appears at the posterior end of the foregut. This dilation gradually becomes prominent in the subsequent larval instars and transforms into the proventriculus of the larva as described for the larval alimentary canal (Figs. 3 & 4).

In the 2nd larval instar the midgut gradually becomes asymmetrically dilated as described before (Fig. 1b). In the subsequent larval instars, i.e. 3rd and 4th instars, the anterior midgut gradually elongates becoming symmetrical and tubular with an antero-lateral bulge. Coiling of the posterior midgut starts in the 2nd instar which gradually becomes more and more prominent in the succeeding instars.

Appreciable change has not been observed in the hindgut during larval life, except that it elongates proportionately and convolutes on itself with the development of the larva.

b) Changes during larva to pupa moult

Drastic and remarkable changes are observed during transformation of the larva to the pupal stage. At the end of the larval life, just before formation of the pupa, the full grown larva suddenly stops feeding. This is the time of evacuation of the gut contents. In this condition, the larva becomes narrower anteriorly, straightens itself, and the rudiments of wings and other structures become prominent. Mean while, the very long and capacious alimentary canal of the larva changes its shape and structure to become the pupal alimentary canal through the last larval moult. This advancement or the preparation for the pupal gut formation is the "prepupal alimentary canal". This is quite dissimilar to the larval as well as the pupal alimentary canal; hence, it is described separately.

Alimentary canal of the prepupa

During prepupal condition, the foregut does not change much, except that it reduces slightly in length and width.

Radical changes, however, are observed in the midgut during prepupal condition. During this period, the midgut shortens considerably in length, which is accompanied by the shrinking and folding of the midgut wall. Due to this the anterior midgut becomes very short, less than half its

length in the active full grown larval stage. Close observation reveals that the folding of the anterior midgut is not uniform throughout its length (Fig. 6).

The folding of the wall is found to be more compact where the midgut was previously (i.e. during active period of 4th instar larva) slightly wider and the internal epithelial secretions were profuse. The distribution of the folds are less compact where the gut wall was previously less distensible or was constricted and the internal epithelial secretion was less. Thus, the dilated part of the midgut with profuse epithelial secretion made the gut wall thinner, which ultimately shrank during prepupal condition. This probably occurred during the onset of prepupal condition due to the peristaltic movement of the alimentary canal.

Shortening in length of the posterior midgut during prepupal condition is not due to folding of the gut wall as in the anterior midgut. A few superficial transverse striations appear due to the formation of fine transverse ridges and grooves of the gut wall. There is no distinct shrinking areas to be seen as in the anterior midgut. The surface of the posterior midgut appears more or less uniform.

During prepupal condition the hindgut also shortens considerably, becoming almost half of the hindgut of full grown larva. Moreover, the twisting of the ileo-colon

reduces and it tends to be straight. The whole hindgut wall is irregularly dilated, probably caused by the accumulation of discharged matters running through the hindgut lumen during this time.

Transformation of the prepupal gut into the pupal gut

Drastic changes have been observed during the last larval moult. The prepupal alimentary canal, just described above, changes to the pupal alimentary canal, as described earlier (see pp. 25-32). During pupal stage, the shortening of the midgut still continues. But this is a very slow process as compared to the prepupal condition, and is not accompanied by any folding or transverse striations of the gut wall (Fig. 8).

During the pupal stage, the foregut and the hindgut elongates slightly, as opposed to the prepupal condition. The elongation of the hindgut is more pronounced than that of the foregut.

c) Changes during pupa to adult moult

With the onset of the last moult, i.e. the pupal moult, the pupal alimentary canal transforms into the adult alimentary canal, described earlier (see pp. 33-44). In the adult, mesenteric diverticula appear on the surface of the midgut. The midgut becomes shorter than the pupal midgut (Fig. 10).

The larval proventriculus is carried over to the adult insect through the pupal stage, which becomes prominent during the last moult. The length of the foregut increases slightly with the increase in length and diameter of proventriculus from the pupal stage.

The hindgut also elongates during pupa to adult transformation. The larval cryptonephridial system continues in the adult hindgut.

Thus, it is evident that the foregut and the hindgut gradually increase in length during pupal and adult stages. On the otherhand, the midgut abruptly decreases in the prepupa which continues to decrease gradually in length during the pupal stage and in just emerged adult which still remains within the pabulum of the pulse.

III. A. 2(a). ANATOMY OF THE LARVAL GUT OF C. chinensis

The gross anatomy of the alimentary canal of C. chinensis larva is identical with that of C. analis except that it is smaller in dimension than the later. th
The length of the larval alimentary canal of C. chinensis at different instars is shown in the Table 5 for comparison with C. analis (vide p. 10).

Table 5. Measurements of the body and the gut lengths in different instars of C. chinensis.

Instars	Body (mm)	Gut (mm)	<u>Proportion</u> Body : Gut
1st	0.60	1.20	1 : 2.00
2nd	0.80	2.04	1 : 2.55
3rd	1.00	3.02	1 : 3.02
4th	2.25	8.27	1 : 3.67

The foregut is more or less similar to that of C. analis, which is divisible into a pharynx and an oesophagus in the early larva (1st & 2nd instar); or into pharynx, oesophagus, and a proventriculus in the later instars. The proventriculus thus makes its appearance just after the 2nd larval moult. Unlike that

of C. analis, the anterior end of the midgut does not form the two lateral lobes around the posterior end of the proventriculus in C. chinensis. Here the proventriculus appears like a simple bulb in fresh dissections (Fig. 5). Lengths of the different regions of the foregut of C. chinensis in different instars have been shown in the Table 6 (cf. C. analis, p. 13).

Table 6. Measurements of the body and foregut lengths in different instars of C. chinensis.

Instars	Body (mm)	Foregut (mm)			
		Pharynx	Oesopahgus	Proventri- culus	Total
Ist	0.60	0.04	0.05	-	0.09
2nd	0.80	0.06	0.07	-	0.13
3rd	1.00	0.12	0.09	0.09	0.30
4th	2.25	0.24	0.20	0.16	0.60

As in C. analis, the midgut of the larva of C. chinensis is very long as compared to the fore-and hindgut. It is clearly distinguishable into a long anterior swollen part (called the anterior midgut) and a posterior short and narrow tubular part (called the posterior midgut). The measurements of these regions in the different instars are given in the Table 7 (cf. C. analis, p. 18).

Table 7. Measurements of the body and the midgut lengths in different instars of C. chinensis.

Instars	Body (mm)	Midgut (mm)			
		Anterior	Posterior	Total	Post.Mg.:Ant.Mg.
Ist	0.60	0.38	0.28	0.66	1 : 1.36
2nd	0.80	0.92	0.38	1.30	1 : 2.42
3rd	1.00	1.45	0.51	1.96	1 : 2.84
4th	2.25	4.65	1.24	5.89	1 : 3.75

The hindgut is distinguishable into four distinct regions. Like that of C. analis, six independent Malpighian tubules originate from the pylorus of C. chinensis. The ileum is provided with a characteristic "S"-shaped convolution, and the colon bears a cryptonephridial system. The rectum is very simple, which appears as a narrow cone-shaped structure. The measurements of the length of different regions of the hindgut of C. chinensis in different instars are shown in the Table 8 (cf. C. analis. p. 21).

Table 8. Measurements of the body and the hindgut lengths in different instars of C. chinensis

Instars	Body (mm)	Hindgut (mm)				Total
		Pylorus	Ileum	Colon	Rectum	
1st	0.60	0.07	0.11	0.16	0.11	0.45
2nd	0.80	0.08	0.15	0.23	0.15	0.61
3rd	1.00	0.09	0.23	0.28	0.16	0.76
4th	2.25	0.19	0.57	0.66	0.36	1.78

III. A. 2(b). ANATOMY OF THE PUPAL GUT OF C. chinensis

In general appearance, the pupal alimentary canal of C. chinensis is also very similar to that of C. analis. Among the three divisions of the foregut, the pharynx is bent cephalad. The midgut is shortened considerably in the pupa (cf. larval midgut), but it still consists of two distinct divisions as in C. analis. Presence of "yellow bodies" in the midgut lumen can be seen through the semi-transparent gut wall. The hindgut bears characteristic ileal convolution, and cryptonephridial arrangement in the colon. The length of the pupal alimentary canal of C. chinensis is shown in the Table 9 (cf. C. analis p. 25).

Table 9. Lengths of the different regions of the alimentary canal of C. chinensis pupa.

Body (mm)	Foregut (mm)	Midgut (mm)	Hindgut (mm)	Total length (mm)
2.35	0.40	1.77	1.33	3.50

III. A. 2(c). ANATOMY OF THE ADULT GUT OF C. chinensis

The adult alimentary canal of C. chinensis is more similar to that of the pupa than the larva. It is identical with that of C. analis in gross anatomy (Fig. 12) but differ slightly in dimension from the later (cf. C. analis, pp. 33-44). However, the following measurements (Table 10) on the adult alimentary canal of C. chinensis easily distinguishes it from that of C. analis.

Table 10. Lengths of the different regions of the alimentary canal of adult C. chinensis.

Body (mm)	Foregut (mm)	Midgut (mm)	Hindgut (mm)	Total (mm)
2.15	0.42	1.32	1.53	3.27

III. A. 2(d). METAMORPHIC CHANGES (ANATOMICAL) IN THE
ALIMENTARY CANAL OF C. CHINENSIS

Anatomical changes of the alimentary canal of C. chinensis during metamorphosis is quite similar to that observed in C. analis (vide pp. 45-50). These changes in C. chinensis are described very briefly.

a) Changes of the alimentary canal during larva to larva moults is not very remarkable. The main anatomical changes are :

(i) the alimentary canal (especially the midgut), increase in length and diameter which is proportional to the growth and development of the larval body during successive instars;

(ii) the proventriculus which was absent in the early larva (1st & 2nd instar), appear as a very small dilation of the posterior end of the oesophagus just after the 2nd larval moult. This simple proventriculus gradually becomes more prominent during next (4th) instar (i.e. after the 3rd larval moult).

b) Changes of the alimentary canal during prepupal stage, larval-pupal moult, and pupal period is very remarkable and distinct, but similar to that of C. analis. The midgut suddenly becomes shrunken during prepupal condition and thereby shortens the midgut. Later during

early pupal period this shortening still continues but is gradual. The shortening stops during the mid-pupal stage. As the shortening of the midgut stops its wall becomes uniformly thin and more or less transparent. At this time "yellow bodies" become clearly visible in the lumen.

c) The changes in the alimentary canal of C. chinensis during pupal-adult moult, and early adult condition is feeble. However, the following main metamorphic changes have been observed during this time :

(i) numerous "crypts" develop on the surface of the midgut. They first appear as very simple dots on the surface of the gut, soon after the pupal-adult moult. These dot-like structures of the midgut gradually become prominent with the advancement of the adult life; and

(ii) the foregut and the hindgut slightly increase in length with the development of the early adult, which still remains within the pabulum of the pulse. Similar elongation of the foregut and the hindgut, and shortening of the midgut during metamorphosis was observed in C. analis and other beetles, e.g., Lytta nuttalli Say (Morgan et al. 1970; and Prionus laticollis (Benham, 1970).

B. HISTOLOGY

III. B. 1(a). HISTOLOGY OF THE LARVAL GUT OF C. ANALIS

Essentially the histological structures of the foregut and hindgut are similar and quite distinct from that of the midgut. A peritrophic membrane was never found in the gut of C. analis larva.

Foregut

The cellular pattern of the foregut epithelium in all the different larval instars is almost similar. Histologically, the foregut of the 1st, 2nd and 3rd instars are distinguishable into pharynx and oesophagus; whereas in the 4th instar a third region, viz., proventriculus, is revealed which is distinguishable in gross anatomy also. The oesophageal invagination, which was not so prominent in gross anatomy, is clearly distinguishable in the histological preparations. The sequence of the layers of histological elements in the foregut beginning from the lining of the gut lumen outwards, consists of the following :- (i) intima, (ii) epithelium, (iii) basement membrane, and (iv) muscle layers.

Pharynx

The pharynx of the larva is provided internally with a cuticular intima. It covers the entire inner surface of the pharynx and continues into the oesophagus.

The intima of the pharynx is not uniformly thick. It is very thin at the base of adjacent epithelial folds whereas it thickens gradually towards the apex of the folds. In the thick portion, the intima is much cuticularized and thrown into many spine-like projections, which may be called the "pharyngeal spines" or "pharyngeal teeth" (Fig. 13). These spines are very prominent structures with broad bases and pointed tips, extending from the thick intima of the epithelial folds. The intima is 0.5-1 μm thick and the spines are 2-4 μm long. In a transverse section, 4-7 such spines are seen in each of the epithelial folds (Fig. 13). These spines are backwardly directed. They, probably, help in shredding the larger food particles into smaller pieces, while passing through the pharynx.

In sections, the pharyngeal epithelium looks like a thin film of cells with indistinct cell boundaries, attached end to end in the form of a ribbon. It is lined internally by an intima and externally rests on a basement membrane. The cytoplasm of the epithelium appears to be homogenous and transparent. The epithelial cells are flat cuboidal and almost isodiametric but with barely discernible nuclei. The cell boundaries are visible only in well stained preparations and when viewed under high magnification. The nuclei are round or spherical with very fine chromatin granules. The epithelium is 2-5 μm

thick and the nuclei 1-2 μ m in diameter. The epithelium along with the intima is projected longitudinally into the lumen in the form of invaginations. These are known as the pharyngeal epithelial folds. There are six such longitudinal epithelial folds in the pharynx. These folds are moderately high, regular and equidistant. Towards the anterior end of the pharynx all the folds are almost equal in height. Two of these folds gradually shortens or diminish in height towards the posterior end and ultimately disappear at the entrance of the oesophageal region. The remaining four folds of the pharynx, gradually arrange themselves in a regular manner and become continuous with the four folds of the oesophagus.

The basement membrane is very thin, remains closely attached to the epithelium. It is almost indistinguishable and is not easily recognizable.

The muscle layers include a few bands of isolated longitudinal muscles, grouped together, and two strands of closely attached circular muscle layers. The longitudinal muscles are very thin, arranged longitudinally near the bases of the epithelial folds along with the connective tissue mass, which are not easily distinguishable. The circular muscle layer is prominent. It is 3-5 μ m thick. The two strands of muscles encircle the epithelium.

Oesophagus

The intima of the oesophagus is irregularly thickened. There are no intimal spines as seen in the pharynx, but the inner projected parts of the epithelial folds are provided with thick intima whose surface is irregularly folded (Figs. 14 & 74). The normal oesophageal intima is 1-2 μm thick which becomes 2-5 μm in the lobed portions.

The uniform ribbon-like oesophageal epithelium is similar to that of the pharynx. The cells are flat cuboidal. The nuclei are round to oval, slightly larger than those of the pharynx. The epithelial cells are 4-5 μm high and 7-8 μm wide, and their nuclei are 2.5-3.5 μm by 2.5-3.0 μm . There are four regular longitudinal epithelial folds in the oesophagus as opposed to six in the pharynx. These folds are almost equal in height. The epithelium is encircled by a thin basement membrane which can not be recognized separately. There are spaces between the bases of epithelial folds and the circular muscle layer. These spaces are partially filled with connective tissue masses. In this tissue masses, a few bands of very thin longitudinal muscles remain embedded. In sections, they appear as very simple dot-like structures.

Just surrounding the epithelium, there is a layer of circular muscle which is thinner than that of the pharynx. It consists of one or two layers and is 2.5-4.0 μm thick.

Proventriculus

A proventriculus is absent in the early larval instars. Only a weakly developed proventriculus could be seen in the 4th larval instar which also lacked well developed cuticular spines, teeth, hairs or needle-like processes. The lumen of the larval proventriculus is spacious and the epithelial wall is lined by a thick cuticular intima. A large amount of food is retained in the proventriculus which press against the intima.

The intima is pleated into numerous short and blunt processes in the form of regular waves, as revealed in longitudinal sections (Figs. 16,17 &76). Actually these blunt processes are heavily cuticularized, provided with minute spines known as the "proventricular spines" and are almost uniformly distributed throughout the whole inner surface of the epithelium. The intima of the proventriculus is 1-2 μ m in thickness and the proventricular spines are 2-3 μ m in length.

The shape and size i.e. the pattern of the proventricular epithelium is almost similar to the oesophageal epithelium. In this case, the epithelium is thrown into eight regular and very short folds. Of these, four folds are shorter than the other four folds and they alternate with each other. The cells, cytoplasm, and nuclei of the epithelium are more or less similar to that of the oesophagus. The epithelial cells measure

5-8 μm in height and 5-8 μm in width, where the nuclei measure 3.5-5 μm in diameter. The basement membrane on which the epithelium of the proventriculus rests on, is also identical with that of the oesophagus.

The musculature of the proventriculus is well developed. One to two strands of circular muscles encircle the epithelium. The circular muscles layer is 2-3 μm in thickness.

A few scattered bands of longitudinal muscles adhere to the basement membrane near the epithelial folds and the circular muscle layer. However, due to their negligible dimensions, the exact structural condition could not be ascertained.

Oesophageal or proventricular invagination

The oesophageal invagination is an extension of the oesophageal epithelium into the anterior end of the midgut. The epithelium thus introduced is always accompanied by the other essential histological elements. The oesophageal invagination may be formed directly from the oesophagus or from a modified part of the oesophagus, like the proventriculus. Both the conditions are found in the larva of C. analis. The first case, which may be regarded as an oesophageal invagination, is seen in the 1st, 2nd, and 3rd instar larvae. Whereas the second case, which is a modified condition, may be regarded as the

proventricular invagination, is observed in the 4th instar larva only. Both these types of invaginations are separately described below.

Oesophageal invagination : The first type of invagination or the oesophageal invagination is just a turn out process of the oesophageal epithelium, in the case of C. analis larvae (Figs. 15). The epithelial folds of the oesophagus (i.e. the projected epithelium) extends a little distance to meet the epithelium of the anterior bulging area of the midgut. The oesophageal epithelium along with other histological elements then turns straight towards the peripheral side (i.e. reflexed outer epithelium of the foregut) and meet with the midgut epithelium (Figs. 15 & 75). In the oesophageal invagination, the intima is much folded and elongated which extends a little distance into the midgut lumen. The projected epithelium of this region is similar to the epithelium of the oesophagus. This projected epithelium along with the intima is 25-30 μm high, where the nuclei are 3-4 μm . The oesophageal invagination does not form any true "oesophageal valve".

A few cells at the junction of the foregut epithelium and the midgut epithelium, on the peripheral side, resemble neither the oesophageal cells nor the midgut cells. These are the transitional cells. They

are smaller, with imperfect and indistinct cell boundaries. Their nuclei are also smaller than the nuclei of other adjoining cells. The transitional cells are 8-10 μm high by 4-5 μm wide and the nuclei are 2.5-3.5 μm . These transitional cells do not possess any intima or brush-border. Besides these cells, there is a cluster of large cells at the anterior end of the midgut epithelium, behind the smaller transitional cells. In this cluster, 2-3 cells are very large and prominent as compared to the others. They bear large nuclei. The largest cell of the cluster is 35-40 μm high and 20-25 μm wide at the apex and 8-10 μm at the base. Its nucleus is 8-10 μm in diameter.

There are a few muscle bands, haphazardly arranged around the neck of the oesophageal invagination, in addition to the normal musculature of the oesophageal epithelium. These extra muscles form what is known as the sphincter muscles of the oesophageal invagination, which is rather poorly developed (Fig. 15). The normal circular muscle layer of the oesophageal invagination is 1-1.5 μm thick.

Proventricular invagination : In the second type of invagination or the proventricular invagination, the posterior end of the proventriculus becomes considerably constricted to make a narrow neck (Figs. 16, 17 & 76). The

neck is provided with many strong circular muscles, arranged at random which acts as an effective sphincter. Internally, the epithelium along with other histological elements arch outwards which ultimately meet with the midgut epithelium as in the oesophageal invagination of the early larval instars. Intima of the invaginated proventriculus also extends a little distance into the midgut. A few transitional cells which resemble neither the cells of the foregut nor those of the midgut, at the junction of the foregut and midgut epithelium are also observed in the proventricular invagination. These transitional cells do not possess distinct adjoining cell boundaries and are always devoid of intima or brush-border. They are more or less compactly arranged in a row (Fig. 17).

A few columnar epithelial cells of the midgut, of which two to three cells are very distinct, and two to three of their adjacent cells were seen to proliferate enormously during the pupal stage. Their daughter cells ultimately form the "anterior imaginal ring". Therefore, these cells may be regarded as representing the "anterior imaginal ring" (Figs. 15 & 17).

Midgut

The histological elements of the midgut throughout the whole larval life is almost identical except a few

minor differences. The elements consists of the following from the gut lumen outwards :- (i) epithelium, (ii) basement membrane, (iii) circular muscle, and (iv) longitudinal muscle. Histologically, the anterior midgut and the posterior midgut are quite distinct. The epithelium of the anterior midgut consists mainly of the two types of columnar cells with infolding of the epithelium whereas the posterior midgut consists of only one type of columnar cells and without any infolding of the epithelium.

Anterior midgut

The epithelium of the anterior midgut is not uniform. Depending on the structural peculiarities of the epithelial cells, the anterior midgut of the larva can be distinguished into two sections; the anterior and the posterior.

As stated earlier a few cells at the anterior tip of the midgut which meet with the foregut epithelium are quite distinct and forms an anterior imaginal ring. The midgut epithelium behind this ring consists of narrow and tall columnar cells, whose distal ends appear rather dilated (Figs. 16,17,20 & 76). These cells measure 45-55 μm in height and 20-28 μm in width near their apex and 5-8 μm towards their base. This type of cells are abundantly seen towards the late larval stages. They appear to be

very active in secretion. They have large and rounded nuclei situated towards the middle of the cells or slightly distal. The nuclei are 6-10 μm in diameter. When the cells do not show secretory activity their brush-borders can be seen distinctly. At this time it measures 2-2.5 μm in height. In the early larval instars (1st and 2nd) the tall epithelial cells are shorter and appear more cuboidal than columnar (Figs. 18 & 77). In the early larval stages they measure 22-25 μm in height and 18-22 μm in width. Their nuclei measure 12-15 μm in diameter and brush-border 2-3 μm in height. These shorter cells appear to be less active in secretion than the taller cells of the late larval instars. Cytoplasm of these epithelial cells are uniformly and finely granular. Their nuclei contain numerous chromatin granules of various shapes and sizes. The epithelium rests on a thin basement membrane which is encircled by a single layer of circular muscle, about 2 μm thick. External to the circular muscle layer there are a few bands of longitudinal muscles at regular intervals.

The regenerative cells of the anterior section of the anterior midgut epithelium are situated between the bases of the tall columnar cells. They remain scattered and hidden in the epithelium. In the tall columnar epithelium, the inner contour does not show any sign of the presence of regenerative cells (Fig. 20). The

regenerative cells are arranged in clusters, known as nidi. Each nidus is a short conical structure. The broad end of the nidus remain attached to the basement membrane and the narrow end is directed towards the gut lumen. Each nidus bears 1-2 free nuclei in the early larvae (Figs. 18 & 77) and 2-3 nuclei in the late larvae (Fig. 20). The nuclei of the nidus are smaller than the nuclei of the adjacent columnar epithelial cells. In the young larva, the nidi are 12-16 μm high and 22-28 μm wide at the base. The nuclei are round, about 7-9 μm in diameter. In the late larva, the nidi measure 15-20 μm in height and 20-25 μm in width at the base, and the nuclei are 6-7 μm in diameter.

There are some infoldings of the epithelium within the anterior section of the anterior midgut. In this case, the epithelium is thrown into the gut lumen as very short villiform processes (Figs. 16, 17, 19 & 76). The cells in this region are exceptionally tall, their distal parts are broader than the basal parts. The cells measure 30-45 μm in height and 15-20 μm in width at their apex and 3-7 μm at their basal end. The nuclei of these cells are round, distally placed and with larger chromatin granules (Fig. 19). They measure 5-10 μm in diameter. The epithelial cells in the unfolded region adjacent to the folds are comparatively shorter and their nuclei also smaller. The base of the epithelial folds always has regenerative cells

in the form of a nidus where it meets the unfolded epithelium (Fig. 19). The cells of the epithelial folds show distinct brush-border which is $1-2\text{ }\mu\text{m}$ thick. The circular muscle layer does not enter into the folds but surrounds the epithelium, thereby leaving a space at the base of the folds (i.e. the villiform process). These spaces are filled with connective tissues masses. The circular muscle layer at this region is $1.5-2\text{ }\mu\text{m}$ thick.

The posterior section of the anterior midgut bears short and wide columnar epithelial cells and without any fold (Figs. 21 & 78). These cells are large and wider than their height and are not dilated distally as observed in the anterior end (cf. Fig. 20). The cells are $15-20\text{ }\mu\text{m}$ high and $20-30\text{ }\mu\text{m}$ wide. The nuclei are large and oval which measure $12-15\text{ }\mu\text{m}$ by $8-10\text{ }\mu\text{m}$. The cytoplasm is coarsely granular and normally with small vacuoles. The brush-border of these cells are tall and very distinct, which measure $2.5-3.5\text{ }\mu\text{m}$ in height. The epithelium as usual rests on a thin basement membrane, encircled by single layer of circular muscle which is $2-2.5\text{ }\mu\text{m}$ thick.

The tips of the nidi are not covered by the epithelium in this part of the midgut so that their contour is clearly visible in section (Fig. 21).

Posterior midgut

The posterior midgut of the larva includes both columnar and regenerative cells. The columnar epithelial cells of the posterior midgut are narrow and smaller than the cells of the anterior midgut (Fig. 22 & 23). The epithelial cells are 18-25 μm high and 7-10 μm wide. The cells are almost uniform in height and width, thus their inner contour is almost uniform. The cells rest on a thin basement membrane uniformly. The nuclei are round and small, and are slightly basal in position. The nuclei are 4-6 μm in diameter. The brush-border is distinct which is 1-1.5 μm thick. The circular muscle layer is very thin with sparsely arranged external bands of longitudinal muscles. The circular muscle layer is 1-1.5 μm thick.

The regenerative cells are in the form of nidi as found in the anterior midgut. But they are very few in number as compared to the anterior midgut. Each conical nidus measures 7-9 μm in height and 7-9 μm in width at the base. The nidi remain attached to the basement membrane at the base of the epithelium and can not be easily recognized. The nuclei of the nidi measure 3.5-4.0 μm in diameter.

Hindgut

The hindgut epithelium is made up of cuboidal cells except in the pylorus. Though the epithelium of the

pyloric chamber consists of highly specialized epithelial cells, yet it resembles more closely the hindgut than the midgut cells. The stratification of the histological element of the hindgut is similar to that of the foregut.

Pylorus

The pylorus is a transitional part between the midgut and the hindgut. The epithelial cells of this region resemble neither the cells of the midgut nor that of the hindgut. Actually they are specialized condition of the normal hindgut cells.

The Malpighian tubules open at the wide anterior end of the pylorus. Six Malpighian tubules originate independently from six zones of undifferentiated cells located at the bases of six epithelial folds (Figs. 24 & 79). The basal cells of each epithelial fold of the pylorus thus transform into a group of circularly arranged cells which are covered externally by the basement membrane. These are the basal cells of Malpighian tubules which push to bulge the external surface of the anterior end of the pylorus, and are continued with the epithelium of the Malpighian tubules. As a result the circular muscle layer becomes discontinuous around the anterior end of the pylorus. The epithelial folds are very tall at the anterior end of the pylorus which

almost fill up the gut lumen, leaving a little space at the centre, thus acting together as an imperfect valve of the pylorus; but it can not be regarded as a "true pyloric valve". Similar structure was observed by Ameen & Rahman (1973) in T. castaneum larva.

The pyloric epithelium is thrown into six prominent longitudinal folds. The folds are very distinct at the anterior end of the pylorus where the Malpighian tubules make their origin. Towards the posterior end of the pylorus, the epithelial folds become gradually shorter with lateral extensions, thus increasing the lumen (Fig. 25). The epithelium of pylorus towards the anterior part consists of densely packed columnar cells with indistinct cell boundaries (Fig. 24). The epithelial cells are gradually transformed into an irregular cuboidal type towards the posterior end of the pylorus, which ultimately continue into the ileum. The nuclei of the pyloric epithelial cells are almost round or oval with fine chromatin granules. The cytoplasm appears to be finely granular. The intima is poorly developed in the pyloric epithelium.

The muscle layer is well developed towards the posterior end of the pylorus, where a few strong bands of circular muscles firmly encircle the wall of the pylorus and act as a "sphincter of the pylorus", which is common in many insects.

Ileum

The intima of the ileal epithelium is almost uniformly thick. It is a comparatively thin structure, lining the inner side of the epithelium. The intima is 1.5-2.5 μm thick.

The epithelium is thrown into six longitudinal folds which are continuous with the six folds of the colon. Of these six folds, three folds are shorter and alternate with the remaining three taller epithelial folds (Fig. 26). The cells are of irregular cuboidal type. The cells of the tip of the epithelial folds are slightly larger than those near the base, i.e., all the cells are not iso-diametric. The cells of the epithelial folds are 4-5 μm high by 4-5 μm width whereas those near the base are 2-2.5 μm high and 3-3.5 μm wide. The nuclei are round to oval-shaped with distinct nuclear membrane and variously stained chromatin granules. The nuclei of the basal cells are 1-1.5 μm , whereas the nuclei of the epithelial fold cells are 2.5-3.0 μm . The cytoplasm of the cells is finely granular. Externally, the infolded epithelium is surrounded by a thin basement membrane. It is closely allied and continuous with the bases of the epithelial cells.

The musculature of the ileum is not so prominent. Usually one strand of circular muscle band encircles the gut with a few thin strands of longitudinal muscles

attached with the circular muscle layer, near the bases of the epithelial folds. The circular muscle of the ileum is 2-3 μm thick.

Colon

Histology of the larval colon is described in two steps :- firstly, the anteriormost part, about one-fifth of the total length of the colon, which does not take active part of the cryptonephridial system; and secondly, the posterior major part, about four-fifths of the total colon length, which takes part in the formation of the cryptonephridial system (Figs. 27, 28, 29 & 80).

In the first case, the histological structure is quite identical with the ileum. Thus it hardly needs any elaborate description. However, the epithelial cells are comparatively regular and larger than the ileum. The cells are wider than tall and almost iso-diametric. These cells are 5-8 μm high by 6-10 μm width. The nuclei are also larger than those of the ileum. They are round to oval in shape. The nuclei measure 3-4 μm . The intima of the anterior colon is 1-2 μm and does not form any extra structure. The circular muscle layer is more prominent than the ileal part which consists of two to three strands of circular muscles. The circular muscle layer is 3.5-7 μm thick. The distal parts of the Malpighian tubules just adhere with the colon in a

straight line. The brush-border of the Malpighian tubules still persists in this region (Fig. 27).

In the second case, where the cryptonephridial system is involved, the distal parts of the Malpighian tubules enter into a separate chamber around the colon wall (Figs. 28, 29 & 30) known as "cryptonephridia". In C. analis larva, there is a limited space surrounding the colon known as the "cryptonephridial chamber" or "perinephric space". It is lined by a thin membrane known as the "perinephric membrane". The Malpighian tubules after following a straight course through the first part of the colon, gradually proceeds towards the posterior side of the colon in a convoluted manner within the cryptonephridial chamber. The epithelium of the colon within the cryptonephridial chamber is slightly different from that of its anterior free portion. In this case, it consists of flat cuboidal cells. The epithelial cells of the early larva are 5-9 μm high by 16-20 μm width, where the large rounded nuclei are 6-8 μm in diameter (Fig. 28). The cells of this region in late larva are 6-8 μm high by 10-15 μm width, where the small oval or slight elongated nuclei are 2-3 μm by 4-7 μm (Fig. 29). The epithelial folds are very poorly developed. It rests on a thin basement membrane which adheres closely to the circular muscle except a few little gaps between the

bases of the folds and the circular muscle layer. The intima is almost similar to the other parts of the colon. It is 1-2 μm thick. The circular muscle is thin and weak as compared to the anterior colon region. The circular muscle layer of the posterior colon region is 1-1.5 μm thick. This layer is a single stranded structure. The lumen of the posterior colon region, i.e., the cryptonephridial part of the colon is very spacious as compared to the other parts of the hindgut.

Rectum

Internally, the rectum of the larva of C. analis is characterised by the presence of spiny intima and "complex diagonal muscles", described by Reinecke et al. (1973) around the normal circular muscle in the larva of Manduca sexta (L.).

The intima of the rectum is modified into projected spine-like structures, which may be called "rectal spines" like the pharyngeal spines of the same insect. The rectal spines are present at the top of each epithelial folds. They are strong and stout with broad base and pointed tips, identical with the internal spines of the pharynx. They are projected into the lumen and directed slightly backwardly. The intima with spines are 5-10 μm high of which intima is 1-1.5 μm thick (Figs. 30 & 81).

The epithelium is pleated into six longitudinal folds. The folds are prominent and unequal in height. The basal parts of the folds remain free from the circular muscle layer whereas the other side of the epithelium remain attached to the circular muscle layer. The cells are irregular cuboidal type with curved outer margin which remain entangled with the basement membrane. These cells are 3.5-5.5 μm in height and 3.5-5.5 μm in width. The nuclei are small and round with distinct nuclear membrane and dot-like chromatin granules. The nuclei are 2.5-4 μm in diameter. The cytoplasm is finely granular.

The musculature of the larval rectum is very characteristic because it is developed in a complex form unlike many other insects (Fig. 30). It consists of inner circular muscle layer, middle independent longitudinal muscle bands, and outer interwoven diagonal muscles. The circular muscle layer is a single stranded structure just surrounding the epithelium. It is 2-3.5 μm thick. There are six very prominent and isolated bands of longitudinal muscles present outside the circular muscle layer. These muscles are multinucleated and are situated near the bases of the epithelial folds. Thus, these six bands of such longitudinal muscles represent six epithelial folds of the rectum. This type of longitudinal muscles are 5-6 μm by 10-15 μm . There is a thick layer of complex muscles surrounding the circular and the longitudinal muscles.

This layer consists of 4-6 strands of muscles, loosely arranged on themselves in a sewing manner thus making a complex system of musculature (Fig. 30). The diagonal muscle layer of C. analis larva is 15-25 μ m thick. Since the circular muscle layer is poorly developed in the rectum of the larva, it needs additional support to squeeze out the waste products from the lumen of the rectum. It is probable that this type of complex muscles may exert pressure upon the gut wall and thus propel out the faecal matters to the exterior.

III.B. 1(b). HISTOLOGY OF THE PUPAL GUT OF C. ANALIS

It is very difficult to describe the typical histological structures of a pupal alimentary canal since it is always in a state of metamorphic changes. However, attempts have been made to describe the alimentary canal of an advanced (late) pharate pupa.

Foregut

Essentially the histological elements of the foregut represent the intermediate condition of the late larva and the adult. The intima of the foregut is relatively well developed than that of the larva and is closely similar to that of the adult. The epithelium of the foregut consists of undifferentiated cells, sometimes resembling a syncytial epithelium.

Pharynx

The intima which sloughed off and discharged out of the lumen during pre-pupal and early pupal condition, reappeared now in the late pharate pupa, observed in the present investigation (Fig. 43 & 93). This intima is a newly formed structure developed during pupal stage. It is thickest at the apices of epithelial folds and projected into the lumen as a few weakly developed "pharyngeal spines" which are unequal in shape and size. The

epithelial folds are identical with the folds of the adult epithelium. The epithelial cells are not distinguishable from each other due to their indistinct adjacent cell boundaries. The whole pharyngeal epithelium apparently looks like a thin, vacuolated film of cytoplasmic contents with sparsely distributed nuclei which appear almost syncytial. The nuclei are distinct, rough and irregularly round and with various chromatin granules. The basement membrane is not easily recognizable. Muscle layer is similar to that of the adult but they are rather loosely arranged. Longitudinal muscles are not visible. Pharyngeal dilator muscles remain attached with the wall of the pharynx (Fig. 43).

Oesophagus

The oesophageal intima towards the anterior and the middle part is almost uniform and without any projection. Whereas in the posterior part of the oesophagus, it is projected into the lumen as long spiny structures, comparable to the "oesophageal spines" of the adult, but are less developed (Fig. 43). The nature of the epithelium and its nuclei are almost similar to that of the pharyngeal region. The structural pattern of the epithelial folds are similar to those of the adult. The circular muscle layer is not so prominent as in the pharynx. It is single-layered structure. Longitudinal muscles are not visible.

Proventriculus

Proventricular intima is more developed than that of the larval proventriculus. The intima of the anterior half of the proventriculus is modified into prominent lobes, each of them representing the adjacent cell of the epithelium. Each lobe is provided with a long sclerotized intimal projection, nearly identical with the "proventricular needles" of the adult, developed from the "proventricular spines" of the larva. But these "proventricular needles" are structurally weaker, therefore, these can be considered as developing proventricular needles (Fig. 43). These structures are not so tiny as the "proventricular spines" of the larva or such a long structures as the "proventricular needles" of the adult of C. analis (cf. Figs. 16, 17, 35, 36, & 37).

Posterior half of the proventriculus does not bear any intimal lobe or any intimal projection. The intima of this region is found to be continuous with the anterior part but does not line closely with the epithelium. The epithelium is quite identical with the oesophageal epithelium. Muscle layer includes one to two sets of circular muscle bands surrounding the epithelium.

Proventricular invagination

Proventricular invagination of the pupa is very distinct and resembles that of the adult (Fig. 43).

It is characterised as follows :- (i) The proventricular epithelium has not yet formed any "proventricular valve" as observed in the adult (cf. Fig. 37), but the epithelium is more extended towards the lumen which ultimately develops as the "proventricular valve". (ii) The imaginal ring cells which were more active during pre-pupal and early pupal conditions, still continue to be active which is proliferating in the form of cellular "droplets". These cells ultimately help in developing the midgut epithelium. (iii) The intima of the proventricular invagination is very thick. It extends a little distance into the anterior midgut and blocks the passage, probably, to restrict the entrance of "yellow bodies" from the midgut into the foregut. (iv) The neck of the proventricular invagination is very prominent which extends a long distance into the midgut. A narrow space between the proventricular epithelium and its reflexed outer epithelium is filled with a few very weakly developed sphincter muscles. (v) "Transitional cells" between the foregut and midgut epithelium, are very distinct. These cells are lined towards the lumen by a stream of proliferating cells, originated from the anterior imaginal ring cells (Fig. 43).

Midgut

The characteristic features of the pupal midgut are the following :-

(i) The midgut lumen remains filled with "yellow bodies" as observed by many workers, viz., Dufour (1846), Weismann (1864), Ganin (1876), Kowalewsky (1887), van Rees (1888), Perez (1910a), Mansour (1927), Gray (1931), Bushnell (1936), Pajni (1967), Kathuria (1971), and Strambi (1976). In *C. analis*, the yellow bodies are actually the remnants of the larval midgut epithelium along with the digestive products, which are produced in the form of secretory droplets and are ultimately deposited in the lumen in a modified condition. These yellow bodies consist of large bodies, which bear distinct large nuclei, smaller bodies without nuclei, and dot-like granular bodies. These are round structures; numerous in number, and remain distributed in a fluid medium of the midgut lumen. They are the nourishing products for the future imaginal (developing) cells. It was observed that the "yellow bodies" gradually decrease in amount with the development of the adult epithelium and ultimately disappear from the lumen of the midgut.

(ii) The midgut epithelium does not show any structural differentiation though there is a morphological distinction. The cells are columnar but wider and irregular in shape i.e. their inner margin are often

irregularly protruded towards the lumen. Brush-border is not visible. The cytoplasm is coarsely granular and often with small vacuoles. The nuclei are comparatively large, and centrally placed. They are apparently round but with rough surface and possess various chromatin granules (Figs. 44 & 94). The cells measure 8-15 μ m in height and 6-10 μ m in width, and the nuclei measure 4-7 μ m in diameter.

(iii) The regenerative cells are not easily recognizable in the late pharate pupa, but they are clearly distinguishable in the non-pharate pupa, as observed in a cross section (Figs. 45 & 95). In this case, the regenerative cells occur singly in the form of nidi, which are identical with the nidi of the larva, but they are always found to be uninucleated. These cells would soon become multinucleated to form the future adult regenerative cells in the form of crypts.

(iv) A stream of proliferated cells from the anterior imaginal ring cells near the entrance of the foregut into the midgut (Fig. 43) and a stream of proliferated cells from the posterior imaginal ring cells near the entrance of the midgut into the hindgut (Figs. 46 & 96) still exist.

(v) A single layer of moderately developed circular muscle encircles the whole midgut epithelium.

Hindgut

The pupal hindgut is almost identical with the foregut in its essential histology except the pylorus.

Pylorus

The pyloric epithelium consists of anterior tall and narrow columnar cells which gradually become shorter and wider towards the posterior end to become cuboidal, nearly identical with the ileal cells. There is no intima at the anterior end whereas it is more or less developed towards the posterior end of the pylorus. All muscles including the "pyloric sphincter muscles" are weakly developed as compared to the pylorus of the larva (Fig. 46).

Ileum

The ileal intima is a lamellated structure which is thrown into the lumen as wrinkles. In gross appearance, the ileal epithelium looks syncytial since cell boundaries are very indistinct. Six epithelial folds are prominent, almost covering the lumen of the gut. The nuclei are often very large and distinct. Basal space of each epithelial fold is comparatively large and without any connective tissue masses or traces of longitudinal muscles unlike those of the larva and the adult (Figs. 47 & 97).

Colon

The colon is internally provided with a more or less thick intima unlike those of the larva and the adult (Fig. 48). The epithelial cells are distinct and are more or less regularly arranged. Round to oval nuclei are also distinctly visible and with numerous chromatin granules. The muscle layer comprises of a poorly developed circular muscle band which separates the colon epithelium from the surrounding cryptonephridial chamber (Figs. 48 & 98).

Rectum

The intima of the pupal rectum is irregularly thick and continuous throughout its length. It is provided with a few "rectal spines" towards the posterior part of the rectum. The epithelial folds are comparatively shorter towards the anterior part and taller towards the posterior part of the rectum (Fig. 49), which indicate that the folds of the anterior rectum are developing as "rectal pads" and the folds of the posterior rectum as "rectal papillae" of the future adult. The epithelium appears to be syncytial due to indistinct adjacent cell-boundaries. Round nuclei are distinctly visible in the rectal epithelium. There is a thin layer of circular muscles; outside this, a complex of poorly developed and haphazardly arranged "diagonal muscles" are also seen in the pupal rectum (Figs. 49 & 99).

III.B. 1(c). HISTOLOGY OF THE ADULT GUT OF C. ANALIS

Histologically the fore-and hindgut are more or less identical, but they are quite distinct from the midgut. Like that of larva, a peritrophic membrane was not found in the gut of adult C. analis.

Foregut

Among the three regions of the foregut of adult C. analis, the histological appearance of the pharynx and the oesophagus are almost identical except a few minor differences. The proventriculus is highly modified into a complex structure due to the presence of numerous needle-like intimal projections, which have been termed here as the "proventricular needles".

Pharynx

The intima of the pharynx is irregularly thick and continuous with the intima of the oesophagus. It is thickest at the apices of the epithelial folds and thinnest near the bases of adjacent folds. Intima of the anterior pharynx is provided with a few "pharyngeal spines" (Figs. 31 & 82), whereas the posterior part of the pharynx lacks such pharyngeal spines (Figs. 32 & 83). These spines are shorter than the pharyngeal spines of the larva. Similar spines were also observed by Sinha (1958b)

in four species of tenebrionid beetles, where the spines were concentrated on a part of the dorsal surface of the pharynx. Khatoon (1983) found hair-like "pharyngeal spines" in the dung beetle, Catharsius sagax (Quens.). Hussain (1983) observed needle-like "pharyngeal and oesophageal spines" in Alphitobius diaperinus (Panzer), but unlike that of C. analis they were very few in number. The intima of the posterior pharynx is rather extended at the apex of each epithelial fold, where it measures about 10-15 μm thick (Fig. 32). In the anterior pharynx, the intima with spines measures about 5-10 μm , of which the intima alone measures 2-3 μm in thickness (Fig. 31).

The epithelium of pharynx apparently looks like a ribbon of haphazardly arranged unequal cells, attached end to end. The cell-boundaries of adjacent cells are often very indistinct. The cells are of cuboidal type, which measure 3-6 μm in height and 3-6 μm in width. The nuclei are round to oval which measure 2-4 μm in diameter. The nuclei show distinct nucleoli and many chromatin granules. The cytoplasm of the cells appears to be homogenous and non-granular. The pharyngeal epithelium is thrown into the lumen as six alternately taller and shorter folds towards the anterior part of the pharynx, which gradually become almost equal in height towards the posterior end of the pharynx (cf. Figs. 31 & 32). There is a gap between the base of an epithelial fold

and the outer circular muscle layer, which is filled with connective tissues. A few bands (usually 2-4 bands, observed in each section) of strongly developed longitudinal muscles remain embeded in this connective tissue mass. The outermost layer consists of 2-3 strands of circular muscles. They are well developed and are closely attached with each other. The circular muscle layer measures 8-13 μm in thickness.

Oesophagus

The intima of the anterior oesophagus is similar to that of the posterior pharynx, but it is less thick. This intima gradually becomes more sclerotized towards the posterior oesophagus, where this layer is modified into spines, known as the "oesophageal spines" (Figs. 33 & 34). These oesophageal spines are morphologically almost similar to the pharyngeal spines but are slightly taller. Oesophageal spines are continuous with the needle-like intimal processes of the proventriculus (Fig. 37). The intima along with the oesophageal spines measure about 6-12 μm in length. Similar sclerotized oesophageal intima with "intimal hairs" continuous with the proventriculus or "gizzard" was observed in the beetle, Cyaneolytta acteon Lap. (Goel, 1969).

The six tall epithelial folds of the anterior oesophagus gradually become shorter and broader and

transform into eight very short and flat folds, comparable with the proventricular epithelial folds (Fig. 33). The oesophageal epithelium is morphologically identical with that of the pharyngeal epithelium. The epithelial folds are taller towards the two ends of the oesophagus and shorter in the middle. The epithelial cells and their nuclei are almost identical with the cells and nuclei of the pharyngeal epithelium. The cells are 4-7 μm in height and 4-7 μm in width, the nuclei measure 2.5-5 μm in diameter. The epithelium is encircled by a thin basement membrane, outside which are one or two bands of circular muscles. A few bands of poorly developed longitudinal muscles remain embedded in the connective tissue masses near the bases of the epithelial folds. The circular muscle layer measures 4-7 μm in thickness in the middle of the oesophagus.

Proventriculus

Histologically, the proventriculus is distinguished into two sections :- an anterior and a posterior, which are marked by a slight constriction at the middle of the proventriculus (Figs. 37 & 87).

The intima of the proventriculus is sclerotized into a narrow strip of uniform thickness which lines the inner surface of the epithelium (Figs. 35 & 85). From this lining numerous needle-like structures originate and

project into the lumen. These are the "proventricular needles" which develop around the inner surface of the anterior section of the proventriculus (Figs. 35, 36 & 37). These needles are very long and directed backwards. Probably, the needles of the proventriculus together act as a sieve. However, the exact function of these type of proventricular needles is not determined. Strambi (1974) found small chitinous spines in the proventriculus of a catopid beetle, Trogloedromus bucheti gaveti Dev. which are involved in crushing of the food. Balfour-Browne (1934) observed "proventricular teeth" or "proventricular hairs" in the Dytiscidae which act as a mill for crushing partly-digested food materials or as a filter respectively. Mathur & Goel (1966) found numerous bristles in the proventriculus or "gizzard" of Cybister tripunctatus asiaticus Sharp. Numerous bristle-like spines were found in the proventriculus of Calandra granaria L. (Dennell, 1942), which together act as a sieve of the proventriculus. The sclerotized zone of intima in the anterior proventriculus is 1-1.5 μm thick. The proventricular needles are found to be 15-35 μm in length.

Posterior section of the proventriculus does not possess any "proventricular needles" or any other extra structures (Fig. 37). The intima in this case, is very prominent and folded towards the tip of the epithelial folds where it measures 5-9 μm in thickness.

The epithelium of the anterior part of proventriculus is irregularly thick and ribbon-like. The epithelium is thrown into the lumen as eight regular folds, of which four folds are shorter than the remaining four folds (Fig. 36). Similar tall and short proventricular epithelial folds were found in the beetles, Epicauta cinera marginata F. (Everly, 1936); Macrobasis unicolor Kirby (Judd, 1947); Cyaneolytta acteon Lap. (Goel, 1969); and many others. The cells are 4-6 μm in height and 4-6 μm in width. The nuclei are round to oval, which measure 2-4 μm in diameter. The epithelium of the posterior part of the proventriculus is taller than the anterior part, where the cells are 6-8 μm in height and 8-10 μm in width. Their nuclei measure 3-7 μm in diameter.

Muscle layers include a few bands of poorly developed longitudinal muscles situated in the connective tissue masses at the bases of epithelial folds, and a layer of outer circular muscle consisting of a single strand, measuring 4-6 μm in thickness.

Proventricular invagination

The proventricular invagination is very distinct in the adult. It looks like a valve introduced into the anterior end of the midgut (Fig. 37). The intima of the proventricular invagination is not sclerotized and does not form any extra structure like the proventricular

needles, spines, hairs, teeth and bristles etc. The intima is very thick and folded, which extends into the anterior midgut. This extended part measures 8-12 μm in thickness.

The epithelium of the proventricular invagination is almost similar to that of the proventriculus, except that a layer of cells in the reflexed outer epithelium is transformed as "transitional cells" between the foregut and midgut epithelium. The transitional cells possess neither intima nor brush-border. Their nuclei are smaller than the nuclei of the proventricular cells. The transitional cells measure 5-8 μm in height and 5-8 μm in width. Their nuclei measure 3-4.5 μm in diameter.

The epithelium of the proventricular invagination is distinctly folded anteriorly into lobe-like structures which may act like valves (Fig. 37). These folds together can close and open the lumen of this region and thereby these can be regarded as "proventricular valves", which are absent in the larva of C. analis.

Besides the normal musculature of the proventricular region and the anterior midgut region, the narrow neck of the invagination is invested by numerous extra muscles which are known as the "sphincter muscles" of the proventricular invagination. These are prominent structures in the adult (Figs. 36 & 37).

Midgut

The epithelial cells of the midgut of adult of C. analis are typically tall columnar cells except the anteriormost lobed part surrounding the invaginated proventriculus, where the cells are comparatively shorter (Figs. 36 & 86). There is no other differentiation of the epithelial cells between the anterior and posterior midgut, even among their subregions, as observed in the larval midgut. Only, the cells of the posterior midgut are comparatively smaller than those of the anterior midgut. The cells of both regions of the midgut are narrow and almost cylindrical which are more or less uniform in diameter, during non-secretory condition. Most of the times during the present investigations, the cells were found more or less in a secretory condition (Figs. 38 & 88). Due to their constant secretory condition the exact structure of the brush-borders and their measurements were not possible. The cells of the anterior midgut measured 20-25 μm in height and 3.5-4 μm in width, whereas the cells of the posterior midgut measured 15-20 μm in height and 3.0-3.5 μm in width. The nuclei are elongated with numerous prominent chromatin granules of various shapes and sizes. Nuclear membrane is also distinct. The nuclei of the anterior midgut cells measured 6-10 μm by 3-5 μm , whereas the nuclei of posterior midgut cells measured 4-7 μm by 2.5-3.0 μm .

Unlike the larval midgut, the regenerative cells remain isolated from the normal epithelial cells. In this case, the cells remain in the evaginated part of the gut wall, like a short tubular outpocket, known as the crypt of the midgut. Each crypt bears a series of regularly arranged, very short cells with prominent nuclei. The cells are of conical to slightly cuboidal type. The nuclei of these cells cover almost the whole interior of each cell. A few cells near the opening of the crypts into the lumen bear large round nuclei which are quite identical with the crypt cells (Fig. 38). The nuclei of the crypt cells are large and round, with numerous, variously-shaped chromatin granules. The crypt cells measure 8-12 μm in width at the base and their nuclei are 5-8 μm in diameter. The brush-borders of the epithelial cells are often found to be very indistinct. This lining is about 1.5 μm in thickness.

A thin basement membrane encircles the whole epithelium including the crypt cells. In the gut epithelium the basement membrane is not easily distinguishable but in the crypt it is clearly visible. The circular muscle layer is a poorly developed single-stranded structure. It does not cover the outer margin of the crypts, i.e., the crypts are always devoid of any external musculature. The circular muscle layer measures about 1.5-2 μm in thickness. There are a very

few weakly developed and isolated bands of longitudinal muscles resting on the outer side of the circular muscle layer. They are set on the circular muscle layer at regular intervals, and are normally placed near the adjacent sides of the basal parts of crypts. The crypts are always invested externally by a peritoneal membrane (Fig. 38). A peritrophic membrane is never found in the adult midgut.

Hindgut

The histology of the adult pylorus is quite similar to that of the larva, and, therefore, does not require separate description. Some structural peculiarities were observed in the adult rectum which have been elaborated below.

Ileum

The thin ileal intima measuring $1.5-2\ \mu\text{m}$ in thickness, lines the lumen side of the epithelium. The epithelium is pleated into six regular longitudinal folds, where three folds are taller and alternate with three shorter folds. The epithelium consists of cuboidal cells which are not iso-diametric. They remain attached to each other in an irregular pattern (Figs. 39 & 89). The cells near the base of the epithelial folds are comparatively smaller than the cells of the folds proper.

These cells measure 5-9 μm in height and 5-9 μm in width. Their cytoplasm is finely granular and the nuclei are distinctly visible which are round to oval in shape. They measure 4-6 μm in diameter. Each nucleus contains a distinct nucleolus and numerous deeply stained chromatin granules of variable shape and size. Nuclear membrane is also visible. The epithelium rests on a basement membrane which is a thin structure but is distinctly visible in most of the sections.

The muscle layers include mainly a ring of single stranded circular muscle band surrounding the epithelium. It measures 2-3 μm in thickness. Besides, a few isolated bands of very poorly developed longitudinal muscles remain embedded in the connective tissue matrix near the base of the epithelial folds.

Colon

As in the larva, the colon of the adult C. analis is also associated with the cryptonephridial system, where the colon is invested externally by convoluted distal parts of the Malpighian tubules within a chamber. This is a common occurrence in many beetles and lepidopteran larvae as described by Saini (1964). The detailed account of the cryptonephridial system is given in a separate chapter on Malpighian tubules.

Unlike the larval colon, there is no histological differentiation between the anterior and the posterior regions of the adult colon. However, the six epithelial folds gradually become shorter towards the posterior end and thereby gradually increase the diameter of the lumen (Figs. 40 & 90).

The colon intima is variable in thickness, measuring 1.5-2.5 μm . The epithelium consists of cuboidal cells which are almost regularly arranged in the form of a ribbon as seen in cross sections (Fig. 40). The cells are nearly iso-diametric and usually slightly wider than tall. The cells measure 5-8 μm in height and 7-10 μm in width. The nuclei are round to oval and have distinct chromatin granules of various shapes and sizes, and are often seen with distinct nucleoli and nuclear membrane. The nuclei measure 3.5-7 μm in diameter. A thin basement membrane is also distinct.

The circular muscle layer, which encircle the colon epithelium, marks the boundary between the colon and the "cryptonephridial chamber". "Leptophragma" of the cryptonephridial chamber is found to be continuous with the gut lumen through this circular muscle layer (Fig. 40). The circular muscle layer is poorly developed and single-stranded which measures 1-1.5 μm in thickness. Longitudinal muscle bands are not found in the colon, probably, due to their negligible size.

Rectum

Some structural peculiarities are observed in the rectum of adult C. analis. Histologically, the rectum is clearly distinguishable into two sections :- the anterior "rectal pad" region, and the posterior "rectal papilla" region.

The initial part of the rectum, just behind the cryptonephridial system, bears six regularly arranged, very flat epithelial folds with thick intima, and wide lumen. These short epithelial folds may be regarded as the "rectal pads" (Figs. 41 & 91). Hussain (1983) also recognized such rectal pads in Alphitobius diaperinus but unlike that of C. analis, the pads were associated with the trachae. Immediately behind the rectal pad region, the epithelial folds become exceedingly narrow and elongated, almost closing the lumen, which may be called "rectal papillae" (Figs. 42 & 92). But these two structures differ slightly from the description of Snodgrass (1935). According to Snodgrass (1935, pp. 381 & 382), "The rectal pads are areas of the wall of the rectum on which the epithelium is composed of high, columnar cells forming elongate oval bodies elevated on the sides toward the lumen. The intima is usually thin over the surface of each pad but forms a thickened rim around its margin. Typically the rectal pads are six in number, equally spaced around the anterior part

of the rectal sac, with their long axes longitudinal". Again he noted, "The rectal papillae are hollow, conical invaginations of the intestinal wall. The position of each is marked externally by a pit, which is penetrated by ramifying tracheal branches". Snodgrass (1935) mentioned that the rectal pad is absent in Coleoptera. But according to Palm (1949) and Reichenbach-Klinke (1952), these structures are found, among Coleoptera, only in the Silphidae and some Adepagan families. However, the present investigation showed the presence of "rectal pads" and "rectal papillae" only in the adult C. analis, a representative of the family - Bruchidae.

In the present material, the anteriormost part of the rectum or the rectal pad region (called "rectal sac" by Snodgrass, 1935) consists of six very simple rectal pads formed by the normal epithelium with moderately thickened rim of intima. The lumen is very spacious which accommodates a large amount of faecal matters. The intima is 2-3 μ m thick. The epithelial cells are large and apparently look like glandular cells, which are of irregular cuboidal type (Fig. 41). These cells measure 4-7 μ m in height and 4-7 μ m in width. The cells of the apices of the folds are usually larger than the cells of the basal parts of the folds. The nuclei are also large, round or oval, and measure 3-6 μ m in diameter.

The posterior part of the rectum or the rectal papillae region (called "rectum proper" by Snodgrass, 1935),

consists of very narrow, elongated and flexible epithelial folds, which extend upto the centre of the lumen (Figs. 42 & 92). There are six such very long folds which alternate with six shorter folds. The intima of these folds are thicker than those of the anterior pad region and are provided with a few "rectal spines" which are almost similar to the spines of the larval rectum. The spines are 4-8 μm in length and the intima is 2-5 μm thick. The cells are slightly wider than tall. These cells and their nuclei are comparatively smaller than those of the anterior region. The cells measure 3-5 μm in height and 4-6 μm in width, where the nuclei are 2-4 μm in diameter.

The functional significance of the "rectal pads" and "rectal papillae" in the adult of C. analis is rather uncertain and a matter of later investigation. Yet, it is probable that the rectal pad region facilitates temporary storage of a large amount of faecal matters. Afterwards, these are slowly passed through the posterior rectum by the undulatory movements of the rectal papillae and ultimately thrown out of the rectum. During this course, the greater surface area of the rectal papillae help to conserve more water from the outgoing faecal matters. Wigglesworth (1932 & 1972) considered these structures as the sites of water conservation by reabsorption from the faeces and inorganic ion reabsorption.

III.B. 1(d). METAMORPHIC CHANGES (HISTOLOGICAL) IN THE
THE ALIMENTARY CANAL OF C. ANALIS.

Major histological changes in the alimentary canal of C. analis do not take place during the various larval-larval moults. However, some drastic changes occur during larval-pupal and pupal-adult moults. In addition, some major histological changes take place, specially in the midgut region, towards the end of the last larval instar. This is also accompanied by some changes in the external appearance of the last larval stage. These have been described earlier (vide pp. 45-50). This part of the last larval stage has been earlier referred to as the "prepupal stage" for the convenience of description. However, it should be emphasized that no moulting (apolysis) was observed between the last larval and prepupal stages. In otherwards, "prepupa" of C. analis is not a distinct instar separated by apolysis from the last larval and pupal instars. It is just a part of the last larval instar when some changes in external appearance accompanied by internal histological reorganization of some of the organ systems take place.

The histological changes in the alimentary canal of C. analis during metamorphosis are discussed below under two headings for the ease of description, viz. ;

- i) Histological changes during larval-larval moults, and
- ii) Histological changes during prepupal (i.e. last larval) stage, prepupal-pupal, and pupal-adult moults.

i) Histological changes during larval-larval moults

There is no drastic metamorphic change like histolysis or degeneration and regeneration of the entire gut epithelium during the whole larval life except during the prepupal condition of the last larval stage. Only the normal growth and development of the histological elements proceeds through successive larval instars. However, the following histological changes were observed in the larval alimentary canal of C. analis.

Development of the proventriculus begins in the early 3rd instar larva just after the 2nd larval moult, with the addition of epithelial cells from the posterior end of the oesophageal invagination. The cells of the proventriculus are quite similar to those of the posterior oesophagus. Intima becomes more sclerotized in the form of a thin and uniform strip lining the lumen side of the epithelium but still devoid of any thickening or spines. Towards the beginning of the 4th instar larva (i.e. just after the 3rd larval moult), the

proventricular epithelium becomes adorned with thickened intima with short spines. The lumen becomes more spacious than that of the 3rd instar condition.

Any remarkable structural changes of the foregut and the hindgut epithelia throughout the larval life was not observed. These larval epithelia, derived in the 1st instar from the embryonic condition, continue to grow in size only without alteration of the basic pattern of the cells. The epithelial cells thus gradually attain their full grown condition, as described earlier (vide pp. 59-68 & 73-80).

The epithelial cells of the anterior midgut of the early larvae (1st and 2nd instars) are more or less similar throughout its whole length. This epithelium gradually becomes differentiated into an anterior part with tall and narrow columnar cells and a posterior part with short and wide columnar cells in the 3rd and 4th instars, as described earlier (vide pp. 68-71)

There is no active role of the regenerative cells in the formation of a new epithelium or regeneration of the old epithelium in any stage of the larval life except in the prepupa. The regenerative cells are always found in the form of nidus, remaining anchored as usual at the base of the epithelium. During early stages of the larva (1st and 2nd instars), each nidus bears 1-2 nuclei. But in the later stages (3rd and 4th instars) each nidus

normally bears 2-4 nuclei. The basic structural pattern of the nidi remain identical throughout the whole larval life except during the prepupal condition.

ii) Histological changes during prepupal (i.e. last larval) stage, prepupal-pupal, and pupal-adult moults

Drastic and remarkable histological changes in the alimentary canal of C. analis are observed during the transformation of the larva into the adult form. A detailed account of the various metamorphic changes of the three regions of the alimentary canal in the prepupa, pupa and the early adult of C. analis are described separately below.

Foregut

Prepupal changes : With the advent of the prepupal condition in the late larva of C. analis, the lumen of the foregut begins to diminish in volume. With the shortening and straightening of the foregut, the epithelium along with other histological elements also modify. The lumen becomes empty. The intima begins to separate from the epithelium which is ultimately discharged into the lumen and then gradually disintegrates along with the "yellow body".

Pupal changes : After the last larval moult, as observed in 1-2 days old pupa, the foregut epithelium

does not bear any intima. The detached intima remains aggregated in the centre of the lumen (Figs. 50 & 100). It is continuous throughout the length of the foregut lumen. It is aggregated mostly in the "proventricular invagination" region and it temporarily blocks the lumen (Figs. 51 & 101). Meanwhile, a stream of proliferated cells from the anterior imaginal ring make an "anterior plug" in the foregut-midgut junction. In C. analis, the proventricular invagination is neither retracted out nor does it straighten from the larval condition. There is a non-nucleated part at the posterior end of the proventricular invagination which bears a homogenous cytoplasmic content (Fig. 50). The foregut epithelium still remains cuboidal with indistinct cell boundaries and with a little loss of its cytoplasmic content. The muscles begin to histolyse.

In 5-6 days old pupa, there is no trace of any intima or its processes in the foregut lumen which has disintegrated already. The entrance from the foregut into the midgut is now completely obstructed by the proliferated cells of the anterior imaginal ring (Figs. 52 & 102). The "anterior plug" thus formed restricts the entrance of yellow bodies from the midgut into the foregut. The foregut epithelium is almost uniform in height. The cells lose a good amount of cytoplasm during and after the detachment of the intima. Due to

this loss, the cells become narrower, laterally compressed, and intercellular spaces appear between them. Nuclei become slightly elongated some of which touch the two lateral cell boundaries. The circular muscles and the sphincter muscles become completely histolysed. A few transitional cells (Figs. 51 & 52) near the proventricular invagination divide and give rise to a multinucleated structure which ultimately occupy the non-nucleated area (Fig. 50), and becomes packed with nuclei. These nuclei migrate towards the growing epithelial folds of the proventricular invagination and ultimately form the "proventricular valve" as in the adult insect. Intima along with very delicate processes are often found to develop in the late pupal foregut (Figs. 43 & 93).

Early adult changes : After the pupal moult, the epithelial cells of the foregut regain their normal shape and size in the new adult, i.e., the cells become cuboidal as described in adult histology (vide pp. 89-95). Soon after this, the intima gradually appears as a very thin and uniform layer. This new adult intima becomes gradually thicker and develops numerous spines. The proventricular needles make their full appearance before the emergence of the adult from the pabulum. The musculature also develops along with these changes.

Midgut

Prepupal changes : During early prepupa, when the larva becomes straight, the epithelial cells of the midgut become more crowded on the dorsal side than on the ventral wall (Figs. 16 & 17). The wall shows several infoldings of the epithelium which become more prominent on the dorsal side. The cells of these folds show more secretory activities than those situated in the unfolded areas of the epithelium. Due to this profuse secretion by the dorsal folded epithelium (Figs. 16 & 76), the region soon shrivels and loses its folded appearance.

A few cells at the extreme anterior and posterior ends of the midgut proliferate very actively and thus they act as an anterior and a posterior imaginal ring, respectively. The larval midgut continues to contract due to the exhaustion of the secretory epithelial cells which detach from the epithelium and are thrown into the lumen.

In the late prepupa the midgut cells secrete actively. A large number of secretory vesicles deposit in the gut lumen. Along with the secretion droplets, parts of epithelial cells also detach and mix up with the yellow body. Just before the last larval moult, the secretion and the detachment of cells stop for a while. The cells become short columnar type (Fig. 53). The lumen still contains degenerating cells and secretion vesicles. At this time, the cells of the anterior and

the posterior midgut become uniform and similar without any folding of the epithelium. Pajni (1967) also observed similar prepupal midgut epithelium in Callosobruchus maculatus (F.).

Pupal changes : With the onset of the pupal life, the above described midgut cells begin to degenerate rapidly and continuously upto the middle aged pupa (about 5-6 days old). The pupal period upto this time may be regarded as the histolysing and degenerating period of the midgut, and the time after this may be considered as the reconstructive and remodelling period.

In the beginning of the pupal life, the midgut epithelial cells begin to degenerate by a holocrine type of secretory process along with the detachment of the epithelial cells, as was observed in the prepupal condition. The distal ends of the cells dilate, the cytoplasm becomes vacuolate, and ultimately various secretory vesicles detach from the epithelium into the lumen. Along with those processes, sloughing off of individual cell or part of the epithelium also takes place which are thrown into the lumen (Figs. 54 & 55). These cells ultimately degenerate. The secretory products and the cast off epithelial cells mix up in a physiological process and ultimately form a mass of dense heterogenous product, generally known as the

"yellow body". Thus, in 1-2 days old pupa, the lumen of the midgut remains filled with pieces of yellow body. The larger bodies are round to oval with large nuclei which stain deeply with haematoxylin. The smaller bodies usually do not bear distinct nuclei. Besides these, there are many other smaller granules which remain suspended in a fluid medium.

Meanwhile, the anterior imaginal ring and posterior imaginal ring proliferate into numerous daughter nuclei which migrate with the stream of cytoplasm towards the middle from the two ends of the midgut for a short distance. These migrating cells form the plugs which block the two ends of the midgut. Thereby, the midgut lumen becomes a physiological chamber for metamorphosis of the midgut epithelium. The regenerative cells divide and become multicellular. These cells in the form of nidus remain adherent to the basement membrane. The degenerating epithelial cells still bear variously shaped nuclei at their bases. But very soon these are also thrown into the lumen and become degenerated. The remnants of the larval epithelium persist for a while until a new epithelium is formed.

In 4-5 days old pupa, the regenerative cells from the nidus constitute a very thin pupal epithelium representing almost a "regenerative syncytia", described

by Bushnell (1936) in a bruchid beetle, Acanthoscelides obtectus (Say). Soon these cells divide and form groups of imaginal cells at regular intervals (Fig. 55). These imaginal cells then divide mitotically and their daughter cells constitute the new adult midgut epithelium.

In 6-7 days old pupa, the epithelial cells of the flattened epithelium become tall and wide which now appear cuboidal. Yellow bodies of the gut lumen begin to decrease in volume with the growth of the new epithelial cells. The regenerative cells are uninucleated nidi, in the form of short cones (Figs. 45 & 95)

In 8-9 days old pupa, the epithelial cells are almost fully developed but they still remain short and wide (Fig. 56). The nuclei of the epithelial cells are prominent, round to oval, often with rough surface (Figs. 44 & 94). The regenerative cells multiplied to form a multinucleated nidus. The nuclei of this nidus are round, some of which are smaller than the others (Fig. 56).

Early adult changes : Just after the pupal-adult moult, the regenerative cells which were previously set in a nidus at the base of the pupal epithelium (Fig. 56), now continuously divide and give rise to a series of new cells. These cells push the basement membrane and the thin layer outwards. Thus the epithelium evaginates and ultimately forms a short pocket-like structure, known as

the "crypt" (Figs. 57 & 103). With the advancement of the adult stage, the crypts become more prominent.

It was clearly observed that there was a flow of the digested matters from the "yellow bodies" towards the developing crypts (Fig. 56), which indicates that "yellow bodies" have an important role in the development of the crypts. Besides, the gradual decrease in the volume of "yellow body" is proportionate to the development of the adult midgut epithelium. It was also observed that as soon as the adult midgut epithelium was formed, no trace of any yellow body or proliferated cells of the imaginal rings remain in the gut lumen. All these observations indicate that the yellow bodies are definitely a kind of nourishing material for the developing imaginal midgut epithelium in C. analis.

Hindgut

Prepupal changes : At the beginning of the prepupal life, the undigested food materials are passed from the midgut into the hindgut and are ultimately thrown out to the exterior. The lumen then becomes empty and narrower. The intima begins to separate from the epithelium. It is cast off in the lumen in the form of a thin ribbon. It is mostly aggregated at the posterior part of the colon, just in front of the rectum.

Pupal changes : In 1-2 days old pupa, the hindgut lumen becomes narrower and the epithelium appears to be uniform in thickness throughout its whole length. The detached intima of the larval epithelium still persists in the centre of the lumen. The muscle layers are histolysing. The cells of the posterior imaginal ring begin to proliferate.

In 3-4 days old pupa, the larval intima still persists in the lumen in the form of a thin membrane. It is most abundant in the pylorus and the rectum. Muscle layers histolyse completely; but the "pyloric sphincter muscles" and "diagonal rectal muscles" are found to be loosely and irregularly arranged (Figs. 58, 59, 104 & 105). Epithelial cells lose a certain amount of their cytoplasmic contents during the detachment of the intima, consequently the normal cell structure becomes changed. The cells become laterally compressed and intercellular spaces appear between them. The nuclei are round to oval with rough surface. A stream of proliferated cells from the posterior imaginal ring make a "posterior plug" at the midgut-hindgut junction. It restricts the entrance of yellow bodies from the midgut into the hindgut (Figs. 58 & 104). Two zones are recognizable in the posterior plug : a loose cellular zone in front and a densely packed nucleated zone on the posterior face. The zone in front bears a loosely arranged cell mass in a

cytoplasmic matrix. This stream of cells has a tendency to flow towards the anterior of the midgut lumen. The cells are comparatively very large and round, often with distinct large nuclei, comparable with the larger yellow bodies. These cells gradually become larger towards the anterior side and ultimately are set free from the stream into the lumen along with the yellow bodies. The densely packed nucleated zone bears numerous elongated nuclei which are arranged in a very compact manner in a thick cytoplasmic matrix. They together form a transverse row which make a strong barrier at the entrance of the pylorus, just in front of the origin of the Malpighian tubules.

Beside this, a thin and noncellular transverse septum is developed at the junction of the colon and the rectum which clearly separates the cryptonephridial part of the colon from the rectum (Figs. 59 & 105). This septum probably makes a temporary partition wall to protect and restrict the passing out of the histolysed matters of the major parts of the hindgut including cryptonephridia into the rectum.

In a 6-7 days old pupa, the epithelial cells of the hindgut become narrower with intercellular spaces. Thus the cells become laterally compressed. This is due to the loss of cytoplasmic contents during detachment of intima from the epithelium. The bases of the epithelial

folds remain empty i.e., there is no trace of any connective tissue or muscles (Figs. 47 & 97a,b).

The greater amount of the proliferated cells are found to migrate from both the anterior and posterior plugs region towards the midgut side of the lumen. There is no clear distinction between the two zones of the posterior plug cells since the nuclei of the densely packed nucleated zone gradually transform into the normal proliferated cells similar to those in the anterior loose cellular zone of the plug (Figs. 60, 61, 106 & 107).

In a 8-9 days old pupa, there is an uniform distribution of the proliferated cells in the posterior plug region. The cells are mixing with the yellow bodies. The digestion or dissolution and absorption of these cells by the epithelial cells is continued from this time till the formation of the new adult epithelium. Meanwhile, a few cells of the extreme anterior end of the pylorus, near the base of the Malpighian tubules, become flattened and extend into the lumen and form a shelf-like projection. This structure obstructs the back flow of the stream of cells and directs them to move forward towards the midgut lumen (Figs. 60, 61, 106 & 107). The epithelial cells begin to regain their normal shape and size. The cells gradually become cuboidal in the ileum, colon and rectum; and more or less columnar in the pylorus. Intercellular spaces still persist in the epithelium. In the anterior end, the rectal

epithelium begins to differentiate into rectal pads by the widening of the epithelial folds, whereas in the posterior region rectal papillae develop by the greater extension of the epithelial folds into the lumen (Figs. 49 & 99). This remodelling of the rectal epithelium into two distinct regions continue upto the adult stage. The muscles which histolysed earlier begin to reappear.

Early adult changes : After the pupal adult moult, the hindgut epithelium regain their normal shape and size, probably, by the absorption of the digested matters from the gut lumen. Meanwhile, the septum separating the colon from the rectum disappears and consequently a continuous passage is formed again. The intima makes its first appearance as a very thin layer lining the epithelium. It attains its full dimension including the spines in the rectum within a short time while the adult remains still in the pabulum. The muscles including the "sphincter muscles" of the pylorus and "diagonal muscles" of the rectum are reconstituted in a regular pattern as described in the histology of the adult hindgut.

III.B. 2(a). HISTOLOGY OF THE LARVAL GUT OF C. CHINENSIS

The essential histological elements of the larval alimentary canal of C. chinensis is almost identical with that of C. analis. The main difference is that the epithelial cells of respective regions of the gut of these two insects vary in dimension. The cells in C. chinensis appear smaller than those in C. analis. Since there is no special distinguishable histological difference in the larval gut of C. chinensis and C. analis, attempts have been made to describe them only briefly to avoid repetition.

The foregut epithelium is folded, and consists of cuboidal cells which are very similar to that of C. analis (vide pp. 59-67). The intima also bears "pharyngeal spines" and "proventricular lobes with simple spines". The oesophageal or proventricular invagination is quite similar to that of the non-lobular portion of the invagination of C. analis. However, measurements of some of the histological elements at different regions of the foregut of C. chinensis is shown in the Table 11 (cf. C. analis, pp. 60-66).

Table 11. Measurements of different histological elements of the foregut of 4th instar of C. chinensis.

Regions of alimentary canal	Epithelium (μm)			Intima (μm)	Circular muscle (μm)
	Cell		Nucleus		
	Height	Width	Diameter	Thickness	Thickness
Pharynx	1.5-4	2-5	1-2	0.5-2.5	2-4
Oesophagus	3-4	5-6	2-3	0.5-1.5	1.5-3
Proventriculus	4-6	4-6	2.5-3.5	0.5-1.5	1.5-3

The anterior midgut epithelial cells of C. chinensis are mostly of tall columnar type. But, a few cells towards the posterior end of the anterior midgut are comparatively wider than the others. The cells of the posterior midgut are also tall columnar but they are smaller in dimension than those of the anterior midgut. Similar midgut epithelial cells were also found in the midgut of C. analis. The regenerative cells in the form of nidi are scatteredly distributed in the gut epithelium. These are also identical with those of C. analis. The midgut cells of C. chinensis have been measured and tabulated below (Table 12) (cf. C. analis, pp. 68-72).

Table 12. Measurements of different histological elements of the midgut of 4th instar of C. chinensis.

Midgut regions	Epithelium (μm)			Brush border (μm) Thick- ness	Regenerative cells (μm)			Circular muscle layer (μm) Thick- ness
	Cell		Nucleus Diameter		Height	Width (at the base)	Nucleus Diam- eter	
	Height	Width						
Anterior midgut : Ant. section	30-40	12-20 (apex) 4-6 (base)	4-7	1.5-2	7-10	13-18	4-5	1-1.5
Post. section	14-17	9-13	5-8	1.5-2	7-10	13-18	3-5	1-1.5
Posterior midgut :	12-17	5-7	3-5	1.5-2	4-7	4-7	2-3	1-1.5

The histology of the larval hindgut of C. chinensis is also similar to that of C. analis. The cells of the pyloric epithelium are narrower than the cells of the other parts of the hindgut. Whereas, the epithelial cells of the ileum, colon and the rectum are typically cuboidal with slight variation in dimension as shown in the Table 13. Other histological elements of the hindgut are also similar to those of C. analis (vide pp. 72-80).

Table 13. Measurements of different histological elements of the hindgut of 4th instar of C. chinensis.

Hindgut regions	Epithelium (μm)		Intima (μm)	Circular muscle layer (μm)	
	Cells				Nucleus
	Height	Width	Diameter	Thickness	Thickness
Ileum	2-4	2.5-4.5	1-2.5	1-2	1.5-2
Colon (anterior)	3-6	3.5-7	2-3	1-2	2-2.5
Colon (posterior)	4-7	5-9	2-3.5	1-2	1-2
Rectum	2.5-4	2.5-4	2-3	1-2	1.5-2.5

III.B. 2(b). HISTOLOGY OF THE PUPAL GUT OF C. CHINENSIS

As in C. analis, the pupal fore-and hindgut of C. chinensis consist of a characteristic shrivel type of epithelium. This is due to loss of certain amount of cytoplasmic contents along with the histolysis of intima during prepupal condition and larval-pupal moult. The intima, if it is found, never bears any processes like the "pharyngeal or proventricular or rectal spines". The musculature is very weakly developed.

The early pupal midgut of C. chinensis consists of a degenerating epithelium, as a result the anterior and posterior midgut can not be distinguished from each other from their histological appearance. The regenerative nuclei remain distributed in a thin film of cytoplasm along with the basement membrane. But soon, the adjacent cell-boundaries appear and the epithelial cells gradually become distinguishable into a definitive pupal epithelium as described in C. analis (vide pp. 85-86). In this condition, the epithelial cells measure 6-10 μm in height and 4-6 μm in width, and the nuclei measure 2.5-5 μm in diameter. In the late pupal epithelium, the regenerative cells are found in clusters as in C. analis.

III.B. 2(c). HISTOLOGY OF THE ADULT GUT OF C. CHINENSIS

The histology of the adult alimentary canal of C. chinensis is also identical with that of C. analis. They differ only slightly in dimension of cells and thickness of different layers (e.g. muscle layer and intima).

The foregut epithelium is folded with typical cuboidal cells of different dimensions in different regions. The foregut intima of C. chinensis is provided with short "spines" in the pharynx and oesophagus; and long "needles" in the proventriculus, which are morphologically very similar to those of C. analis, but shorter than the later (vide pp. 89-93). Other essential histological structures are also similar to those of C. analis. A table showing the measurements of the cells in different regions of the foregut of C. chinensis is given in Table 14 (cf. C. analis). Histologically, the proventricular invagination is also identical with that of C. analis.

Table 14. Measurements of different histological elements of the foregut of adult C. chinensis.

Foregut regions	Epithelium (μm)		Intima (μm)	Circular muscle (μm)	Spines/ needles (μm)	
	Cells Nucleus					
	Height	Width	Diameter	Thickness	Thickness	Length
Pharynx	2-4	2-5	1.5-3	1.5-2.5	5-7	2-4
Oesophagus	2-4	2-5	1.5-3	1.5-2.5	3-5	3-5
Proventri- culus	2-4	2-5	2-4	1.5-2.5	3.5-5	10-20

Similar to that of C. analis, there is no basic difference between the epithelium of the anterior and posterior midgut in C. chinensis. The cells are typically columnar. They are much longer in the anterior midgut than in the posterior midgut. Normally, brush-border is not discernable due to continuous secretory activity of the epithelial cells. The nuclei are elongated in the epithelial cells but round in the crypt cells. Besides these, other histological elements, viz., muscles, basement membrane etc. are also quite similar to that of C. analis. Peritrophic membrane is not found in C. chinensis also. The Table 15 showing the measurements of the midgut epithelial cells and other structures is given below to compare with those of C. analis (vide pp. 96-97).

Table 15. Measurements of different histological elements of the midgut of adult C. chinensis.

Midgut regions	Epithelium (μ m)		Brush border (μ m)	Circular muscle layer (μ m)	Crypt (μ m)			
	Cells				Cells	Nucleus		
	Height	Width	Diameter	Height			Width	Diameter
Anterior midgut	12-16	3-3.5	2.5-6	1-1.5	1-1.5	6-8	6-8	3-6
Posterior midgut	9-12	2.5-3.5	2-5	1-1.5	1-1.5	5-7	5-7	2.5-5

The epithelium of the adult hindgut of C. chinensis consists of cuboidal cells of various dimensions in different regions. The pyloric epithelial cells are narrower whereas the others are cuboidal, as described in C. analis (vide pp. 98-103). But these cells (in C. chinensis) differ from those of C. analis in their dimensions only. The musculature, epithelial foldings, intima and its projections are also similar to those of C. analis. Measurements of the histological elements of the adult alimentary canal of C. chinensis is given in the Table 16 (cf. C. analis).

Table 16. Measurements of different histological elements of the hindgut of adult C. chinensis.

Hindgut regions	Epithelium (μm)		Intima (μm)	Circular muscle (μm)	
	Cells				Nucleus
	Height	Width	Diameter	Thickness	Thickness
	Ileum	3.5-6	3.5-6	3-5	1-1.5
Colon	3.5-6	4-7	3-5	1-1.5	1-1.5
Rectum	2.5-5	2.5-5	2.5-5	1.5-2.5	1.5-2.5

III.B. 2(d). METAMORPHIC CHANGES (HISTOLOGICAL) IN THE
ALIMENTARY CANAL OF C. chinensis

Since the histo-metamorphic changes in the alimentary canal of C. chinensis is quite similar to that of C. analis, they have been they have been described very briefly.

a) The histological changes in the alimentary canal of C. chinensis during larva to larva moult is not very prominent. There is no degeneration or any remodelling of the epithelial cells, nor there is any drastic change in other histological elements (viz., muscles, intima, etc.) during this course. However, similar to that of C. analis, the following changes were noticed in C. chinensis (cf. C. analis).

(i) The alimentary canal, specially the midgut region, increases considerably in length and diameter during successive instars. This is due to increase in the number of cells by division in the growing epithelium during development of the larva.

(ii) As in C. analis, the proventriculus also develops in C. chinensis in the same manner at the same time (i.e., after 2nd larval moult) (cf. C. analis).

b) The histological changes in the alimentary canal of C. chinensis during prepupal condition, larval-pupal moult, and pupal stage is very remarkable and quite similar

to those of C. analis. However, the following important aspects can be mentioned here (cf. C. analis).

(i) During prepupal condition, the midgut epithelial cells begin to degenerate. This process of epithelium degeneration continues upto the early pupal condition. As a result the gut becomes very short and its wall appears very thin, in the middle aged pupa.

(ii) Meanwhile, the regenerative cells multiply and distribute numerous daughter nuclei over a thin film of cytoplasm. But soon adjacent cell-boundaries appear thus forming a thin cellular epithelium on the basement membrane.

(iii) During pupal period, the midgut lumen remains filled with "yellow bodies" as described earlier in C. analis (vide pp. 110-113).

(iv) In the late pupa, the epithelial cells of the midgut become prominent but these cells are still devoid of brush-borders.

(v) The fore- and hindgut epithelium does not degenerate but cytoplasmic diminution occurs during this time. The intima and its processes histolyse completely.

c) The following histological changes have been observed in the alimentary canal of C. chinensis during pupal-adult moult and early adult condition (cf. C. analis).

(i) As in C. analis, the fore-and hindgut epithelium of C. chinensis redifferentiate by regaining their lost amount of cytoplasm and re-appearance of the intima.

(ii) The "crypts" develop during early adult condition (until it remains in the pabulum of the pulse), from the cluster of regenerative cells. These cells multiply to give rise to the daughter cells which ultimately push the basement membrane outward and thus form the crypt, as in C. analis (vide pp. 113-114).

III.C. DISCUSSION

ALIMENTARY CANAL

Foregut and hindgut

Ameen (1969) while comparing the metamorphosis of the foregut and the hindgut of P. albimana (Diptera : Ptychopteridae) with those of other insects, distinguished two main types of epithelial changes which take place during larval to the adult transformation, viz., (a) cellular redifferentiation, and (b) regeneration from specialised imaginal ring after degeneration of the larval epithelium. He also mentioned that both the processes may take part in varying degrees. However, in C. analis and C. chinensis, the metamorphosis of the foregut and the hindgut is very simple which follows the 1st type with certain exceptions. The metamorphosis of the foregut and the hindgut are almost identical and therefore, are discussed together with that of other beetles and insects of other orders.

Coleopteran insects

In Hydrophilus (Deegener, 1900) the whole epithelium of the foregut degenerates completely during metamorphosis; the anterior imaginal ring becomes very active which proliferates numerous daughter cells. These proliferated cells ultimately form the adult foregut epithelium in place of the larval epithelium. The

metamorphosis of most of the hindgut epithelium is similar to that of the foregut, where the posterior imaginal ring becomes active and proliferates numerous daughter cells. These cells ultimately replace the major part of the anterior hindgut epithelium. Only, a little portion at the end of the hindgut is remodelled by the division of the persisting larval cells.

In Cybister roeseli Curtis (Deegener, 1904) the larval foregut epithelium does not degenerate, rather it redifferentiates. During this redifferentiation, the anterior imaginal ring supply a few proliferated cells which make an addition to the larval epithelial cells. The larval hindgut epithelium also redifferentiates into the adult epithelium without cellular breakdown or degeneration. As in the foregut, the proliferated cells from the posterior imaginal ring make a small addition to the larval epithelial cells during metamorphosis.

In Calandra oryzae (Murray & Tiegs, 1935) the larval foregut epithelium degenerates completely. It is replaced by a new epithelium formed by the proliferated cells of the anterior imaginal ring. The larval hindgut epithelium also degenerates completely and therefore replaced by a new epithelium formed by the proliferated cells of the posterior imaginal ring.

In Tenebrio molitor (Rengel, 1896) the larval foregut epithelium is remodelled by the cells of the

posterior end of the foregut. The remains of the larval epithelium are absorbed by the developing adult epithelium. However, Rengel did not regard the posterior foregut cells as the anterior imaginal ring. As in the foregut, the hindgut epithelium is also remodelled by the cells of the anterior end of the hindgut. But these cells were not regarded as the posterior imaginal ring. The remains of the larval hindgut epithelium are also absorbed by the developing adult epithelium.

In Galerucella (Poyarkoff, 1910) and Leptinotarsa decemlineata (Say) (Patay, 1939) the larval foregut epithelium does not degenerate or destroy. The epithelium simply redifferentiates with a little loss in cytoplasmic contents of the cells during histolysis. Chromatin globules also liberate out from the cells after detachment of the intima. As a result the epithelial cells change in shape and size. In certain condition nuclei also divide. Ultimately, a new adult foregut epithelium becomes established. The same procedure is also followed by the hindgut epithelium during metamorphosis in these beetles.

Insects orders other than Coleoptera

In the ant Lasius flavus (Karawaiew, 1898) the larval foregut epithelium does not degenerate. A few cells at the posterior end of the larval foregut divide, make an addition to the normal epithelium and ultimately

redifferentiate into the adult epithelium. Karawaiew did not regard these type of cells as the anterior imaginal ring. The larval hindgut epithelium does not degenerate during metamorphosis. Most of the hindgut epithelium redifferentiates. In this case, the larval colon epithelium is characterised by the presence of alternate lamellae of small and large cells. During metamorphosis, the large cells degenerate whereas the small cells redifferentiate into the adult colon epithelium.

In another ant Formica (Perez, 1902) the anterior and posterior parts of the larval foregut degenerate whereas the oesophageal region does not degenerate. The anterior part of the adult epithelium is developed from the special histoblasts of the developing mouth parts and the posterior part from the anterior imaginal ring. The oesophagus only redifferentiates during metamorphosis. The anterior part of the hindgut epithelium develops from the posterior imaginal ring, middle part from the smaller persisting cells, and the posterior part from the epidermal invagination during metamorphosis.

In the honey bee (Oertel, 1930) certain imaginal cells at the posterior end of the foregut representing the "anterior imaginal ring cells" are responsible for the development of the foregut epithelium. Similarly, a few cells at the anterior end of the hindgut representing

the "posterior imaginal ring cells" are responsible for the formation of the hindgut epithelium during metamorphosis.

In many dipteran insects the anterior and the posterior imaginal rings are mostly responsible for the development of the adult foregut and hindgut epithelium respectively. In addition to these rings other histoblastic cells also take part in the formation of the adult foregut and hindgut epithelium. These type of cells have been called "anal disc" in the muscid flies (Kowalewsky, 1887); "imaginal disc" of the pharynx in Gastrophilus (Vaney, 1902) and muscid flies (Perez, 1908a, 1908b & 1910a, 1910c); "Labial bud" of the foregut and "ectodermal imaginal disc" in Drosophila melanogaster (Robertson, 1936).

In P. albimana (Ameen, 1969) the adult foregut epithelium develops from the three centres of the larval foregut. The anterior part develops from the imaginal disc of the mouth parts (labrum & labium), middle part from the persisting larval cells, and posterior part from the anterior imaginal ring. The hindgut similarly develops from three centres. The anterior part from the posterior imaginal ring, middle part from the persisting larval cells and the posterior part from the anal disc.

From the study of the metamorphosis of the foregut and the hindgut of some mosquitoes, viz., Culex (Thompson, 1905), Aedes dorsalis (Richins, 1938), Culex pipiens (Berger, 1938), and Aedes aegypti (Risler, 1961), it was revealed that the foregut epithelium of the adult mosquitoes develops mostly from the persisting larval cells. The anterior imaginal ring makes an addition in the formation of oesophageal invagination and diverticula. Similarly, the hindgut epithelium is developed mostly from the persisting larval epithelial cells or products of the cells (due to successive division), but not from the posterior imaginal ring. The imaginal ring and the anal disc make only an addition to the formation of the anteriormost part of the hindgut (pylorus) and the posteriormost part of the hindgut (rectum) respectively.

The above review reveals that the metamorphosis of the foregut of C. analis and C. chinensis closely resembles those of two other beetles, viz., Galerucella and Leptinotarsa, described by Poyarkoff (1910) and Patay (1939) respectively. However, in C. analis and C. chinensis, the larval foregut epithelium is never destroyed or degenerated completely. But during histolysis, along with the detachment of the intima, a little amount of the cytoplasmic contents of the epithelium is lost. As a result the cells become narrower with intercellular spaces appearing during pupal period. The cells attain

their normal adult condition by absorbing the contents of the lumen as soon as the pupal-adult moult is over, i.e., during early adult condition. Thus, the foregut epithelium is redifferentiated except at the proventricular valve region. The proventricular valve is formed by the division of a few cells at the extreme posterior end of the proventriculus (i.e. the posteriormost end of the foregut). The newly formed cells push the posterior part of the proventricular epithelium to become infolded so as to form the "proventricular valve" in the adult.

Similar foregut epithelium and proventricular valve formation during metamorphosis was observed by Dobrovsky (1951) in Apis mellifera L., and Nitschmann (1959) in Myrmica ruginodis. According to Dobrovsky, "the foregut undergoes a cytoplasmic depletion followed by a cellular rejuvenation without an epithelial breakdown", and "the posterior part of the proventriculus gives rise to the entire proventricular valve of the adult". Kathuria (1972) regarded the "proventricular valve" as the "oesophageal valve" in Papilio aristolochiae F. According to him the "anterior imaginal ring" is of foregut origin. This ring divides mitotically and extends forward in the posterior region of the foregut in prepupa and early pupa and later forms the oesophageal valve. Meanwhile, other two structures also develop from this imaginal ring, viz., proventriculus and ventral oesophageal diverticulum.

Thus, the cellular region towards the posterior end of the foregut which has the power of division or multiplication to constitute or reconstitute or to make an addition to the developing foregut epithelium, have been variously named by many workers, viz., as "regenerative region" by Deegener (1908), Bordas (1911), and Hufnagel (1918); as a "persistent embryonic region" by Perez (1910a); as an "anterior interstitial ring" by Henson (1931); as "histoblastic disc" by Gray (1931); and an "anterior imaginal ring" by Ameen (1969) and Kathuria (1972), and many other workers. Among all these terms the "anterior imaginal ring" is well established now. It was first observed by Kowalewsky (1887) in muscid flies. In the present investigation on C. analis and C. chinensis, a few cells at the extreme anterior end of the midgut which have great proliferating power have been considered as the anterior imaginal ring, which only contribute to the midgut metamorphosis. In addition, some of the transitional cells (vide pp. 108-109) just in front of the anterior imaginal ring divide several times, but not as fast as the anterior imaginal ring cells. The daughter cells of these transitional cells contribute greatly to the formation of the proventricular valve only.

Like Galerucella (Poyarkoff, 1910) and Leptinotarsa (Patay, 1939), there is no appreciable

metamorphic change in the histology of the larval hindgut to the adult hindgut in C. analis and C. chinensis. Similar to that of the foregut, the hindgut epithelial cells lose some amount of cytoplasmic contents during detachment of the larval intima. The larval cells gradually redifferentiate into the adult form by absorbing the lumen contents during metamorphosis. Meanwhile, the rectal epithelium differentiates into an anterior "rectal pad" region and a posterior "rectal papillae" region as described earlier.

Midgut

In insects the larval midgut epithelium is normally replaced by an adult epithelium during metamorphosis. This involves a simultaneous degeneration of the old epithelium and a reconstitution or regeneration of a new epithelium. There are mainly two types of larval cells involved in the replacement of the old epithelium. During metamorphosis these cells multiply repeatedly and constitute the new adult epithelium.

(i) Special cells at the two ends of the midgut which have retained the characteristics of embryonic cells, i.e., the power of rapid cell proliferation. These groups of cells ultimately form the "anterior imaginal ring" and "posterior imaginal ring".

(ii) The regenerative cells either singly or in the form of nidi or crypts, scattered over the entire larval midgut epithelium.

The adult midgut epithelium may be developed directly from the larval cells with a single degeneration of the larval epithelium. The reconstituting cells (i.e., the regenerative cells or imaginal ring cells) may constitute an intermediate definitive pupal epithelium, e.g. in Callosobruchus maculatus F. (Pajni, 1967), Pieris rapae L. (Yu, 1980), and in the present investigation on C. analis and C. chinensis. In a single degeneration process, the reconstituting cells may not constitute a definitive (transitional) pupal epithelium, e.g. in Ptychoptera albimana F. (Ameen, 1969). In these instances, the degeneration of the epithelium during metamorphosis takes place only once i.e., during the larval-pupal moult. After that the epithelium grows independently without any degeneration and even regeneration.

The adult midgut epithelium may also develop indirectly from the reconstituting larval cells (i.e., regenerative cells or imaginal ring cells) with double degeneration of the epithelium. It includes first the degeneration of the larval and then the pupal epithelium respectively. First, the larval epithelium degenerates during larval-pupal moult and then the pupal epithelium degenerates during pupal-adult moult. When the pupal epithelium degenerates completely, it may be called as

"complete double degeneration process", e.g. in Acanthoscelides obtectus (Say) (Bushnell, 1936) and Chilomenes sexmaculata (Kathuria, 1971). But, when the pupal epithelium degenerates partly or incompletely, it may be called as "incomplete double degeneration process", e.g. in Nasonia (Tiegs, 1922).

Comparison with two bruchid beetles

Metamorphosis of the midgut of C. analis and C. chinensis is very interesting because it shows certain contrasting features when compared to two other allied beetles. It closely resembles that of Callosobruchus maculatus, described by Pajni (1967), except a few minor differences. But it differs greatly from that of Acanthoscelides obtectus, described by Bushnell (1936).

Pajni's (1967) short description of the metamorphosis of the midgut of C. maculatus is very inadequate to describe the process of larval epithelial degeneration as well as reconstitution of a pupal epithelium and its subsequent development into the definitive adult form. He did not explain the process of development of the crypts in the adult epithelium. All these need more elaborate description to explain the metamorphic changes of the midgut of C. maculatus. In C. analis a precise description has been given to explain the metamorphic processes involved. Moreover, Pajni (1967) did not mention the fate and role of any imaginal ring. During the present investigation, both

the anterior and posterior imaginal rings were clearly observed. The proliferated cells of these rings do not take direct and active part in the adult epithelium formation. These cells initially act as the "plug cells" and later as "nourishing cells" to make up the developing adult midgut epithelium of C. analis and C. chinensis.

Bushnell (1936) described the metamorphosis of the midgut of another bruchid beetle, A. obtectus. In this context it is noteworthy to discuss the following points :-

(i) The epithelial degeneration takes place twice in A. obtectus, first during larval-pupal and second during pupal-adult moult. Whereas, in C. analis and C. chinensis (present investigation), and in C. maculatus (Pajni, 1967), epithelial degeneration takes place only once during the larval-pupal moult.

(ii) As a result of "pseudo reduction" (called by Bushnell), the "smaller nuclei" of the larval epithelium with a little amount of cytoplasm form a "regenerative syncytium" which is the sole agent of adult epithelium formation. The "larger nuclei" and "smaller nuclei" of this "regenerative syncytium" ultimately form the pupal and adult epithelium respectively. This type of metamorphic change is not known to occur in any other beetles; and it also does not agree with the present investigation.

(iii) Regarding the crypt formation, Bushnell (1936) pointed out that these (called mesenteric caeca

by him) are formed by the aggregation of small spindle-shaped cells which proliferate from the anterior and posterior imaginal rings. This type of crypt formation is very unusual. In the present investigation in C. analis and C. chinensis, it was found that the stream of proliferated cells from the anterior and posterior imaginal rings never flow through the base of the epithelium or touch at any point of the gut, which may form an epithelium, unlike that of A. obtectus. Pajni (1967) also did not report any such occurrence in C. maculatus; rather his short comment regarding the crypt formation supports the present investigation on C. analis and C. chinensis.

In C. analis and C. chinensis the crypts develop from the persisting larval regenerative cells. Some of the daughter regenerative cells instead of forming the adult epithelium become isolated and distribute over the basement membrane. During late pupal period they divide to form the multinucleated nidi. With the onset of the adult life (just after the pupal-adult moult) the nuclei of each nidus multiply, become cellular, and pushes the basement membrane outwards in an evaginating manner and thus form the crypt of the midgut.

Comparison with other beetles

In a coccinellid beetle, Chilomenes sexmaculata (Kathuria, 1971), degeneration of the epithelium takes place twice, following a "sloughed off" or "strip off" process during each of the larval-pupal and pupal-adult

moult. The regenerative cells replace the old epithelium. Similar replacement of epithelium during larval-pupal and pupal-adult moults was also observed in Anthrenus (Möbusz, 1897), Cybister roeseli (Deegener, 1904), Galerucella (Poyarkoff, 1910), and Leptinotarsa (Patay, 1939).

In many beetles, degeneration of the larval epithelium takes place as a secretory process instead of its being "sloughed off", e.g. Tenebrio molitor (Patay, 1942), Choleva fagniezi J., Spheophyes lucidulus and Trogloedromus bucheti gaveti Del. (Strambi, 1976). In C. analis and C. chinensis, the larval epithelium was found to degenerate through a holocrine type of secretion.

Metamorphosis of the midgut in Tenebrio molitor L. is controversial. Rengel (1896) first observed that the adult epithelium is derived from larval regenerative cells. But Patay (1942) observed that the adult epithelium in this insect is derived from the migrating proliferated cells of the anterior imaginal ring. The imaginal ring developed from the posterior end of the foregut, which Henson (1946) regarded as the "reactivated oral half of the blastopore".

The anterior imaginal ring is very active in many beetles. The imaginal ring proliferate numerous daughter cells which gradually migrate to form ultimately an adult epithelium, e.g. in Galerucella (Poyarkoff, 1910),

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Calandra oryzae (Mansour, 1927), Calandra (Murray & Tiegs, 1935), and Leptinotarsa (Patay, 1939).

In C. analis and C. chinensis the anterior and posterior imaginal rings were found to be very active in cell proliferation. But the proliferated cells were never found to take direct part in the formation of the gut epithelium. Stream of proliferated cells first form two "plugs" at the two ends of the midgut which converted the midgut lumen as a "physiological chamber". A small portion of the stream of proliferated cells from the anterior and posterior imaginal rings were found to migrate towards the proventricular invagination and anterior end of the pylorus respectively. But they were never found to touch the adjacent epithelium. With the development of the adult epithelium the proliferated cells became digested in the lumen along with the "yellow body" and absorbed by the new epithelium.

Insects of orders other than Coleoptera

In Pieris rapae L. (Yu, 1980), the adult epithelium develops from the larval regenerative cells. The regenerative cells multiply during histolysis of larval gut epithelium and reconstitute a perfect pupal epithelium. The adult epithelium is directly formed from this pupal epithelium without passing through the second degenerative process during pupal-adult moult.

et al.

In Bombyx mori L. (Verson, 1905; Waku & Sumimoto, 1971 ; and Kobayashi, 1978), the regenerative cells of the larval midgut proliferate rapidly during larval-pupal moult which replaces the larval epithelium, thus establishing a new pupal epithelium, consisting of one type of cells. The pupal epithelium degenerates again during pupal-adult moult. A new adult epithelium is constituted by persisting regenerative cells. A similar picture was also observed in Galleria mellonella L. (Tchang, 1929), and in Hyalophora cecropia (L.) (Judy & Gilbert, 1970).

In Ptychoptera albigana (Ameen, 1969) most of the adult midgut epithelium is derived from the larval regenerative cells, but a little portion towards the anterior and posterior ends of the midgut are derived from the migrating cells of the anterior and posterior imaginal ring cells, respectively. In this case, a definitive pupal epithelium was wanting.

In Calliphora erythrocephala (de Priester, 1972, and Bautz, 1979), the regenerative cells replace the larval epithelium. The larval epithelial cells remain aggregated with the cellular granules in the gut lumen, which are ultimately digested and absorbed during adult epithelium formation.

Total replacement of the adult midgut epithelium is very common in hymenopteran insects. Replacement of

the epithelium is made by the larval regenerative cells. The replacement of the epithelium occurs twice during larval-pupal and pupal-adult moults. This type of metamorphic changes have been observed in Apis mellifera (Dobrovsky, 1951); Solenopsis fugax Latr. (Glöckner, 1958); Myrmica ruginodis (Nitschmann, 1959); and in Formica polyctena (Schmidt, 1964). However, in Nasonia (Tiegs, 1922), a pupal epithelium develops from the larval regenerative cells after total degeneration of the larval epithelium. Then the adult epithelium is developed from the posterior or basal half; whereas the anterior half of the pupal epithelium degenerates completely which never takes part in adult epithelium formation.

In odonates, e.g. Aeschna cyanea (Andries, 1972, 1975, & 1976), the larval midgut epithelium undergoes histolysis and ultimately degenerates in the lumen. A new imaginal epithelium is developed from the regenerative cells of the nidi. The imaginal epithelium undergoes redifferentiation by cellular growth and multiplication of certain cytoplasmic organelles.

"Yellow body"

The term "yellow body" is commonly used to denote the midgut contents of certain metamorphosing insects during pupal condition. The lumen of the pupal midgut

contains a stage-specific emulsion consisting of various distinct nucleated matters and debris shed from the degraded larval midgut epithelium.

Yellow body was first described by Dufour (1846). Later on, the term was used by many subsequent workers, viz., Ganin (1876); Kowalewsky (1887); van Rees (1888); Perez (1910a & 1910c); Mansour (1927); Gray (1931); and Bushnell (1936), and many others.

Butler & Leone (1966) in Tenebrio, and Seong (1981) in Bombyx mori L., observed that the midgut of the pupa contains a stage-specific protein known as the "pupal protein". Again, stage-specific proteins remain correlated with "pupal gene sets" as observed by Thomson (1975), Chen (1978), and Seong (1981). Thus, it is evident that pupal gut contents definitely bear certain special characteristics for the development of the adult gut epithelium during metamorphosis.

In C. analis and C. chinensis yellow bodies are prominent which fill up the whole lumen of the pupal midgut. These can be differentiated into three categories as follows:- large and nucleated bodies, small and non-nucleated bodies, and minute granular bodies, which remain mixed up in a fluid medium of the midgut lumen. The yellow bodies combine with the proliferated cells of the anterior and posterior imaginal rings towards the end of the pupal life. These are absorbed together by the developing adult epithelium.

The amount of yellow bodies along with the proliferated cells gradually decrease which is proportional to the advancement of the adult epithelium. The mixture of the yellow bodies and the proliferated cells disappear as soon as the adult epithelium is fully formed. Thus in this case, the yellow bodies together with the proliferated cells may be considered as a "special nourishing matter" for the developing adult midgut epithelium. Kathuria (1971) also observed yellow bodies in the midgut of a coccinellid beetle, Chilomenes sexmaculata and considered them as to provide nourishment to the growing imaginal cells.

IV. MALPIGHIAN TUBULES

A. ANATOMY

IV.A. 1(a). ANATOMY OF THE LARVAL MALPIGHIAN TUBULES OF C. ANALIS

Six cryptonephridic Malpighian tubules are present in the larva of C. analis. Depending on their structural peculiarities and distribution in the body cavity they can be grouped into three pairs, viz., 1st, 2nd and 3rd pairs of Malpighian tubules. Almost similar three pairs of Malpighian tubules were represented in a figure in the larva of C. maculatus (Vats, 1976).

Free tubules

In gross appearance, the 1st and 2nd pairs of Malpighian tubules are almost identical, whereas the 3rd pair is quite dissimilar and are easily distinguishable from the other Malpighian tubules (Figs. 1a, 1b, 2, 3 & 4).

The 1st and 2nd pairs of Malpighian tubules are very long, extend anteriorly upto the anterior end of the midgut then turn back posteriorly and follow a zigzag and convoluted course. These tubules are wider and irregularly dilated, translucent and powdery-white in colour, excepting a little portion towards the two ends, i.e., near the origin and the insertion point. These end-parts closely resemble the 3rd pair of Malpighian tubules. The

1st pair of Malpighian tubules are slightly longer than the 2nd pair and are distributed on the dorso-lateral sides of the midgut. The 2nd pair of Malpighian tubules are shorter than the 1st pair and are distributed on the ventro-lateral aspect of the midgut. The free portion of the 1st pair of Malpighian tubules measure 1.6 to 1.8 mm in length in the early 2nd instar, and 8.0 to 10.0 mm in the 4th instar. The second pair of Malpighian tubules measure 0.8 to 1.1 mm in length in the 2nd instar, and 4.6 to 5.0 mm in the 4th instar. The diameter of these tubules measure (at the wider part) 0.02 to 0.03 mm in the 2nd instar, and 0.06 to 0.1 mm in the 4th instar larva.

The 3rd pair of Malpighian tubules are very short in comparison with the other two pairs. They are narrower and almost uniform in diameter throughout their length, and opaque-white in colour. These tubules instead of running forward, extend directly posteriad along the sides of the ileum and then meet with the colon, thus appearing like two side-strings of the convoluted ileum. Excluding the cryptonephridial portions, their free tubules measure 0.3 to 0.35 mm in length and 0.015 to 0.03 mm in diameter in the 2nd instar; and 1.6 to 1.7 mm in length and 0.04 to 0.06 mm in diameter in the 4th instar larva.

All these three pairs of Malpighian tubules originate independently from the anteriormost end of the

pylorus, i.e., from the midgut-hind junction. They arise symmetrically from the pylorus and are equidistant in position. In a fresh dissection, when a portion of the midgut and the hindgut is pulled out in opposite direction, it automatically tears up through the posterior end of the midgut leaving all the tubules in attached condition with the pyloric end of the hindgut. In this case, the arrangement of the Malpighian tubules on the pylorus looks like a whorl of a flower in an acropetal succession. There is an arrangement of Malpighian tubules entering into the gut via ampullae or common stalks in various insect larvae, e.g., in coleopteran insect, Phyllophaga auxia (Berberet & Helms, 1972); in lepidopteran, Corcyra cephalonica Staint (Khare, 1975), Elasmopalpus lignosellus (Beals & Berberet, 1976), Calogramma festiva (Mohamed & Murad, 1977 & 1979a); and in orthopteran larvae, Schistocerca gregaria (Savage, 1956) and Carausius morosus (Savage, 1962). However, there is no such ampular arrangement or common stalk system of Malpighian tubules near the origin or opening into the gut in C. analis larva. This is similar to those observed in the larvae of the following beetles, e.g., Placoma, Lichnanthe, Dichelonyx and Polyphylla (Areekul, 1957); Prionus laticollis (Benham, 1970); and Tribolium castaneum (Ameen & Rahman, 1973).

After passing through the haemocoel, the three pairs of Malpighian tubules adhere to the colon in three successive annulated areas. Thus, the three pairs representing their entrance into the cryptonephridial chamber through three separate circlets, nearer to each other, and the two Malpighian tubules of a pair are placed to the opposite sides of the colon. Hence, six Malpighian tubules are inserted into the outer envelope of the colon independently. There is no extra structure like "common chamber" as observed in the beetle, Mylabris pustulata (Mohamed & Murad, 1975).

Cryptonephridia

The cryptonephridial system of C. analis larva includes the distal parts of the Malpighian tubules which remain adherent to the surface of the colon through the cryptonephridial chamber. The parts of the Malpighian tubules within this chamber are known as the cryptonephridia. In this case, the cryptonephridia initially extend as more or less straight tubules, then convolute in a serpentine manner. The convolutions are regular and gradually become prominent towards the posterior end of the colon. With the gradual increase in convolutions of the cryptonephridia, the cryptonephridial chamber also becomes proportionately wider towards the posterior end of the colon. Later,

the cryptonephridia end blindly just in front of the junction of the colon and the rectum. The cryptonephridial chamber (including the convoluted cryptonephridia) measures 0.3 to 0.35 mm in length in the 2nd instar, whereas it measures 0.7 to 0.8 mm in the 4th instar.

All the cryptonephridia are uniform in diameter throughout their length, and appear to be equal in length. The cryptonephridia measure 0.02 to 0.025 mm in diameter in the 2nd instar larva, whereas they measure 0.05 to 0.06 mm in the 4th instar. There is no modification, i.e. dilation or any lateral extension of these tubules like the "nephric buds" as observed in the beetle, Mylabris pustulata (Mohamed & Murad, 1975); or any other complex convolutions and ramifications of the cryptonephridia, described in the various lepidopterous larvae (Ishimori, 1924).

IV.A. 1(b). ANATOMY OF THE PUPAL MALPIGHIAN TUBULES
OF C. ANALIS

Free tubules

The three pairs of Malpighian tubules, viz., 1st, 2nd and 3rd pairs which have already been described in the larva of C. analis, still persist during the pupal stage except a few minor anatomical changes in their free parts. The tubules never disappear or degenerate either completely or partially throughout the whole late larval (prepupal condition) and pupal life. Their proximal ends still remain attached to the anterior end of the pylorus; their distribution in the body cavity, and insertion into the cryptonephridial chamber remain as they were in the larva. But, the tubules are narrower than that of the larva, and more or less uniform in diameter throughout their whole lengths. These Malpighian tubules had began to become narrower and uniform in diameter just before the larval-pupal moult (i.e. during prepupal condition) (Fig. 6) and continued upto the middle-aged pupa. Thus, a typical Malpighian tubule of the pupa is a very long, thread-like, thin and semi-translucent organ which closely resembles the adult Malpighian tubules (Figs. 7 & 8). The free parts of the 1st pair of Malpighian tubules measure 5.0 to 5.5 mm in length, whereas the 2nd and the 3rd pairs measure 3.5 to 4.5 and 0.9 to 1.0 mm, respectively. All these tubules

measure 0.01 to 0.02 mm in diameter. The cryptonephridial chamber (including the convoluted cryptonephridia) measure 0.7 to 0.8 mm in length.

Cryptonephridia

The cryptonephridial system as described earlier in the larva, exists in the pupa. The distribution pattern of the cryptonephridia into the cryptonephridial chamber also remains unaltered.

IV.A. 1(c). ANATOMY OF THE ADULT MALPIGHIAN TUBULES
OF C. ANALIS

Free tubules

As in many beetles, there are six cryptonephric Malpighian tubules in C. analis. The Malpighian tubules of the adult are very similar to those of the boll weevil, Anthonomus grandis Boheman (Sundman & King, 1964). On the basis of arrangement and distribution of these tubules, like that of the larva, they can be grouped into three pairs, viz., 1st, 2nd and 3rd pairs. The origin, distribution, and insertion of these tubules into the cryptonephridial chamber are identical with the larval Malpighian tubules. All the adult Malpighian tubules are uniform in diameter (0.03-0.05 mm), thread-like, and opaque-white in colour (Figs. 9, 10 & 11). In general appearance, they are quite similar to each other but greatly variable in length as in the larva. The 1st pair, i.e., the longest pair of the Malpighian tubules extend anteriorly upto the anterior end of the midgut then turn back posteriorly and meet with the cryptonephridial chamber of the colon. They are distributed to the dorso-lateral sides of the midgut, and follow a zigzag convoluted course, similar to that of the larva. The 1st pair of the Malpighian tubules measure 3.7 to 3.9 mm in length. The 2nd pair of Malpighian tubules are

slightly shorter than the 1st pair. They also extend forward and then backward like the 1st pair, but remain situated in the ventro-lateral sides of the midgut. They are 2.4 to 2.6 mm in length. The shortest pair of the Malpighian tubules i.e. the 3rd pair, instead of running forward extend posteriad through the two lateral sides of the convoluted ileum in the form of strings, and then meet with the cryptonephridial chamber of the colon like that of the larva. The 3rd pair of Malpighian tubules measure 0.8 to 0.9 mm in length. It appears that the free part of the longest Malpighian tubule (i.e. 1st pair) is about four and a half times longer than the shortest Malpighian tubule (i.e. 3rd pair), which is more or less similar to the observation of Sundman & King (1964).

Cryptonephridia

Similar to that of the larva, the cryptonephridia of the adult also follow a serpentine convoluted course through the cryptonephridial chamber of the colon. The convolutions gradually become much prominent posteriorly and ultimately the distal tips of the cryptonephridia end blindly just in front of the junction of the colon and the rectum. Due to the greater accommodation of convoluted cryptonephridia into the posterior part of

cryptonephridial chamber, the posterior part of colon with the chamber appears to be more wider than the anterior end. Similar cryptonephridial system was observed in the beetles by many workers, viz., Woods (1918), Potts (1927), Davidson (1931), Miller (1931), Burgess (1932), Kontkanen (1932), Auten (1933), Brubaker (1934), Parkin (1936), Pradhan (1936, 1939, 1942), Patterson (1937), Mattingly (1938), Saksena (1951), Hafez & El-Zaidy (1952), Umeya (1960), Evans (1961), Hafeez & Gardiner (1964), Saini (1964), Hafez & Makky (1965), Grimstone et al. (1968), Benham (1970), Morgan et al. (1970), Ameen & Rahman (1973), Mohamed & Murad (1975), and many others.

However, the cryptonephridial system also observed in many lepidopterous larvae, by many workers, viz., Ishimori (1924), Saini (1964), Durr (1967), Judy & Gilbert (1969), Beals & Berberet (1976), and many others.

IV.A. 1(d). METAMORPHIC CHANGES (ANATOMICAL) IN THE
MALPIGHIAN TUBULES OF C. ANALIS

a) Changes during larva to larva moults

There is no appreciable changes in appearance of the Malpighian tubules during larva to larva moults. The 1st, 2nd, and the 3rd pair of Malpighian tubules, present in the 1st instar larva, gradually increase in length and diameter with the development of the larva during succeeding larval instars upto the prepupal condition of 4th instar larva. There is no other anatomical changes of the tubules which may be pointed out. Thus, for example, the 1st pair of Malpighian tubules which was found to be 2.0 mm in length and 0.015 mm in diameter, in the early 2nd instar larva, became 10.0 mm in length and 0.90 mm in diameter in the full grown (4th instar) larva. The other two pairs of Malpighian tubules also correspondingly develop during larval life of C. analis.

b) Changes during prepupal, larval-pupal moult,
and early pupal condition

During prepupal condition, the 1st and 2nd pair of Malpighian tubules gradually become uniform in diameter. This is due to the gradual decrease in diameter of the middle free parts of the 1st and 2nd pairs of Malpighian tubules, whereas the proximal parts adjacent to the origin and distal parts (cryptonephridia + a little portion near to the insertion) remain unaltered in diameter. The 3rd pair of Malpighian tubules do not change in diameter and

remain unaltered during prepupal condition of the larva. All these tubules still retain their irregular dilations throughout their middle region and powdery-white in colour. This is due to the elimination of the waste products of the lumen and partial histolysis and degeneration of the epithelial cells during prepupal condition, prior to the larval-pupal moult.

With the onset of the pupal life (just after the last larval moult), the Malpighian tubules without undergoing any degenerating process, become more narrower and thinner than the prepupal condition. It continues upto the middle aged pupa. In this condition, the Malpighian tubules become very narrow, semi-translucent and thread-like and without any recognizable dilations in the middle part of the tubules i.e., the surface of the tubules become more or less uniform. This thread-like structure of the tubules remain unchanged upto the pupal-adult moult.

c) Changes during pupal-adult moult and early adult condition

The changes of the Malpighian tubules during this period is very feeble. As soon as the pupal-adult moult is over, the Malpighian tubules slightly change in general appearance, during the early adult condition. The tubules appear to be slightly wider in diameter, become opaque-white in colour, and over all seems to be more rejuvenescent than the pupal condition.

IV.A. 2(a). ANATOMY OF THE LARVAL MALPIGHIAN TUBULES
OF C. CHINENSIS.

In gross anatomy, the larval Malpighian tubules of C. chinensis are very similar to those of C. analis. These are briefly described below.

As in C. analis, of the six cryptonephridic Malpighian tubules, two pairs are very long in comparison with the 3rd pair. After originating independently from the anterior end of the pylorus, the long tubules extend cephalad upto the anterior end of the midgut and then run caudad, whereas the short tubules directly extend caudad and meet with the cryptonephridial chamber of the colon. The long tubules are slightly wider in the middle part, whereas the short tubules are almost uniform in diameter as described in C. analis (vide pp. 150-153). In the late larva, free portion of the long tubules measure 6.0 to 7.5 mm in length, and their widest part measure 0.04 to 0.6 mm in diameter; whereas the short tubules measure 1.2 to 1.5 mm in length and 0.03 to 0.04 mm in diameter.

The cryptonephridial parts (i.e. cryptonephridia) of the Malpighian tubules follow a characteristic serpentine convoluted course within the cryptonephridial chamber as described in C. analis. The cryptonephridial chamber including the cryptonephridia measure 0.5 to 0.6 mm in length in the 4th instar.

IV.A. 2(b). ANATOMY OF THE PUPAL MALPIGHIAN TUBULES OF C. CHINENSIS

Pupal Malpighian tubules of C. chinensis are quite similar to those of C. analis. The tubules are much thinner than those of the larva. All Malpighian tubules are similar and uniform in diameter throughout their entire length. Their cryptonephridial arrangement remain unaltered as in the larva. The long and short Malpighian tubules measure 3.0 to 4.0 mm and 0.6 to 0.7 mm in length, respectively. Both of them measure 0.01 to 0.02 mm in diameter.

IV.A. 2(c). ANATOMY OF THE ADULT MALPIGHIAN TUBULES OF C. CHINENSIS

In gross appearance and distribution, the Malpighian tubules of the adult C. chinensis are quite identical with those of C. analis. They differ with those of the larvae in gross anatomy; but similar in origin, distribution, and cryptonephridic arrangement. The adult tubules resemble more closely with those of the pupa except that they are wider than the later.

As in C. analis, four long tubules (i.e. two long pairs) first extend forward and then backward in

a zigzag way; whereas the two short tubules (i.e. one short pair) directly extend backward after originating from the pylorus (Fig. 12) (for C. analis, vide pp. 157-158). The long tubules of the adult C. chinensis measure 2.0 to 2.6 mm, whereas the short tubules measure 0.5 to 0.6 mm in length. All these tubules measure 0.02 to 0.03 mm in diameter (cf. C. analis).

The cryptonephridia of the adult remain unaltered in structure and arrangement within the cryptonephridial chamber as seen in the larva and pupa, which are also similar to those of C. analis (vide pp. 158-159).

IV.A. 2(d). METAMORPHIC CHANGES (ANATOMICAL) OF THE
MALPIGHIAN TUBULES OF C. CHINENSIS

Anatomical changes of the Malpighian tubules of C. chinensis are similar to those of C. analis.

a) As in C. analis, there is no remarkable anatomical changes in the Malpighian tubules of C. chinensis during larva to larva moults. The tubules only increase in length and diameter which is proportional to the development of the larval body during successive instars.

b) During prepupal condition, larval-pupal moult, and pupal stage, as seen in C. analis (vide pp. 160-161), the two pairs of long Malpighian tubules shorten considerably and become narrower, and ultimately appear as thread-like in the late pupa.

c) The narrow pupal tubules become more prominent during pupal-adult moult and early adult condition, as seen in C. analis (vide p. 161).

B. HISTOLOGY

IV.B. 1(a). HISTOLOGY OF THE LARVAL MALPIGHIAN TUBULES
OF C. ANALIS

Histologically, there is no basic difference between the long (1st and 2nd pairs) and the short (3rd pair) Malpighian tubules; and even, among the different regions (i.e. proximal, middle, and distal parts) of the free tubules except a few minor ones; but these differ from that of the cryptonephridial part.

Free tubules

Each Malpighian tubule of the larva of C. analis is composed of a layer of epithelial cells serially arranged on a thin basement membrane (Figs. 24, 62, 63, 64, 65, 66 & 79). Externally these cells are bounded by a peritonal layer in association with traces of muscles, connective tissue mass, finer branches of tracheae, and often with fat bodies. The cells are irregular cuboidal type which more closely resemble the epithelial cells of the hindgut than that of the midgut.

In cross sections, each of the free Malpighian tubule of the 1st and the early 2nd instar larva is found to consist of 8-16 epithelial cells in the middle region, and 4-6 cells towards the two ends which are arranged surrounding a central lumen (Figs. 62 & 63). Likewise,

the long Malpighian tubules (i.e. 1st and 2nd pairs) were found to be constituted by 8-12 cells, and the short tubules (i.e. 3rd pair) by 6-10 cells respectively, in the 3rd and early 4th instar (Figs. 65, 66, & 67). The cells of the Malpighian tubules of the younger larvae (Figs. 62, 63 & 64) are comparatively shorter and wider than those of the full grown larvae (Figs. 65, 66 & 67). Thus, the cells of the Malpighian tubules of an early 2nd instar larva measure 15-25 μm in height and 20-35 μm in width. The nuclei are large, round to oval, and contain numerous prominent chromatin granules. The nuclei measure 8-15 μm in diameter and the brush-border measures 1-2 μm in thickness. The epithelial cells of the 3rd and the 4th instar are wider at the base, and tall and narrow towards the lumen. In general appearance, the cells look like short and blunt finger-like or conical (Figs. 65, 66 & 67). The cells of the middle part of the long tubules measure 15-25 μm in height and 15-25 μm in width at the base, and their nuclei measure 5-8 μm in diameter. The cells of the proximal part of the Malpighian tubules measure 20-25 μm in height and 15-25 μm in width at the base, and the nuclei measure 6-9 μm in diameter. The cells of the short tubules measure 12-16 μm in height and 12-16 μm in width at the base. The nuclei of these cells measure 5-8 μm in diameter. The cytoplasm of the cells of the Malpighian tubules are coarsely granular. These granular materials

are actually the mitochondria and certain dense cytoplasmic spherules or "《concretions》" as observed under the electron microscope in several insects (Beams et al. 1955; Wigglesworth & Salpeter, 1962; Byers, 1971; Mello & Dolder, 1977; Dolder & Mello, 1979; Ryerse, 1979; Souza et al. 1981; and many other recent workers).

The lumen of the Malpighian tubules of the larva contain a large amount of waste products which almost fill up the space. It is comparatively wider at the middle region and gradually narrower towards the two ends of a long Malpighian tubule (1st and 2nd pair).

Cryptonephridia

The cryptonephridial part of the Malpighian tubules is rather simple. The cryptonephridia consist of normally two large cells with prominent nuclei as observed in cross sections (Figs. 28, 29 & 80). They appear to be very flat cells which encircle a very limited and narrow lumen. The cells are always devoid of brush-border. The cell-boundaries between the two adjacent cells are very indistinct. The nuclei are very prominent with numerous prominent chromatin granules. The nuclei of the young larval cryptonephridia (Fig. 28) are comparatively very large than that of the late larva (Fig. 29). The nuclei thus measure 8 to 12 μm in diameter and 6 to 9 μm , in the early larva and late larva respectively.

All the cryptonephridia are similar in structure. They are associated with the colon or remain adherent to the wall of the colon by an outer peritoneal layer. The tubules are very loosely arranged in the cryptonephridial chamber thus leaving short spaces between the adjacent tubules. These spaces often remain filled with connective tissue mass. There is no trace of any leptophragma in the cryptonephridial system of the larva of C. analis as seen in lepidopterous larvae (Saini, 1964).

IV.B. 1(b). HISTOLOGY OF THE PUPAL MALPIGHIAN TUBULES OF C. ANALIS

Free tubules

Histologically, all the pupal Malpighian tubules are similar in appearance. In cross sections, the free tubules are seen to consist of 2-4 cells (Fig. 68). The cell are serially arranged on a thin basement membrane. The lumen of the Malpighian tubule is almost obliterated in the pupal stage (Figs. 46, 69 & 96). The epithelium appears to be syncytial, since the adjacent cell boundaries are very indistinct; and the brush-border is not visible. Numerous very distinct, comparatively large and oval nuclei remain irregularly distributed in the epithelium. The epithelium contains very little amount of cytoplasm surrounding the nuclei. The epithelium measures 6-9 μm in height where the nuclei are 5-8 μm

in diameter. Numerous very distinct chromatin granules are present in the nuclei of the epithelium.

Cryptonephridia

The structure and arrangement of the cryptonephridia within the cryptonephridial chamber remain unaltered from that of the larva. But, the cytoplasmic content of the epithelial cells of the cryptonephridia is considerably lost, as a result the cells appear, as if shrinkage took place (Figs. 48, 59, 98 & 105). The nuclei are still prominent which measure 6-9 μ m in diameter. The chromatin granules are distinct.

IV.B. 1(c). HISTOLOGY OF THE ADULT MALPIGHIAN TUBULES OF C. ANALIS

The histological elements of all the three pairs of Malpighian tubules of adult C. analis are almost identical. The epithelial cells throughout the free portion of the tubule are more or less similar in appearance, whereas they are dissimilar to that of the cryptonephridial part of the Malpighian tubule. The epithelium of the free tubule of this insect resembles closely that of a beetle, Mylabris pustulata Thumb (Mohamed & Murad, 1975), but differ much in the Cryptonephridia.

Free tubules

The middle and the proximal parts of all Malpighian tubules consist of 8-10 cells, whereas the distal ends (nearer to the insertion into the cryptonephridial chamber) consist of 4-6 cells, as observed in several cross sections (cf. Figs. 71, 73a & 73b). The cells in the proximal and the middle parts of the free tubules are quite similar in appearance; whereas, they differ slightly from those of the distal part, where the cells gradually modify to be adjusted with that of the cryptonephridia. However, typical epithelial cells of the free Malpighian tubules are short and blunt finger-like to slightly conical structures. The cells bear very prominent, round to oval nuclei which contain deeply stained chromatin granules. The cytoplasm of the cells is coarsely granular. The brush-borders are very thin and indistinct and are always found in contact with the waste materials of the lumen. The lumen is more or less uniformly wide throughout the whole length of the free tubule (Figs. 70, 71 & 72). These cells measure 6-10 μm in height and 8-12 μm in width (at the base) in the proximal part of the tubules, and 7-12 μm in height and 9-15 μm in width (at the base) in the middle part of the tubules. The nuclei measure 3-6 μm and 5-8 μm in diameter in the proximal and middle parts of the Malpighian tubules, respectively. The cells of the distal part of the free tubules are comparatively short and

wide and with irregular blunt margin towards the lumen (Fig. 73b). These cells measure 5-9 μm in height and 10-18 μm in width, and their nuclei measure 4-9 μm in diameter.

Cryptonephridia

The six cryptonephridia within the cryptonephridial chamber are very compactly arranged. They fill up the whole chamber.

The epithelial cells are quite large but their nuclei are relatively very small (Figs. 40 & 90). The nuclei measure μm in diameter. The nuclei bear a few chromatin granules. Externally, the cryptonephridia are limited by a peritonal membrane. The lumen of the cryptonephridia are very limited and narrow. Often, simple leptophragma are found. The leptophragma-forming cells, i.e., "leptophragma cells" are hyaline structures and almost semi-lunar type, which touch the outer membrane (i.e. peritoneal membrane) of the cryptonephridial chamber. These are communicated with the interior upto the gut lumen by narrow spaces or cavities or pores (Fig. 40). These leptophragma are ill-defined as compared to those of other insects described by Saini (1964).

IV. B. 1(d). METAMORPHIC CHANGES (HISTOLOGICAL) IN THE MALPIGHIAN TUBULES OF C. ANALIS

Free tubules

a) Changes during larva to larva moults

Appreciable changes in histology of the Malpighian tubules do not take place during larval-larval moults except that the epithelial cells modify in size and increase in number with the development of the larval life. Thus, for example, the cells in the free long Malpighian tubule, which were 15-25 μm in height and 20-35 μm in width in the 2nd instar, become 15-25 μm in height and 15-25 μm in width in the 4th instar larva. The size of the nuclei gradually decrease with the development of the larva. Thus, the nuclei which measured 8-15 μm in diameter in the 2nd instar become 5-9 μm in diameter in the 4th instar larva.

b) Changes during prepupal condition, larval-pupupal moult, and pupal stage

Drastic changes have been observed in the Malpighian tubules especially in the free parts of the long tubules (i.e. 1st & 2nd pairs) during prepupal condition of the late larva. During this preparation, most of the large epithelial cells continue gradual degeneration by liberating numerous cytoplasmic droplets into the lumen which are ultimately thrown out of the Malpighian tubules. The droplets are discharged continually at a time from the distal ends of the larger cells. Due to

elimination of waste products along with the discharged cytoplasmic contents, the lumen of the tubules gradually becomes very narrow. Due to vigorous secretion of the cytoplasmic droplets, some of the large cells become completely degenerated. This phenomenon of degeneration of the epithelial cells continue in the pupa. The boundary walls between the adjacent cells disappear and as a result the epithelium appears to be syncytial. The nuclei of the persisting smaller cells remain distributed in this epithelium. These smaller cells are generally known as the "imaginal cells" (Figs. 64, 65 & 67).

The degeneration process of the Malpighian epithelial cells (i.e. the larger cells of the epithelium) still continue during pupal condition. Meanwhile, the "imaginal cells" become very active. Instead of degeneration, they become enlarged and prominent and sometimes multiply into daughter nuclei. The daughter nuclei also become prominent with a little amount of cytoplasm surrounding them. In this condition, the epithelium becomes filled with prominent multinucleated structures during late pupal stage, just before pupal-adult moult (Fig. 69).

c) Changes during pupal-adult moult and early adult stage

With the onset of the adult life, the epithelium gradually becomes cellular. The cells regain much of their cytoplasmic contents, thus, metamorphosing the epithelium

into a normal adult condition of the Malpighian tubule (Figs. 71, 72, 73a & 73b). The lumen of the tubules gradually become wide and accumulate a great quantity of waste products.

During metamorphosis of the Malpighian tubules of C. analis, it was observed that some of the persisting larval cells at the base of the tubules (in the junction of the midgut-Malpighian tubule becomes very active which divide to give rise to a multinucleated syncytium (Figs. 60 & 106). The nuclei of this zone at the base of the Malpighian tubules soon migrate towards the tubules. Thus, these cells make an addition in the development of tubules during metamorphosis.

Cryptonephridia

The cryptonephridia neither degenerate nor change in structure during successive larval instars. They remain arranged as usual in the cryptonephridial chamber during pupal and adult stages. But, during prepupal and pupal condition, the cryptonephridia become weak in appearance, they loose a large amount of water content at this time (Figs. 48, 59, 98 & 105) with the progress of adult life just after the pupal-adult moult, the cells of the cryptonephridia regain much of the water content which help the tubules to become more prominent in appearance (Figs. 40 & 90).

IV.B. 2(a). HISTOLOGY OF THE LARVAL MALPIGHIAN TUBULES OF C. CHINENSIS

Histological elements of the larval Malpighian tubules of C. chinensis are very similar in structure and arrangement to those of C. analis, but they vary slightly in dimension than those of the latter.

Free tubules

As in C. analis, the free part of each long tubule of C. chinensis consists of 6-8 epithelial cells, as seen in cross sections. The cells are wider at the base and narrower towards the lumen. The cells bear prominent round to oval nuclei. The cells of the free Malpighian tubule of the late larva measure 10-16 μm in height and 10-16 μm in width (at the base), where as the cells of the short tubules measure 8-10 μm in height and 8-10 μm in width (at the base). The nuclei of these epithelial cells measure 3.5-5.5 μm in diameter.

Cryptonephridia

The cryptonephridia consist of 2-3 cells with indistinct cell-boundaries in a cross section. The nuclei are very prominent which measure 4-6 μm in diameter. Other associated histological elements of the larval Malpighian tubules of C. chinensis are also similar to those of C. analis (vide pp. 168-169).

IV.B. 2(b). HISTOLOGY OF THE PUPAL MALPIGHIAN TUBULES
OF C. CHINENSIS

Free tubules

As in C. analis, the epithelium of the free Malpighian tubule of the pupa of C. chinensis is very thin and appears syncytial as the adjacent cell-boundaries are not distinct. The lumen is almost obliterated. But the nuclei are always very prominent. The pupal epithelium of the Malpighian tubule measures 4-6 μm in height, where the nuclei measure 3.5-5.0 μm in diameter. A brush-border is absent in the pupal epithelium.

Cryptonephridia

The cryptonephridial epithelium with their nuclei remain unaltered in the pupa, as seen in C. analis also (vide p. 170).

IV.B. 2(c). HISTOLOGY OF THE ADULT MALPIGHIAN TUBULES
OF C. CHINENSIS

The histological elements of all the Malpighian tubules (i.e. long and short tubules) are similar to each other. They are identical with those of C. analis but differ slightly in the dimension of the cells and nuclei.

Free tubules

Each free tubule consists of a ring of 4-8 cells in cross sections. The shape of these epithelial cells and their nuclei are quite similar to those of C. analis (vide pp. 171-172). The epithelial cells of the Malpighian tubules of C. chinensis measure 4-7 μ m in height and 5-8 μ m in width (at the base), and their nuclei measure 3.5-5 μ m in diameter (cf. C. analis).

Cryptonephridia

The epithelium of a cryptonephridium consists of 2-3 cells in a cross section with distinct nuclei, which are also identical with those of C. analis. The nuclei of these cells measured 4.0-5.5 μ m in diameter. Often, simple leptophragma is seen in the cryptonephridial arrangement which is also similar to that of C. analis (vide p. 172).

IV.B. 2(d). METAMORPHIC CHANGES (HISTOLOGICAL) IN THE MALPIGHIAN TUBULES OF C. CHINENSIS

The histological changes in the Malpighian tubules of C. chinensis during metamorphosis are very similar to those of C. analis. These changes are described only briefly with reference to those of C. analis.

a) The change of the histological elements of the Malpighian tubules of C. chinensis during larva to larva moults is very simple. As in C. analis, the epithelial cells increase in number and size, which is proportional to the growth and development of the larval body.

b) The changes in the epithelium of the Malpighian tubules during prepupal period, larval-pupal moult, and pupal stage are very significant, which are very similar to those of C. analis. However, the following points can be described in a nutshell.

i) During the prepupal condition, most of the large epithelial cells of the larval Malpighian tubules degenerate as described in C. analis (vide pp. 173-174).

ii) The smaller epithelial cells which avoid degeneration during this time (prepupa and early pupa) termed to be the "imaginal cells" or "regenerative cells", which ultimately divide, distribute in the syncytium, and form the adult epithelium as in C. analis (vide p. 174).

c) The thin pupal epithelium of the Malpighian tubules gradually redifferentiate into an adult epithelium by collecting a certain amount of the cytoplasmic contents around them and developing the cell boundaries, during pupal-adult moult and early adult condition when the insect remains within the pabulum of seed, as seen in C. analis (vide pp. 175 - 176).

IV. C. DISCUSSION

MALPIGHIAN TUBULES

Coleoptera

Available literature reveals that there is neither complete degeneration nor any "neo-formation" arising as outgrowths of the Malpighian tubules in the beetles during metamorphosis. A few cells of the larval epithelium degenerate; and the adult epithelium is developed from "imaginal cells" or certain persistent larval cells, or from a combination of these two types of cells in the Malpighian tubules of the beetles.

In Anobium paniceum (Karawaiew, 1899) and Calandra (Murray & Tiegs, 1935), certain small cells which are quite distinguishable from the normal larval epithelial cells and have the power of regeneration, have been considered as the "imaginal cells". These "imaginal cells" of the larval Malpighian tubules partially replace the epithelium during metamorphosis. The cryptonephridia never change in structure in A. paniceum.

In Galerucella (Poyarkoff, 1910), partial degeneration of the larval epithelium takes place by the histolysis of some of the larval cells. The adult epithelium is rebuilt from two sources, viz., "imaginal cells" and certain persistent larval cells which have the power of division during metamorphosis. The cryptonephridial parts of the Malpighian tubules never

degenerate nor there is any remarkable change during metamorphosis.

In Leptinotarsa (Patay, 1939), the larval Malpighian tubules neither degenerate nor there is any histolysis of the epithelium, even there is no loss of material. At the beginning of the pupal life, the larval epithelium becomes atrophic which gradually redifferentiates into the adult epithelium.

In Tenebrio molitor L. (Byers & Bond, 1971), the cryptonephridia or "perirectal Malpighian tubules" being similar in structure and function in both larva and adult, they undergo a sequence of dramatic alteration in subcellular organization. The tubule cells undergo degenerative changes which ultimately progressively redifferentiate into the adult form.

Insects other than Coleoptera

Similar to the origin and successive development of the larval Malpighian tubules in different insects, described by many workers, (e.g. Henson, 1932, 1944 & 1946 ; Savage, 1956 & 1962; Srivastava & Bahadur, 1961; Srivastava & Khare, 1966; and Khare, 1975), the adult Malpighian tubules develop as fresh and new from a zone of undifferentiated cells at the junction of the midgut and hindgut, after complete degeneration of the tubules during metamorphosis.

Ameen (1969) called this type of adult Malpighian tubule formation as "neo-formation". This is very common in many hymenopteran insects, e.g. in Bombinae, Psithyrinae, Vespinae, Polistinae and Sphaecinae (Bordas, 1894); in ants, e.g., Lasius flavus (Karawaiew, 1898), Formica (Perez, 1901 & 1902), and Myrmica (Nitschmann, 1959); in wasp (Anglas, 1901); in bees (Anglas, 1901; Oertel, 1930; Kusmenko, 1941; Dobrovsky, 1951). In Nasonia (Tiegs, 1922), the Malpighian tubules of the adult is developed directly (instead of neo-formation) from the anterior end of the hindgut as an evaginating process, since there are no larval Malpighian tubules.

In many lepidopteran insects e.g. Tineola and Galleria (Cholodkowsky, 1884 & 1887), Galleria mellonella and Monopis crocicapitella (Le Garff, 1974 & 1976), the Malpighian tubules present complete degeneration and new development during metamorphosis. The adult tubules are developed as neo-formations from a special population of larval cells, localized at the anterior end of the hindgut. In these insects, the cryptonephridia which were present in the larva, never develop in the adult condition. In some lepidopteran insects e.g. Heterogenea limacodes (Samson, 1908), Hyponomeuta padella L. (Hufnagel, 1918), Bombyx mori L. (Ito, 1921), sphingid moths (Garbarskaja, 1929), Aglaia (Venessa) (Henson, 1939), Philosamia ricini (Srivastava & Khare, 1966), and Korscheltellus lupulinus

(Le Garff, 1978), the larval Malpighian tubules do not degenerate. They persist integrally during metamorphosis. Thus, the adult Malpighian tubules are derived by simple redifferentiation. Garff (1978) observed that each cell of the Malpighian tubule undergoes a reduction of size and important nuclear alteration during metamorphosis in K. lupulinus.

In dipteran insects, e.g. Musca vomitoria (van Rees, 1888), Chironomus, Simulium and Psychoda (Vaney, 1902), Culex (Thompson, 1905), Calliphora (Perez, 1910a & 1910b), Drosophila (Robertson, 1936), the metamorphic changes of the Malpighian tubules are very feeble. The larval tubules become imaginal without any remarkable change, like complete degeneration or neo-formation of the Malpighian tubules. However, little changes that occur in these tubules are due to progressive redifferentiation of the epithelial cells during metamorphosis. In Ptychoptera albimana (Ameen, 1969), a combination of several processes take part in the metamorphosis of the Malpighian tubules. In the normal tubules, progressive divisions of some of the persistent larval nuclei or certain regenerative nuclei (known as the "imaginal nuclei") give rise to the daughter nuclei which are ultimately carried over to the adult without any morphological change. Whereas, in the saccular tubes (modified larval tubes), the larval

epithelium mostly degenerates which ultimately regenerates by the regenerative nuclei. This is accompanied by cellular redifferentiation of the tubule.

In C. analis and C. chinensis, the larval Malpighian tubules neither degenerate nor there is any "neo-formation" during metamorphosis. The metamorphic changes in these tubules closely resemble those of other coleopteran insects, certain dipteran insects, and a few insects of some other groups as revealed from the above review of literature. However, it is very similar to that of the beetle, Galerucella (Poyarkoff, 1910) except a few minor differences. In C. analis and C. chinensis, some of the larval epithelial cells which are larger in size degenerate and consequently a few smaller cells which may be regarded as the "regenerative" or "imaginal cells" divide, thus making an addition to the normal epithelium of the free middle part of the Malpighian tubules during metamorphosis. Besides, a few undifferentiated nuclei localized at the base (proximal end) of the Malpighian tubules divide and give rise to many daughter cells which ultimately make a small addition to the adult epithelium formation. During this procedure, cytoplasmic diminution takes place during initial condition, as a result the pupal epithelium becomes thin and weak. The loss of the cytoplasmic

contents of the epithelium is compensated with the onset of the adult life, thus establishing the normal adult epithelium of the Malpighian tubules.

The larval cryptonephridia never degenerate either completely or partly during metamorphosis of C. analis and C. chinensis. The arrangement of these tubules also remain unaltered. Only, the cytoplasmic loss which occurred in the prepupal and early pupal stages is regained in the early adult condition. Besides this, a few simple "leptophragma" which were never found in the larval cryptonephridial system, gradually became evident in the adult during metamorphosis.

V. SUMMARY

The larval alimentary canal of C. analis consists of a short and straight foregut, distinguishable into pharynx, oesophagus, and proventriculus (only in the late larva); long and wide midgut, divisible into an anterior and a posterior midgut; and a narrow convoluted hindgut, differentiated into a short pylorus, a characteristic "S"-shaped ileum, a cryptonephridia-invested colon, and a short rectum.

In the pupal stage the midgut shortens considerably and the so-called "yellow bodies" can be seen easily through the thin wall. The fore- and hind-gut remain almost unchanged except that the foregut is bent cephalad.

In the adult the fore- and hind-gut are slightly longer than in the pupa. "Crypts" appear along the entire midgut, and the anterior midgut becomes slightly swollen and irregular in shape.

The anatomy of the alimentary canal of C. chinensis and its changes during metamorphosis are very similar to those of C. analis.

The foregut epithelium of C. analis consists of cuboidal cells resting on a basement membrane and surrounded by longitudinal and circular muscles. The epithelium is invested by an intima internally which

bears spines in the pharyngeal and proventricular regions. The structure of the hindgut is more or less similar to that of the foregut and its intima bears spines in the rectal region. In addition to the normal musculature the rectum bears a "complex of diagonal muscles".

The larval midgut epithelium contains three types of epithelial cells in addition to the regenerative cells (i.e. nidi) : tall and narrow columnar in the anterior part of the anterior midgut, short and wide columnar in the posterior part of the anterior midgut, and simple narrow columnar in the posterior midgut. Both anterior and posterior imaginal ring cells occur at the junction of the midgut with foregut and hindgut respectively. In addition there are a few undifferentiated "transitional cells" in front of the anterior imaginal ring cells.

The pupal foregut and hindgut epithelial cells become squeezed, the intima detach and histolyse completely. The musculature shows degenerative changes. The pupal midgut epithelium is thin and uniform all along its length; its lumen contains "yellow bodies" towards the middle, and "proliferated cells" from the anterior and posterior imaginal rings at the two ends.

"Pharyngeal and oesophageal spines" and "proventricular needles", and "proventricular valves" appear in the adult foregut. "Rectal pads" and "rectal papillae" differentiate in the adult hindgut.

During metamorphosis the larval midgut epithelium degenerates completely with simultaneous regeneration and neo-formation of the pupal epithelium from regenerative cells.

The pupal midgut epithelium redifferentiates into the adult epithelium by absorbing digested yellow bodies and the cells that were proliferated from the anterior and posterior imaginal rings.

The epithelium of the foregut and hindgut never degenerate but redifferentiate during metamorphosis.

The histology and metamorphic changes of the alimentary canal of C. chinensis are similar to those of C. analis.

In C. analis, there are six cryptonephridic Malpighian tubules. Two of them are very short, extend caudad and differ slightly from the longer four which initially extend cephalad. All the Malpighian tubules of the pupa and adult are similar, unlike that in the larva.

The Malpighian tubules shorten and become narrower in the pupa, which widen again in the adult. Their distribution is similar in both the pupa and the adult. The anatomy and disposition of the Malpighian tubules of C. chinensis are similar to those of C. analis.

The epithelium of the free Malpighian tubules is composed of cuboidal cells with large nuclei and distinct brush-border. There are some "imaginal cells" in addition to the active cells, which remain scattered in the epithelium. During metamorphosis some larval Malpighian epithelial cells degenerate and a new epithelium is formed by the growth and differentiation of the "imaginal cells".

There is no appreciable change in the cryptonephridia or its arrangement in the cryptonephridial chamber during metamorphosis except that a few indistinct "leptophragma" become apparent in the adult.

The anatomy, histology, and metamorphosis of the Malpighian tubules of C. chinensis are identical with those of C. analis.

VI. REFERENCES

- ✓ Ameen, M. 1969. Metamorphosis of some of the organ systems in the fly Ptychoptera albimana F. Trans. R. ent. Soc. Lond. 121(6) : 235-279
- ✓ Ameen, M. 1970. Absorption of dyes in different parts of the midgut of two phytophagous beetles : Epilachna vigintioctopunctata (F.) and Aulacophora foveicollis Kust. Dacca Univ. Studies (Pt. B) 18 : 31-44
- ✓ Ameen, M. & Imam, F. 1976. Alimentary canal of Sphaerodema rusticum (F) (Hemiptera : Belostomatidae). J. nat. Hist. 10 : 545-568.
- ✓ Ameen, M. & Rahman, M.F. 1973. Larval and adult digestive tracts of Tribolium castaneum (Herbst) (Coleoptera : Tenebrionidae). Int. J. Insect Morphol. & Embryol. 2(2) : 137-152.
- ✓ Andries, J. C. 1972. Remaniements de l'intestin moyen chez les odonates au cours de la métamorphose. Bull. Soc. zool. Fr. 97 : 377-385
- ✓ Andries, J. C. 1975. Cellular differentiation and death during midgut metamorphosis of Aeschna cyanea larva. J. microsc. Biol. Cell 24 : 327-350.
- ✓ Andries, J. C. 1976. Dégénérescence et rejet des cellules mésentériques des larves d'insectes. Bull. Soc. zool. Fr. 101 : 1-7

- ✓ Anglas, J. 1901. Observations sur les metamorphoses internes de la gûepe et de l'abeille. Bull. Scient. Fr. Belg. 34 : 363-473.
- ✓ Areekul, S. 1957. The comparative internal larval anatomy of several genera of Scarabaeidae (Coleoptera). Ann. ent. Soc. Am. 50(6) : 562-577
- ✓ Arora, G.L. & Pajni, H.R. 1957. Some observations on the biology and oviposition of Bruchus analis F. (Bruchidae : Coleoptera). Res. Bull. Punjab Univ. 128 : 453-470.
- ✓ Auten, M. 1933. The structure of the digestive system in Bolitotherus cornutus. Ohio Jour. Sci. 33 : 280-287
- ✓ Bahadur, J. & Kathuria, O.P. 1971. Development of ventral oesophageal diverticulum in Papilio aristolochiae F. (Lepidoptera : Papilionidae). Int. J. Insect Morphol. & Embryol. 1(1) : 21-27
- ✓ Balfour-Browne, F. 1934. The proventriculus in Dytiscidae (Coleoptera) as a taxonomic character. Stylops 3 : 241-244.
- ✓ Bautz, A. M. 1979. La dégénérescence des cellules larvaires intestinales au cours de la métamorphose chez Calliphora erythrocephala (Insecte; Diptère). Comparaison avec la dégénérescence des cellules épidermiques. Arch. Zool. exp. gén. 120 : 183-194

- ✓ Beals, G.R. & Berberet, R. C. 1976. Anatomy and histology of selected systems in larval and adult Elasmopalpus lignosellus (Lepidoptera : Pyralidae). Ann. ent. Soc. Am. 69(6) : 1105-1119
- ✓ Beams, H.W., Tahmisian, T. N., & Devine, R. L. 1955. Electron microscope studies on the cells of the Malpighian tubules of the grasshopper (Orthoptera, Acrididae). J. biophys. biochem. Cytol. 1 : 197-202.
- ✓ Becton Jr., E. M. 1930. The alimentary tract of Phanaeus vindex MacI. (Scarabaeidae). Ohio Jour. Sci. 30 " 315-323.
- ✓ Begum, A. & Rahman, M. S. 1974. Biology of Callosobruchus analis Fabricius. Bangladesh J. Zool. 2 : 171-177
- ✓ Benham, G.S. 1970. Gross morphology and transformation of the digestive tract of Prionus laticollis (Coleoptera : Cerambycidae). Ann. ent. Soc. Am. 63(5) : 1413-1419.
- ✓ Berberet, R. C. & Helms, T. J. 1972. Comparative anatomy and histology of selected systems in the larval and adult Phyllophaga anxia (Coleoptera : Scarabaeidae). Ann. ent. Soc. Am. 65(5) : 1026-1053.
- ✓ Berger, C.A. 1938. Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, Culex pipiens. Contr. Embryol. Carnegie Inst. Wash. 27 : 209-234.

- ✓ Bigham, J.T. 1931. The alimentary canal of Asaphes memnonius Herbst. Ohio Jour. Sci. 31 : 386-395.
- ✓ Bordas, L. 1894. Les tubes de Malpighi des Hyménoptères. Bull. scient. Fr. Belg. 26 : 402-441.
- ✓ Bordas, L. 1911. L'appareil digestif et les tubues de Malpighi des larves des Lépidoptères. Ann. Sc. nat. Zool. 9(15) : 191-273.
- ✓ Brubaker, R.W. 1934. The alimentary tract of Penthe pimelia Fabr. (Coleoptera : Dacnidae). Ohio Jour. Sci. 34 : 46-56.
- ✓ Burgess, E.D. 1932. A comparison of the alimentary canal of the active and hibernating adults of the Mexican bean beetles, Epilachna corrupta Muls. Ohio Jour. Sci. 32 : 249-261.
- ✓ Bushnell, R. J. 1936. The development and metamorphosis of the mid-intestinal epithelium of Acanthoscelides obtectus (Say) (Coleoptera). J. Morph. 60 : 221-241.
- ✓ Butler, J.E. & Leone, C. A. 1966. Antigenic changes during the life-cycle of the beetle Tenebrio molitor. Comp. Biochem. Physiol. 19 : 699-711.
- ✓ Byers, J. R. & Bond, E. F. 1971. Surface specialization of the hindgut cuticle of lepidopterous larvae. Can. J. Zool. 49 : 867-876.

- ✓ Chen, P.S. 1978. Protein synthesis in relation to cellular activation and deactivation. In : Biochemistry of Insects (Ed. Rockstein, M.). Academic Press, New York. pp. 145-203.
- ✓ Cholodkowsky, M.N. 1884. Contributions à l'anatomie et la morphologie des vaisseaux malpighiens des Lépidoptères. C. r. hebd. Séanc. Acad. Sci., Paris 99 : 816-819.
- ✓ Cholodkowsky, M.N. 1887. Sur la morphologie de l'appareil urinaire des Lépidoptères. Archos Biol. 6 : 497-515.
- ✓ Davidson, R.H. 1931. The alimentary canal of Criocer
asparagi Linn. Ohio Jour. Sci. 31 : 396-405.
- ✓ Deegener, P. 1900. Entwicklung der Mundwerkzeuge und des Darmkanals von Hydrophilus. Z. wiss. Zool. 68 : 113-168.
- ✓ Deegener, P. 1904. Die Entwicklung des Darmkanals der Insekten während der Metamorphose. I. Cybister roeseli Curtis. Zool. Jb., Anat. 20 : 499-676.
- ✓ Deegener, P. 1908. Die Entwicklung des Darmkanals der Insekten während der Metamorphose. II. Malacosoma castrensis (Lepid.). Zool. Jb., Anat. 26 : 45-182.
- ✓ Dennell, R. 1942. The structure and functions of the mouthparts, rostrum and foregut of the weevil, Calandra granaria L. Phil. Trans. R. Soc. Lond. (B) 231 : 247-291.

- ✓ Dobrovsky, T. M. 1951. Postembryonic changes in the digestive tract of the worker Honeybee (Apis mellifera L.). Mem. Cornell Univ. agric. Exp. Stn. 301 : 1-45
- ✓ Dolder, H. & Mellow, M.L.S. 1979. Ultrastructure of the Malpighian tubule cells of Panstrongylus megistus Burmeister. J. submicr. Cytol. 11(3) : 385-394.
- ✓ Dufour, L. 1846. Memoire sur les voisseux billieires on le foides insectes. Ann. Sci. Natur. Ser. 19 : 145-182.
- ✓ Dürr, H.J.R. 1967. The structure of the hindgut and associated structures of the larva of Sesamia calamistis Hamson (Lepidoptera : Noctuidae). S. Afr. J. Agr. Sci. 10 : 723-734.
- ✓ Evans, M.E.G. 1961. The alimentary canal of Atomaria ruficornis (Marsham) (Coleoptera : Cryptophagidae). Entomologist 11 : 263-273.
- ✓ Everly, R. T. 1936. The alimentary tract of the margined blister beetle, Epicauta cinera marginata F. (Coleoptera : Meloidae). Ohio Jour. Sci. 36 : 204-216.
- ✓ Fletcher, F. W. 1930. The alimentary canal of Phyllophaga gracilis Brum. Ohio Jour. Sci. 30 : 109-119.

- ✓ Ganin, M. 1876. On the development of the Insects [in Russian] . [Abstract in English in Am. Nat. (1877) 11 : 423-430, from the Transactions of the fifth meeting of Russian Naturalists in Warsaw; sections on Zoology and Comparative Anatomy] .
- ✓ Garbarskaja, M. 1929. Ueber das verhalten der Malpighi'schen Gefasse einiger Sphingidae-Arten während der Metamorphose, unter Berücksichtigung der veränderungen des Zellkernes. Zool. Jb., Anat. 51 : 63-110.
- ✓ Glöckner, W. E. 1958. Histologische Untersuchungen an der Diebameise Solenopsis fugax Latr. während der Metamorphose. Studia ent. Petropolis (N.S.) 1 : 529-544
- ✓ Goel, S. C. 1969. Studies on the digestive tract of Cyaneolytta acteon Lap. (Coleoptera : Maloidae). Bull. Ent. 10 : 18-26.
- ✓ Goncalves, I.S. 1979. Anatomia do tubo digestivo de Rugitermes niger Oliveira (Isoptera : Kelotermitidae). Rev. bras. Ent. 23: 229-243.
- ✓ Gray, J. 1931. The post-embryological development of the digestive system in Homaledra subalella Chambers (Lepidoptera : Cosmopterygidae). Ann. ent. Soc. Am. 24 : 45-107.

- ✓ Grimstone, A. B., Mullinger, A.M., & Ramsay, J.A. 1968.
Further studies on the rectal complex of the meal worm
Tenebrio molitor L. (Coleoptera, Tenebrionidae).
Phil. Trans. R. Soc. Lond. (B) 253 : 343-382.
- ✓ Gupta, A. P. 1965. Digestive and reproductive systems of
the Meloidae (Coleoptera) and their significance in the
classification of the family. Ann. ent. Soc. Am.
58 : 442-474.
- ✓ Hafeez, M. A. & Gardiner, B.G. 1964. The internal morphology
of the adult Tribolium anaphe Hinton (Coleoptera :
Tenebrionidae). Proc. R. ent. Soc. Lond. 39 : 137-145.
- ✓ Hafez, M. & El-Ziady, S. 1952. On the histology of the alimentary
canal of Hyperaspis vinciguerrae Capra (Coleoptera :
Coccinellidae). Bull. Soc. Fouad I. Ent. 36 : 294-310.
- ✓ Hafez, M. & Makky, A. M. 1965. On the histology of the alimentary
canal of Adesmia bicarinata Klug. (Tenebrionidae :
Coleoptera). Bull. Fac. Sci. Cairo Univ. 40 : 155-172.
- ✓ Haridas, E.T. & Ananthakrishnan, T.N. 1981. Functional
morphology of pylorus and rectal glands in Reduviidae
(Insecta-Heteroptera). Proc. Indian Acad. Sci.
(Anim. Sci.) 90(4):483-493.
- Henson, H. 1929. On the development of the mid-gut in the
larval stages of Venessa urticae (Lepidoptera).
Q. Jl. microsc. Sci. 73 : 87-105.
- ✓ Henson, H. 1931. The structure and post-embryonic development
of Venessa urticae (Lepidoptera). The larval alimentary canal.
Q. Jl. microsc. Sci. 74 : 321-360.

- ✓ Henson, H. 1932. The development of the alimentary canal in Pieris brassicae and the endodermal origin of the Malpighian tubules of insects. Q. Jl. microsc. Sci. 75 : 283-305.
- ✓ Henson, H. 1939. The structure and post-embryonic development of Vanessa urticae (Lepidoptera). The Malpighian tubules of the pupa and imago. Proc. zool. Soc. Lond. (B). 109 : 357-373.
- ✓ Henson, H. 1944. The development of Malpighian tubules of Blatta orientalis. Proc. R. ent. Soc. Lond. 13 : 79-91.
- ✓ Henson, H. 1946. The theoretical aspect of insect metamorphosis. Biol. Rev. 21 : 1-14.
- ✓ Huda, M.S. & Rahman, R. 1982. Control of pulse pests by gamma radiation : A study of Callosobruchus chinensis (Coleoptera : Bruchidae). Acta ent. bohemoslov. 79 : 344-350.
- ✓ Hufnagel, A. 1918. Recherches histologiques sur la métamorphose d'un Lépidoptère (Hyponomeuta padella L.) Arch. Zool. exp. gén. 57 : 47-202
- ✓ Hussain, S. 1983. A study of the adult and larval alimentary canal of Alphitobius diaperinus (Panzer) (Coleoptera : Tenebrionidae). M.Sc. Thesis, Deptt. Zool., Univ. Dhaka : iv + 93 pp.

- ✓ Ishimori, N. 1924. Distribution of the Malpighian vessels in the wall of the rectum of Lepidopterous larvae. Ann. ent. Soc. Am. 17 : 45-84.
- ✓ Ito, H. 1921. On the metamorphosis of the Malpighian tubes of Bombyx mori L. J. Morph. 35 : 195-211.
- ✓ Judd, W.W. 1947. The proventriculus of Macrobasis unicolor Kirby (Coleoptera : Meloidae). Ann. ent. Soc. Am. 40 : 518-521.
- ✓ Judy, K. J. & Gilbert, L. I. 1969. Morphology of the alimentary canal during metamorphosis of Hyalophora cecropia (L.) (Lepidoptera : Saturniidae). Ann. ent. Soc. Am. 63 : 1438-1446.
- ✓ Judy, K. J. & Gilbert, L.I. 1970. Histology of the alimentary canal during metamorphosis of Hyalophora cecropia (L.). J. Morph. 131 : 277-300.
- ✓ Kathuria, O.P. 1971. Metamorphosis of the midgut of a six-spotted Ladybird beetle, Chilomenes sexmaculata (Coleoptera : Coccinellidae). Int. J. Insect. Morphol. & Embryol. 1 : 87-93.
- ✓ Kathuria, O.P. 1972. Metamorphic changes in posterior region of foregut of Papilio aristolochiae F. (Lepidoptera : Papilionidae). Int. J. Insect Morphol. & Embryol. 1 : 163-168.
- ✓ Karawaiew, W. 1898. Die nachembryonale Entwicklung von Lasius flavus (Hymenoptera). Z. wiss. Zool. 64 : 385-478.

- ✓ Karawaiew, W. 1899. Ueber Anatomie und Metamorphose des Darmkanals der Larve von Anobium paniceum. Biol. Zbl. 19 : 122-130, 161-171, 196-202.
- ✓ Khare, M.K. 1975. The development of larval Malpighian tubules in Corcyra cephalonica Staint. (Lepidoptera : Pyralididae). Zoologische Beiträge 21 : 483-492.
- ✓ Khattar, N. 1972. Anatomy of the digestive organs of Schizodactylus monstrosus (Drury) (Orthoptera). J. nat. Hist. 6 : 575-588.
- ✓ Khatoon, S. 1983. Anatomy and histology of the alimentary canal of the dung beetle, Catharsius sagax (Quens.) (Coleoptera : Scarabaeidae). M.Sc. Thesis, Deptt. Zool., Univ. Dhaka : vii + 59 pp.
- ✓ Kobayashi, M. 1978. Structural changes of midgut epithelial cells during metamorphosis in the silkworm, Bombyx mori L. The Cell 10 : 15-23.
- ✓ Kontkanen, P. 1932. On the larval digestive tract of Acanthocinus aedilis L. (Co. Ceramb.). Ann. Bot. Soc. Zool. -Bot. Fenn. "Vanamo" 12 : 91-98.
- ✓ Kowalewsky, A. 1887. Beitrage zur Kenntnis der nachembryonalen Entwicklung der Musciden I. Z. wiss. Zool. 45 : 542-594.
- ✓ Kusmenko, S. 1941. Herkunft der Malpighischen Gefasse der Honigbiene. Zool. Jb., Anat. 67 : 271-292.

- ✓ Le Garff, B. 1974. Contribution à l'étude de la métamorphose du système malpighien de Galleria mellonella L. (Lepid. : Pyralides). Bull. Soc. zool. Fr. 99(3): 377-390.
- ✓ Le Garff, B. 1976. Organogénèse de l'appareil malpighien imaginal de Monopis crocicapitella (Clem.) (Lepid. (Tinéides). Evolution d'un disque imaginal. Bull. Soc. zool. Fr. 101(3) : 399-405.
- ✓ Le Garff, B. 1978. Evolution du système malpighien lors de la métamorphose d'un Lepidoptera primitif : Korscheltellus Lupulinus L. (Hepialides). Bull. Soc. zool. Fr. 103(1) : 59-65.
- ✓ Lewis, H.C. 1926. The alimentary canal of Passalus. Ohio Jour. Sci. 26 : 11-24.
- ✓ MacGown, M.W. & Sikopowski, P.P. 1981. Digestive anatomy of the adult boll weevil, Anthonomus grandis grandis Boheman (Coleoptera : Curculionidae). Ann. ent. Soc. Am. 74(1) : 117-126.
- ✓ Mansour, K. 1927. The development of the larval and adult mid-gut of Calandra oryzae (Linn.) : The Rice Weevil. Q. Jl. microsc. Sci. 71 : 313-352.
- ✓ Mathur, S.N. & Goel, S.C. 1966. The anatomy and histology of the alimentary canal of Cybister tripunctatus asiaticus Sharp (Coleoptera : Dytiscidae). Zool. Anz., Jena. 177 : 236-247.

- ✓ Mattingly, H.E. 1938. The morphology of the alimentary tract of the blister beetles, Epicanta pennsylvanica Deg. (Coleoptera : Meloidae). Ohio Jour. Sci. 38 : 251-263.
- ✓ Mello, M.L.S. & Dolder, H. 1977. Fine structure of the Malpighian tubes in the Blood-sucking insect, Triatoma infestans Klung. Protoplasma 93 : 275-288.
- ✓ Miller, A. 1961. The mouthparts and digestive tract of adult dung beetles (Coleoptera : Scarabaeidae) with reference to the ingestion of helminth eggs. J. Parasit. 47 : 735-744.
- ✓ Miller, W.C. 1931. The alimentary canal of Maracantha contracta Beauv. (Tenebrionidae). Ohio Jour. Sci. 31 : 143-156.
- ✓ Möbusz, A. 1897. Ueber den Darmkanal der Anthrenus-Larve, nebst Bemerkungen zur Epithel-regeneration. Arch. Naturgesch. 53 : 89-128.
- ✓ Mohamed, U.V.K. & Murad, H. 1975. Morphology and histology of excretory system of Mylabris pustulata Thunb. (Coleoptera : Meloidae). Bull. Ent. 16 : 11-20.
- ✓ Mohamed, U.V.K. & Murad, H. 1977. Studies on the excretory system of the fully grown larva of Callogramma festiva Donovan. (Lepidoptera : Noctuidae). J. Res. Lep. 16 : 213-218.

- ✓ Mohamed, U.V.K. & Murad, H. 1979a. Proctodeal origin of Malpighian tubules in the larva of Callogramma festiva Donovan. Geobios 6 : 156-159.
- Mohamed, U.V.K. & Murad, H. 1979b. Observations on Mucoprotein in the Malpighian tubules of Mylabris pustulata Thunb. (Coleoptera : Meloidae). Zool. Anz., Jena. 203 : 348-353.
- ✓ Morgan, A., Church, N.S., & Rempel, J.G. 1970. The structure and function of the digestive system during postembryonic development in Lytta nuttalli Say (Coleoptera : Meloidae). Can. J. Zool. 48 : 337-350.
- ✓ Mukerjii, D. 1939. Anatomy of the larval stages of the Bruchid beetle Bruchus quadrimaculatus Fabr., and the method of emergence of the larva from egg-shell. Z. angew. Ent. Berlin 25 : 442-460.
- ✓ Mukharji, S.P. & Singh, C.B. 1971. The structure of the alimentary canal of Sitophilus oryzae Linn. (Curculionidae : Coleoptera). Indian J. Zool. 12 : 91-102.
- ✓ Murray, F.V. & Tiegs, C.W. 1935. The metamorphosis of Calandra oryzae. Q. Jl. microsc. Sci. 77 : 405-495.
- ✓ Nitschmann, J. 1959. Die Entwicklung des Darmkanals bei Myrmica ruginodis Nyl. (Hym., Formicidae). Dt. ent. Z. 6 : 453-463.

- ✓ Oertel, E. 1930. Metamorphosis in the honeybee. J. Morph.
50 : 295-339.
- ✓ Palm, N.B. 1949. The rectal papillae in insects.
K. Sallsk. Handl. Lund. (N.F.). 60 : 3-29.
- ✓ Pajni, H.R. 1967. A note on the metamorphosis of the
midgut epithelium in Callosobruchus maculatus (F.)
(Bruchidae : Coleoptera). Res. Bull. Panjab
Univ. 18 : 75-78.
- ✓ Pantin, C.F.A. 1960. Notes on Microscopical Technique
for Zoologists. Cambridge Univ. Press, London.
viii + 77 pp.
- ✓ Parkin, E.A. 1936. A study of the food relations of the
Lyctus powder-post beetles. Ann. appl. Biol. 23 : 269-400.
- ✓ Patay, R. 1939. Contribution à l'étude d'un Coleoptère
(Leptinotarsa decemlineata (Say)). Évolution des
organes au cours du développement. Thesis, Rennes
Fac. Sci. 145 pp.
- ✓ Patay, R. 1942. A propos de l'évolution de l'intestin
moyen de Tenebrio molitor L. (Coléoptère :
Tenebrionidae) au cours de la nymphose.
Bull. Soc. scient. Bretagne 18 : 77-80.
- ✓ Patterson, M.F. 1937. The cellular structure of the
digestive tract of the beetle Passalus cornutus
Fab. Ann. ent. Soc. Am. 30 : 619-640.

- ✓ Perez, C. 1901. Histolyse des tubues de Malpighi et des glandes séricigènese chez la Fourmi roussi.
Bull. Soc. ent. Fr. 1901 : 307-310.
- ✓ Perez, C. 1902. Contribution a l'étude des métamorphoses.
Bull. scient. Fr. Belg. 37 : 195-427.
- ✓ Perez, C. 1908a. Rénovation épithéliale de l'intestin moyen chez les Muscides. C. r. Séanc. Soc. Biol. Paris 64 : 694-695.
- ✓ Perez, C. 1908b. Métamorphose de l'intestin anterieurchez Muscides. C. r. Séanc. Soc. Biol. Paris 64 : 835-836.
- ✓ Perez, C. 1910a. Recherches histologiques sur la métamorphose des Muscides (Calliphora erythrocephala Meig.).
Arch. Zool. exp. gén. (5) 4 : 1-274.
- ✓ Perez, C. 1910b. Métamorphose des tubes de Malpighi chez les Muscides. C. r. Séanc. Soc. Biol. Paris 68 : 42-43.
- ✓ Perez, C. 1910c. Métamorphose de l'intestin posterieur chez les Muscides. C. r. Séanc. Soc. Biol. Paris 68 : 115-116.
- ✓ Potts, S. F. 1927. The alimentary canal of the Mexican bean beetle. Ohio Jour. Sci. 27 : 127-137.
- ✓ Poyarkoff, E. 1910. Recherches histologiques sur la métamorphose d'un Coléoptère (la Galéruque de l'orme).
Archs Anat. microsc. 12 : 333-474.

- ✓ Pradhan, S. 1936. The alimentary canal of Epilachna indica (Coccinellidae : Coleoptera) with a discussion on the activity of the midgut epithelium. Journ. Roy. Asiat. Soc. Beng. 2 : 127-156.
- ✓ Pradhan, S. 1938. Neuromuscular study of the mouth parts of Coccinella septempunctata with a comparison to the mouthparts. Rec. Indian Mus. 40 : 341-358.
- ✓ Pradhan, S. 1939. The alimentary canal and pro-epithelial regeneration in Coccinella septempunctata with a comparison of carnivorous and herbivorous coccinellids. Q. Jl. microsc. Sci. 81 : 451-478.
- ✓ Pradhan, S. 1942. Re-association of the Malpighian tubules in Coccinellid beetles (a hydro-filter device for water-conservation). Indian J. Ent., New Delhi 4 (1) : 11-21.
- ✓ de Priester. 1972. Ultrastructural changes in developing midgut epithelium of Calliphora erythrocephala Meigen. Z. Zellforsch. 129 : 278-289.
- ✓ Rahman, M.S. 1971. Studies on the morphology and biology of Callosobruchus chinensis (L.) and Callosobruchus analis (Fab.) and external damage of pulses. M.Sc. Thesis, Deptt. Zool., Univ. Dacca : viii + 199 pp.
- ✓ Rahman, R., Huda, S., & Hussain, A. 1975. Effects of gamma radiation on the different stages of Callosobruchus analis Fabricius. Bangladesh J. Zool. 3 : 102-109.

- ✓ van Rees, J. 1888. Beiträge zur kenntnis der innen
Metamorphose von Musca vomitoria. Zool. Jb., Anat.
3 : 1-134.
- ✓ Rehman, K.A., Sohi, G.S., & Sapora, A.N. 1942. Biology of
Bruchus analis Fab. and Bruchus chinensis Linn.
Indian J. agric. Sci. 12 : 851-864.
- ✓ Reichenback-Klinke, H.H. 1952. Die Rektal-papillen der
Insekten insbesondere der käfer und ihre Beziehung
zur Stammesages chichichte. Zool. Jahrb. abt. Anat.
Ontog. Tiere 72 : 230-250.
- ✓ Reinecke, J.P., Cook, B.J., & Adams, T.S. 1973. Larval
hindgut of Manduca sexta (L.) (Lepidoptera :
Sphingidae). Int. J. Insect Morphol. & Embryol.
2(4) : 277-290.
- ✓ Reinecke, J.P. & Adams, T.S. 1977. A novel muscle complex
on the hindgut of lepidopteran larvae. Int. J.
Insect Morphol. & Embryol. 6 : 239-254.
- ✓ Reinecke, J.P., Gerst, J., Gara, B.O., & Adams, T.S. 1978.
Innervation of hindgut muscle of larval Manduca
sexta (L.) (Lepidoptera : Sphingidae) by a
peripheral multinucleate neurosecretory neuron.
Int. J. Insect Morphol. & Embryol. 7 (5/6) : 435-453.
- ✓ Rengel, C. 1896. Über die Veränderungen des Darmepithels
bei Tenebrio molitor während der Metamorphose.
Z. wiss. Zool. 62 : 1-60.

- ✓ Rezaur, R. Seal, D.R., & Ahmed, M. 1984. A study on the influence of parental age on the life cycle of Callosobruchus analis Fabricius (Coleoptera : Bruchidae). Acta ent. Bohemica 5 : (In press).
- ✓ Richins, C. A. 1938. The metamorphosis of the digestive tract of Aedes dorsalis Meigen. Ann. ent. Soc. Am. 31 : 74-87.
- ✓ Risler, H. 1961. Untersuchungen zur somatischen Reduktion in der Metamorphose des Stechmück-endarms. Biol. Zbl. 80 : 413-428.
- ✓ Robertson, C.W. 1936. The metamorphosis of Drosophila melanogaster, including an accurately timed account of the principal morphological changes. J. Morph. 59 : 351-399.
- ✓ Ryerse, J.S. 1979. Developmental changes in Malpighian tubule cell structure. Tissue & Cell 11 : 533-551.
- ✓ Saini, R.S. 1964. Histology and physiology of the cryptonephridial system of insects. Trans. R. ent. Soc. Lond. 116 : 347-392.
- ✓ Saksena, R.D. 1951. Observations on the Morphology and physiology of the alimentary canal of Aulacophora foveicollis (Lucas) (Chrysomelidae-Coleoptera). Proc. natn. Acad. Sci. India 21 (B) : 23-32.

- ✓ Samson, K. 1908. Über das Verhalten der vasa Malpighi und die excretorische Funktion der Fettzellen während Metamorphose von Heterogenea limacodes Hufn. Zool. Jb., Anat. 26 : 403-422.
- ✓ Savage, A.A. 1956. The development of the Malpighian tubules of Schistocerca gregaria (Orthoptera). Q. Jl. microsc. Sci. 97(4) : 599-615.
- ✓ Savage, A.A. 1962. The development of the Malpighian tubules of Carausius morosus (Orthoptera). Q. Jl. microsc. Sci. 103(4) : 417-437.
- ✓ Schaefer, P.E. 1931. The alimentary canal of Sphaeroderus nitidicollis Chev. Var. Schaumi Chd. (Coleoptera). Ohio Jour. Sci. 31 : 406-415.
- ✓ Schmidt, G.H. 1964. Histologische Untersuchungen zur Metamorphose des Mitteldarmepithels von Formica ployctena Foerst. (Ins. Hym.). Biol. Zbl. 83 : 717-724.
- ✓ Seong Su II. 1981. Changes of protein and nucleic acids during metamorphosis in the midgut of the silkworm, Bombyx mori L. (Lepidoptera : Bombycidae). Appl. Ent. Zool. 16(2) : 138-148.
- ✓ Shukla, G.S. & Awasthi, V.B. 1969. Studies on the alimentary canal of Mylabris pustulata Thumb. (Coleoptera : Meloidae). Indian J. Zool. 10 : 1-12.

- ✓ Shukla, G.S. & Kumar, K. 1969. Studies on alimentary canal of Odoiporus longicollis. I. Morphology. Ann. ent. Soc. Am. 62 : 664-665.
- ✓ Shukla, G.S. & Upadhyay, V.B. 1976. Morphology of the alimentary canal of Dactylosternum hydrophiloides (Macleay) (Coleoptera : Hydrophilidae). Indian J. Zool. 17 : 11-15.
- ✓ Shukla, G.S. & Upadhyay, V.B. 1980. A comparative study of the morphology of alimentary canal in a saprophagous and carnivorous insect. J. Anim. Morph. Physiol. 27 : 132-140.
- ✓ Singh, C.B. & Mukharji, S.P. 1971. The structure of the alimentary canal of flour beetle Tribolium castaneum Herbst. Indian J. Zool. 12 : 103-112.
- ✓ Sinha, R.N. 1958a. The alimentary canal of the adult Tribolium castaneum Herbst (Coleoptera, Tenebrionidae). J. Kans. entomol. Soc. 31 : 118-125.
- ✓ Sinha, R. N. 1958b. Similarities in the history of the gut of some species of Tribolium (Macleay) (Coleoptera, Tenebrionidae) and Oryzaephilus Ganglbauer (Col., Cucujidae). Can. Ent. 90 : 129-145.
- ✓ Snodgrass, R. E. 1935. Principles of Insect Morphology. McGraw-Hill, N. Y. and London, ix + 667 pp.

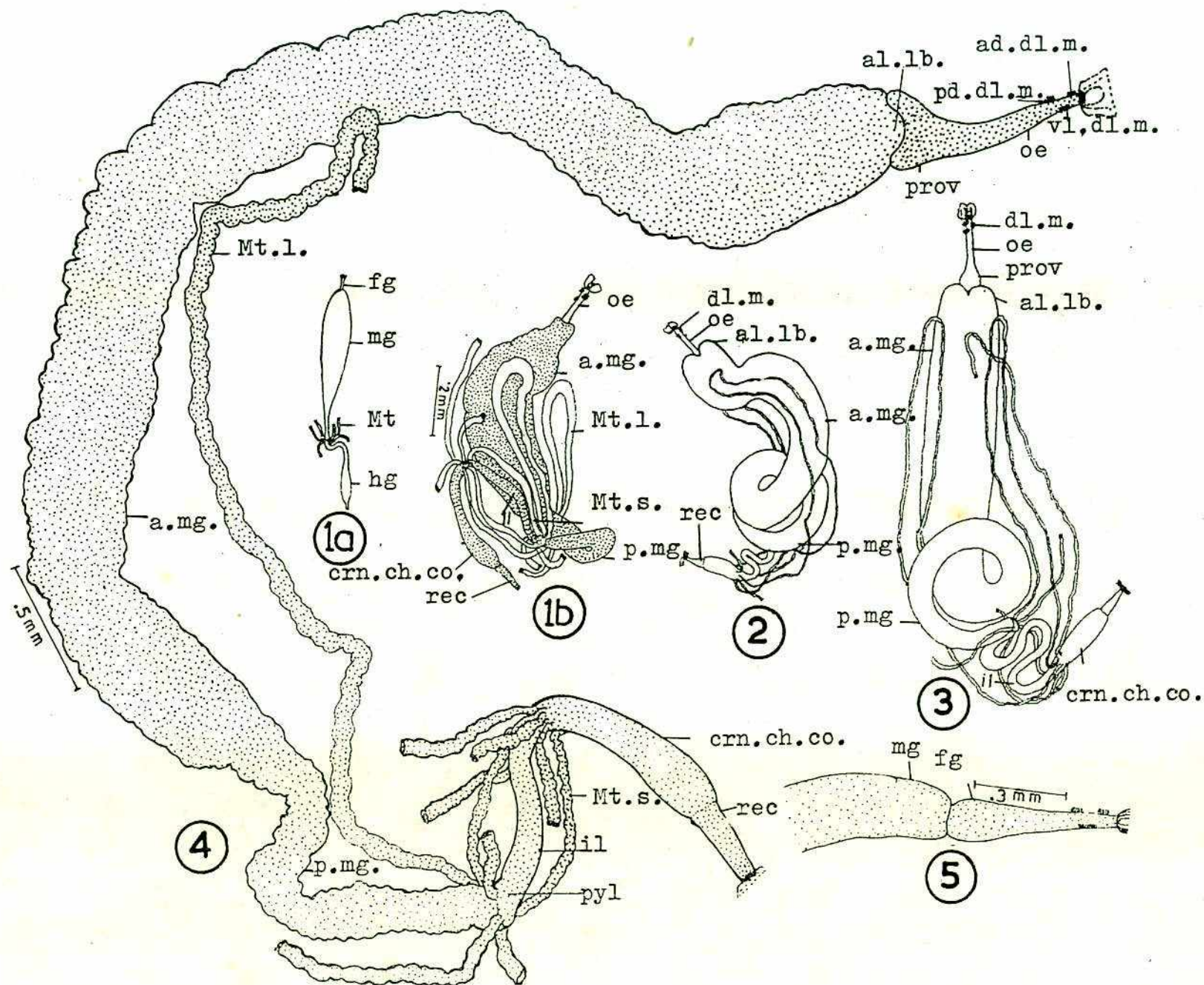
- ✓ Souza, V.B.V., Silva, I., & Freitas, O.T. 1981.
Mitochondrios em células de de tubulos de Malpigh
da larva de Oncideres s. saga (Dalman, 1823)
(Coleoptera, Cerambycidae). Rev. bras. Ent.
25 : 95-101.
- ✓ Srisan, R. 1966. Studies on the use of gamma radiation
in the control of pea weevil, Callosobruchus
chinensis (L). Thai AEC 8 : 18-30.
- ✓ Srivastava, U.S. & Bahadur, J. 1961. The development of
Malpighian tubules in Dysdercus koenigi (Hemiptera :
Pyrrhocoridae). Q. Jl. microsc. Sci. 102 : 347-360.
- ✓ Srivastava, U.S. & Khare, M.K. 1966. The development of
Malpighian tubules and associated structures in
Philosamia ricini (Lepidoptera, Saturnidae).
J. Zool., Lond. 150 : 145-163.
- ✓ Strambi, C. 1970. Anatomie du tube digestif des Coléoptères
Catopidae (imagos). Annls. Spéleol. 25(4) : 851-869.
- ✓ Strambi, C. 1974. Recherches histologiques sur le
stomodeum et le mésenteron des Coléoptères Catopides
(imagos). Archos Biol. 85 : 427-451.
- ✓ Strambi, C. 1976. Contribution a l'étude du tube digestif
des larves des Coléoptères Catopidae. Annls. Spéleol.
31 : 263-270.

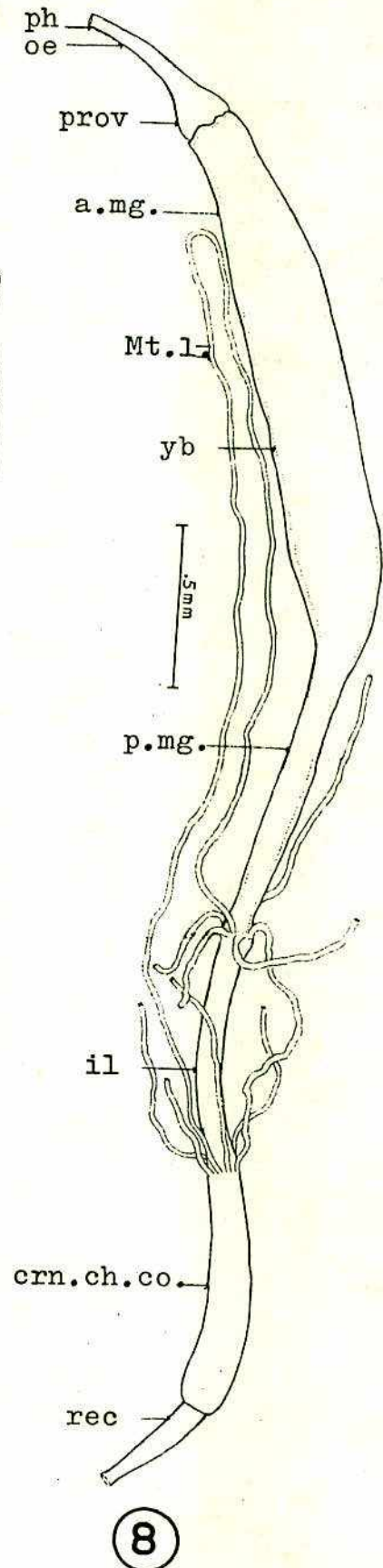
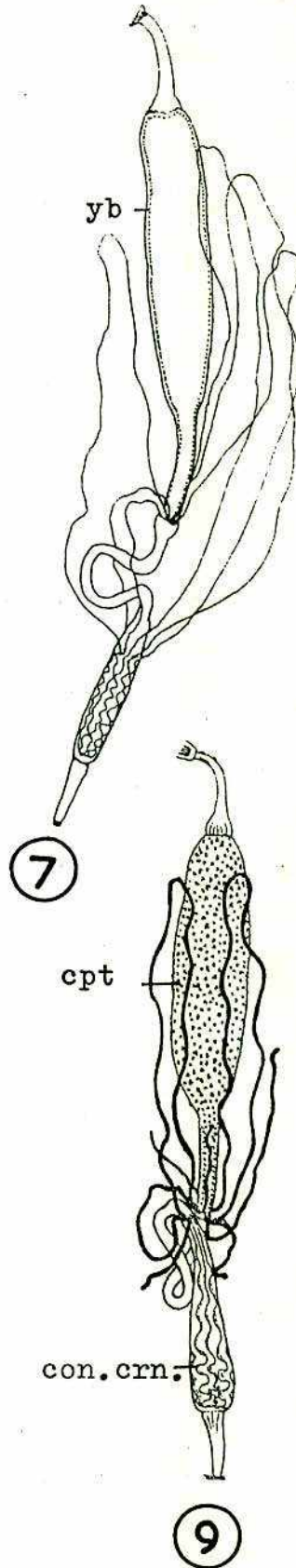
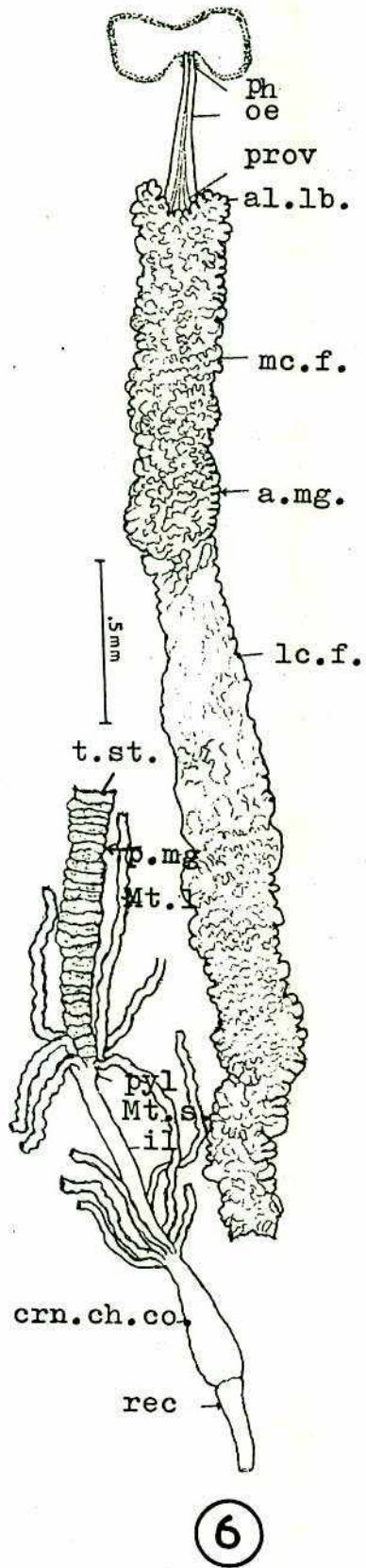
- ✓ Sundman, J.A. & King, D. R. 1964. Morphological, histological and histochemical studies of the alimentary canal and Malpighian tubules of the adult boll weevil, Anthonomus grandis (Coleoptera : Curculionidae). Ann. ent. Soc. Am. 57 : 89-95.
- ✓ Swingle, M. C. 1930. Anatomy and physiology of the digestive tract of the Japanese beetle. J. agric. Res. Washington 41 : 181-196.
- ✓ Tchang, Y-T. 1929. Recherches sur l'histogénese et l'histophysiologie de l'épithélium de l'intestin moyen chez un Lépidoptère (Galleria mellonella L.). Bull. biol. Fr. Belg. 12 : 1-144.
- ✓ Thompson, M.T. 1905. Alimentary canal of the mosquito. Proc. Boston Soc. nat. Hist. 32 : 145-202.
- ✓ Thomson, J.A. 1975. Major patterns of gene activity during development in holometabolous insects. Adv. Insect Physiol. 11 : 321-398.
- ✓ Tiegs, O.W. 1922. Researches on insect metamorphosis. I. On the structure and post-embryonic development of a chalcid wasp, Nasonia. II. On the physiology and interpretation of insect metamorphosis. Trans. R. Soc. S. Aust. 46 : 319-527
- ✓ Umeya, K. 1960. A comparative morphology of the alimentary tract in the adults of Lamellicorn-beetles (Coleoptera). Mem. Fac. Agric. Hokkaido Univ. 3 : 60-113.

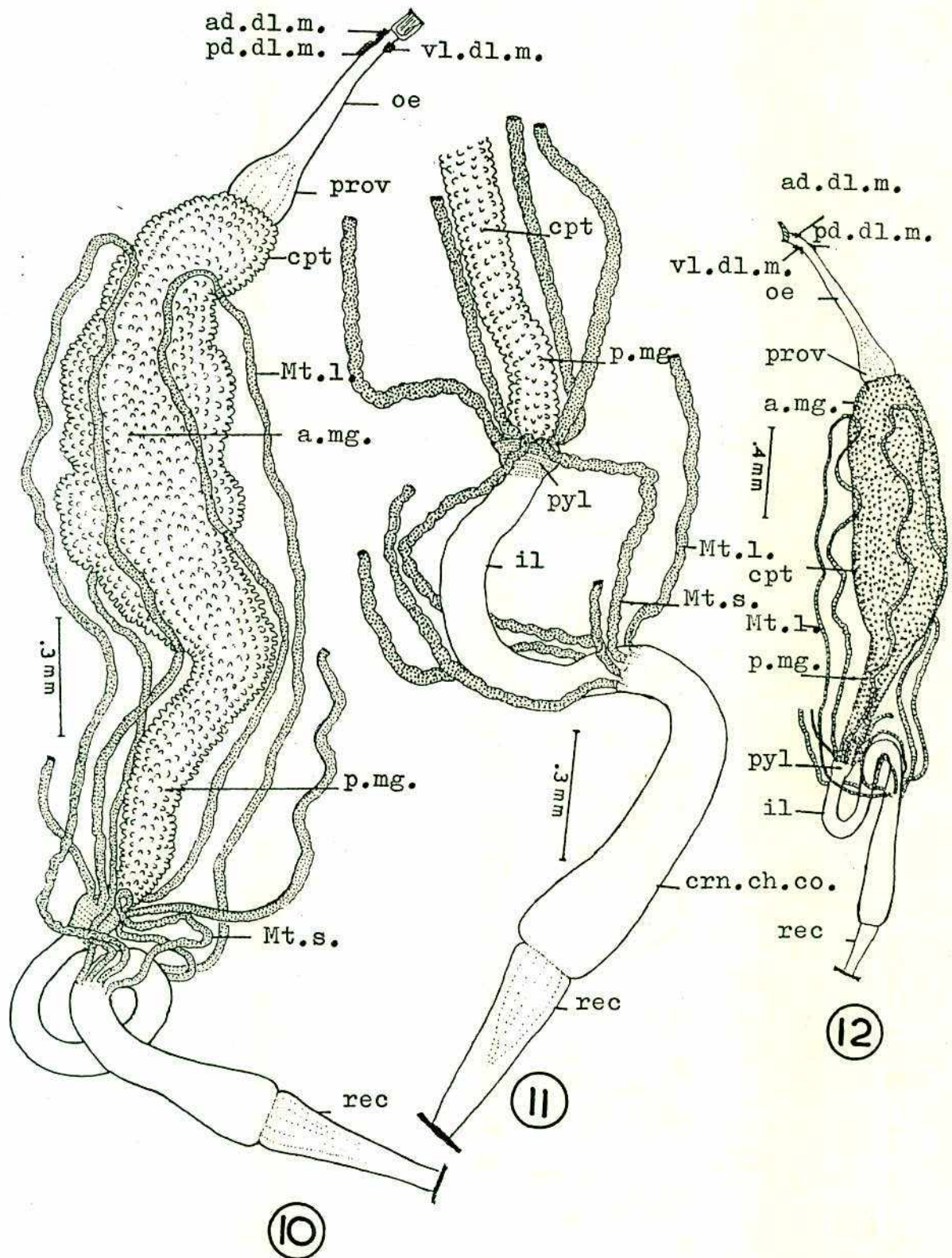
- ✓ Vaney, C. 1902. Contributions à l'étude des larves et des métamorphoses des Diptères. Annales Univ. Lyon (n.s.). 9 : 1-178
- ✓ Vats, L.K. 1976. Alimentary canal in Bruchid larvae (Bruchidae : Coleoptera). Res. Bull. Panjab Univ. 27 : 103-106.
- ✓ Verma, P. S. 1969. The alimentary canal and associated organs of two saprophagous beetles, Onthophagus catta Fabr. and Aphodius moestus Fabr. (Coleoptera : Scarabaeidae). Bull. Ent. 10 : 4-11.
- ✓ Verson, E. 1905. Zur Entwicklung des Verdauungskanal bei Bombyx mori. Z. wiss. Zool. 82 : 523-600.
- ✓ Waku, Y. & Sumimoto, K.I. 1971. Metamorphosis of midgut epithelial cells in the silkworm (Bombyx mori L.) with special regard to the calcium salt deposits in the cytoplasm. I. Light microscopy. Tissue & Cell . 3(1) : 127-136.
- ✓ Weismann, A. 1864. Die nachembryonale Entwicklung der Musciden. Z. wiss. Zool. 14 : 197-336.
- ✓ Wigglesworth, V.B. 1932. On the function of the so-called "rectal glands" of insects. Q. Jl. microsc. Sci. 75 : 131-150.

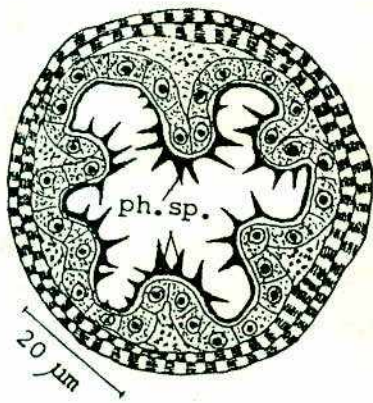
- ✓ Wigglesworth, V.B. 1972. The Principles of Insect Physiology (7th ed.). Chapman and Hall Ltd., London, viii + 827 pp.
- ✓ Wigglesworth, V.B. & Salpeter, M.M. 1962. Histology of the Malpighian tubules in Rhodnius polixus J. Insect Physiol. 8 : 299-307.
- ✓ Woods, W.C. 1918. The alimentary canal of the larva of Altica bimarginata Say (Coleoptera). Ann. ent. Soc. Am. 11 : 283-317.
- / Yu, C.H., Kim, W.K., & Kim, C. W. 1980. Histology of the mudgut during the metamorphosis of Pieris rapae L. Commemoration Papers for Professor C. W. Kim's 60th Birthday Anniv. Korea Univ. pp. 179-186.

VII. ILLUSTRATIONS

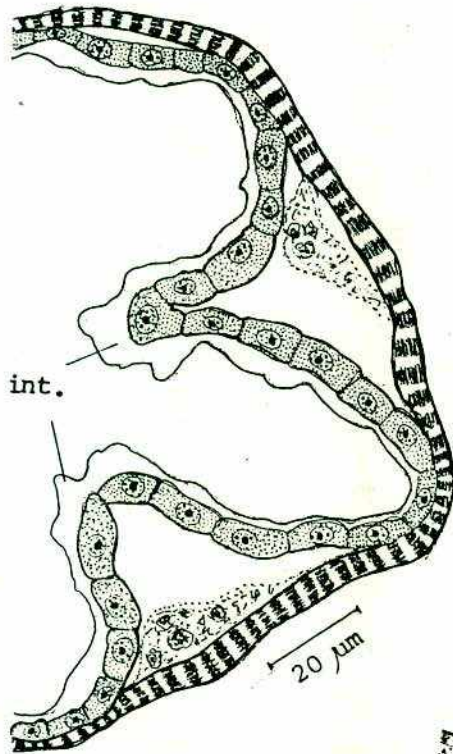




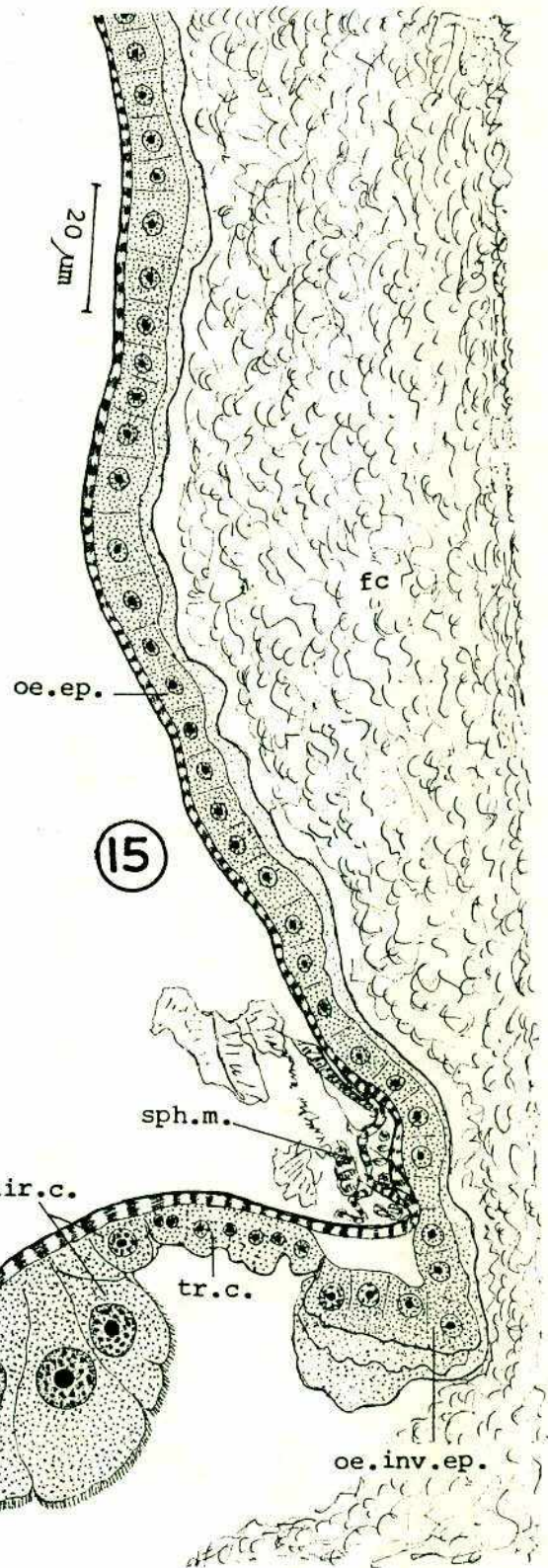




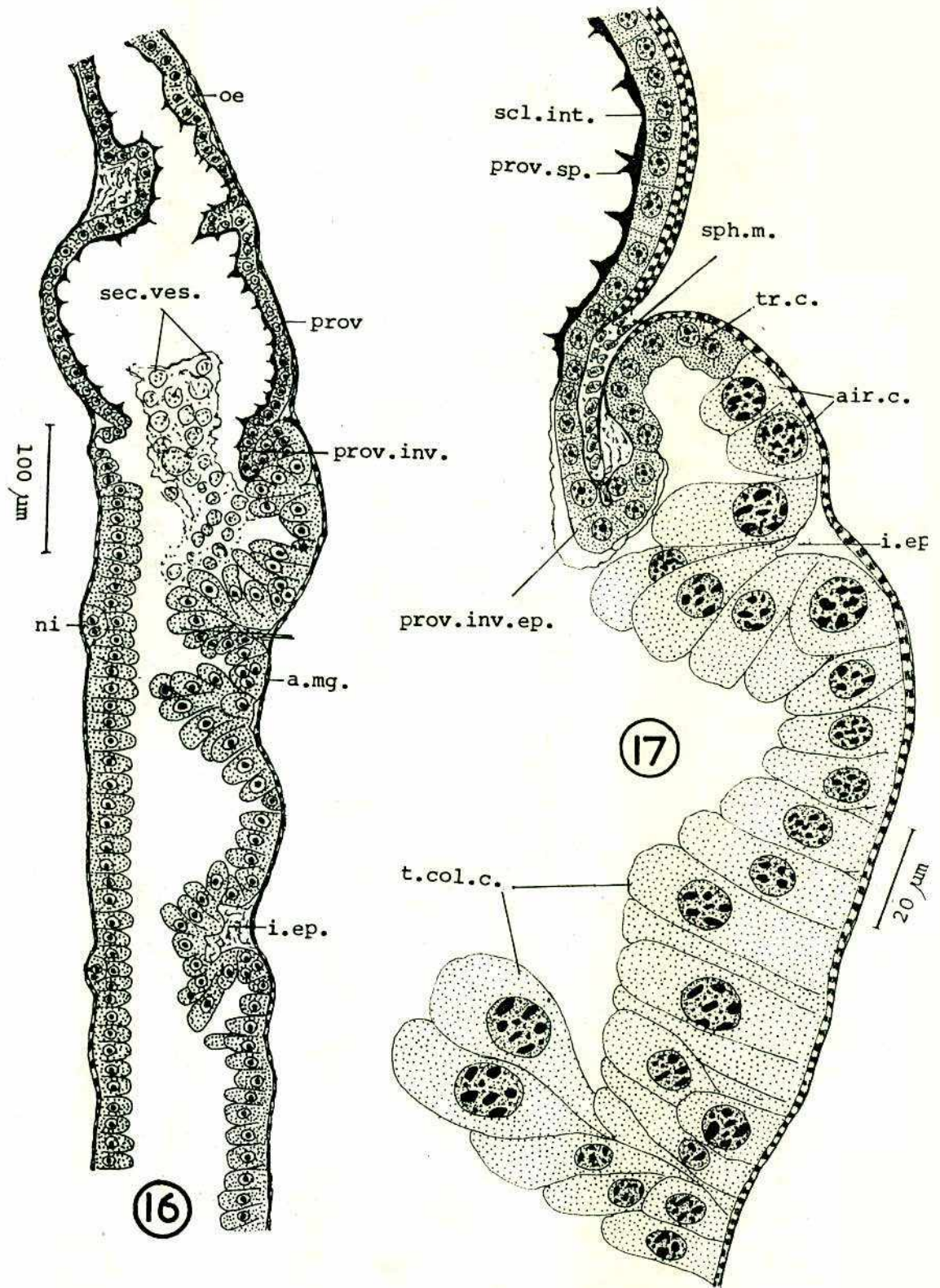
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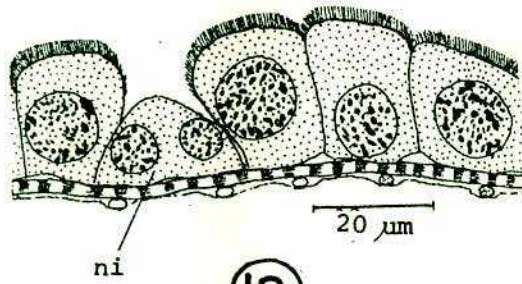


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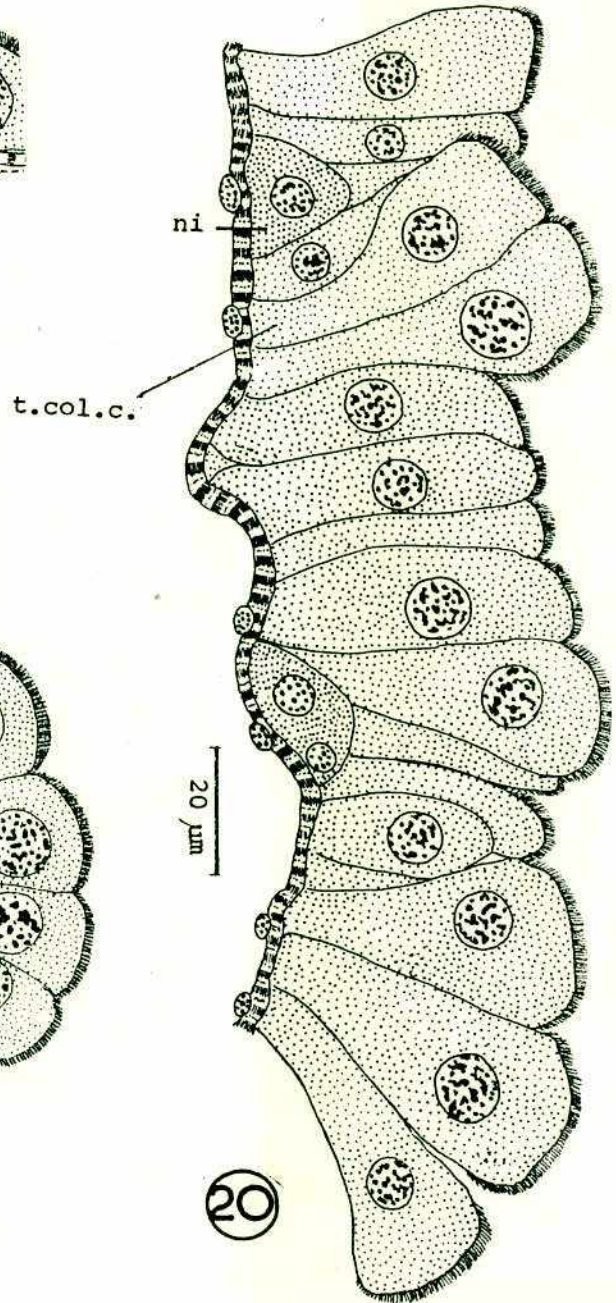


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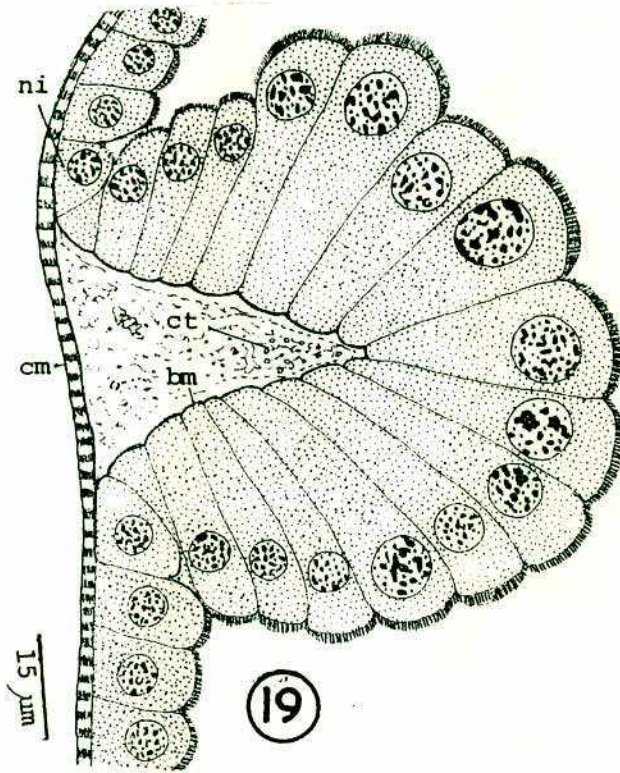




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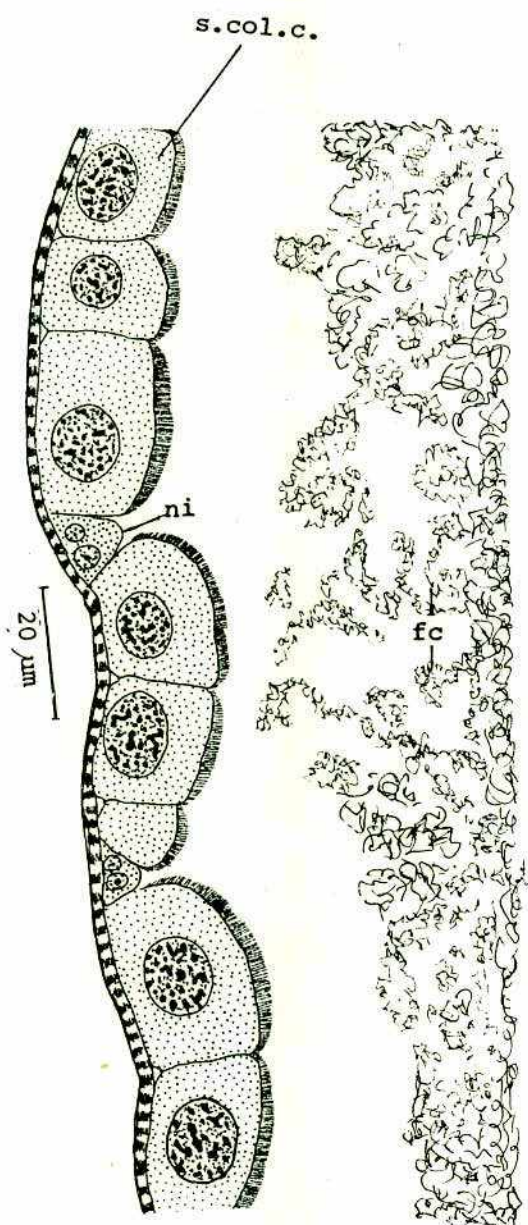
Figs. 21-23. C. analis larva. Different parts of the alimentary canal.

21. Part of L.S. of posterior section of the anterior midgut (2nd instar), showing short and wide columnar epithelial cells.
22. Part of L.S. of posterior midgut (2nd instar).
23. Part of T.S. of posterior midgut (1st instar).

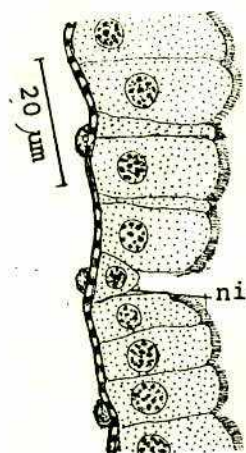
fc = food contents

ni = nidus

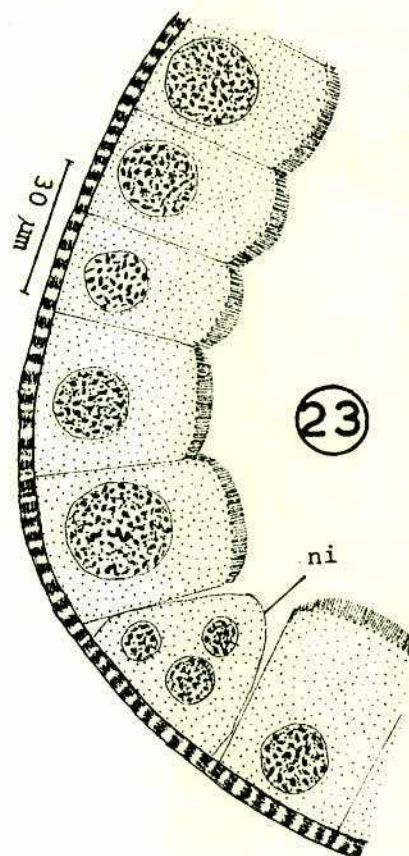
s.col.c. = short and wide columnar epithelial cell



21



22

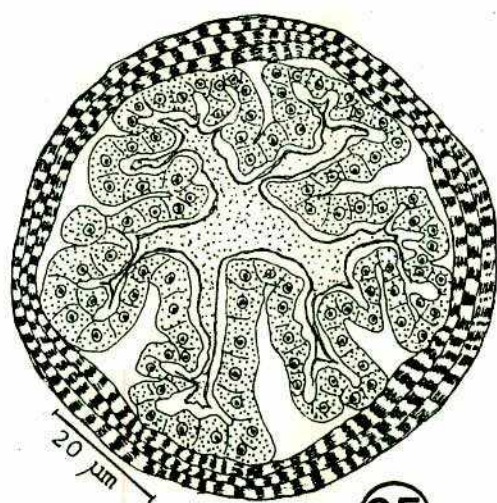
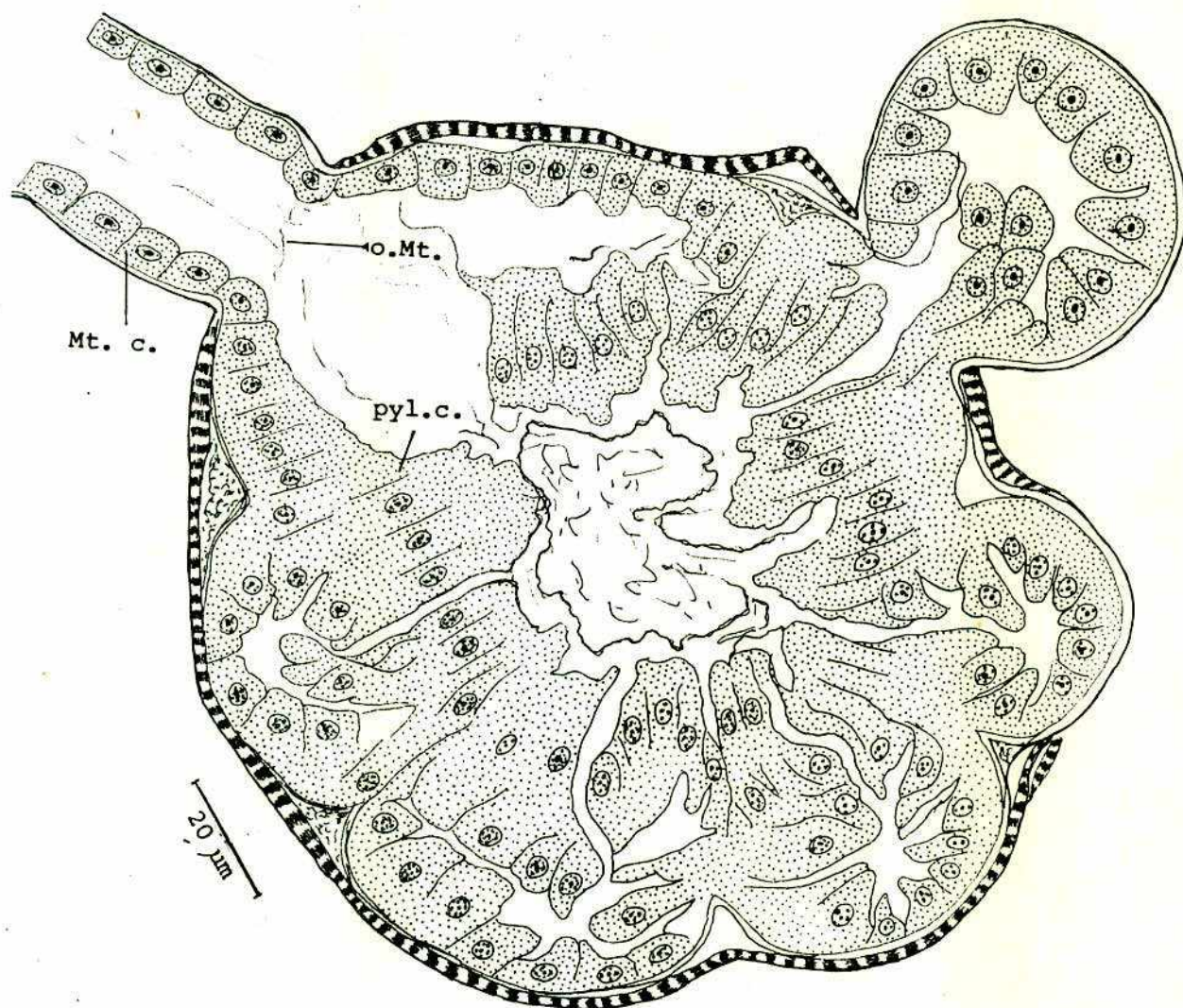


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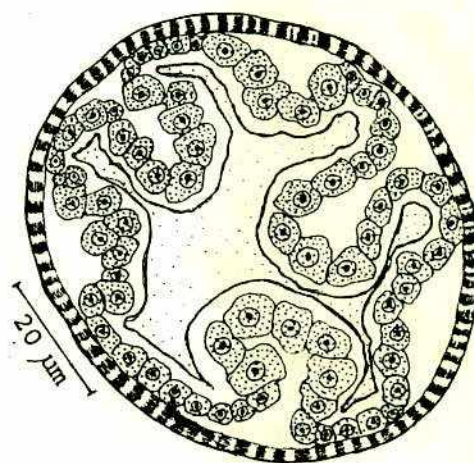
Figs. 24-26. C. analis larva. Different parts of the alimentary canal.

- 24. T.S. of anterior end of the pylorus (2nd instar), showing the opening of the Malpighian tubules.
- 25. T.S. of posterior end of the pylorus (2nd instar).
- 26. T.S. of ileum (2nd instar).

Mt.c. = epithelial cell of Malpighian tubule
o.Mt. = opening of Malpighian tubule
pyl.c. = epithelial cell of pylorus



24



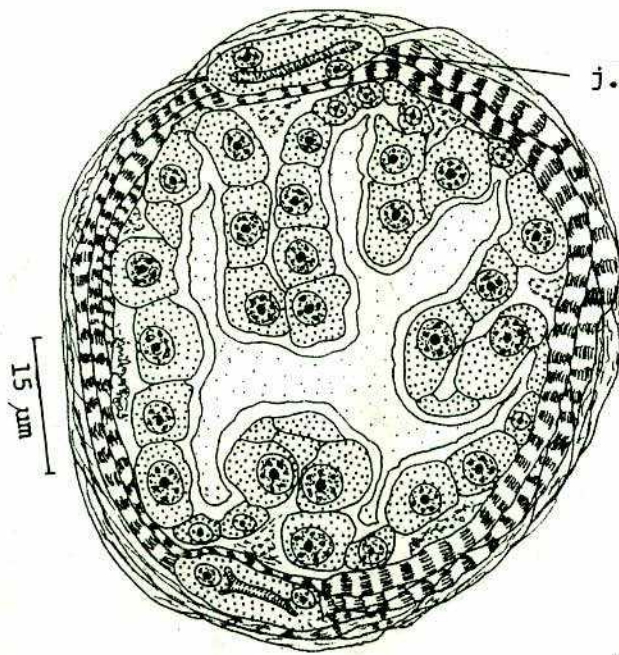
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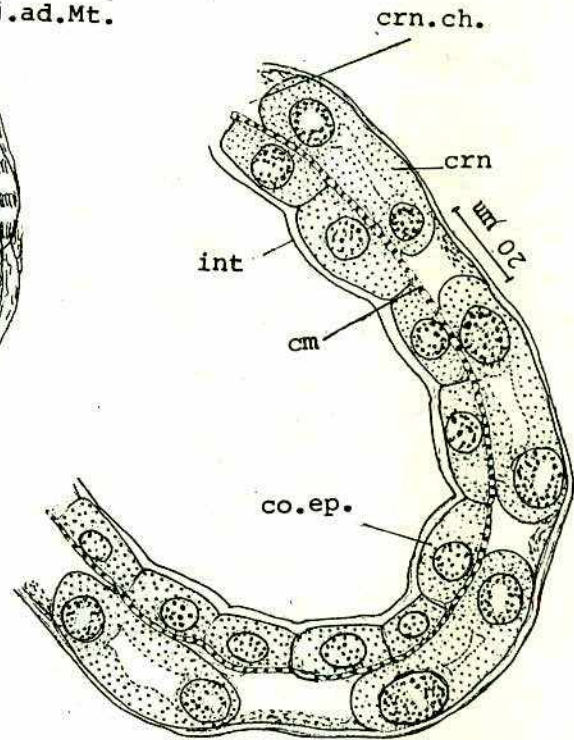
Figs. 27-30. C. analis larva. Different parts of the alimentary canal.

27. T.S. of anterior end of the colon (1st instar) showing initial insertion of two Malpighian tubules.
28. Part of T.S. of colon (1st instar) with cryptonephridial system.
29. T.S. of colon (3rd instar), with cryptonephridial system.
30. T.S. of rectum (2nd instar), showing rectal spines and "diagonal muscles".

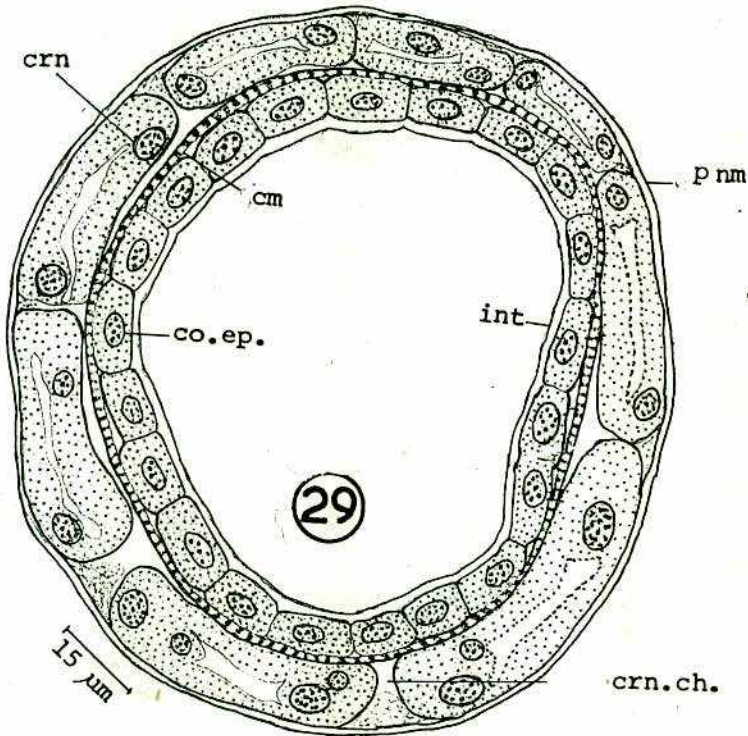
✓cm	=	circular muscle
✓co.ep.	=	colon epithelium
✓crn	=	cryptonephridium
✓crn.ch.	=	cryptonephridial chamber
✓dg.m.	=	diagonal muscle
✓int	=	intima
✓j.ad.Mt.	=	just adrered Malpighian tubule around the colon wall
✓lm	=	longitudinal muscle
✓pnm	=	perinephric membrane
✓rec.sp.	=	rectal spine



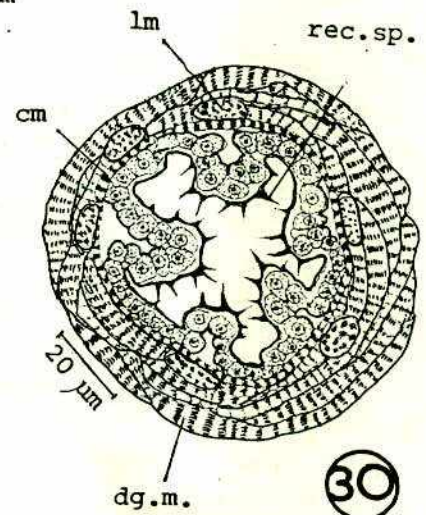
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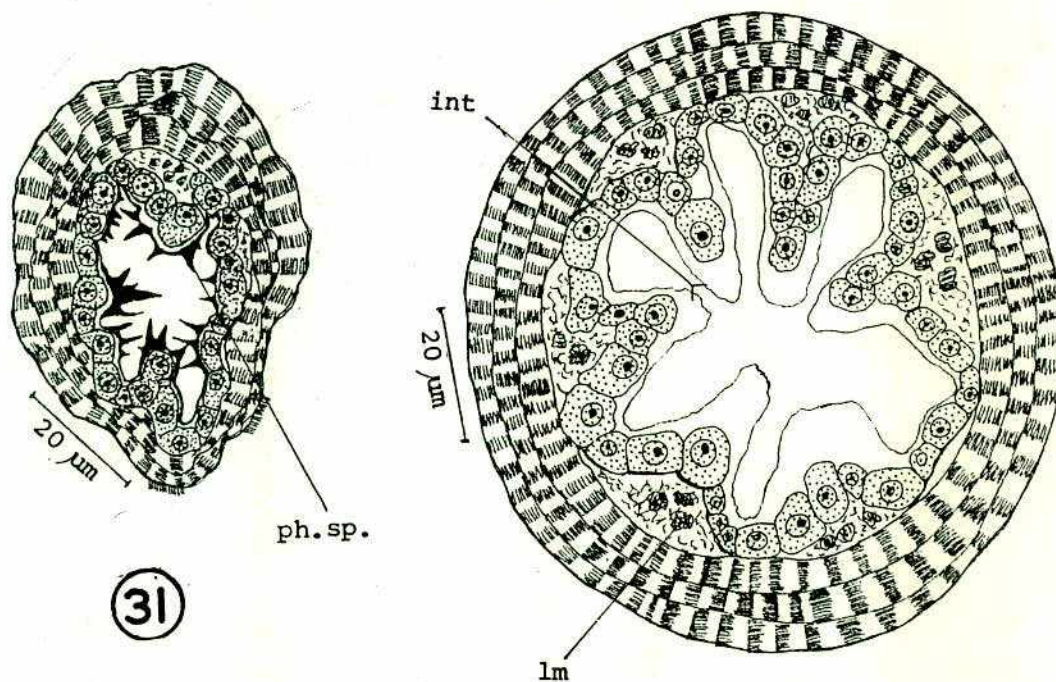


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Figs. 31-34. Adult C. analis. Different parts of the alimentary canal.

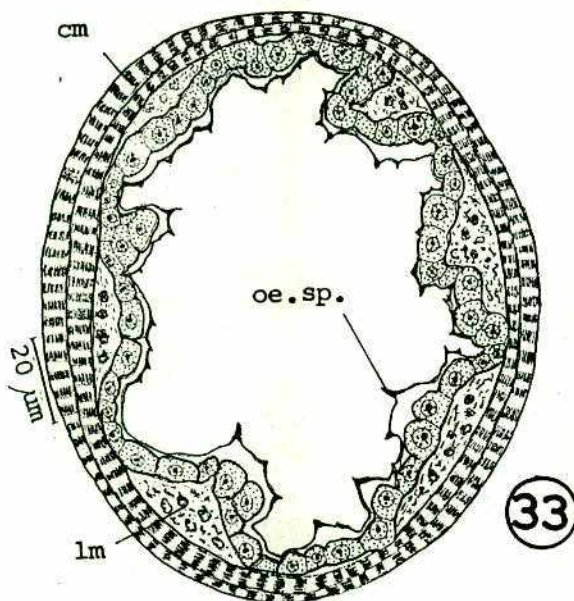
- 31. T.S. of anterior part of the pharynx, showing pharyngeal spines.
- 32. T.S. of posterior part of the pharynx, showing thick intima without spines.
- 33. T.S. of middle part of the oesophagus, showing minute oesophageal spines.
- 34. T.S. of posterior part of the oesophagus, showing prominent oesophageal spines.

cm = circular muscle
 int = intima
 lm = longitudinal muscle
 oe.sp. = oesophageal spine
 ph.sp. = pharyngeal spine

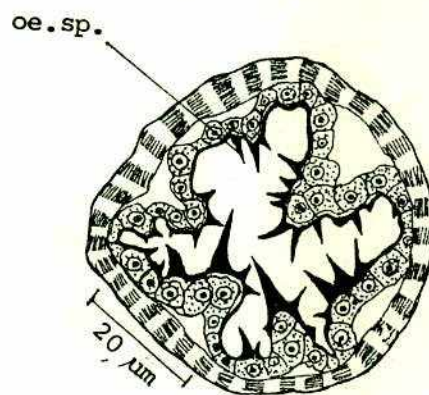


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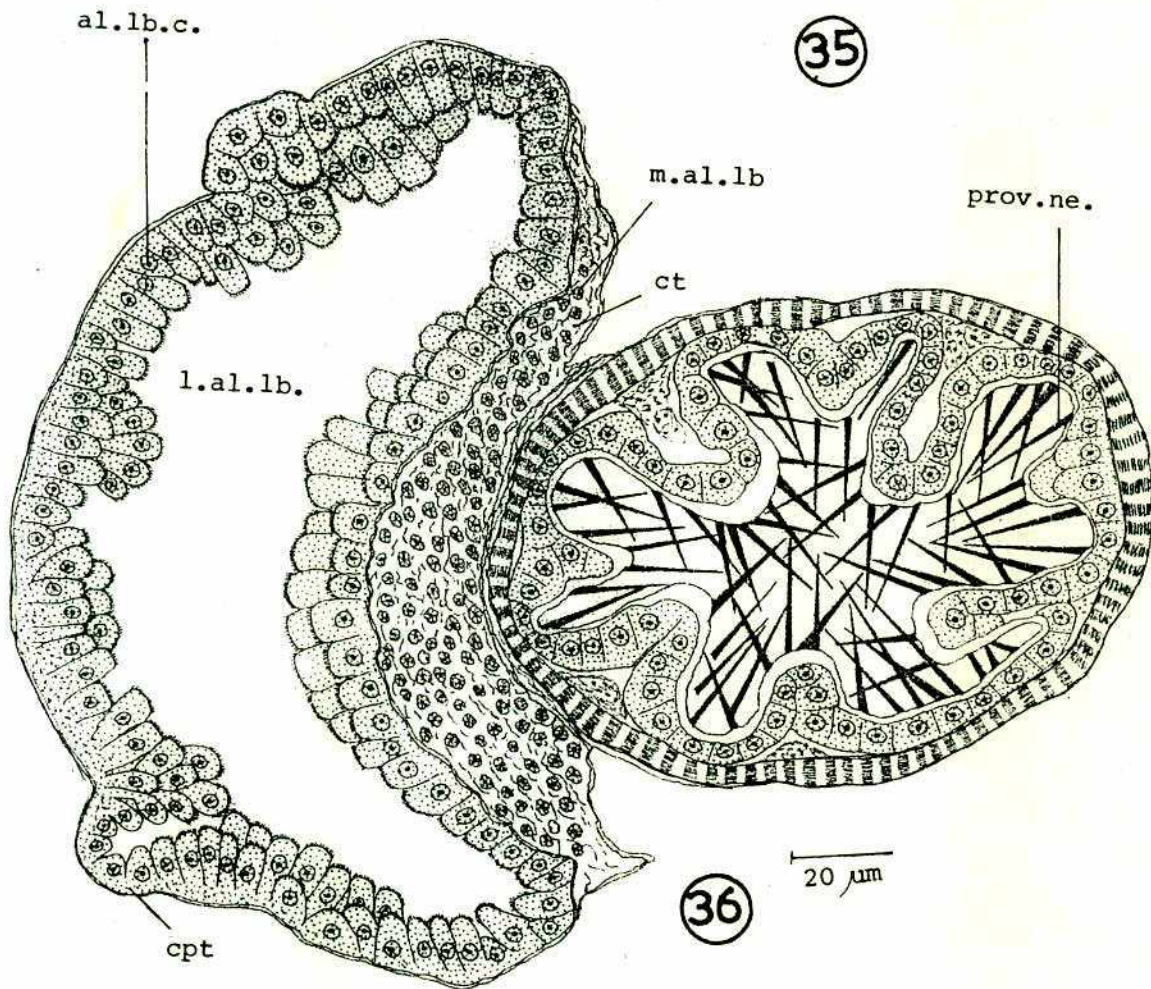
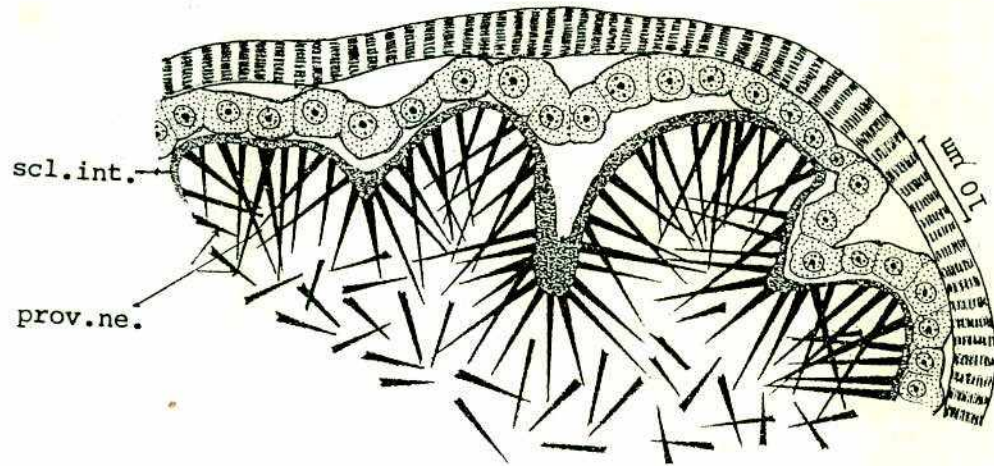


34

Figs. 35 & 36. Adult C. analis. Different parts of the alimentary canal.

- 35. Part of T.S. of anterior end of the proventriculus, showing the stripe of sclerotized intima and proventricular needles.
- 36. Oblique section from foregut-midgut region showing anterior lobed part of the midgut and anterior section of the proventriculus.

- ✓ al.lb.c. = epithelial cells of antero-lateral lobe
- ✓ cpt = crypt
- ✓ ct = connective tissue
- ✓ l.al.lb. = lumen of antero-lateral lobe
- ✓ m.al.lb. = muscles adjacent to the antero-lateral lobe (i.e. in the neck of proventricular invagination)
- ✓ prov.ne. = proventricular needles
- ✓ scl.int. = sclerotized intima

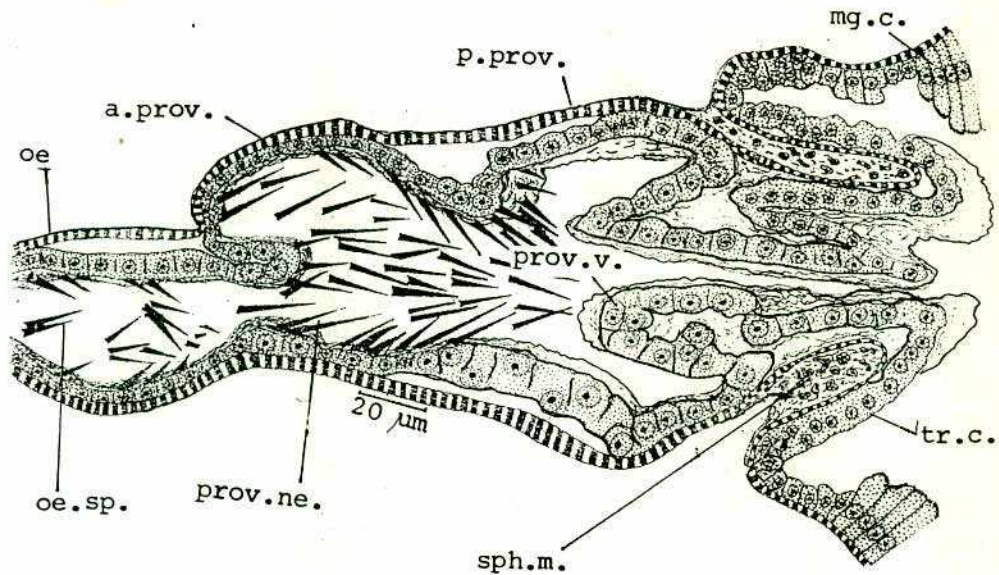


Figs. 37 & 38. Adult C. analis. Different parts of the alimentary canal.

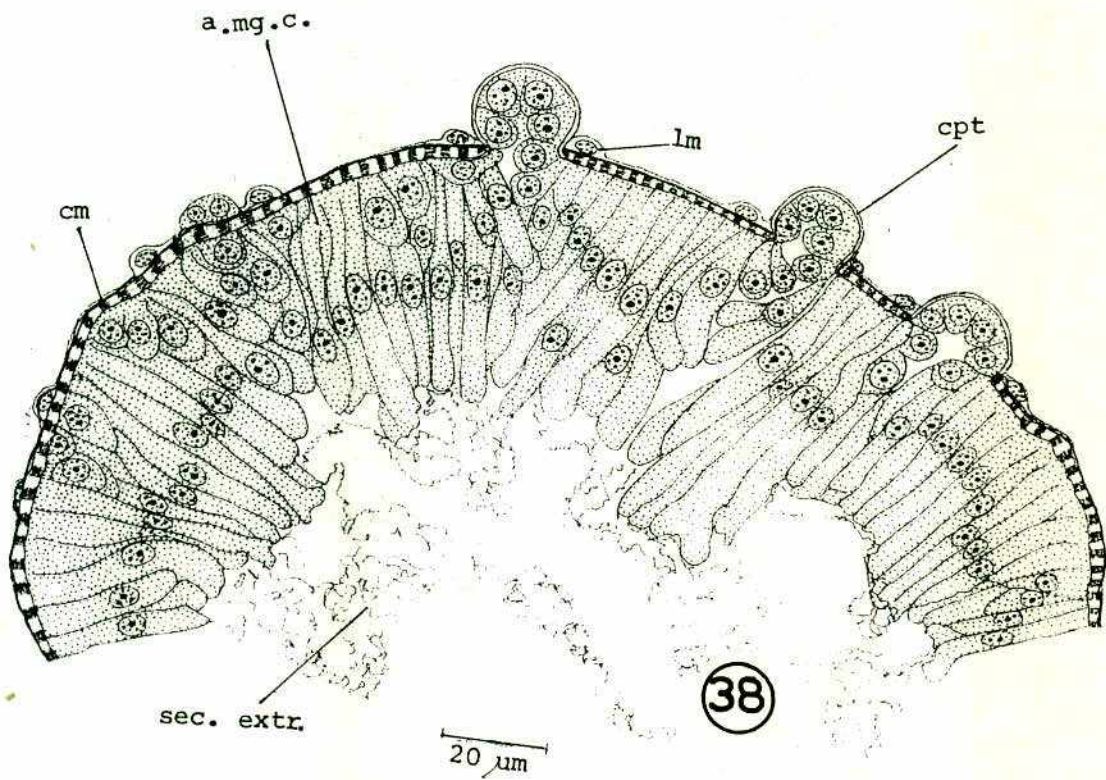
37. L.S. of foregut-midgut junction, showing anterior section of the proventriculus with needles and posterior section with thick intima; proventricular valves and sphincter muscles.

38. Part of T.S. of anterior midgut, showing secretory extrusions of the epithelial cells and crypts.

- ✓ a.mg.c. = anterior midgut (epithelial) cells
- ✓ a. prov. = anterior section of proventriculus
- ✓ cm = circular muscle
- ✓ cpt = crypt
- ✓ lm = longitudinal muscle
- ✓ mg.c. = midgut (epithelial) cell
- ✓ oe = oesophagus
- ✓ oe.sp. = oesophageal spine
- ✓ p.prov. = posterior section of proventriculus
- ✓ prov.ne. = proventricular needles
- ✓ prov.v. = proventricular valve
- ✓ sec.extr. = secretory extrusions
- ✓ sph.m. = sphincter muscle
- ✓ tr.c. = transitional cells



37

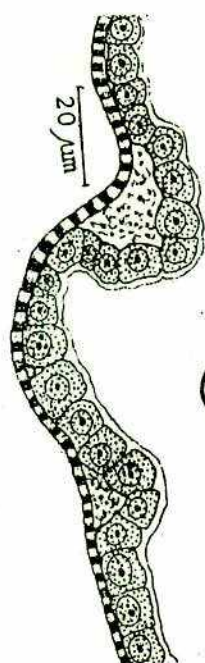


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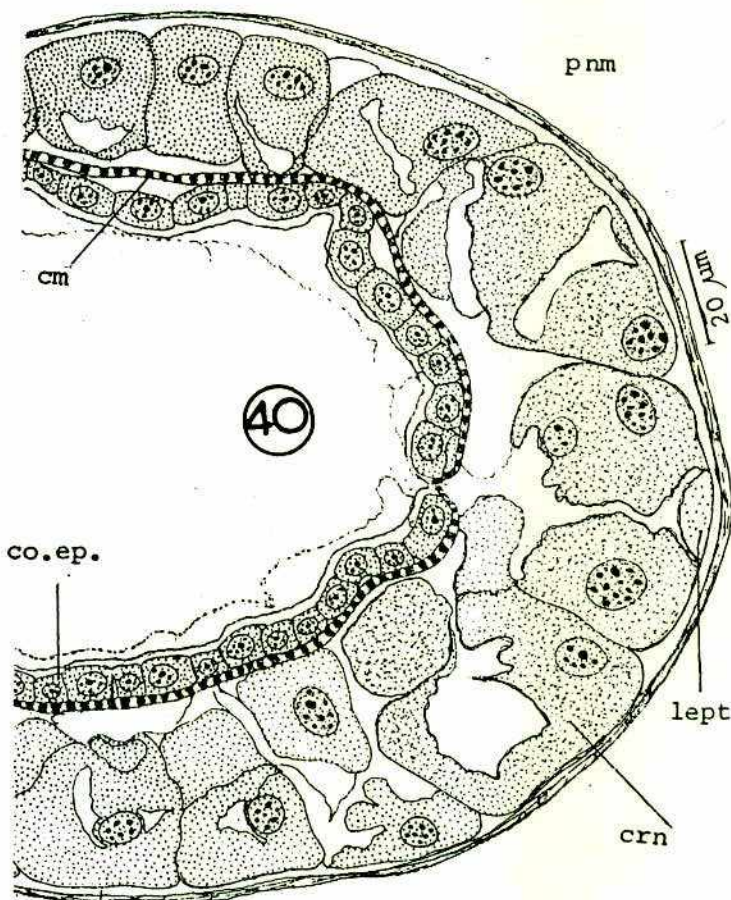
Figs. 39-42. Adult C. analis. Different parts of the alimentary canal.

- 39. Part of L.S. of ileum
- 40. Part of T.S. of colon with cryptonephridial system.
- 41. Part of T.S. of anterior end of the rectum, showing rectal pads.
- 42. T.S. of posterior end of the rectum, showing rectal papillae and rectal spines.

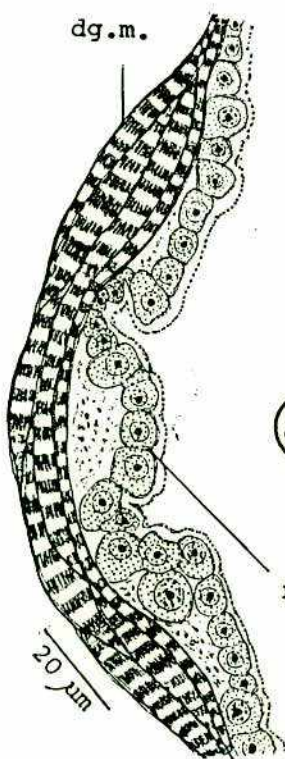
✓cm	=	circular muscle
✓co.ep.	=	colon epithelium
✓crn	=	cryptonephridium
✓crn.ch.	=	cryptonephridial chamber
✓dg.m.	=	diagonal muscle
✓lept	=	leptophragma
✓pnm	=	perinephric membrane
✓rec.pap.	=	rectal papilla
✓rec.pd.	=	rectal pad
✓rec. sp.	=	rectal spine



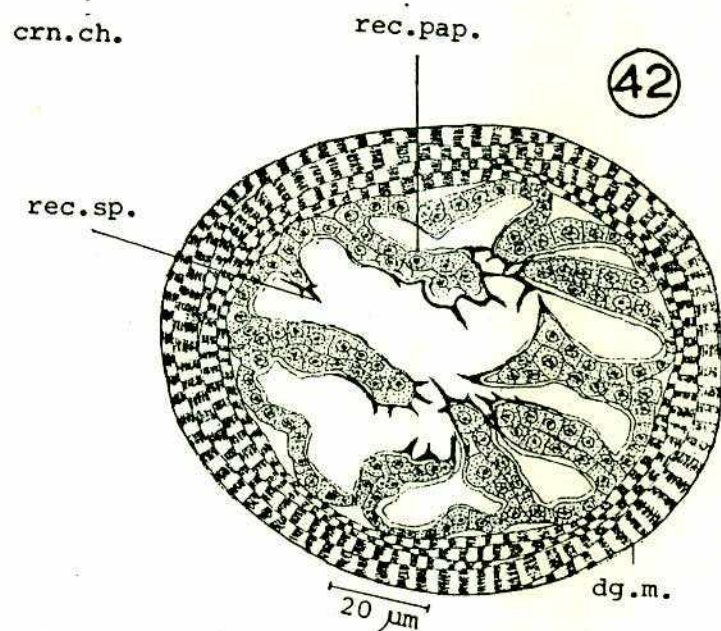
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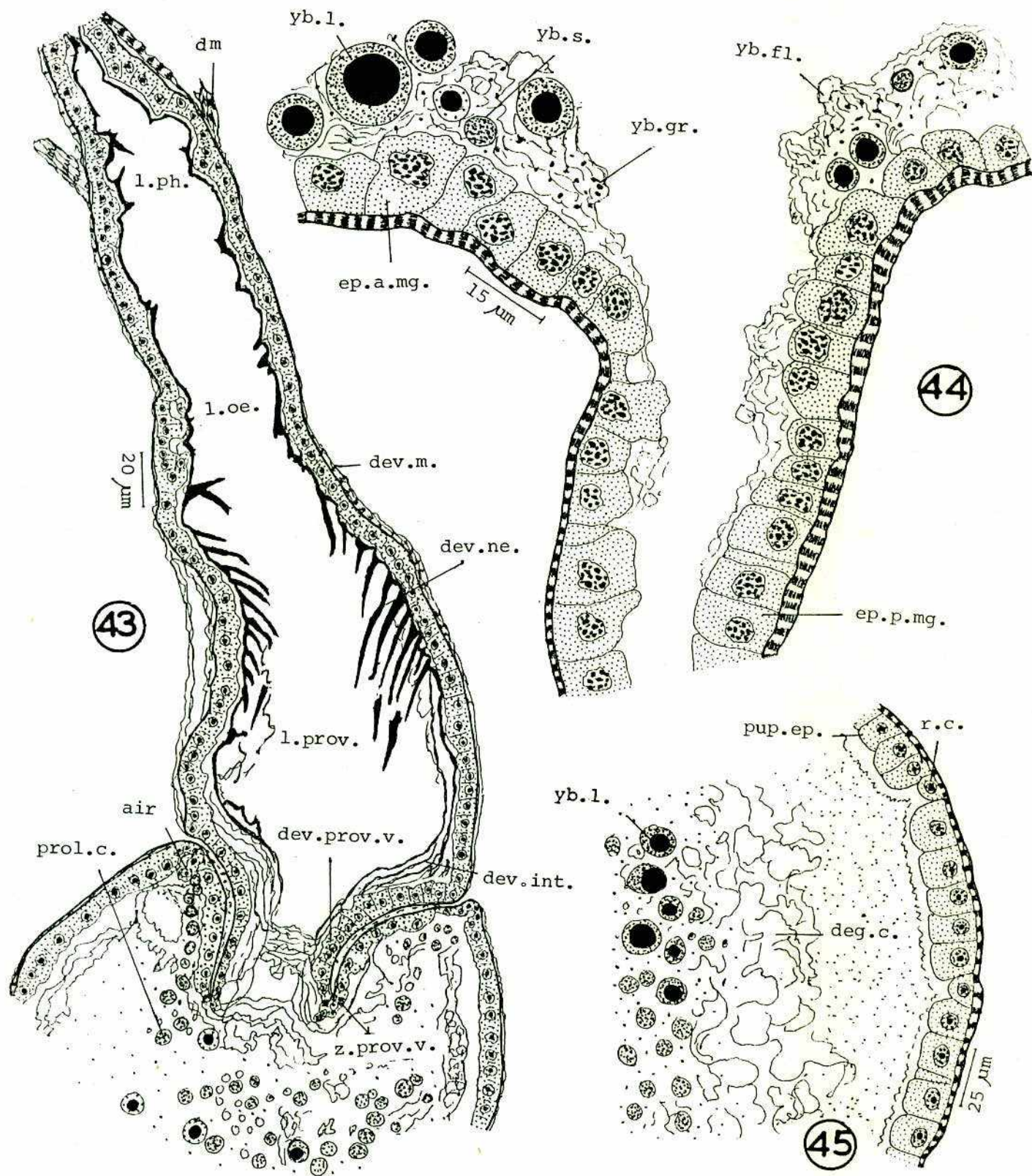


42

Figs. 43-45. Pupa of C. analis. Different parts of the alimentary canal.

43. L.S. of late pupal (6 days old) foregut and foregut-midgut junction, showing developing proventricular needles and valves and stream of proliferated cells from the anterior imaginal ring.
44. L.S. passing through anterior midgut and posterior midgut junction of late pupa (8 days old).
45. Part of T.S. of anterior midgut of the pupa (7 days old), normal pupal midgut epithelium with regenerative cells; yellow bodies and degenerated cells in the lumen.

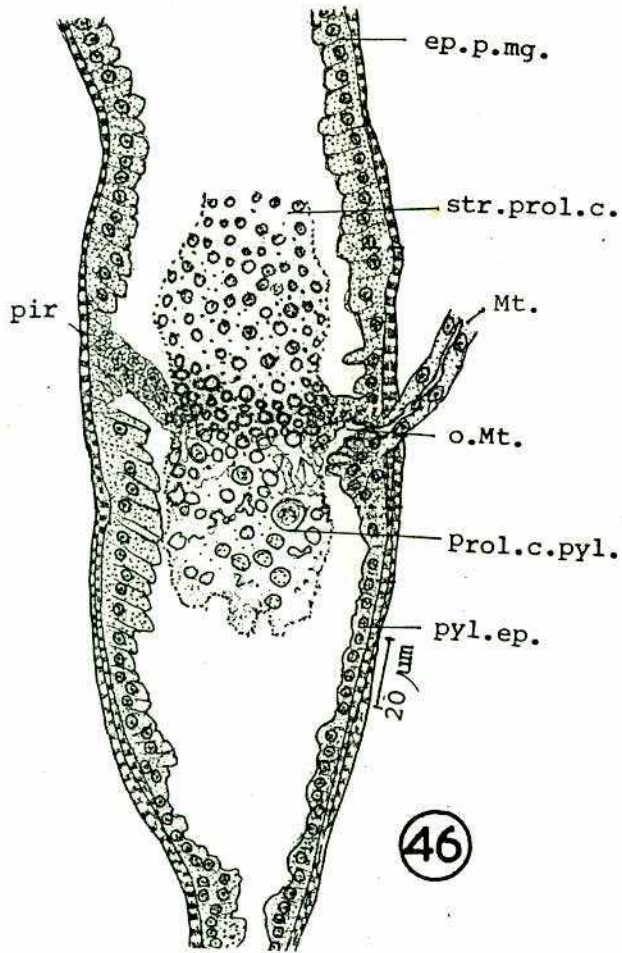
✓ air	=	anterior imaginal ring
✓ deg.c.	=	degenerating cells
✓ dev.int.	=	developing intima
✓ dev.m.	=	developing muscle
✓ dev.ne.	=	developing needles
✓ dev.prov.v.	=	developing proventricular valve
✓ dm	=	dilator muscle
✓ ep.a.mg.	=	epithelium of anterior midgut
✓ ep.p.mg.	=	epithelium of posterior midgut
✓ l.oe.	=	lumen of oesophagus
✓ l.ph.	=	lumen of pharynx
✓ l.prov.	=	lumen of proventriculus
✓ prol.c.	=	proliferated cells
✓ pup.ep.	=	pupal (midgut) epithelium
✓ r.c.	=	regenerative cell
✓ yb.fl.	=	fluid medium which contain yellow bodies
✓ yb.gr.	=	granular yellow bodies
✓ yb.l.	=	larger yellow bodies
✓ yb.s.	=	smaller yellow bodies
✓ z.prov.v.	=	zone of daughter nuclei which form the proventricular valves.



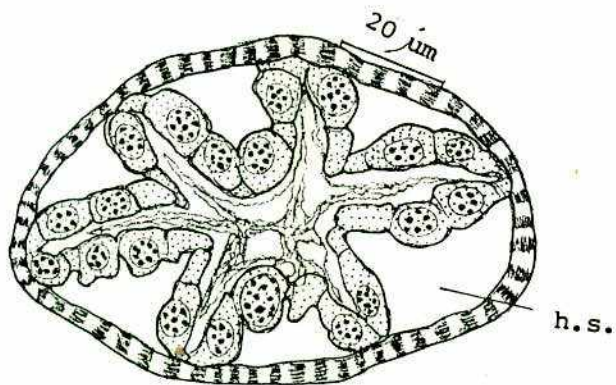
Figs. 46-49. Pupa of C. analis. Different parts of the alimentary canal.

46. L.S. of midgut-hindgut junction of pupa (6 days old), showing stream of proliferated cells from the posterior imaginal ring and opening of a Malpighian tubule.
47. T.S. of pupal (6 days old) ileum, showing hollow space at the basal part of the epithelial fold.
48. L.S. passing through the colon-rectum junction of pupa (6 days old).
49. L.S. of late pupal (8 days old) rectum, showing developing "rectal pads" and "rectal papillae" regions.

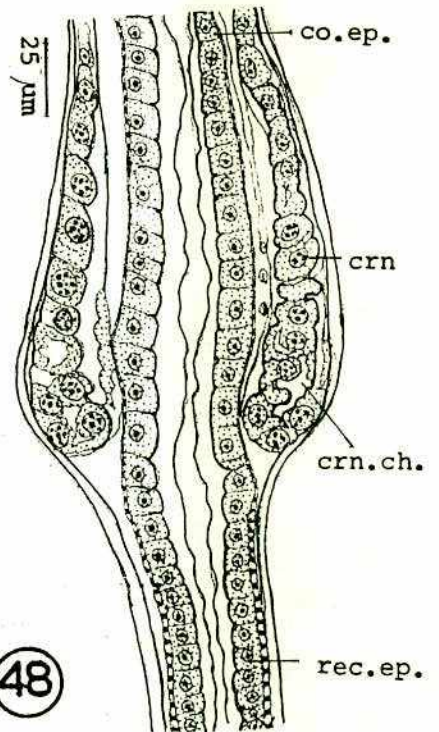
✓co.ep.	= colon epithelium
✓crn	= cryptonephridium
✓crn.ch.	= cryptonephridial chamber
✓dev.rec.pd.	= developing rectal pad
✓dev.rec.pap.	= developing rectal papilla
✓dev.sp.	= developing spine
✓ep.p.mg.	= epithelium of posterior midgut
✓h.s.	= hollow space
✓Mt	= Malpighian tubule
✓o.Mt.	= opening of Malpighian tubule
✓pir	= posterior imaginal ring
✓prol.c.pyl.	= proliferated cells in the pylorus
✓pyl.ep.	= pyloric epithelium
✓rec.ep.	= rectal epithelium
✓str.prol.c.	= stream of proliferated cells



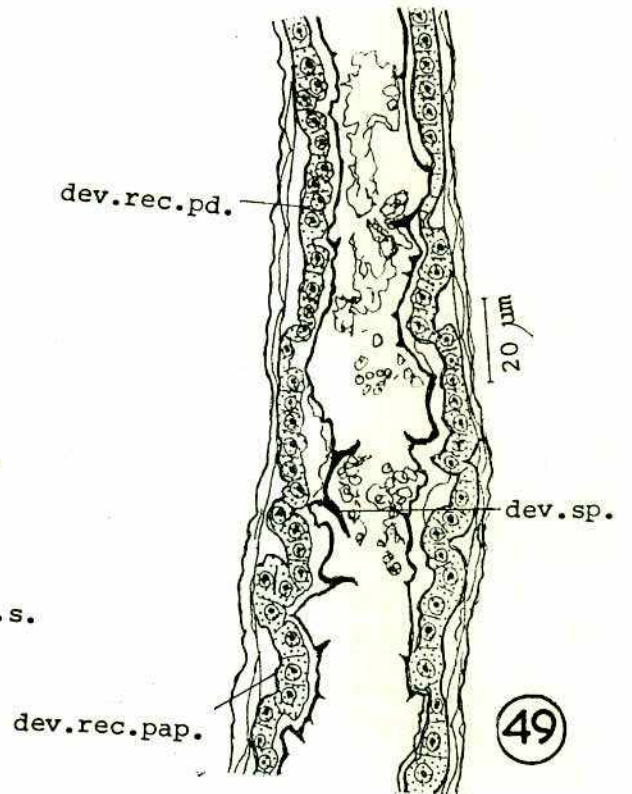
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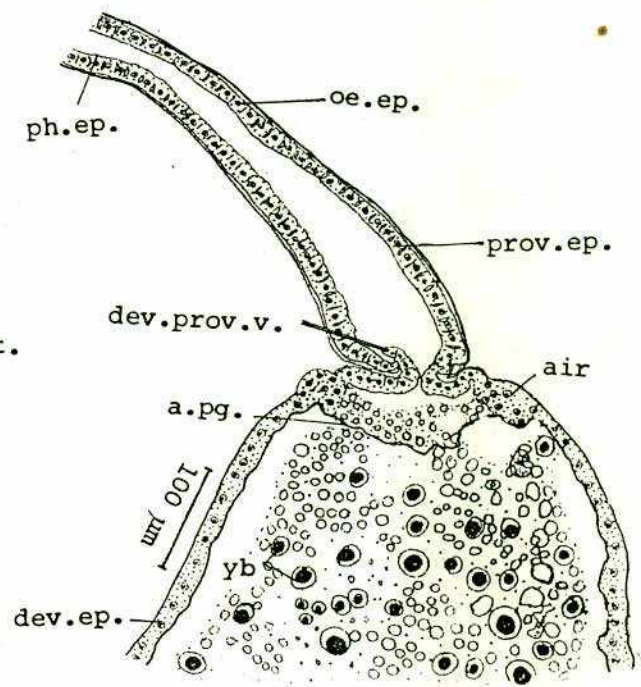
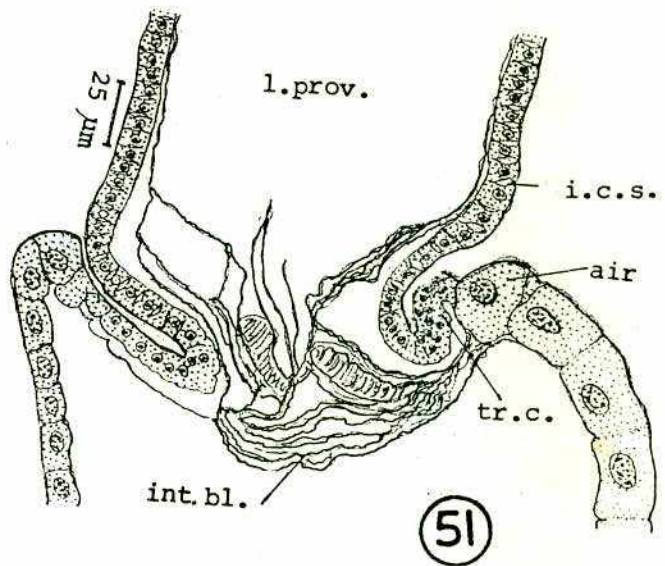
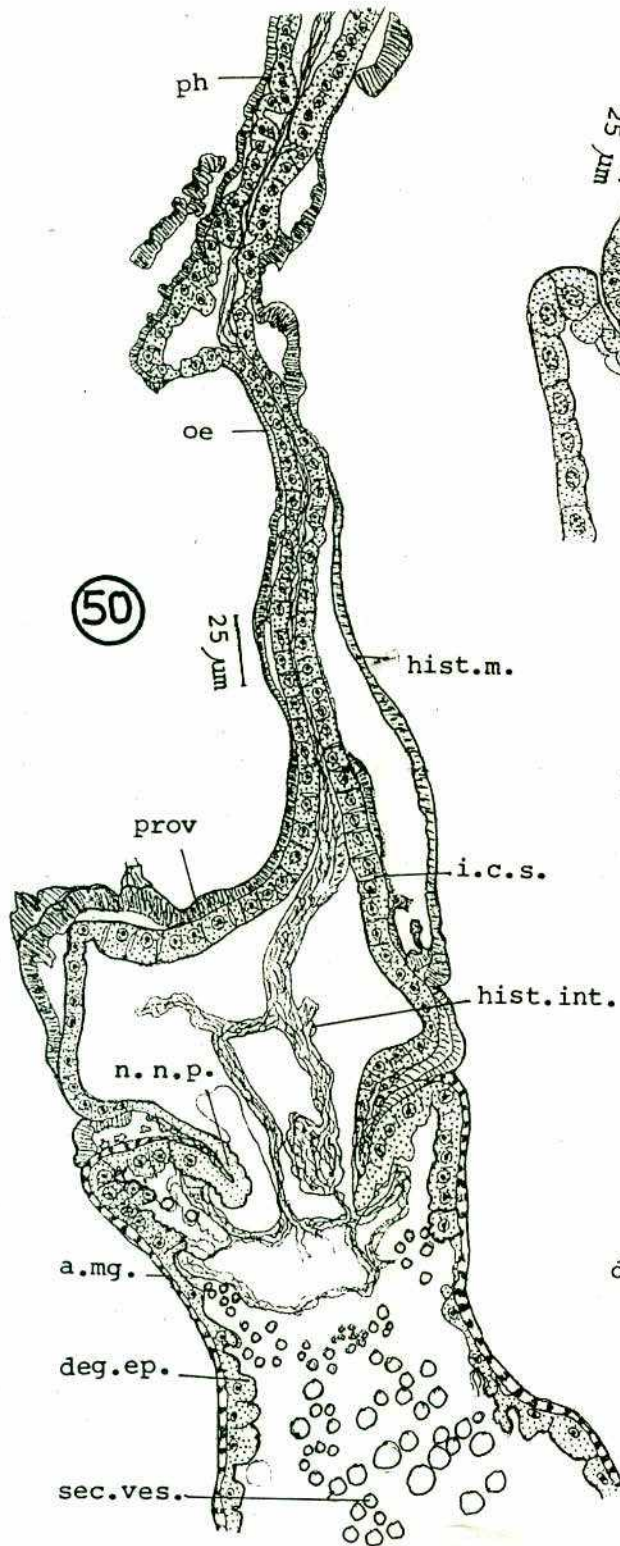


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Figs. 50-52. Pupa of C. analis. Different parts of the alimentary canal.

50. L.S. of foregut and foregut-midgut junction of early pupa (1 day old), showing histolysing intima and muscles, degenerating midgut epithelium and proliferation of cells from the anterior imaginal ring.
51. L.S. of early pupal (2 days old) foregut-midgut junction, showing "intimal block".
52. L.S. of pupal (5days old) foregut and foregut-midgut junction, showing developing proventricular valve and "anterior plug" formed by the proliferated cells from the anterior imaginal ring, and yellow bodies in the midgut lumen.

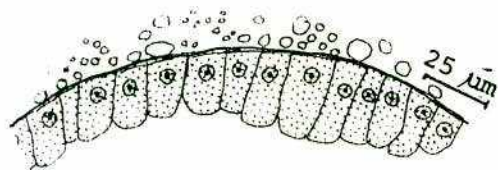
✓air	=	anterior imaginal ring
✓a.mg.	=	anterior midgut
✓a.pg.	=	"anterior plug"
✓deg.ep.	=	degenerating epithelium
✓dev.ep.	=	developing epithelium
✓dev.prov.v.	=	developing proventricular valve
✓hist.int.	=	histolysing intima
✓hist.m.	=	histolysing muscle
✓i.c.s.	=	inter-cellular space
✓int.bl.	=	intimal block
✓l.prov.	=	lumen of proventriculus
✓n.n.p.	=	non-nucleated part
✓oe	=	oesophagus
✓oe.ep.	=	oesophageal epithelium
✓ph	=	pharynx
✓ph.ep.	=	pharyngeal epithelium
✓prov	=	proventriculus
✓prov.ep.	=	proventricular epithelium
✓sec.ves.	=	secretory vesicle
✓tr.c.	=	transitional cells
✓yb	=	yellow body



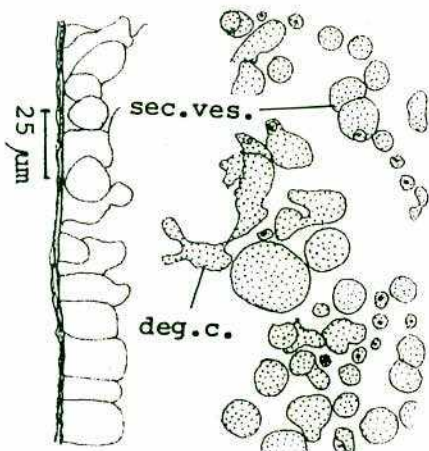
Figs. 53-57. "Prepupa", pupa, and early adult of C. analis.
Different parts of the alimentary canal.

53. Part of T.S. of anterior midgut of the "prepupa" showing almost uniform epithelium after partial degeneration of the larval epithelium.
54. Part of T.S. of early pupal (2 days old) anterior midgut, showing further degeneration of the "prepupal" epithelium after larval-pupal moult.
55. Part of L.S. of middle aged pupal (4 days old) anterior midgut, showing a new imaginal epithelium after complete degeneration of the larval epithelium; the regenerative cells divided into daughter cells in a thin film of cytoplasm.
56. Part of T.S. of late pupal (9 days old) anterior midgut epithelium, showing cluster of daughter regenerative nuclei in the nidus which later form the crypt of the adult midgut.
57. Part of T.S. of anterior midgut of an early adult, showing developing crypt.

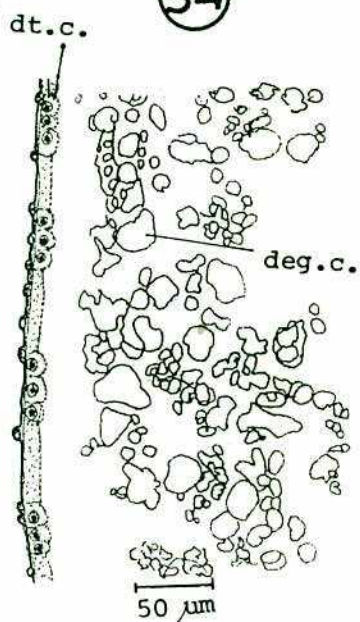
✓deg.c.	=	degenerating cells
✓dt.c.	=	daughter cells
✓dt.n.	=	daughter nucleus
✓dev.cpt.	=	developing crypt
✓mg.c.	=	midgut epithelial cell
✓m.n.	=	mother nucleus
✓sec.ves.	=	secretory vesicle
✓yb	=	yellow body
✓yb.fl.	=	fluid medium which contain yellow bodies
✓yb.gr.	=	granular yellow body
✓yb.l.	=	larger yellow body
✓yb.s.	=	smaller yellow body



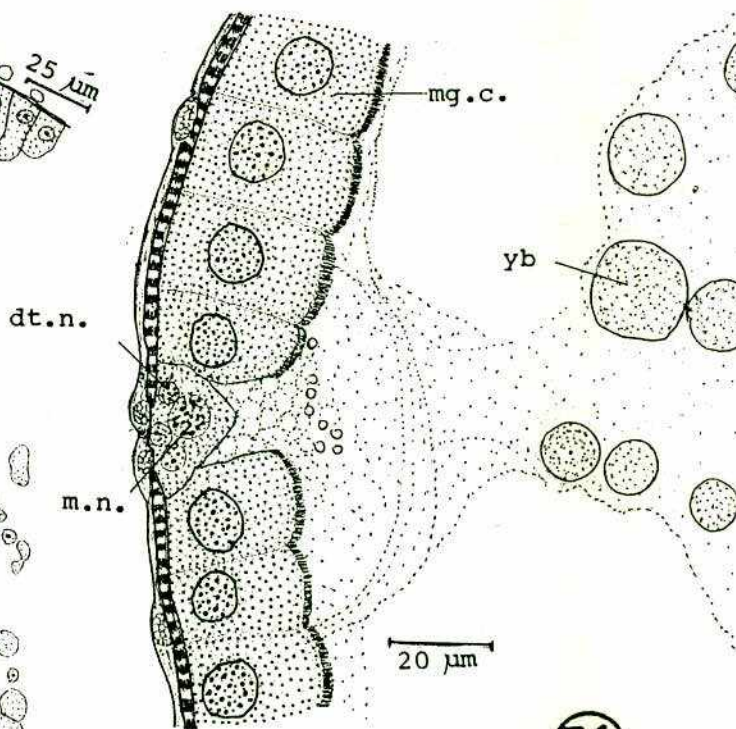
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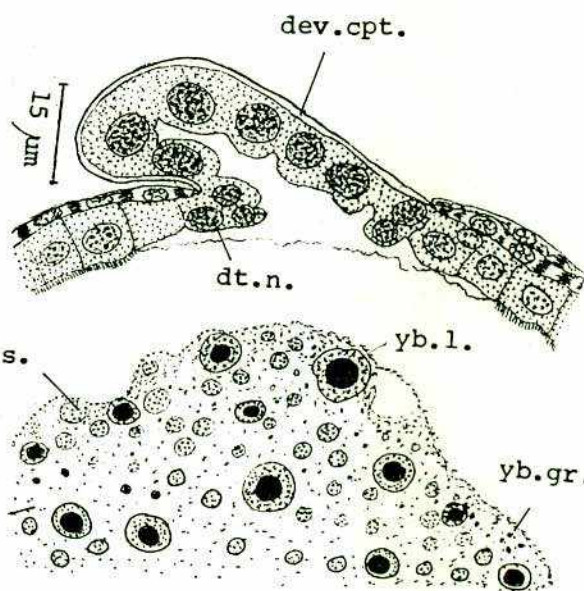
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55



56

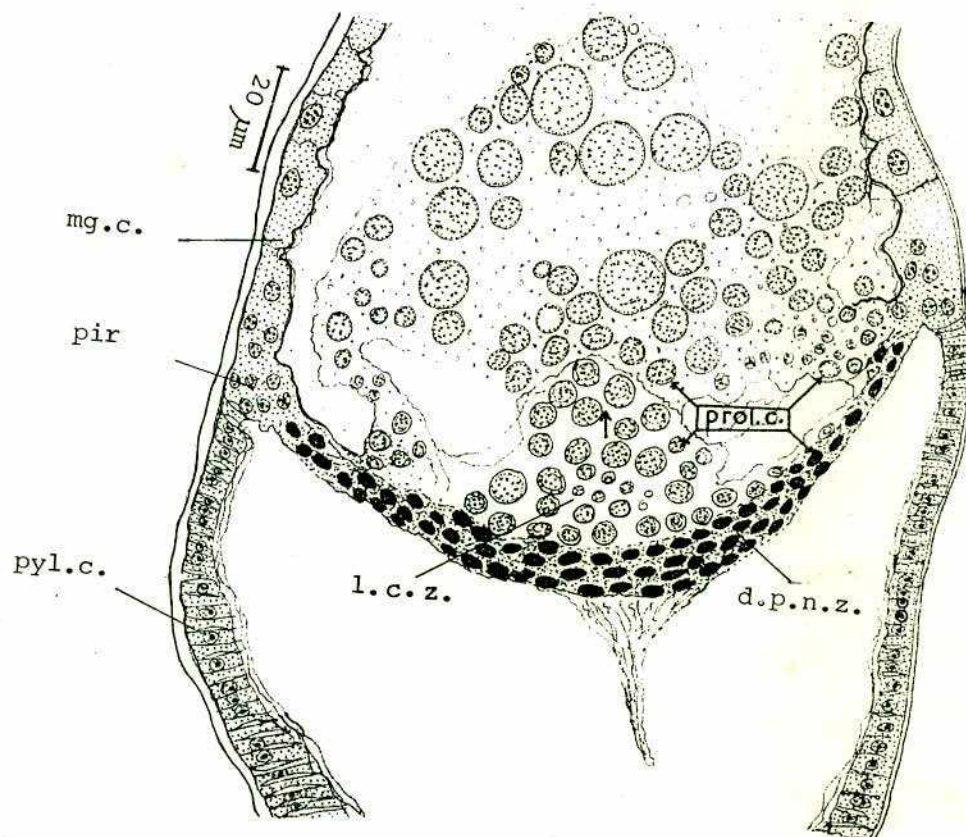


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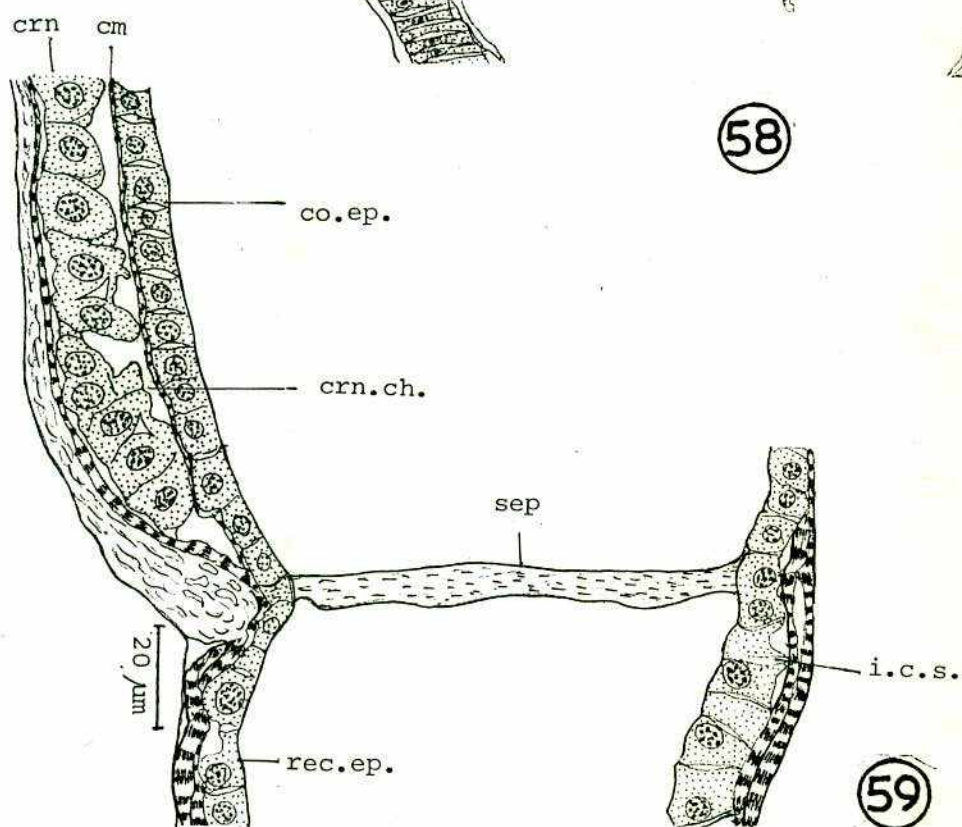
Figs. 58 & 59. Pupa of C. analis. Different parts of the alimentary canal.

58. L.S. of midgut-hindgut junction of an early pupa (3 days old), showing zones of proliferated cells from the posterior imaginal ring.
59. Part of L.S. of colon-rectum junction of the pupa (4 days old), showing a "septum" in the junction, and cryptonephridia.

✓cm	=	circular muscle
✓co.ep.	=	colon epithelium
✓crn	=	cryptonephridium
✓crn.ch.	=	cryptonephridial chamber
✓d.p.n.z.	=	densely packed nucleated zone
✓i.c.s.	=	inter-cellular space
✓l.c.z.	=	loose cellular zone
✓mg.c.	=	midgut (epithelial) cell
✓pir	=	posterior imaginal ring
✓prol.c.	=	proliferated cells
✓pyl.c.	=	pyloric epithelial cell
✓rec.ep.	=	rectal epithelium
✓sep	=	septum



58



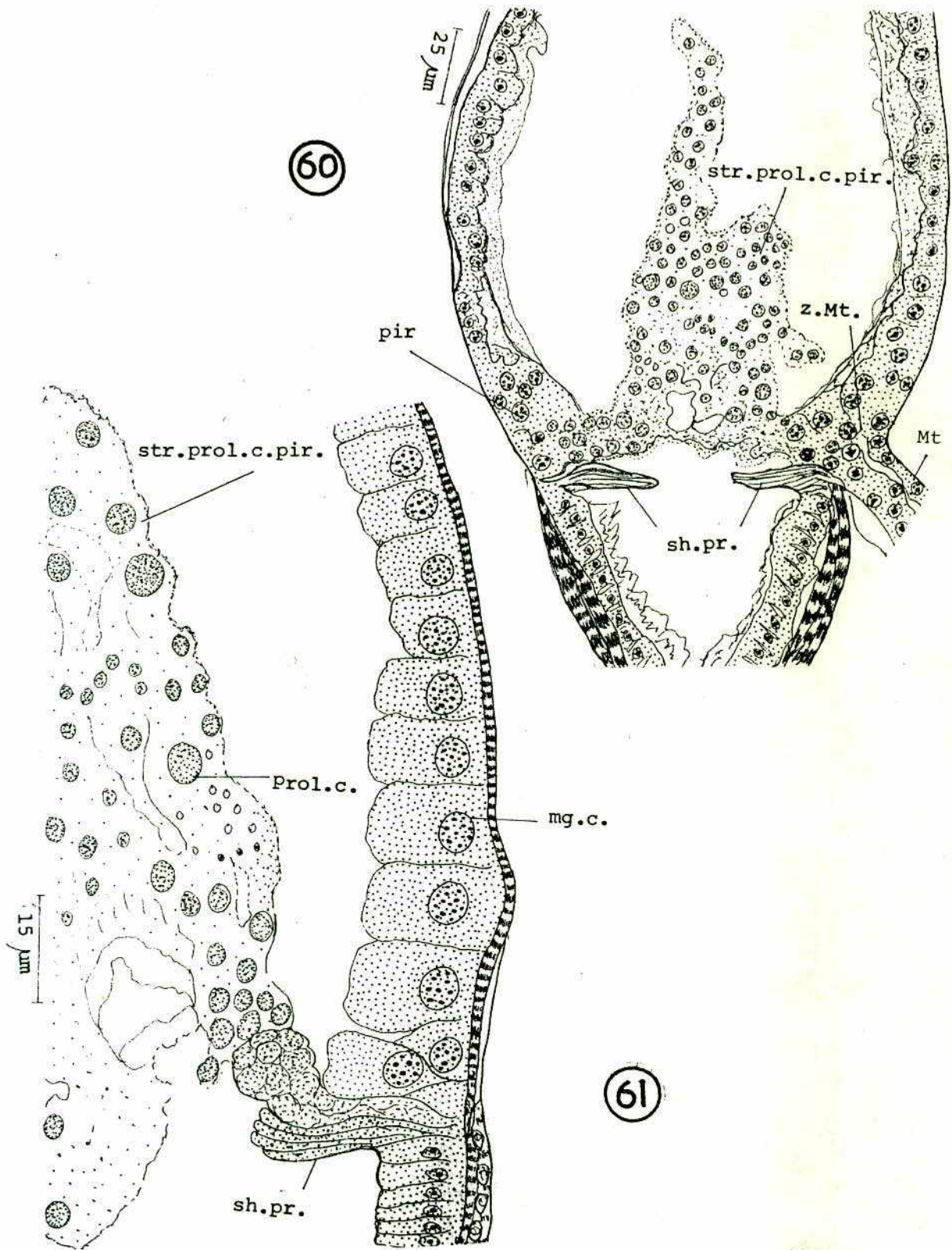
59

Figs. 60 & 61. Pupa of C. analis. Different parts of the alimentary canal.

60. L.S. of midgut-hindgut junction of the pupa (6 days old), showing zone of daughter nuclei which make an addition to the Malpighian tubule formation, stream of proliferated cells from the posterior imaginal ring forming "posterior plug", and a shelf-like structure at the junction.

61. Same as Fig. 60 under higher magnification, showing migration of proliferated cells from the posterior imaginal ring.

✓ mg.c.	= midgut (epithelial) cell
✓ Mt	= Malpighian tubule
✓ pir	= posterior imaginal ring
✓ prol.c.	= proliferated cells
✓ sh.pr.	= shelf-like prolongation
✓ str.prol.c.pir.	= stream of proliferated cells from posterior imaginal ring
✓ z.Mt	= zone of daughter nuclei which make an addition to the formation of Malpighian tubule

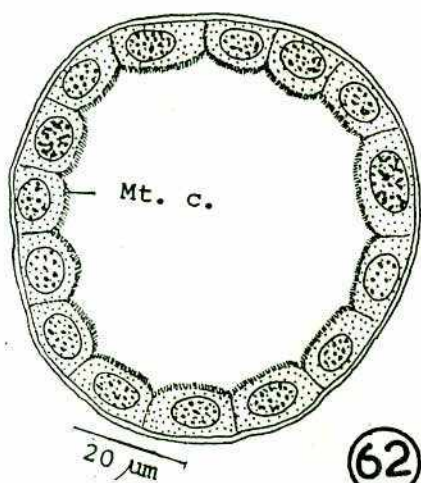


Figs. 62-67. C. analis larva. Different parts of the Malpighian tubules.

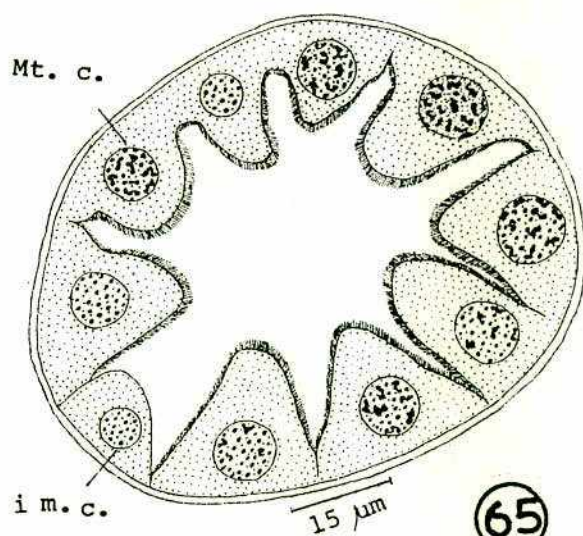
- 62. T.S. of middle region of long Malpighian tubule (1st instar).
- 63. T.S. of proximal part of long Malpighian tubule (2nd instar).
- 64. L.S. of proximal part of long Malpighian tubule (1st instar).
- 65. T.S. of middle part of long Malpighian tubule (3rd instar).
- 66. T.S. of proximal part of long Malpighian tubule (3rd instar).
- 67. T.S. of middle part of short Malpighian tubule (3rd instar).

im.c. = imaginal cell

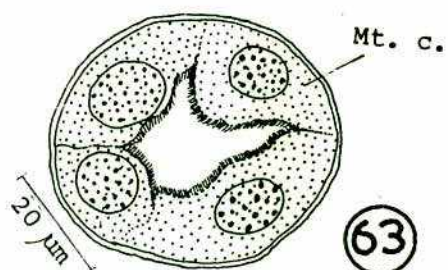
Mt.c. = epithelial cell of Malpighian tubule



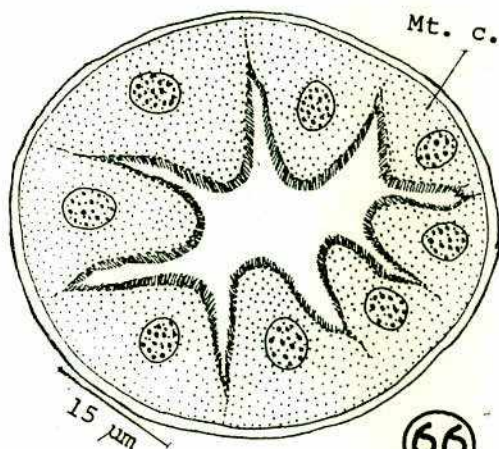
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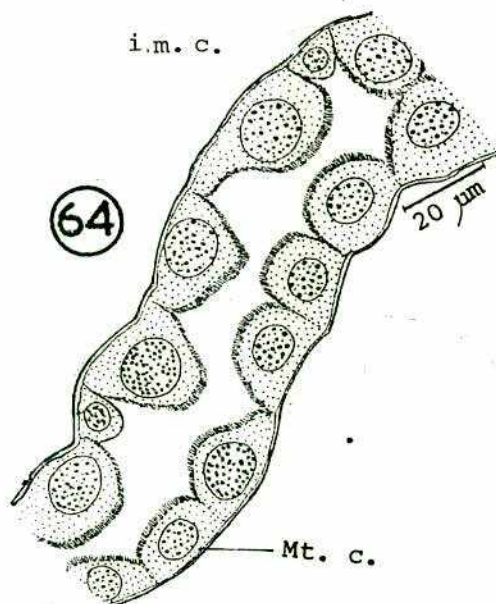
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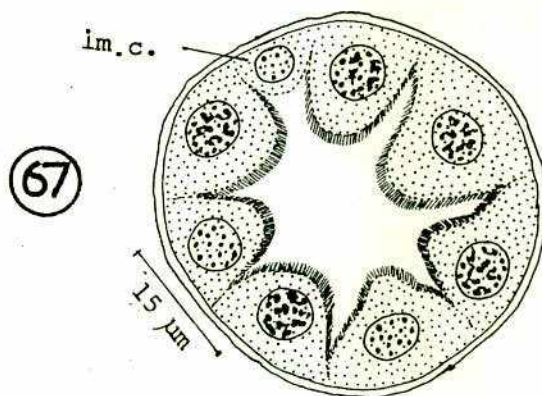
63



66



64



67

Figs. 68 & 69. C. analis pupa. Different parts of the Malpighian tubules.

68. T.S. of middle part of long Malpighian tubule.

69. Part of L.S. of long Malpighian tubule

Figs. 70-73b. Adult C. analis. Different parts of the Malpighian tubules.

70. Part of T.S. of anterior pylorus, showing opening of a Malpighian tubule.

71. T.S. of middle part of a long Malpighian tube.

72. Part of L.S. of a short Malpighian tubule.

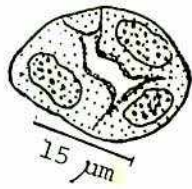
73a. T.S. of proximal part of a long Malpighian tubule.

73b. T.S. of distal part of long Malpighian tubule.

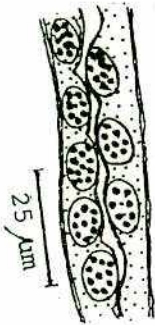
Mt.c. = epithelial cells of Malpighian tubule

o.Mt. = opening of Malpighian tubule

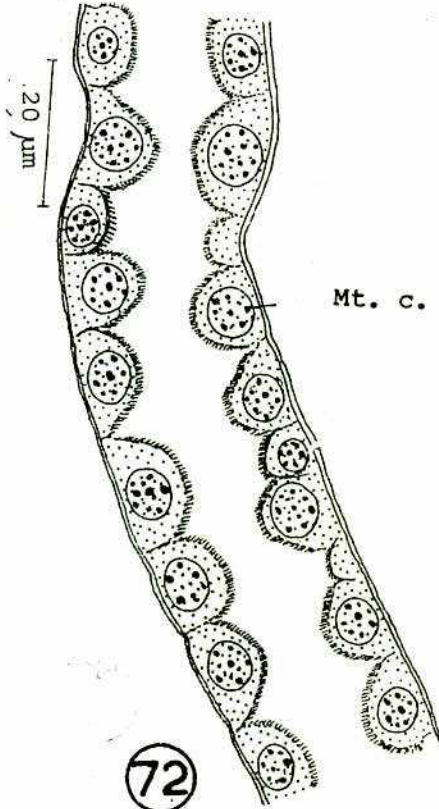
pyl.c. = epithelial cells of pylorus



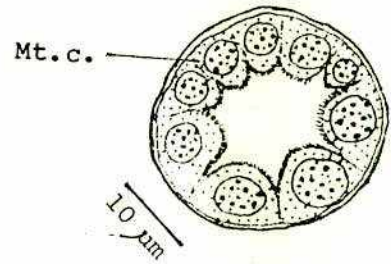
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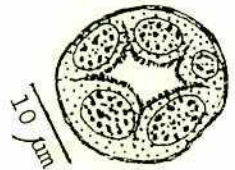
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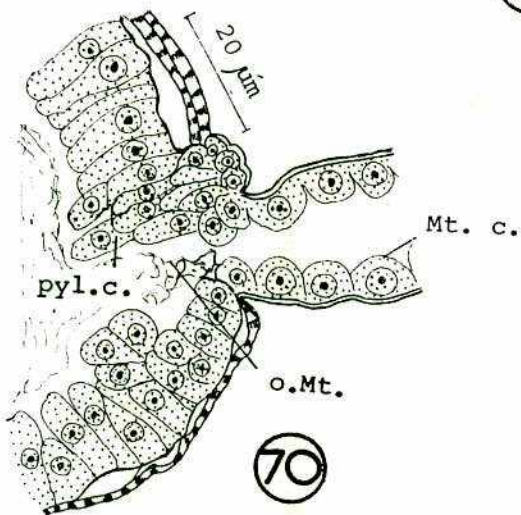
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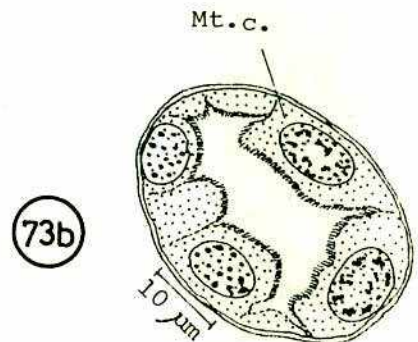
71



73a



70



73b

Fig. 74. C. analis larva (2nd instar). Photomicrograph.
Part of T.S. of oesophagus.

✓ cm = circular muscle
✓ int = intima
✓ oe.ep. = oesophageal epithelium

Fig. 75. C. analis larva (2nd instar). Photomicrograph.
Part of L.S. of foregut-midgut junction,
showing oesophageal invagination.

✓ air.c. = anterior imaginal ring
✓ fc = food contents of the gut
✓ oe. ep. = oesophageal epithelium
✓ oe.inv.ep. = epithelium of the oesophageal
invagination
✓ sph.m. = sphincter muscle
✓ tr.c. = transitional cells.

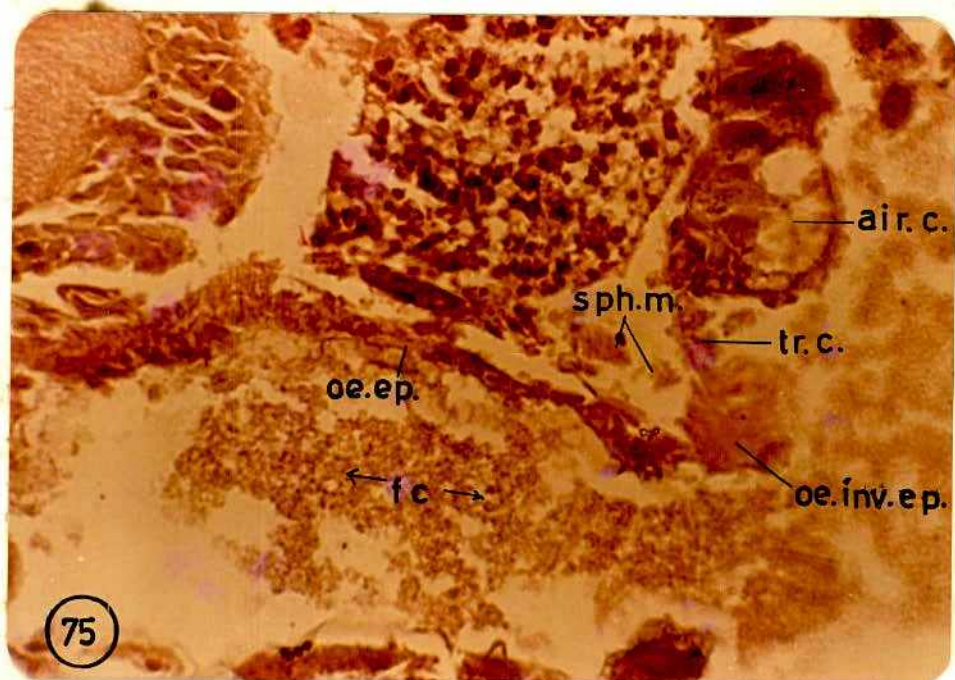
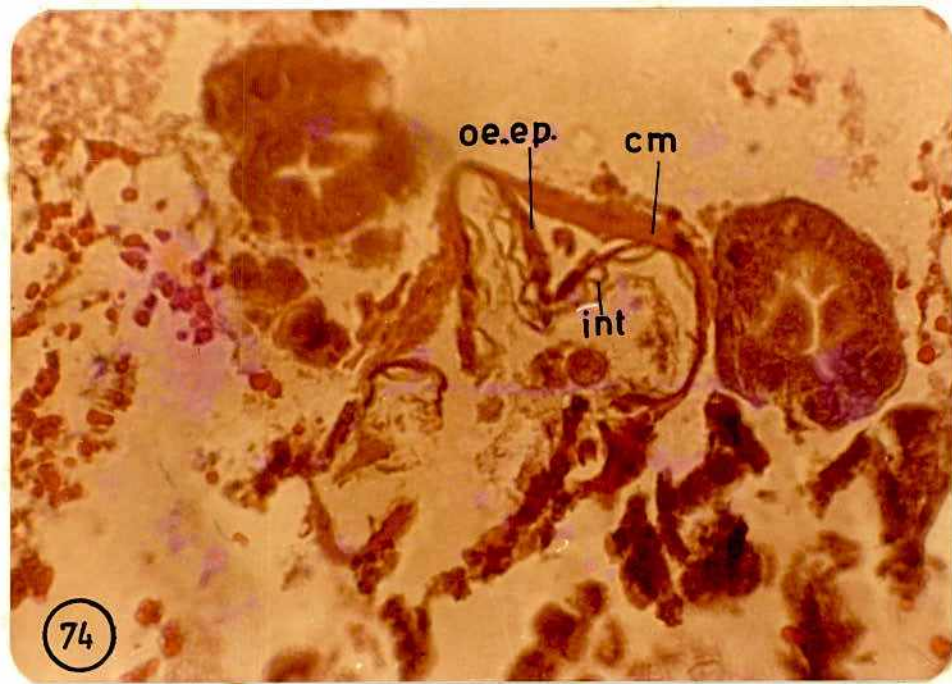


Fig. 76. C. analis larva (4th instar). Photomicrograph.

L.S. passing through the foregut-midgut junction, showing proventricular invagination and tall and narrow columnar cells of anterior section of the anterior midgut.

- | | | | |
|------------|----------------------------------|-------------|----------------------------------|
| ✓ i.ep. | = infolding of midgut epithelium | ✓ prov.inv. | = proventricular invagination |
| ✓ oe.ep. | = oesophageal epithelium | ✓ sec.ves. | = secretory vesicle |
| ✓ prov.ep. | = proventricular epithelium | ✓ t.col.c. | = tall and narrow columnar cells |

Fig. 77. C. analis larva (1st instar). Photomicrograph.

Part of L.S. of anterior midgut.

- ✓ fc = food contents
✓ ni = nidus

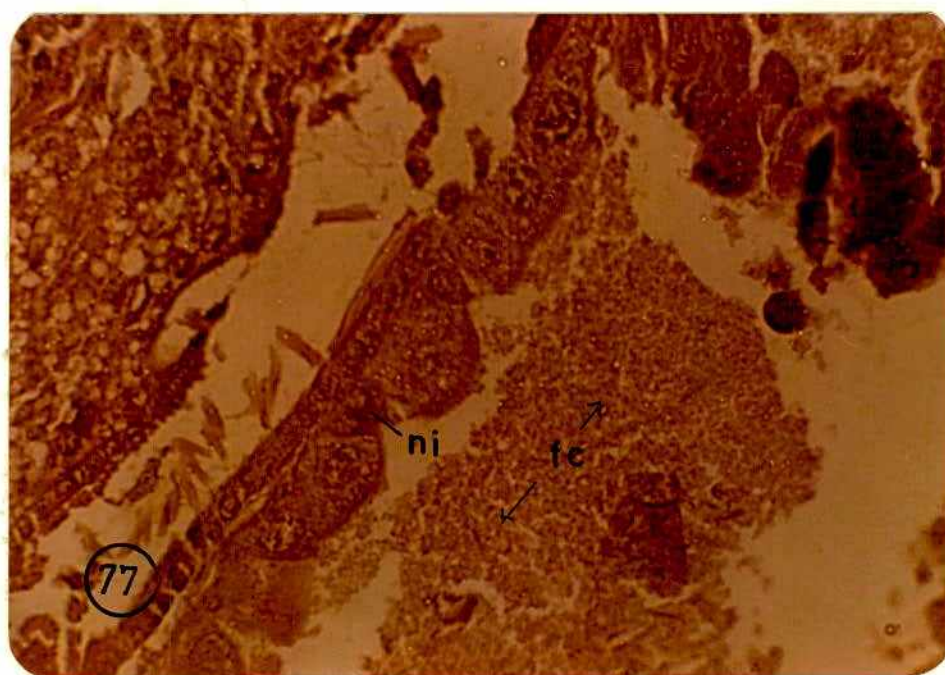
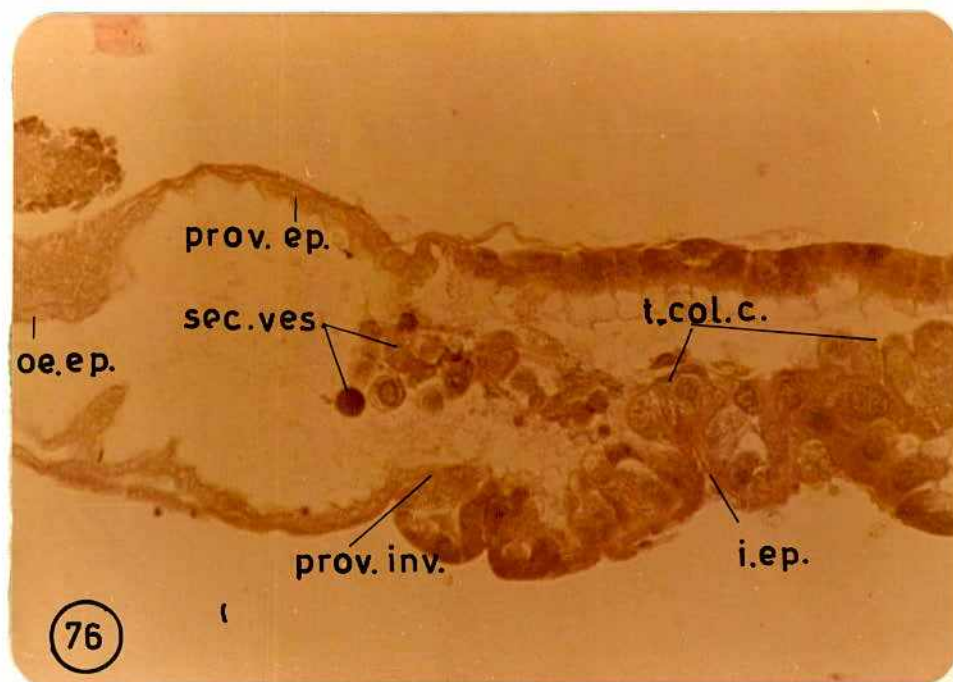


Fig. 78. C. analis larva (4th instar). Photomicrograph.
Part of L.S. passing through the posterior
section of the anterior midgut, showing short
and wide columnar cells.

s.col.c. = short and wide columnar cells
sec. ves. = secretory vesicle

Fig. 79. C. analis larva (2nd instar). Photomicrograph.
T.S. of anterior end of pylorus, showing the
opening of the Malpighian tubules.

Mt.c. = cell of Malpighian tubule
o.Mt. = opening of Malpighian tubule
pyl.c. = pyloric epithelial cell.

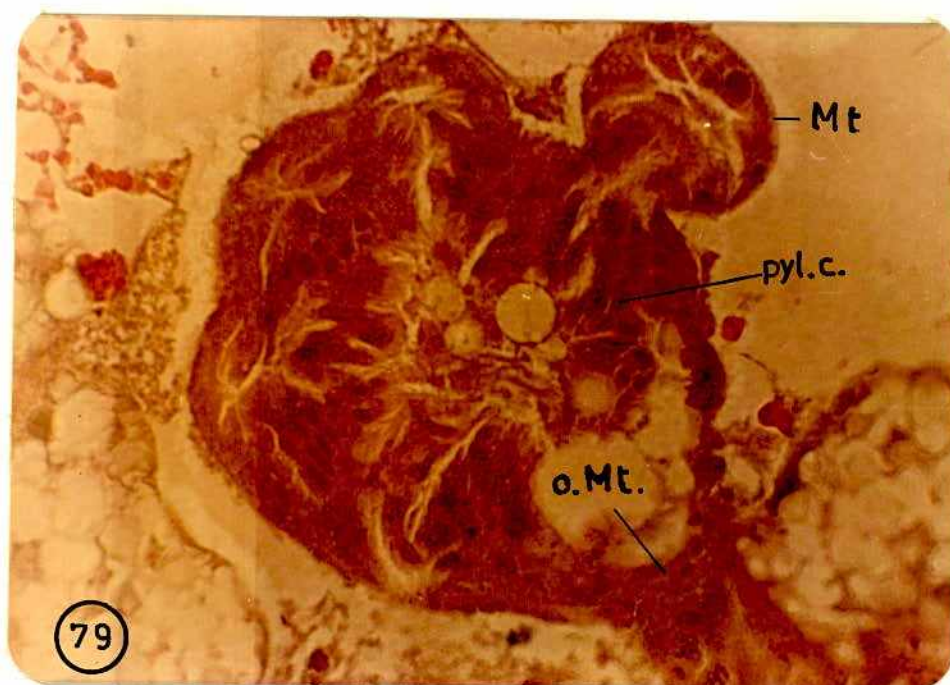
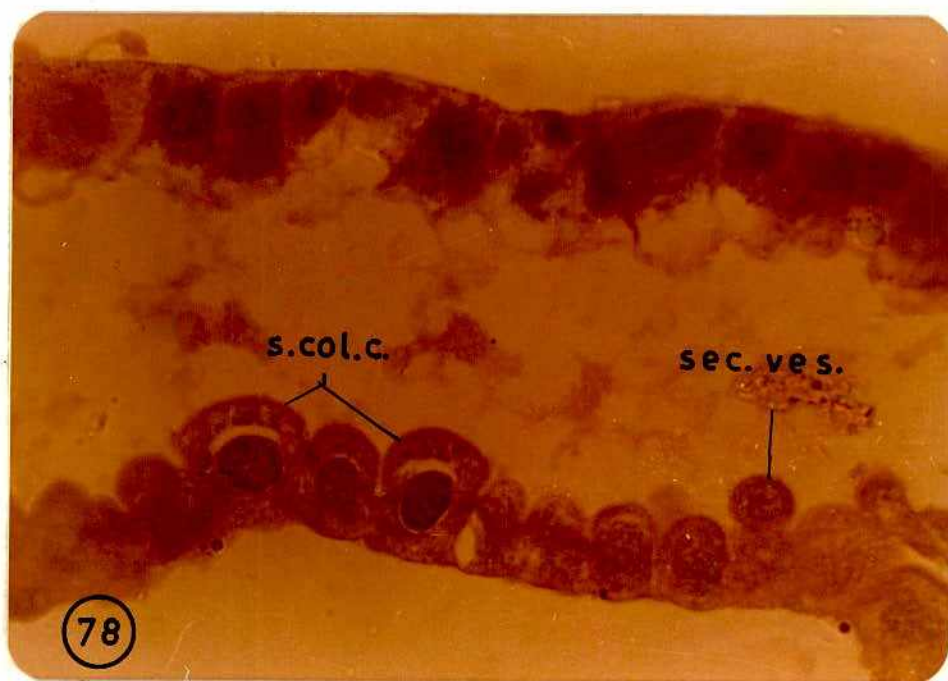


Fig. 80. C. analis larva (3rd instar). Photomicrograph.
T.S. of colon with cryptonephridial system.

crn = cryptonephridium

co.ep. = colon epithelium

Fig. 81. C. analis larva (2nd instar). Photomicrograph.
T.S. of rectum, showing rectal spines,
diagonal and longitudinal muscles.

dg.m. = diagonal muscles

lm = longitudinal muscles

rec.sp. = rectal spines

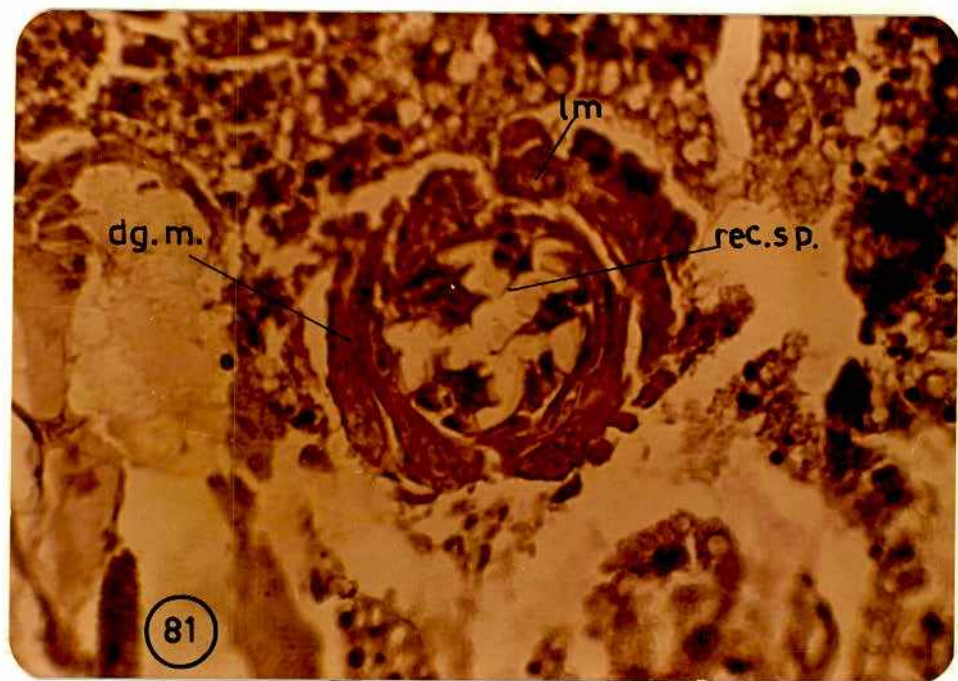
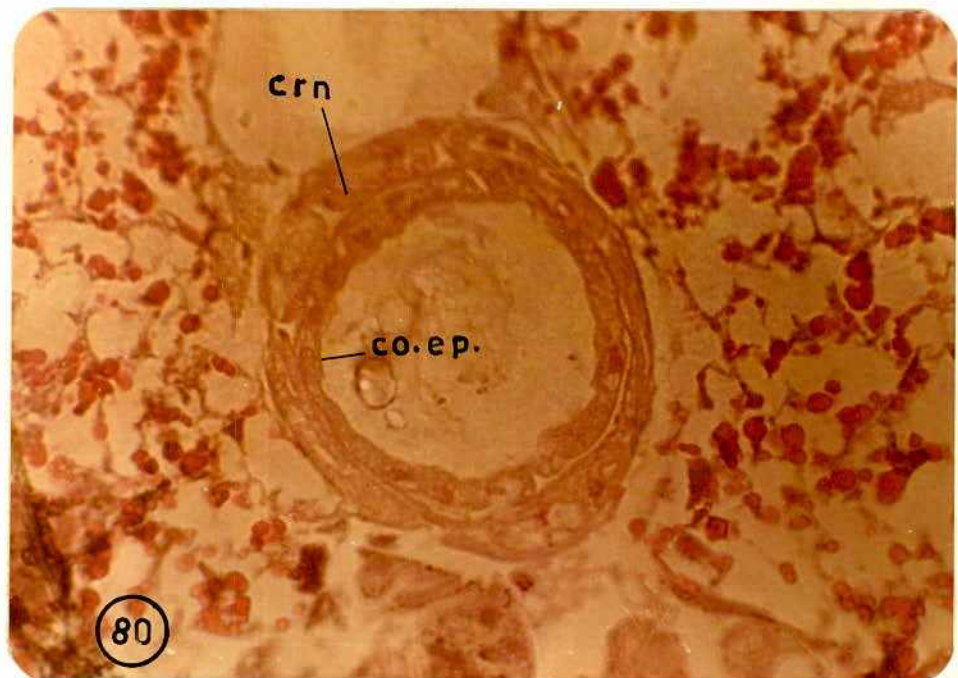




Fig. 82. Adult C. analis. Photomicrograph.
T.S. of anterior part of the pharynx
showing pharyngeal spine.

ph.sp. = pharyngeal spine

Fig. 83. Adult C. analis. Photomicrograph.
T.S. of posterior part of the pharynx,
showing thick intima without spines.

int = intima

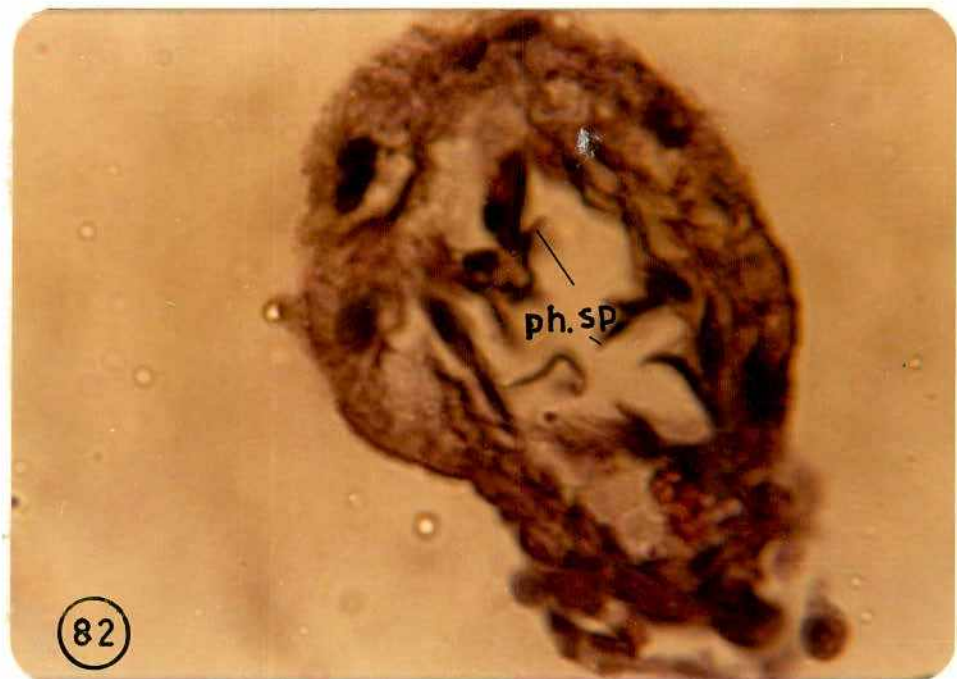




Fig. 84. Adult C. analis. Photomicrograph.

T.S. of middle part of the oesophagus,
showing minute oesophageal spines.

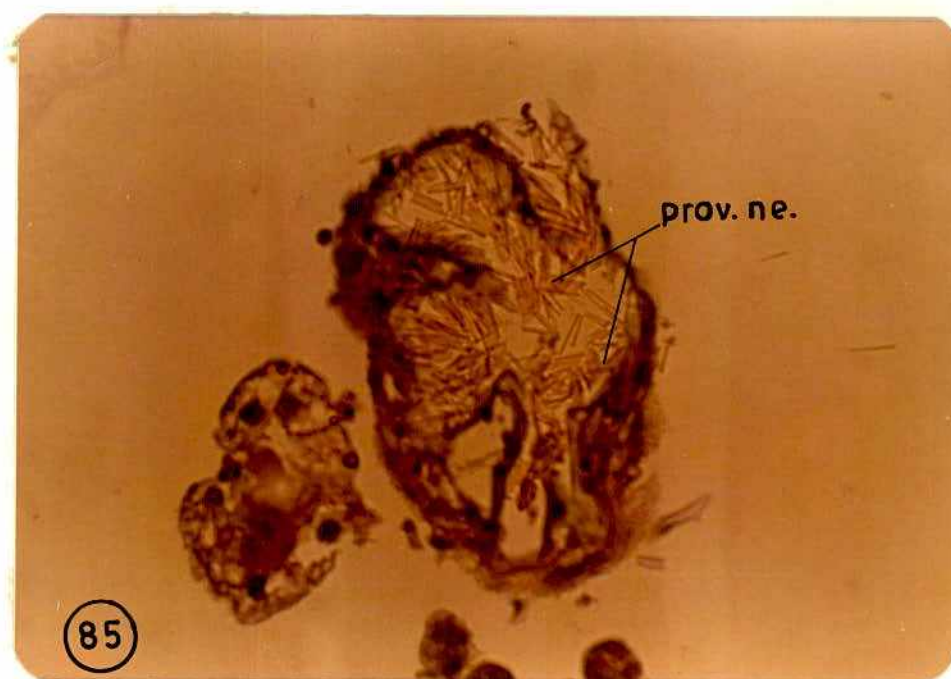
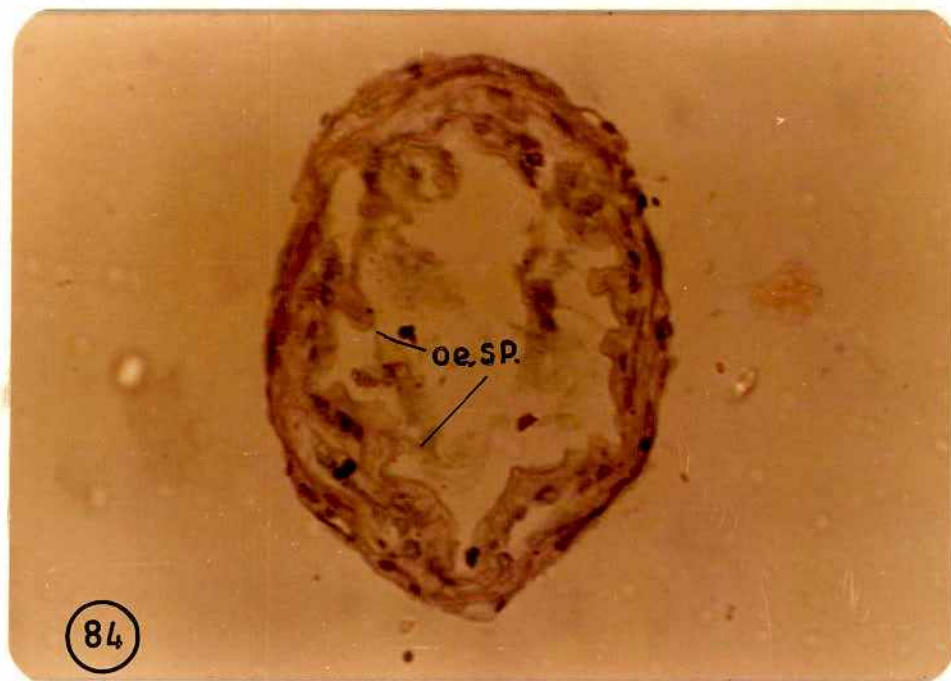
oe.sp. = oesophageal spines

Fig. 85. Adult C. analis. Photomicrograph.

T.S. of anterior end of the proventriculus,
showing proventricular needles.

prov. ne. = proventricular needles

✓



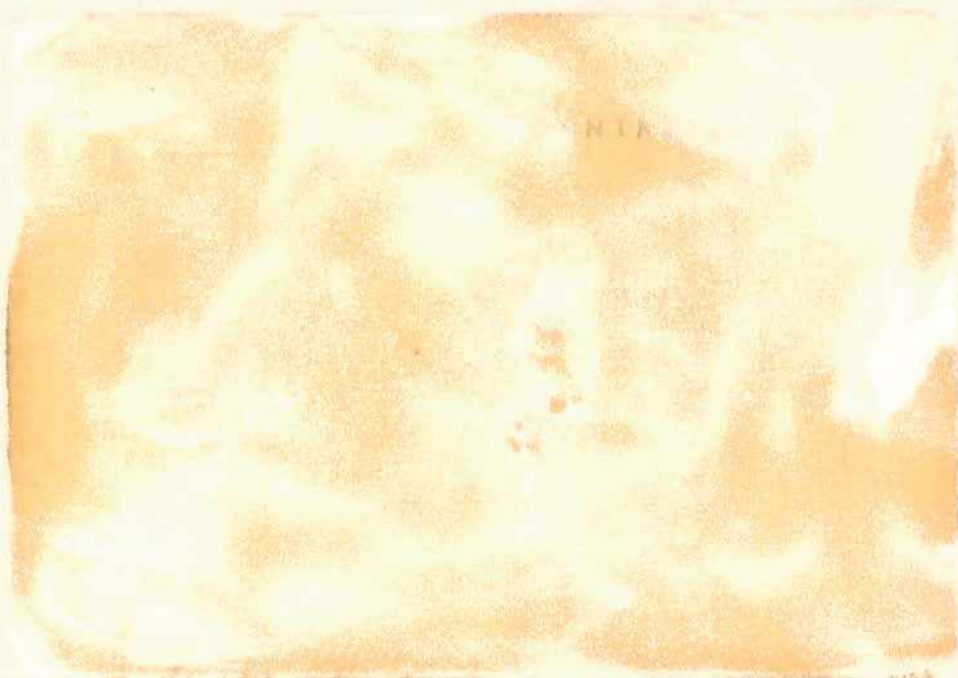


Fig. 86. Adult C. analis. Photomicrograph.

Oblique section from foregut-midgut region, showing proventricular needles in the proventriculus, prominent muscles (sphincter muscles) in between the proventriculus and lobed end of the midgut.

al.lb. = anterior lobed part (antero-lateral lobe) of midgut

prov = proventriculus

prov.ne. = proventricular needles.

cpt = crypt

t/ m.al.lb. = muscles (sphincter muscles) adjacent to the antero-lateral lobe

Fig. 87. Adult of C. analis. Photomicrograph.

L.S. of foregut-midgut junction, showing proventricular valve, needles, and invagination.

prov. inv. = proventricular invagination

prov. ne. = proventricular needles

prov. v. = proventricular valve

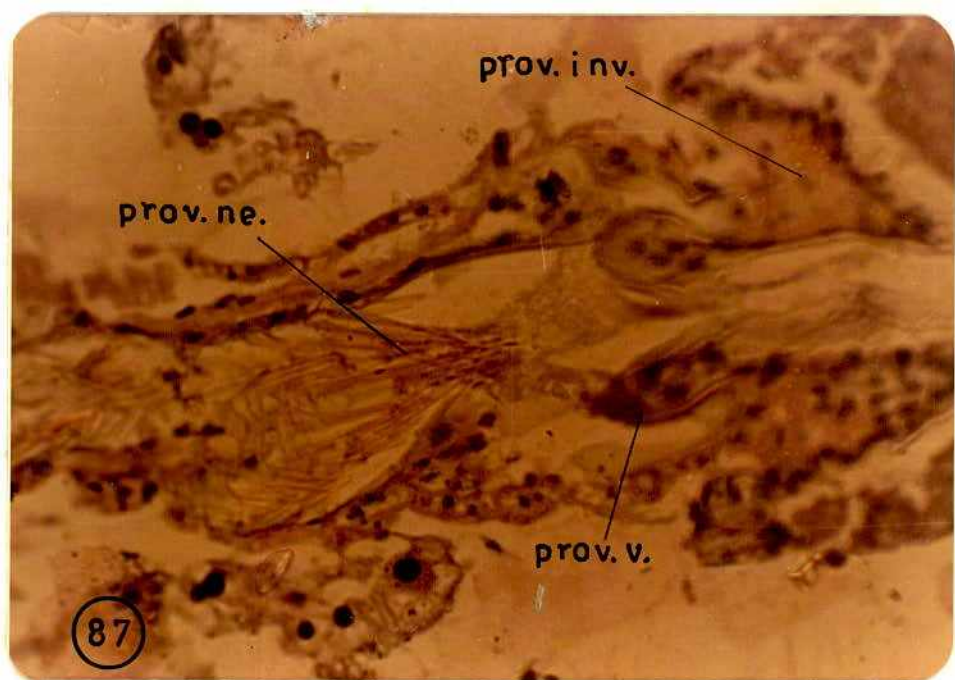
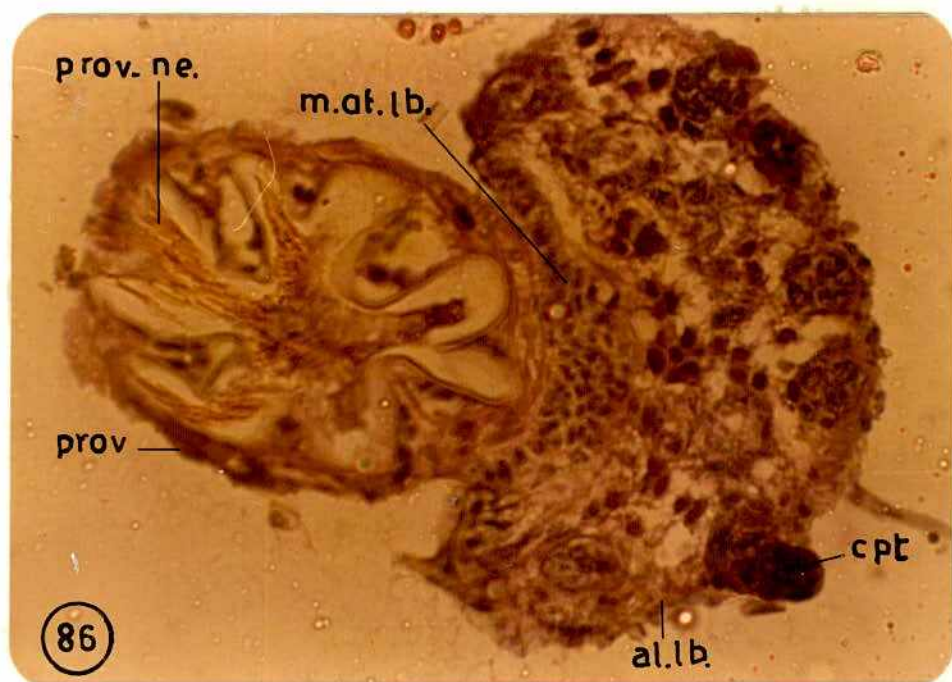




Fig. 88. Adult C. analis. Photomicrograph.

Part of T.S. of anterior midgut, showing
secretory extrusions of the epithelial
cells, and crypts.

cpt = crypt

sec.extr.= secretory extrusions

Fig. 89. Adult C. analis. Photomicrograph.

Part of L.S. of ileum.

il.ep. = ileal epithelium

Mt = Malpighian tubule

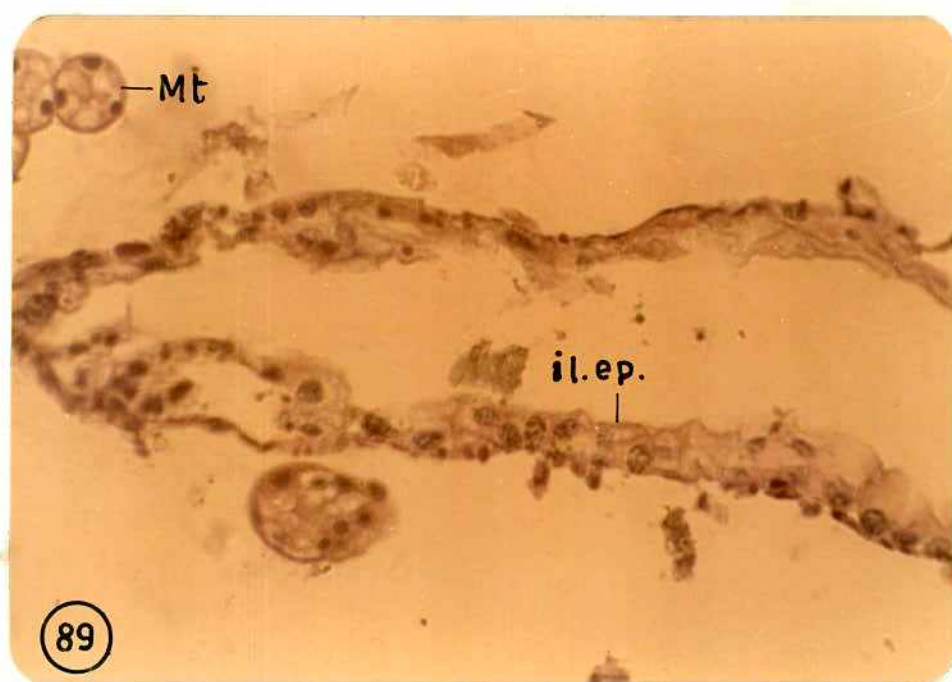
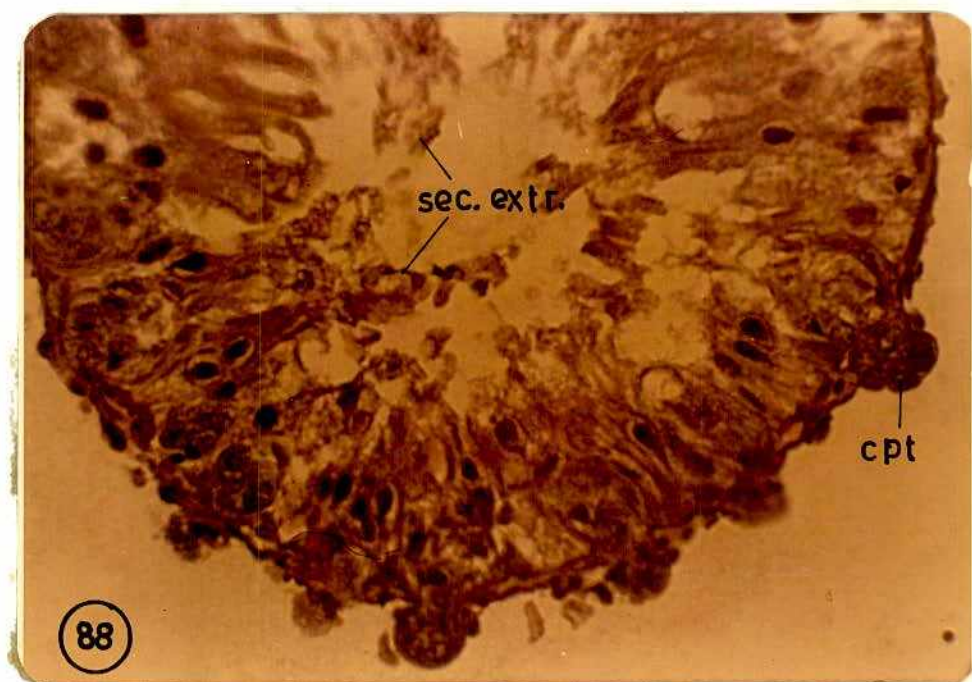


Fig. 90. Adult C. analis. Photomicrograph.

T.S. of colon with cryptonephridial system.

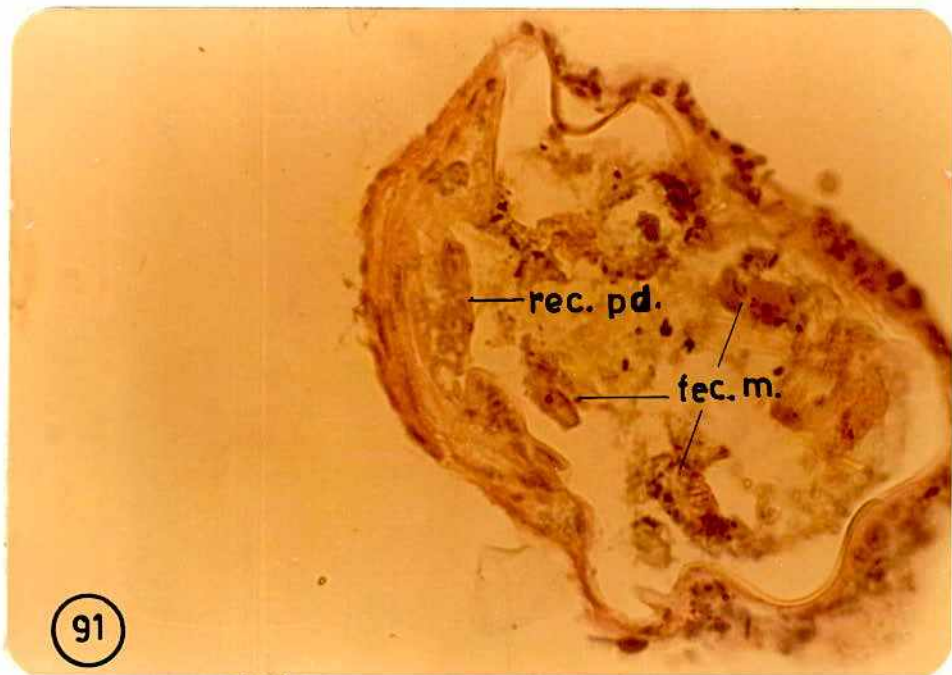
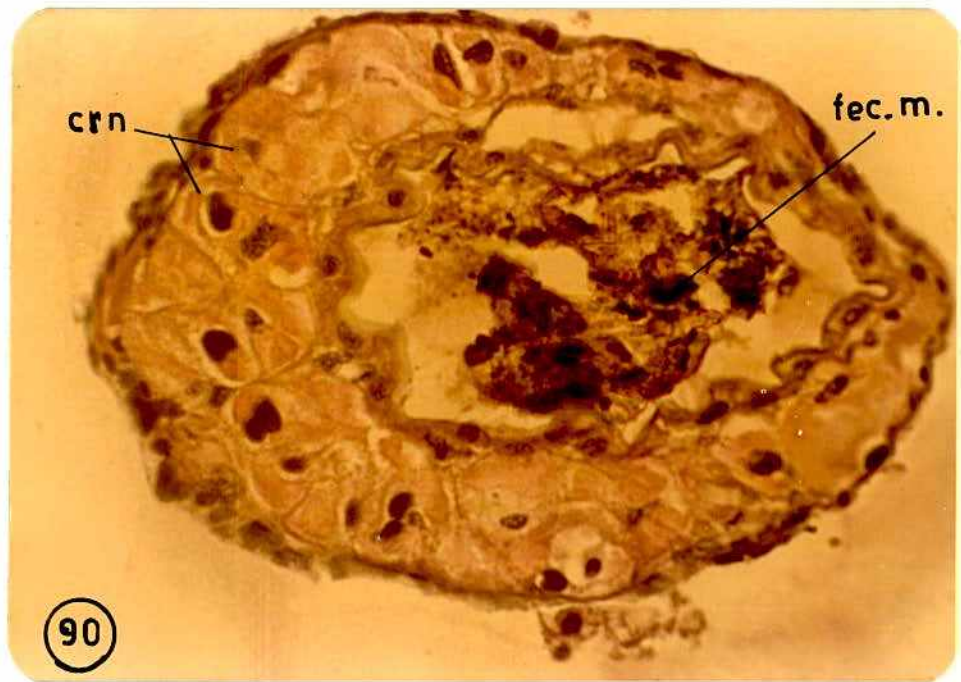
? — co.ep. = colon epithelium
crn = cryptonephridium
fec.m. = faecal matters

Fig. 91. Adult C. analis. Photomicrograph.

T.S. of anterior end of rectum,
showing rectal pad.

rec. pd. = rectal pad

fec.m. = ?



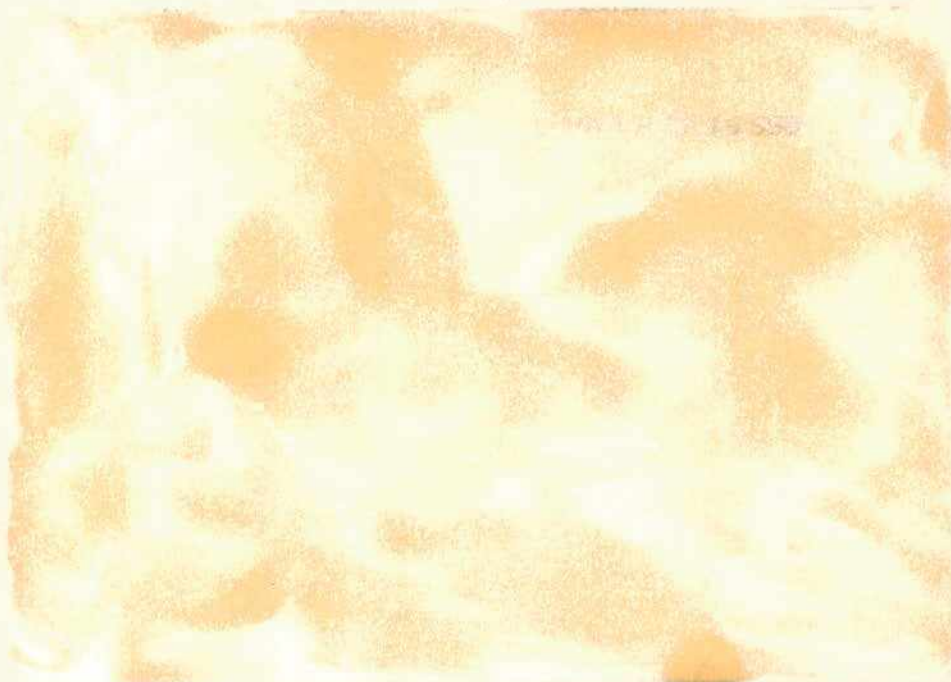
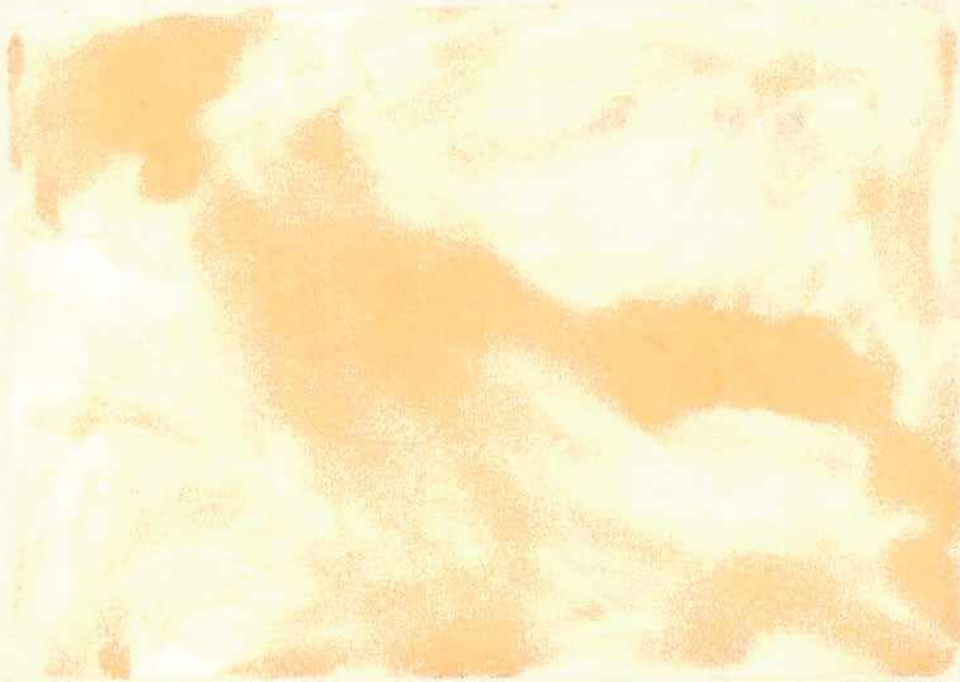


Fig. 92. Adult C. analis. Photomicrograph.

T.S. of posterior end of rectum, showing
rectal papillae with rectal spines.

rec. pap. = rectal papilla

rec. sp. = rectal spine

Fig. 93. C. analis pupa (6 days old). Photomicrograph.

L.S. of foregut-midgut junction, showing
developing proventricular needles, and
proventricular valves.

dev. ne. = developing needles

dev. prov. v. = developing proventricular valve

prol. c. = proliferating cells

oc - ?

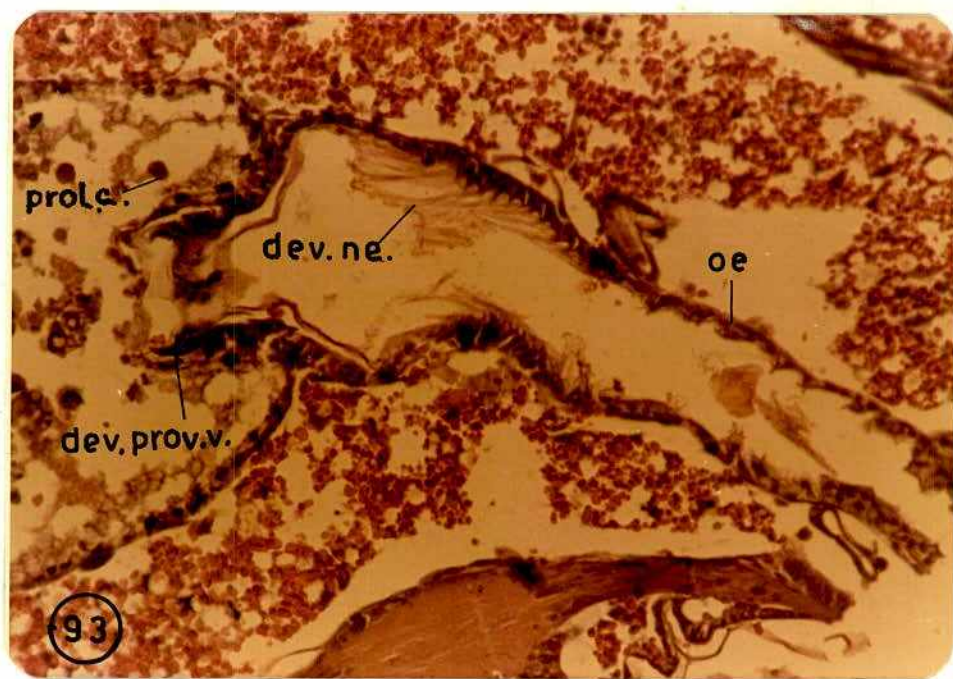
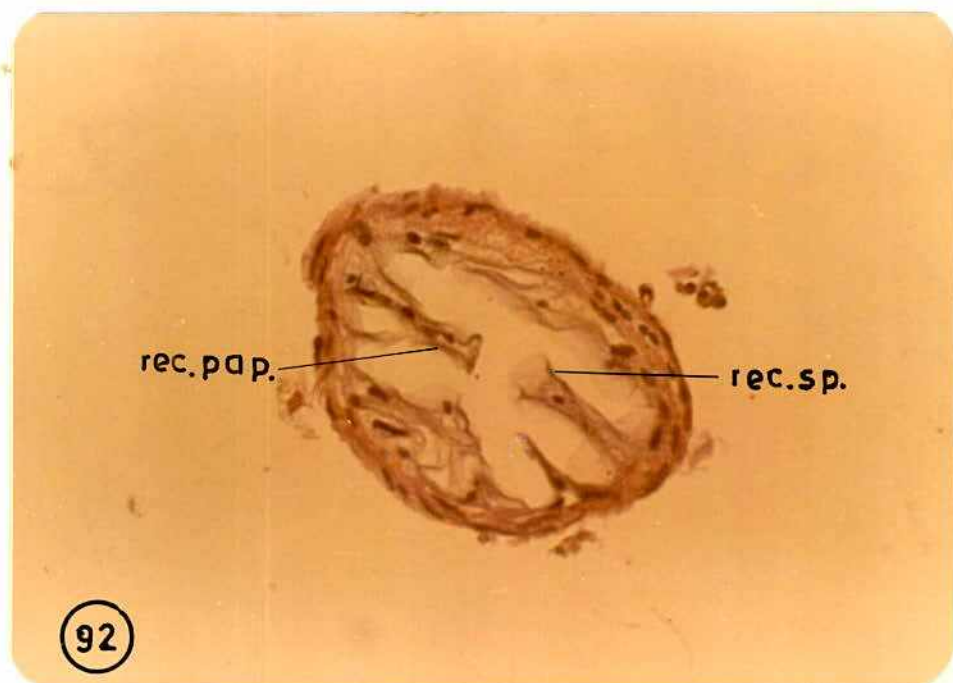


Fig. 94. C. analis pupa (8 days old). Photomicrograph.

L.S. passing through anterior midgut and
posterior midgut junction.

a.mg. = anterior midgut

p.mg. = posterior midgut

yb = yellow bodies

Fig. 95. C. analis pupa (7 days old). Photomicrograph.

Part of T.S. of anterior midgut, showing
normal pupal midgut epithelium, regenerative
cell and yellow bodies in the centre of the
gut lumen.

pup.ep. = pupal epithelium

r.c. = regenerative cell

yb = yellow bodies

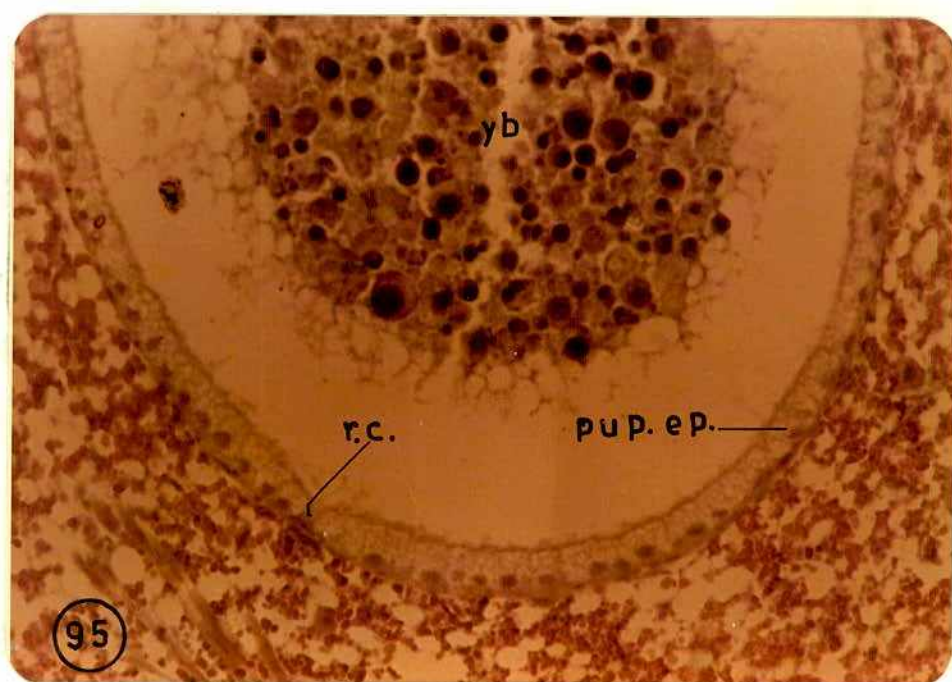
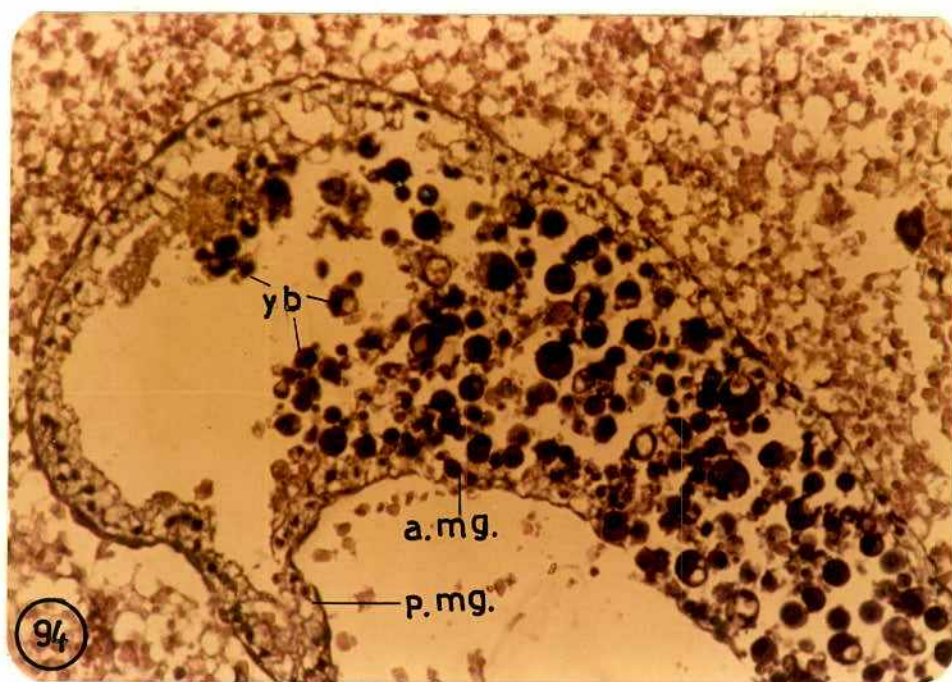


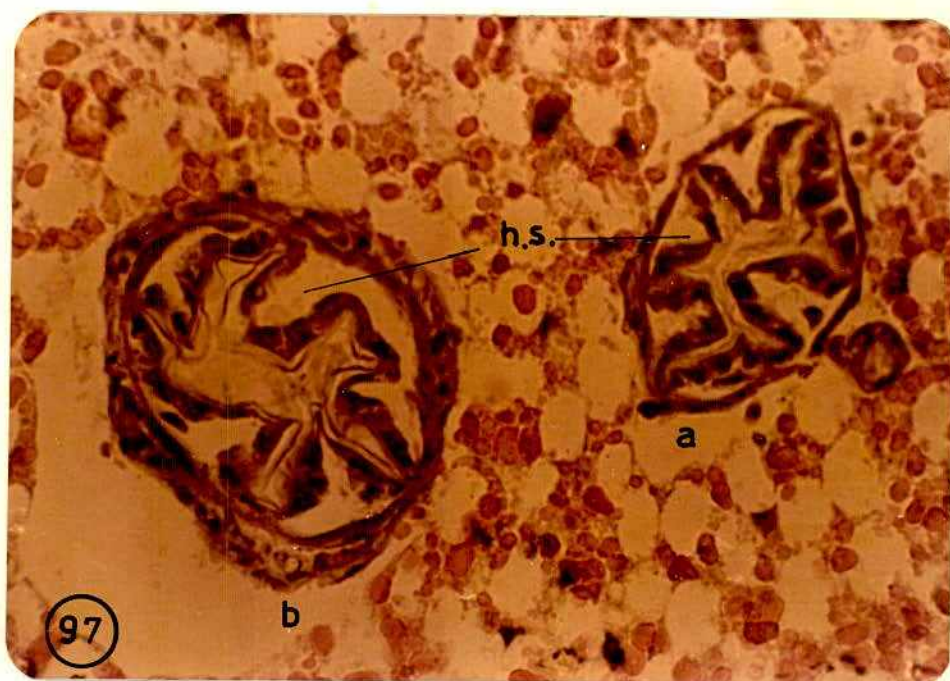
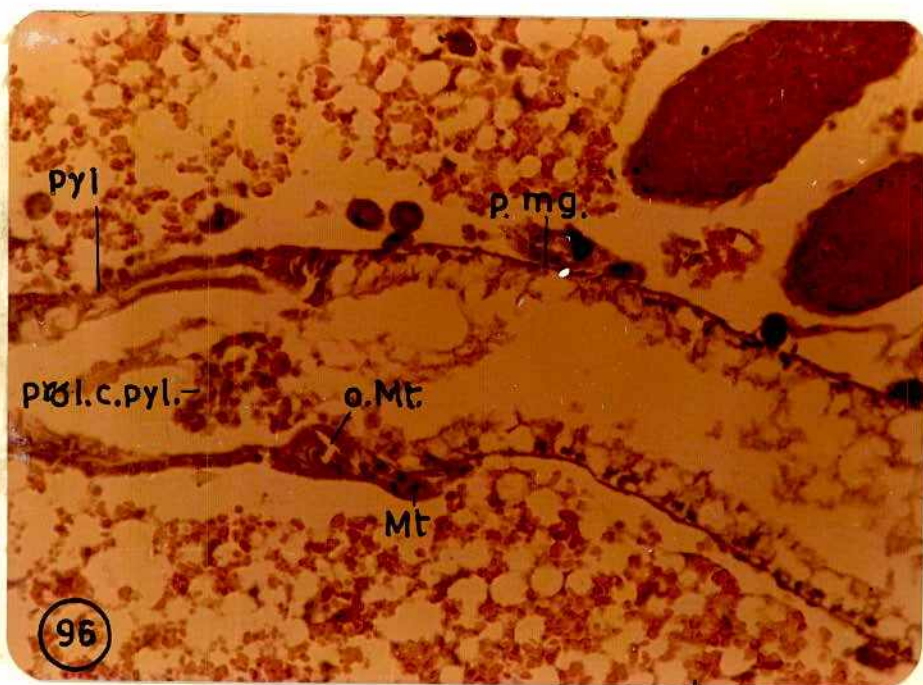
Fig. 96. C. analis pupa (6 days old). Photomicrograph.
L.S. of midgut-hindgut junction.

Mt	=	Malpighian tubule
o.Mt.	=	opening of the Malpighian tubule
p.mg.	=	posterior midgut
prol.c.pyl.	=	proliferated cells (from the posterior imaginal ring) in the pylorus
pyl	=	pylorus

Fig. 97. C. analis pupa (6 days old). Photomicrograph.

- a. T.S. passing through the anterior end of the ileum;
- b. T.S. passing through the anteriormost end of the
colon just in front of the insertion of the
Malpighian tubules into the cryptonephridial
chamber.

h.s. = hollow space at the base of epithelial fold.



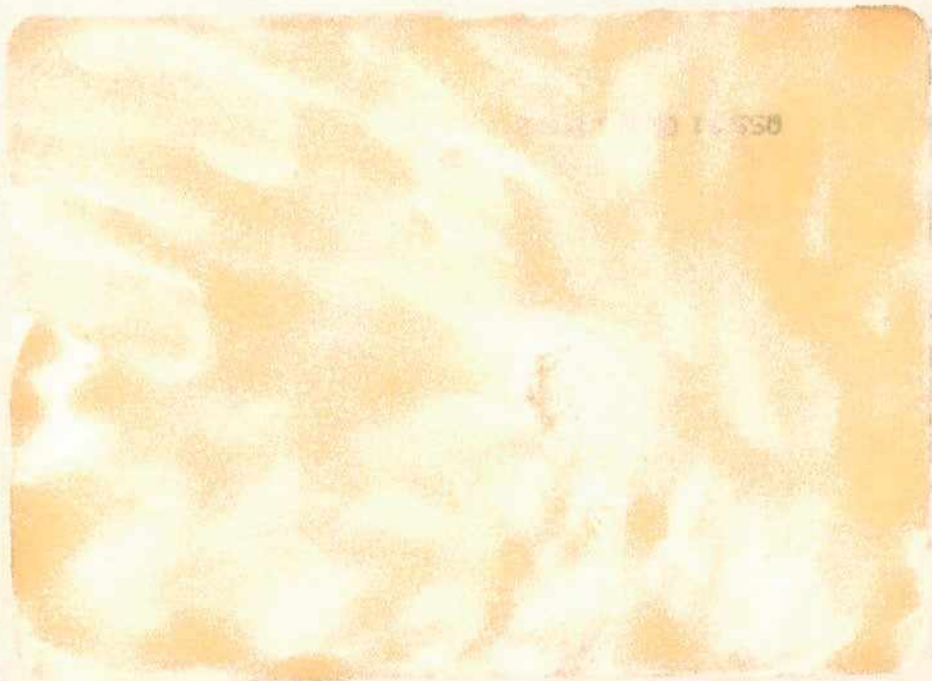


Fig. 98. C. analis pupa (6 days old). Photomicrograph.
L.S. passing through colon-rectum junction.

co.ep. = colon epithelium

crn = cryptonephridium

rec. ep. = rectal epithelium

Fig. 99. C. analis pupa (8 days old). Photomicrograph.
L.S. of rectum, showing developing "rectal pad"
and "rectal papilla" regions.

dev.rec.pap. = developing rectal papilla

dev.rec.pd. = developing rectal pad

dev.sp. = developing rectal spines

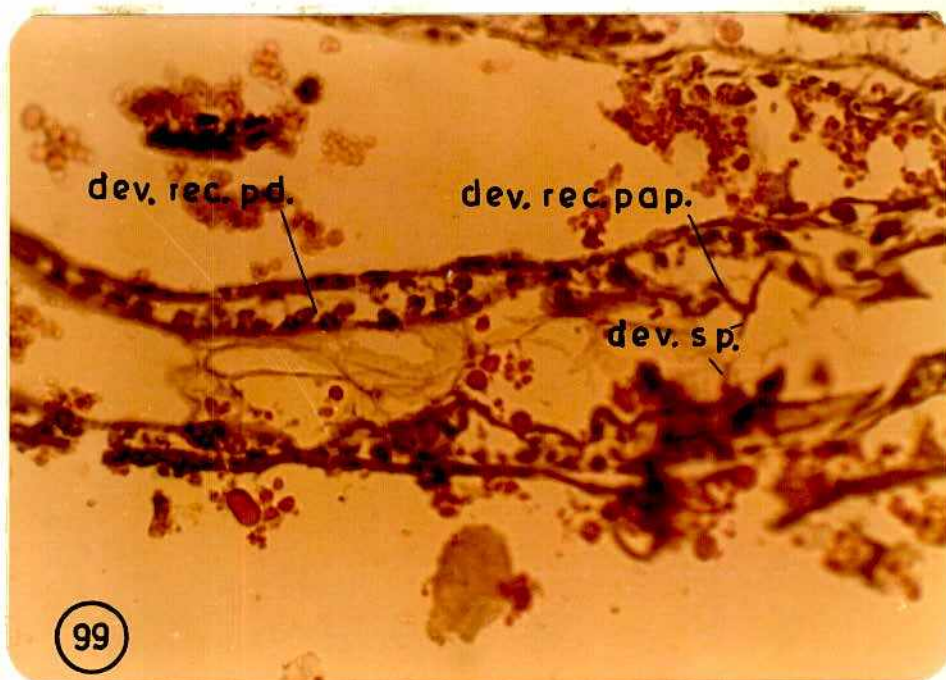
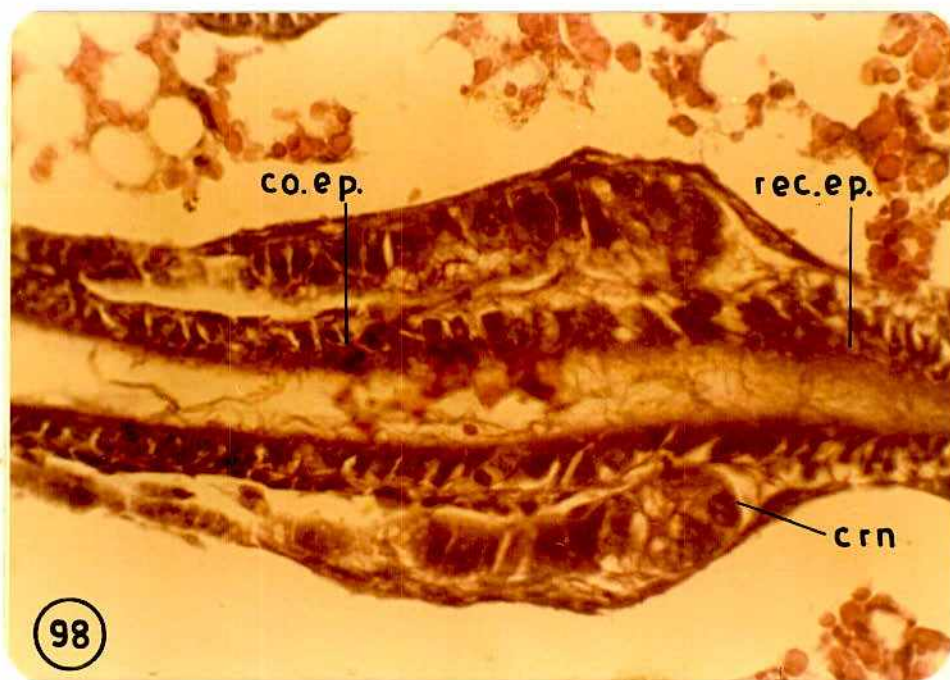


Fig. 100. C. analis pupa (1 day old). Photomicrograph.
L.S. of foregut and foregut-midgut junction,
showing histolysis of intima of the foregut
and degeneration of the midgut epithelial cells.

a.mg.	= anterior midgut	oe	= oesophagus
deg.ep.	= degenerating epithelium	ph	= pharynx
hist.int.	= histolysing intima	prov	= proventriculus

Fig. 101. C. analis pupa (2 days old). Photomicrograph.
L.S. of foregut-midgut junction, showing
"intimal block".

air	= anterior imaginal ring
a.mg.	= anterior midgut
int.bl.	= intimal block
prov	= proventriculus

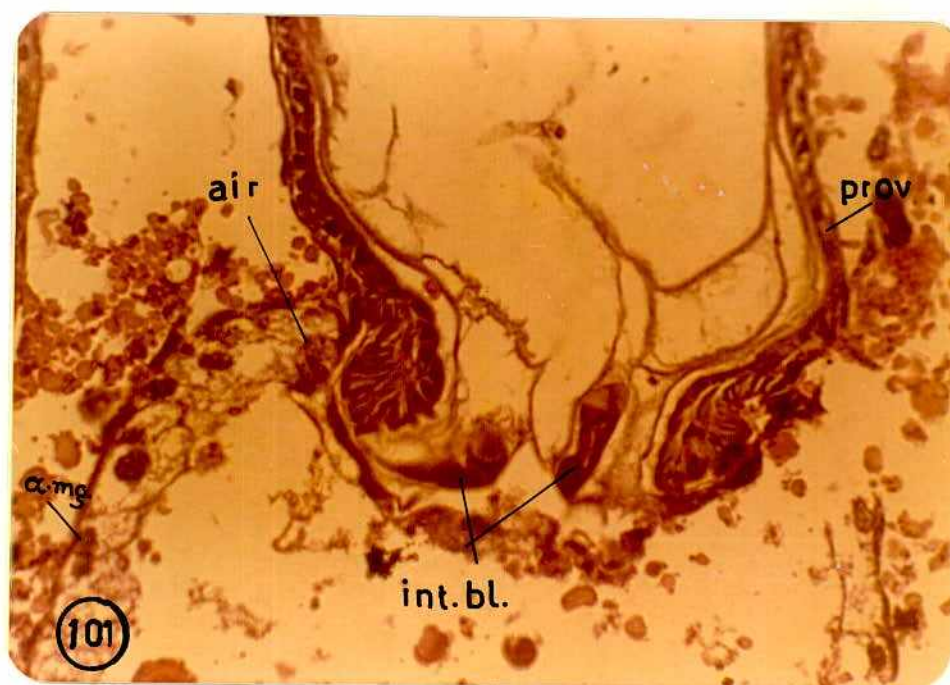
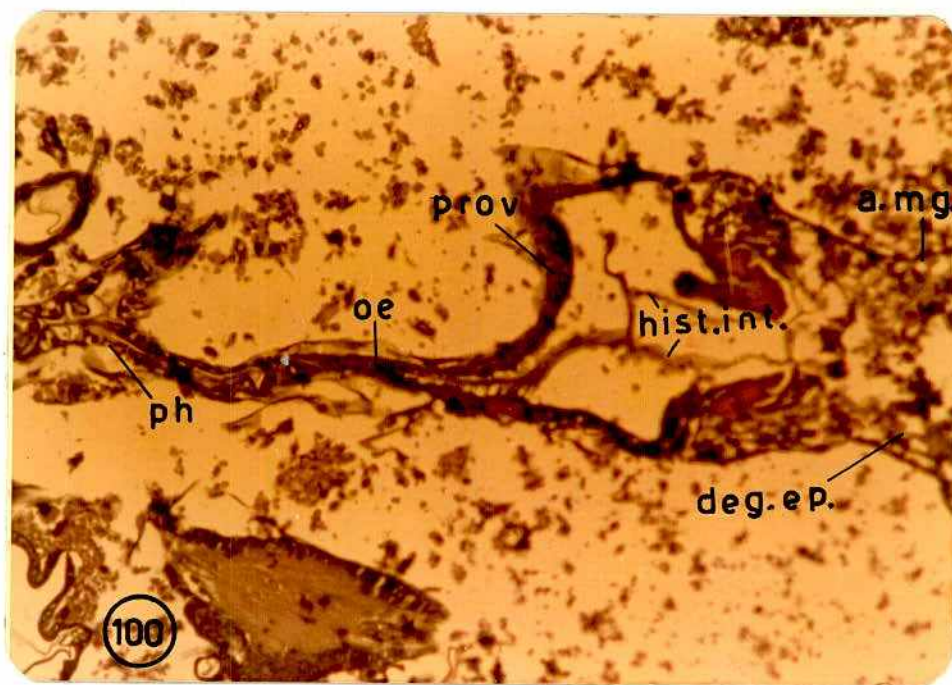
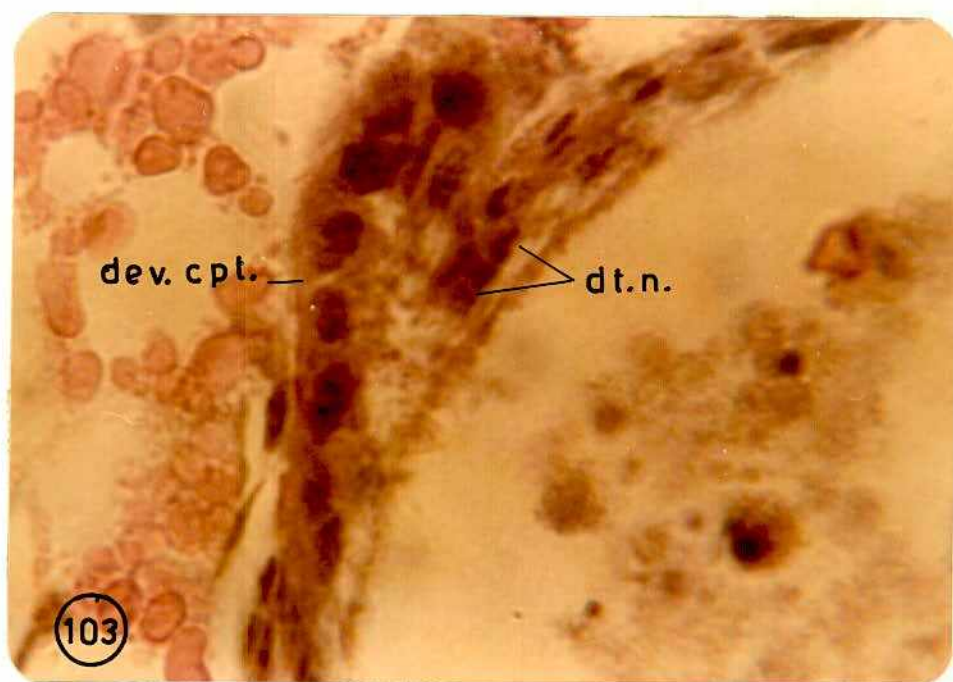
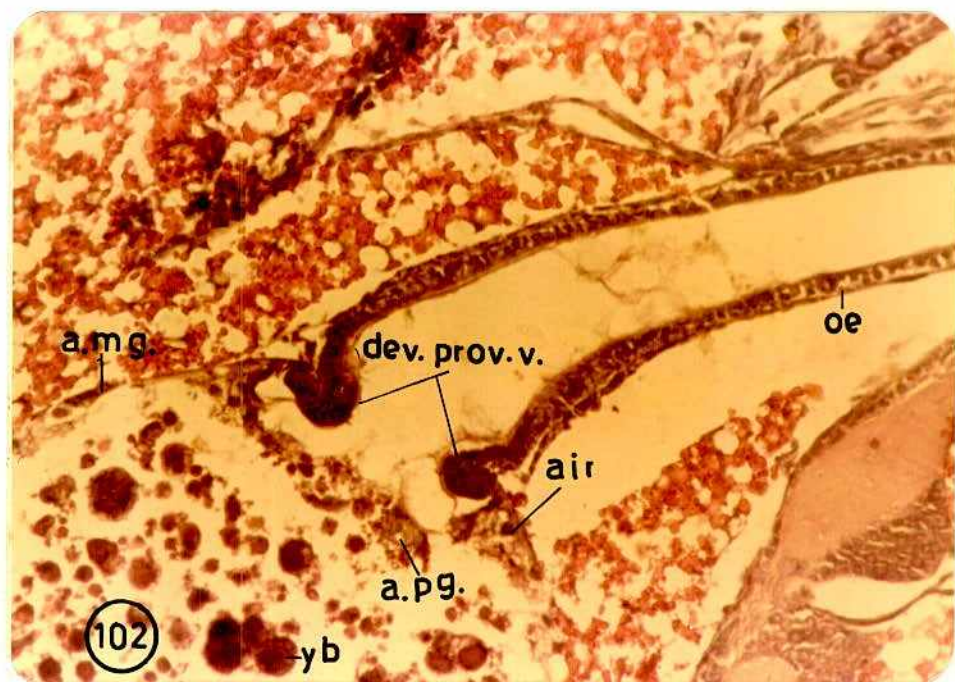


Fig. 102. C. analis pupa (5 days old). Photomicrograph.
L.S. of foregut and foregut-midgut junction,
showing "anterior plug" formed by the proliferated
cells from the anterior imaginal ring.

air	= anterior imaginal ring	dev.prov.v.	= developing proventricular valve
a.mg.	= anterior midgut	oe	= oesophagus
a. pg.	= anterior plug	yb	= yellow body

Fig. 103. Early adult C. analis. Photomicrograph.
Part of T.S. of anterior midgut, showing
developing crypt.

dev.cpt.	= developing crypt
dt.n.	= daughter nuclei from the regenerative cells
? - yb	= yellow bodies



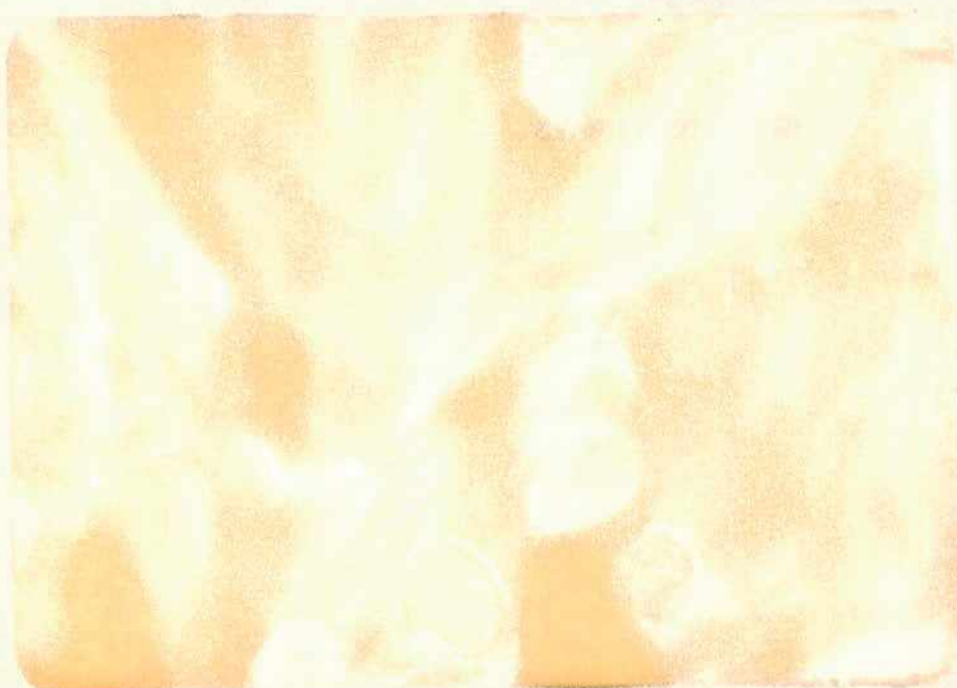
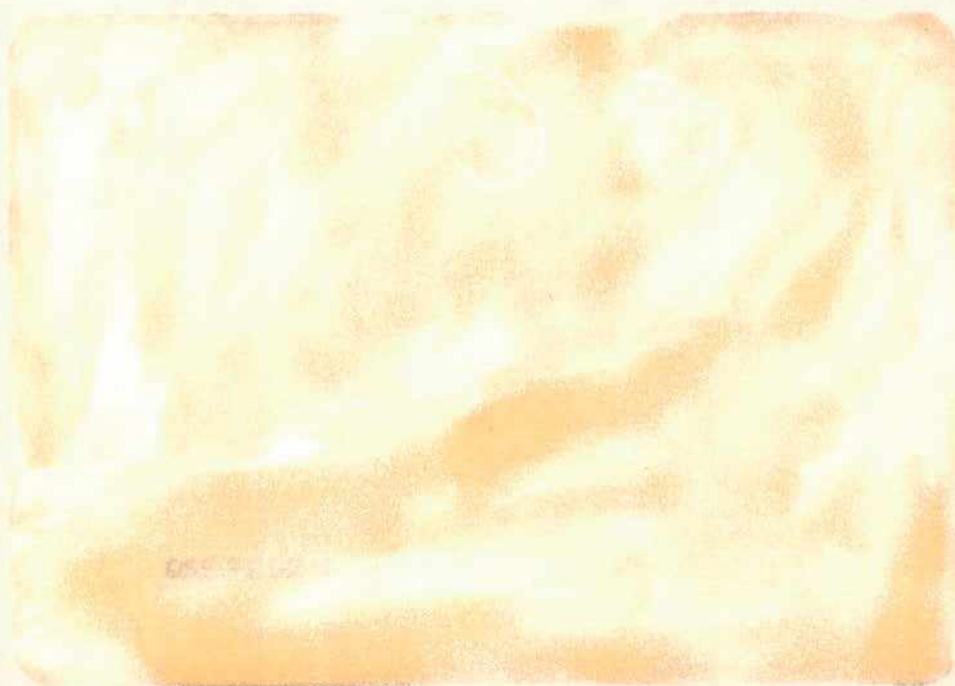


Fig. 104. C. analis pupa (3 days old). Photomicrograph.
L.S. of midgut-hindgut junction, showing two
zones of proliferated cells (from the posterior
imaginal ring) which formed the "posterior plug".

d.p.n.z. = densely packed
 nucleated zone

l.c.z. = loose cellular
 zone

pir = posterior
 imaginal ring

p.mg. = posterior midgut

prol.c. = proliferated
 cells

pyl = pylorus

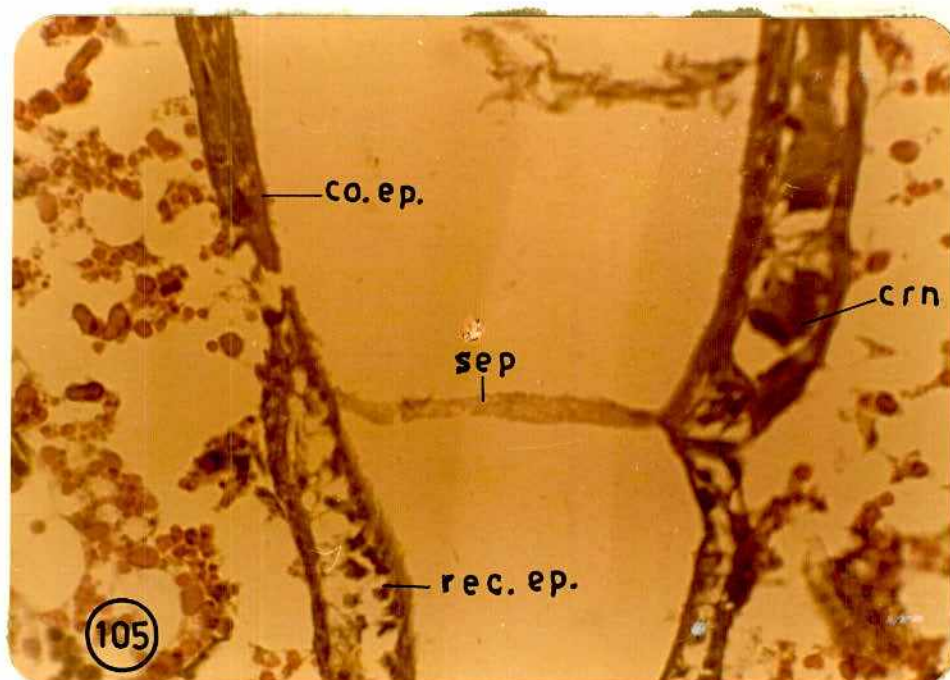
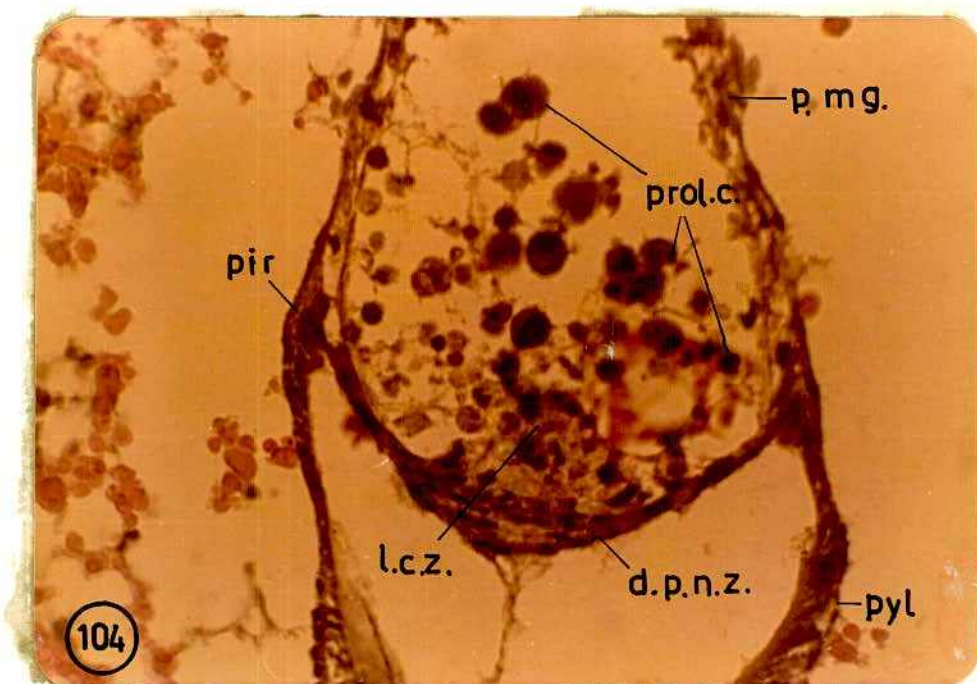
Fig. 105. C. analis pupa (4 days old). Photomicrograph.
Part of L.S. of colon-rectum junction, showing
a "septum" in the junction and cryptonephridia
around the colon.

co.ep. = colon epithelium

crn = cryptonephridium

rec.ep. = rectal epithelium

sep = septum



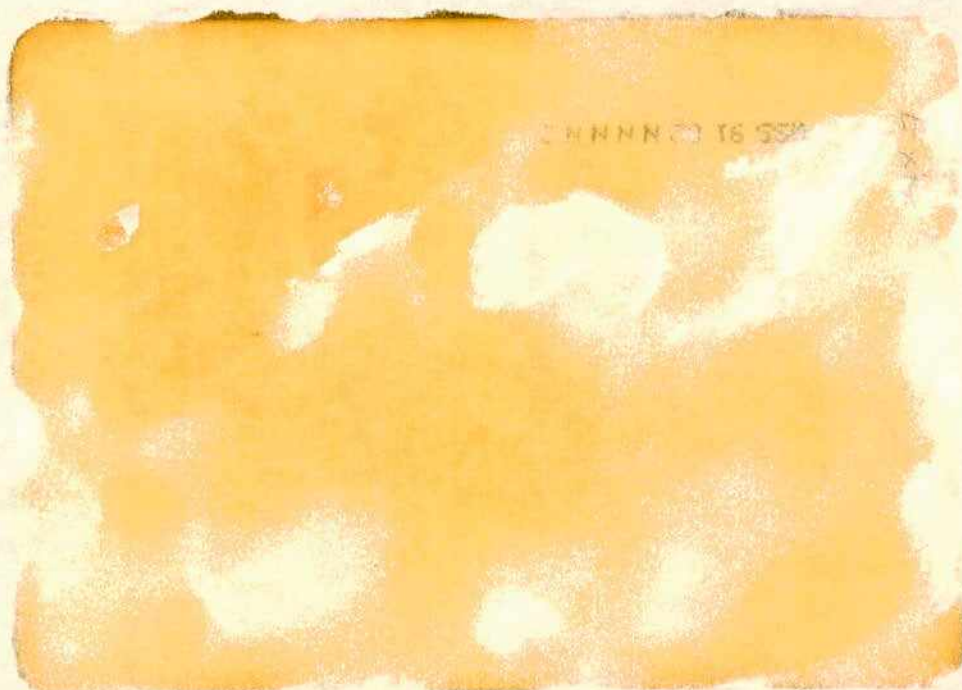


Fig. 106. C. analis pupa (6 days old). Photomicrograph.
L.S. of midgut-hindgut junction, showing the
zone of daughter nuclei which make an addition
to the Malpighian tubule formation, and a stream
of proliferated cells from the posterior imaginal ring.

Mt	= Malpighian tubule	str.prol.c.	= stream of proliferated cells
pir	= posterior imaginal ring	z.Mt.	= zone of daughter cells which make an addition to the Malpighian tubule formation
sh.pr.	= shelf-like prolongation		

Fig. 107. C. analis pupa (6 days old). Photomicrograph.

L.S. of midgut-hindgut junction showing a
prominent "posterior plug" formed by the
proliferated cells of the posterior imaginal
ring, and "shelf-like prolongation" at the
anterior end of the pylorus.

pir	= posterior imaginal ring
p.pg.	= posterior plug
prol.c.	= proliferated cells
sh.pr.	= shelf-like prolongation

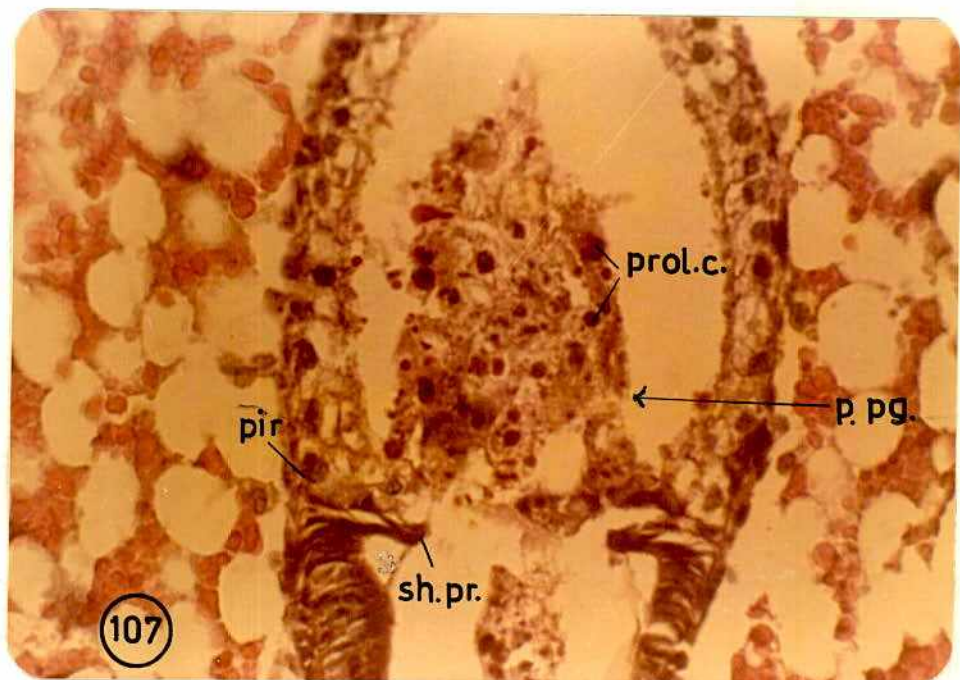
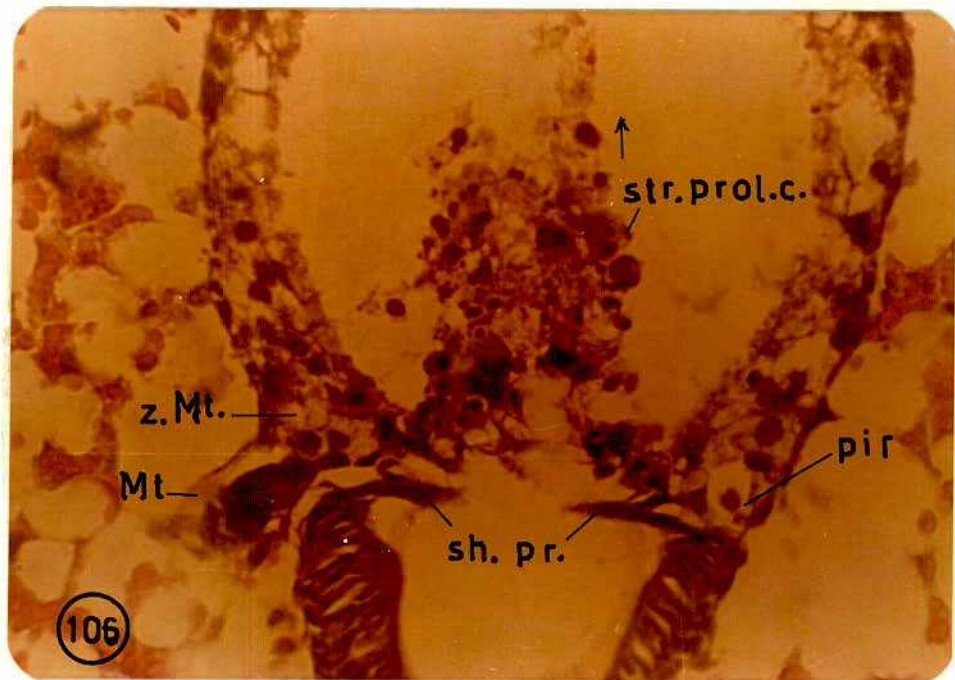
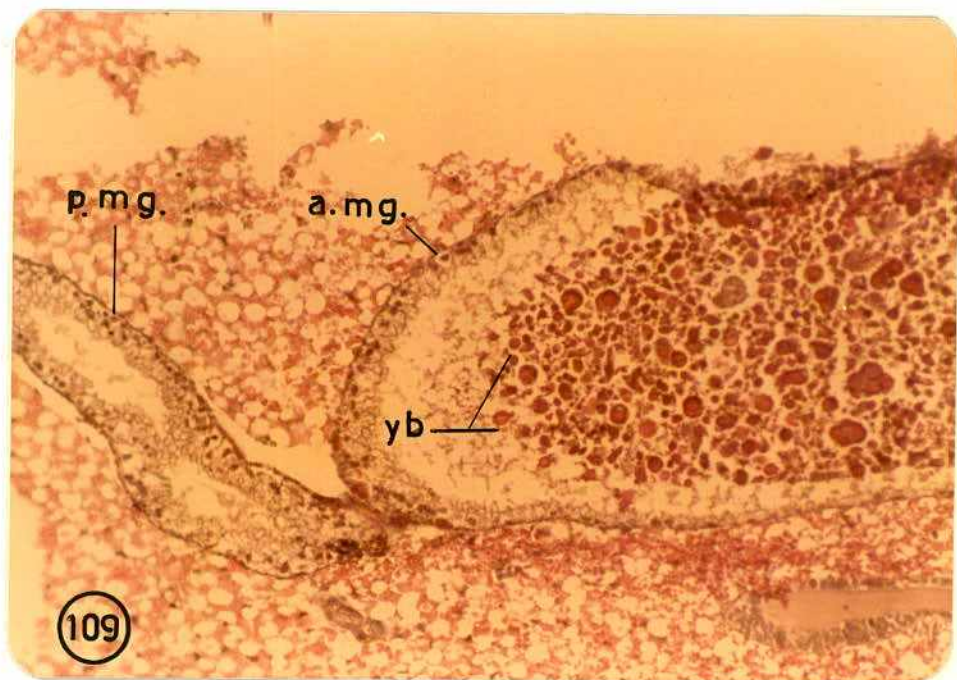
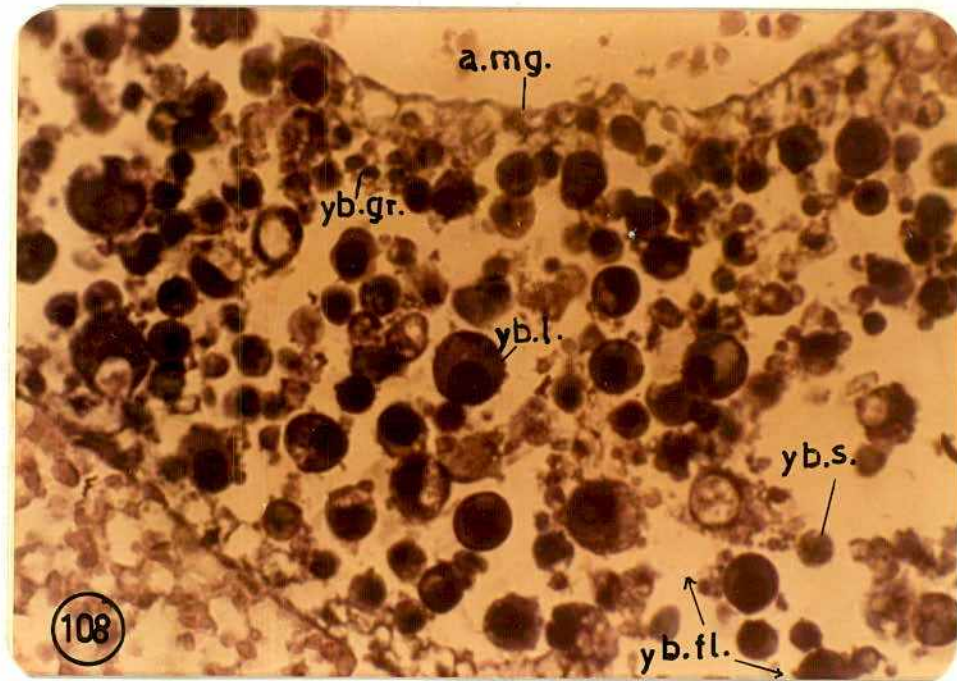


Fig. 108. C. analis pupa (6 days old). Photomicrograph.
Part of L.S. of anterior midgut, showing
different types of yellow bodies in the gut lumen.

a.mg. = anterior midgut
yb.fl. = fluid medium of the gut lumen where
the yellow bodies remain mixed up
yb.gr. = minute, granular yellow bodies
yb.l. = larger, nucleated yellow bodies
yb.s. = smaller, non-nucleated yellow bodies

Fig. 109. C. analis pupa (7 days old). Photomicrograph.
L.S. of anterior midgut and posterior midgut,
showing mass of yellow bodies in the gut lumen.

a.mg = anterior midgut
p.mg. = posterior midgut
yb = yellow bodies



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