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THE HISTOLOGY OF THE MIDGUT EPITHELIUM OF *ONCOPELTUS FASCIATUS* DALLAS (HEMIPTERA : LYGAEIDAE)

by

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ABSTRACT

A detailed histological study of the adult midgut of Oncopeltus fasciatus Dallas (Lygaeidae : Heteroptera) was carried out in the present work. The adult midgut of Oncopeltus fasciatus comprises six anatomically and histologically distinct ventriculi. The third ventriculus was newly recognized here and the sixth (previously called the pylorus) was shown in the present work to be the most posterior region of the midgut on the basis of its histological similarity to the remaining anterior regions. Evidence is brought forward suggesting that the Malpighian tubules are of endodermal origin. Both the oesophageal and the ventriculo-rectal valves were also studied histologically and illustrated. Investigations of the histological changes in the midgut epithelium in progressively older adults were conducted for the first time in the present work. By comparing the histology and cytology of the midgut in normally fed mature specimens with those of starved and or desiccated ones, it was concluded from cytological evidence that digestive enzyme production is accompanied by changes such as cytoplasmic extrusions, vaculation and vesicle formation.

Other aspects on this subject were critically reviewed, including previous views on the correlation between enzymatic secretion and the cytological changes in the midgut epithelium. Some electron microscopical observations have been made, revealing the nature of the ultrastructure of the epithelium, such as the striated border and some of the cellular organelles.

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CONTENTS

GENERAL INTRODUCTION

The Hemiptera are sucking insects obtaining their food by piercing the surface of plants or animals and drawing into their gut the sap or blood. The phytophagous Hemiptera are very destructive to vegetation and crops as are also certain Homoptera some of which also act as vectors transferring the viruses of such diseases as mosaic, leaf roll, yellows ..... etc., from plant to plant by means of their piercing mouth parts. In this respect the phytophagous Hemiptera form one of the most economically important groups of insect.

Oncopeltus fasciatus (Dall.) commonly known as the large milkweed bug belongs to the family Lygaeidae of the suborder Heteroptera. This is a large family and world wide in its distribution. Its members are mostly phytophagous and some are destructive to various cereal crops. Others are important pests on cotton, as the Egyptian Cotton Stainer (Oxycarenus hyalinipennis (Costa). Others are destructive to fruit trees, to grasses and to seeds of various Angiosperms. A high percentage of the seeds attacked by these bugs become sterile, their oil content is reduced and they lose weight, specially when heavily infested. The damage caused to plants by transmission of diseases is far more serious in some cases than that caused by the direct feeding of the bugs on seeds. Oncopeltus fasciatus is a seed-sucking Heteroptera insect and feeds on the milkweeds (Asclepias curassavica and Asclepias syriaca).

The midgut of Heteroptera has been the subject of various studies by a number of workers in the past and more recently. The first major work on the Hemiptera gut was undertaken by Dufour (1833) who dealt with the alimentary anatomy of both Hemiptera and the larger Homoptera. Since that early data it has been known that the alimentary canal of Hemiptera is highly differentiated and may incorporate some unusual structure such as the so called filter-champer found in most Homoptera. Since then studies

have been made of more restricted groups of Hemiptera dealing with various aspects of the subject.

Apart from much information on individual species of which some are listed in the introduction to the first chapter in the present thesis, certain studies of a comparative or review nature may be mentioned. Licent (1912) dealt with the Homoptera Auchenorrhyncha, Glasgow (1914) made a comparative survey of 87 species from a variety of families of Heteroptera. Glasgow was concerned with the peculiar gastric caeca and caecal bacteria which usually occur in the Heteroptera and in this he was followed by Kuscop (1923), Schneider (1940), Rosenkranz (1939) and others who also provided information on the anatomy of the midgut of the species studied. Weber (1930) while working on the Biology of Hemiptera provided much useful information about the anatomy of the Hemiptera gut in a variety of examples representing Heteroptera and Homoptera species of different sizes and feeding habits. Pesson (1933, 1935, 1936, 1941), in a series of works on Coccoidea, and Auclair (1963), who reviewed these structures in Aphidoidea, contributed most information on the smaller Homoptera (Sternorrhyncha).

There have been many attempts at comparative investigation by Elson (1937), Yanai (1952), Yanai and Iga (1956), Parsons(1959), Miyamota (1963), Goodchild (1963, 1965) and others, in which efforts have been made to relate gut structure to the classification and phylogeny of the order. At the same time some special functional problems have arisen in the study of alimentary canal, such as the problems of water disposal and conservation with related structures. Miyamota (1961), Bahadur (1961), Goodchild (1963a) and others discuss these.

Regional differentiation of the midgut is highest in Hemiptera. Accordingly to Saxena (1958) each ventriculus in Dysdercus koenigii plays a specific role in digestion and absorption of different carbohydrates; similar regions of functional differentiation are found in Eurygaster integriceps (Pentatomidae) in which absorption was concentrated in the

third region of the midgut where an absorptive type of cell predominated (Bocharova-Mesner, 1959). Miles (1958) stated that the third section of the midgut of Oncopeltus fasciatus nymphs holds the accumulated residues of food until after the final ecdysis when continuity with the hindgut is established and they are voided. Khan (1961) found that in Dysdercus fasciatus invertase is mainly produced in the first ventriculus of the midgut while amylase is mostly secreted from the salivary glands. Bongers (1970) while working on the digestive enzymes of Oncopeltus fasciatus found that the second and third regions are the main site of carbohydrase production.

Digestion is determined by the functional organization of the digestive system which is closely related to its anatomical structure. Major discussions of this subject have been provided by Snodgrass (1935), Wigglesworth (1972), Day and Powning (1949), Waterhouse and Stay(1955), Waterhouse (1957) and others. Many authors have studied the cytology of intestinal secretion in insects, but there is still considerable doubt about the nature of many of the cell inclusions and their relation to enzyme synthesis and release. Droplets produced by the digestive cells of the midgut are supposed generally to contain enzymes among the secretory products but different and contradictory interpretations occur among various authors. According to Sharif (1937) secretion in Nosopsyllus fasciatus is merocrine, in which cells remain intact through a cyclic process of repeated formation and discharge of their enzymatic products in the form of droplets. Ballentine (1940) observed a similar case of merocrine secretion of protease by cells in the cardiac region in the dragon fly nymph (Odonata). Srivastava (1955) concluded that both types of secretion (holo and merocrine) occurred in Periplaneta americana when feeding normally. According to Duspiva (1939) feeding starved adults of Dytiscus marginalis leads in one minute to the midgut epithelial cells discharging trypsin in the form of

cytoplasmic globules which are passed toward the crop where maximal enzyme concentration is recorded. Sutton (1951), Parsons(1959) and Kanungo (1964) also suggested that the inclusions and droplets produced by digestive cells of the midgut are secretory products. On the other hand quantitative enzyme estimations and cytological investigations on Blattella germanica (L.) made by Day and Powning (1949) have proved that the presence of cytoplasmic globules generally referred to as cytological evidence of secretory activity is not associated with an increase in enzyme concentration in the gut contents. According to Day and Powning, the greatest enzyme concentrations are found when the cytoplasm is cytologically uniform. They concluded that secretory globules are more probably signs of cell breakdown than an indication of secretory activity.

Khan and Ford (1962) while amplifying Day and Powning's results showed that the cytoplasmic extrusions from some of the midgut epithelial cells in Dysdercus fasciatus were greatly increased in number as a result of starvation, that the increase occurred first in the most anterior region of the midgut then progressively along its length and that the arrival of food restored the normal appearance of the epithelium.

Electron microscopical studies have provided information about the phenomena of secretion, absorption and transportation in the midgut epithelium of insects so that some of the physiological properties of the midgut epithelia can now be defined in ultrastructural terms.

Threadgold and Gresson (1962) while working on the midgut and hepatic caeca of Blatta orientalis L. showed epithelial cells in both secretory and absorptive cycles. Many ultrastructural details of the epithelial cells were revealed by their work such as the microvilli, the irregularly shaped projections found on the lateral regions of the cells, various vacuole-like structures Golgi elements, etc. According to Threadgold and Gresson the epithelial cells engaged in absorption differ from secretory cells in the arrangement of the Golgi saccules and vacuoles, in the size of Golgi

vacuoles, distribution of mitochondria and in the possession of a less extensive endoplasmic reticulum.

Meanwhile, of course, many special functional problems related to the ultrastructure of the alimentary canal have also received attention in insects, such as the absorption and transport of water and inorganic ions across epithelia (Berridge, 1970, 1967). Clark and Anstee (1971) investigated by light and electron microscopical study, the effect of the removal of the frontal ganglion on the cellular structure of the fat body and the midgut in Locusta migratoria migratorioides R.and F. in normally fed and starved specimens. Among their findings was the discovery that both frontal ganglion removal and starvation produce changes in the fine structural appearance of cells of the fat body and midgut. But the changes are different in the two treatments; the mitochondria of the operated Locusta are swollen and the cisternae of the endoplasmic reticulum dilated, while mitochondria are shrunken in starved specimens and the endoplasmic reticulum sparse although numerous free ribosomes are present.

Richard et al. (1971) made an ultrastructural examination of the midgut epithelium of Periplaneta americana. This shows numerous axonal ramifications which contain neuroscretory material. They thought axons adjacent to the epithelium liberate neorosecretory material which in turn acts upon specific localized areas to control water movement.

Munk (1968) in light and electron studies made on the filter chamber of Euscelidius variegatus Kbm (Cicadellidae) and related species revealed some of the ultrastructure of the filter-chamber and the midgut epithelium. Recently de Priester (1971, 1972, 1974) described the ultra-structure of the midgut epithelium and its fine structural changes in developing specimens and adults of Calliphora erythrocephala Meigen (Calliphoridae).

Beams and Anderson (1957) made one of the earliest electron microscope studies of the striated border of the intestinal cells of

Malacosoma larva and found that it is composed of Microvilli which are simply papillary elevations of plasma membrane on the free border of the cell. Noirot and Noirot (1972) investigated the brush border of the midgut epithelium in some Dictyoptera and Isoptera. They found distinct microvilli, inside each of which was a very clear axial filament running past an intracytoplasmic rootlet, where a true terminal web was lacking. They also found fibrillar material homologous to a glycocalyx covering the microvilli; histochemical tests indicated the presence chiefly of carboxylated acidic mucopolysaccharide. Marshall et al., (1970) studied the ultrastructure of the Homoptera digestive system and observed an unusual surface coat on the midgut epithelial cells of Fulgora candelaria. This is developed into a plexiform or reticulate structure which almost occludes the gut lumen; it has some of the characteristics of a glycocalyx and topographically resembles a peritrophic membrane. Dallai (1970) found the intercellular space (Zonula continua) is completely filled with electron-dense material and suggests that this structure is related to the continuous regeneration that these epithelia undergo. Pease (1956) worked on the infolded of basal plasma membranes found in epithelia noted for their water transport and Staeubli et al. (1966) on the structural modification of the endoplasmic reticulum of midgut epithelial cells of Mosquitoes in relation to blood intake. The latter workers found that the midgut epithelial cells of starved Aedes aegypti contain extensive, highly organised complexes of rough-surfaced endoplasmic reticulum or whorls, while those of unfed Anopheles species contain apical granules which presumably represent secretion granules (cf earlier work of Bertram and Bird (1961) on Aedes). They found that in Aedes aegypti secretion was linked with rapid break-down of the whorls into microsome-like rough-surfaced vesicles and in Anopheles with the disappearance of the apical granules, and they suggested that these whorls could be regarded as functional analogues of the apical granules in Anopheles and that their purpose would be to synthesise and to store secretion material.

Oncopeltus fasciatus (Dall.) which represents a seed sucking phytophagous Hemiptera insect was chosen here for the present work. This milkweed bug is an excellent example of a highly specific feeder and one which is used increasingly in laboratory studies (Feir, 1974). A detailed study on the anatomy and histology of the midgut of Oncopeltus fasciatus is described in the present thesis; some experiments on starvation and desiccation of the adult, with examination of the correlated changes in the epithelial cells of the midgut during progressively older adult contributed useful information which has not been recorded before.

In the present work some electron microscopical observation on the midgut epithelium of adult specimens belonging to Oncopeltus fasciatus are also provided for the first time.

MATERIALS AND METHODS

The milkweed bug Oncopeltus fasciatus Dall. (Lygaeidae Heteroptera) has been a desirable subject for numbers of laboratory researches. Feir (1974) briefly reviewed some of the literature on this insect showing that Oncopeltus fasciatus is a widely used laboratory and field research animal.

The large milkweed bug was first described as Lygaeus fasciatus by Dallas in 1852; Stål transferred it to the genus Oncopeltus according to Slater (1964). Specimens used in the present work were obtained in October 1975 from the Imperial College Field Station, Silwood Park, Ascot. They were kept and reared in the laboratory at room temperature (approximately 26°C) and relative humidity of about 75% in plastic containers. They were fed on milkweed seed Asclepias curassavica and Asclepias syriaca with moist cotton wool which made the seeds tender and provided water for the insect to drink. The cotton wool was changed every day and the insects were provided with fresh seeds every two days.

After copulation the mature females deposited their egg on the cotton wool which was constantly kept damp by sprinkling water inside the containers. Newly hatched individuals were kept in the original containers until an active large stock culture was obtained; this was maintained during the two year period of this study.

1. Gross anatomy To study the general anatomy of the alimentary canal freshly killed insects of both sexes were dissected in 70% alcohol. All illustrations and measurements were made under the dissecting microscope with the aid of an eye piece graticule and micrometer.

2. Histology For the histological investigations specimens were dissected very quickly under normal saline solution (NaCl - 7.5 gm, KCl - 0.5 gm, H_2O - 1l) or under insect saline solution (NaCl 6.5 gm,

KCl 0.14 gm, $CaCl_2$ 0.12 gm, NAH_2PO_4 0.01g, $NAHCO_3$ 0.1 gm made up with water to 2 l). The midgut was then removed immediately to fixative fluid for killing and fixation. A number of fixatives were used for the observation on the adult midgut, including Bouin's fluid (Bouin, 1897). Zenker's fluid (Zenker, 1894), 10% formal saline, Helly's fluid or Zenker Formal (Helly, 1903). Bouin's fixative was found most satisfactory for general histological purposes. Diethylene dioxide (Dioxan) was used for dehydration of some material instead of graduated alcohols (HUMASON, G.L. 1972) but it was found unsatisfactory, since it caused shrinkage to the tissue, Miller's schedule for Dioxane (1937) used for this purpose. The method of Bhatti et al. (1967) by using glutaraldehyde and Bouin's fluid sequence for fixation of parasitic flat worms for light microscopy was tried on the tissue of the midgut in Oncopeltus fasciatus. This method gave unsatisfactory results with the midgut tissue of Oncopeltus. Generally speaking after using all the above fixative fluids, the material was dehydrated in ethanol, cleared in xylol and embedded in paraffin wax (M.P. 57°C). Sections were cut on a standard Cambridge rocker microtome, from 5 - 8μ in thickness. Sections were mounted serially on albuminized slides and stained with phosphotungstic acid haematoxylin and Mallory's connective tissue stain (DRURY and WALLINGTON, 1967 as modified by R.G. Davies). The midgut of Oncopeltus fasciatus was removed and placed in a solution of aceto-orcein for 15 minutes (Humason, 1972) to count nidi undergoing mitosis. The material was then transferred to a slide and small pieces of the epithelium were cut off and covered with small coverslips. The preparations were squeezed under a blotter and then examined under an oil immersion objective. Mitoses were not clearly visible in such preparations, though many were found in the sectioned material that formed the main basis of the work. The drawings were made with the aid of a binocular microscope and squared eye piece graticule. All measurements were made in micrometers.

3. Postmetamorphic changes For this study a stock culture of Oncopeltus fasciatus was maintained in the laboratory under the condition mentioned above and the insects were fed and watered normally. Freshly emerged adults were regarded as of zero age. Specimens of zero age to 30 days old were dissected and sections prepared by the above procedure for 10 individuals in each age group. Material was fixed with Zenker and Bouin's fixative, the latter used for photography. The data were analysed statistically to appreciate the significant changes by calculating the standard errors of the means according to Bailey (1974).

4. Starvation and desiccation As soon as the adult insect emerged, they were removed from the rearing cage to separate containers and kept under similar conditions of temperature and humidity where they were allowed to remain for an appropriate period. They were supplied or not with food and water according to the experiment involved, as in Table 26. Specimens were dissected and sections prepared by the procedure given above for 10 individuals each in experiment.

5. Electron microscopy Several fixatives were tried, most of them based on glutaraldehyde and or osmium tetroxide and buffered phosphate (Millonig, 1961) with veronal acetate, sodium cacodylate and varying quantities of sucrose and $CaCl_2$ added to regulate the osmolality. These fixatives gave variable and on the whole unsatisfactory results. The most satisfactory results were obtained after primary fixation with paraformaldehyde and 25% glutaraldehyde in a 0.2M cacodylate buffer (pH 7.4) at room temperature and secondary fixation with 5% osmium tetroxide in a similar buffer at $4^{\circ}C$. The midgut was transferred to a shallow container of primary fixative and sliced into 1 mm^2 pieces at room temperature; after 10 minutes it was transferred to fresh fixative surrounded by ice for 1 hour. Then the

fixative was sucked off with a pasteur pipette and washed twice with 0.12M cacohylate buffer, both washes for 30 mins. After removing buffer, the tissue was dealt with by secondary fixation in OsO_4 (2.5%) in 0.1M cacodylate for 1 hour on ice. After osmium treatment tissue was then rinsed in two washes of 10 minutes each with 0.1M sodium acetate in ice. Specimens were then transferred to 0.25% aqueous uranyl acetate for 1 hour at 4°C. and rinsed in two washes of 10 minutes each with 0.1M sodium acetate in the refrigerator. The tissue was then dehydrated, both graded acetones and alochol were used; during dehydration with 70% acetone, 1% phosphotungstic acid (PTA) + 1% uranyl acetate were added at 4°C. Tissues were embedded in Araldite resin. Sections were cut with a Huxley Cambridge ultramicrotome. Thick section for light microscopy observation were cut at $\frac{1}{2}$ - 1μ and stained with 1% toluidine blue in 1% borax; a few thin sections were cut; they were expanded with chloroform vapour and mounted on grids (Type G 100, size 3mm), coated with formvar film (0.3% Formvar in ethylene dichloride or chloroform). Sections were stained in uranyl acetate and lead citrate, Reynolds (1963) and observed in AEI EM6 electron microscope under which they were photographed. The full details of fixation and dehydration follow those given by Sinden (pers. comm.).

SECTION 1

GROSS ANATOMY OF THE ALIMENTARY CANAL

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Introduction

An understanding of the general plan of the anatomy of the digestive system is a necessary prerequisite to the detailed study of the histological features of the various parts of the tract. Apart from various comparative studies on the alimentary canal of the order Hemiptera, there are numerous studies on individual species of the order which will be referred to at the appropriate place throughout the text of the present account. The earliest work on the alimentary canal of Hemiptera was carried out by Dufour (1833), followed by Locy (1884) on the family Nepidae, Pantel and Licent (1910) and Licent (1912) on some Homoptera Auchenorrhyncha. One of the earliest investigations on small species of Homoptera was made by Lubbock (1858) on Coccus hesperidum (Coccidae), followed by Kershaw (1910) on the candle fly Pyrops candelaria.

Terrestrial species of Heteroptera have been studied individually by several authors and may be listed as follows :

Malouf (1933) : Nezara viridula (Linn.) (Pentatomidae)

Breakey(1936) : Anasa tristis Deg. (Coreidae)

Hamner (1936) : Solubea pugnax Fab. (Pentatomidae)

Hood (1937) : Oncopeltus fasciatus Dall. (Lygaeidae)

Harris (1938) : Murgantia histrionica Hahn. (Pentatomidae)

Woolley(1949) : Leptocoris trivittatus Say. (Coreidae)

Servadei (1951) : Heterogaster urticae F. (Myodochidae)

Rastogi (1960) : Lygaeus pandurus Scop. (Lygaeidae)

Bocharova-Messner (1959) : Eurygaster integriceps Put. (Pentatomidae)

Kanungo (1964) : Dysdercus fasciatus Sign. (Pyrrhocoridae)

The aquatic Heteroptera were dealt with by other authors such as Hamilton (1931) on Nepa cinerea L. (Nepidae), Griffith (1945) and Sutton (1951) on Corixidae, Marks (1958, 1959) on various Hydrocorisae (Cryptocerata).

Rastogi (1961) on Sphaerodema rusticum Fabr. (Belostomatidae) and Kurup (1961) on the digestive tract of some other Hydrocorisae. Information on the alimentary canal of the Homoptera has been provided by Kershaw (1913, 1914), Hough (1925) Forbes (1964) and Munk (1967, 1968), while numerous studies on the symbiotic microorganisms of the Heteroptera include much information on their alimentary canal, e.g. Glasgow (1914), Kuskop (1923), Rosenkranz (1939), Schneider (1940) and Buchner (1953). Very useful information on the comparative morphology of the alimentary canal of Hemiptera was provided by Elson (1937) and Miyamota (1961). Goodchild (1952, 1963a, 1963b, 1965), working on the functional anatomy and evolution of the alimentary canal of Hemiptera has become a prominent authority on the subject.

Feir (1974) in her review shows that very little work has been done on the alimentary canal and its physiology in Oncopeltus fasciatus , a part from a brief study on the anatomy and histology of the digestive system of this insect provided by Hood (1937), some investigations on the physiology of its digestive organs were made by Bongers (1968, 1970). There is, therefore, reason to hope that further detailed work on the anatomy and histology of the midgut of Oncopeltus fasicatus will provide a more reliable account of this terrestrial seed-sucking heteroptera species.

The alimentary canal of Oncopeltus fasciatus (Dall.) (Lydaeidae) is of a moderate length, about two and a half times that of the body, and divided as in other insects, into three major portions : the stomodaeum on fore-gut, the mid-gut (mesenteron or ventriculus) and the hindgut or proctodaeum (Fig. 1). The relevant measurements of the length and diameter of each division or subdivision are shown in Table 1.

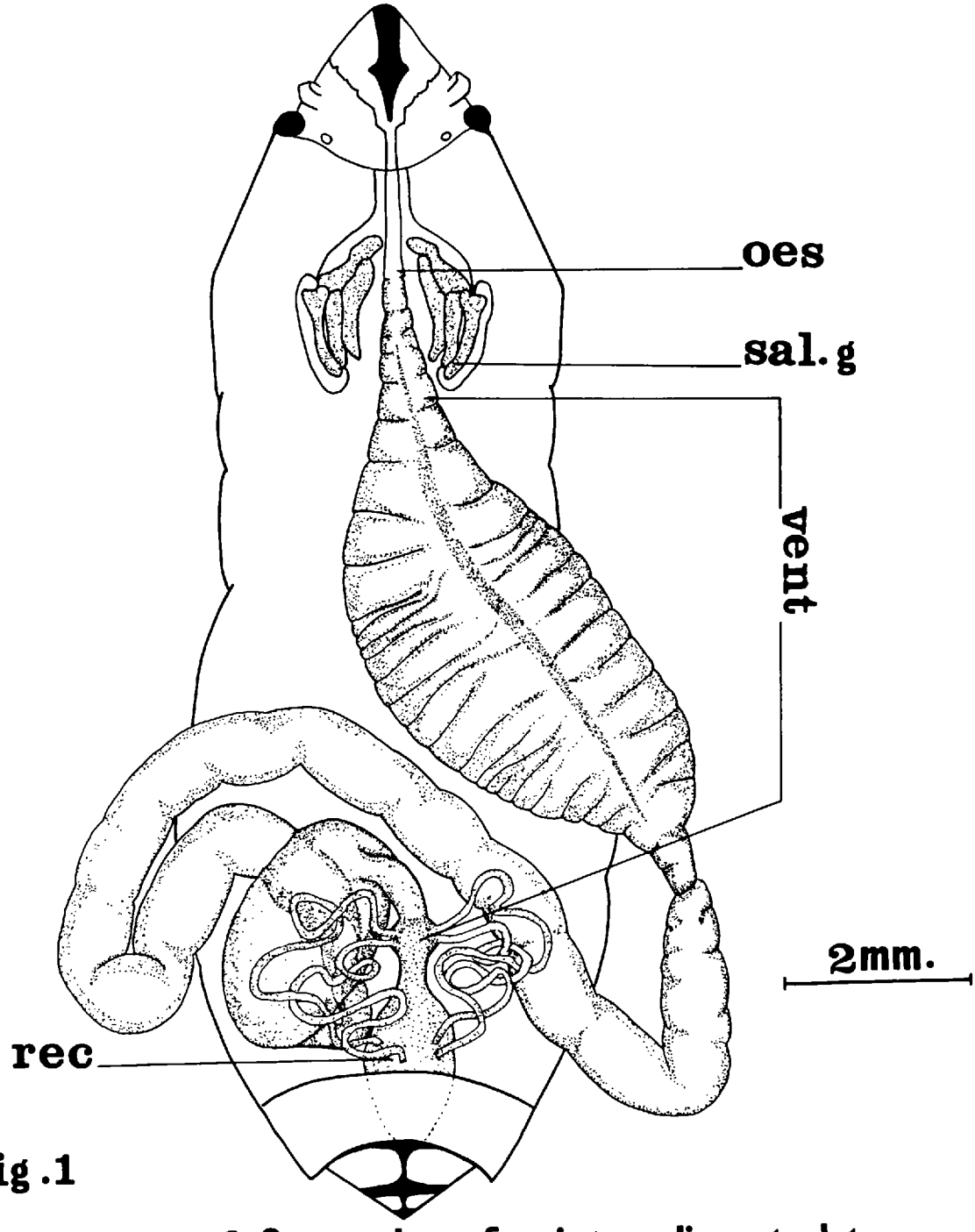


fig .1

Dorsal view of Oncopeltus fasciatus dissected to show the digestive system

Table 1 Dimensions of Body, alimentary canal and parts of ventriculus in Oncopeltus fasciatus Dall.

All measurements are in millimeters; averages are based on about 30 specimens

Specimens	Sex	Average Body length (A)	Average alimentary canal length (B)	Average midgut length	1st. Ventriculus		2nd. Ventriculus		3rd. Ventriculus		4th. Ventriculus		5th. Ventriculus		6th. Ventriculus		Ratio B/A
					length	maximum diameter	length	maximum diameter	length	maximum diameter	length	maximum diameter	length	maximum diameter	length	maximum diameter	
Adult Oncopeltus fasciatus	Female	13.2	32.8	29.79	10.12	2.64	14.64	0.62	0.67	0.34	2.42	1.89	1.12	0.42	0.82	0.66	2.48
	Male	11.22	28.05	26.53	9.10	1.87	12.30	0.42	1.06	0.49	2.34	1.66	0.99	0.26	0.74	0.58	2.5

The proboscis of Oncopeltus fasciatus Dall. is a typical hemipteran type, formed of a 4-segmented labium. The mandibles and maxillae are stylet-like structures that together with the labium and labrum form the proboscis. Both mandibles and maxillae lie in a deep groove on the anterior surface of the labium. The mandibular stylets or bristles are the chief piercing organs and always lie lateral to the maxillary stylets. Each maxillary stylet is provided with two grooves on the inner surface. The apposed surfaces of the maxillary stylets thus form the dorsal food canal and ventral salivary canal. Basally both the mandibular and maxillary stylets are more or less thickened and connected by a lever. The stylets converge along the sides of the hypopharynx and form a fascicle (Matsuda, 1965).

The development of the mouth parts in Oncopeltus fasicatus has been traced by Butt (1949) and Newcomer (1948). According to Newcomer the mandibular lever in Oncopeltus fasciatus is secreted by the wall of the mandibular setal sac after hatching, while the lever of the maxillary stylet is produced from a simple diverticulum of the maxillary setal sac.

The hypopharynx in Hemiptera is a well developed structure arising from the venter of all the gnathal segments (Heymons, 1899). According to Newcomer the hypopharynx arises from the three gnathal and the intercalary segments in Oncopeltus fasciatus. It forms the ventral floor of the food canal medially and a greatly developed hypopharyngeal wing laterally on either side of the small hypopharyngeal lobe. The salivary syringe in Hemiptera is a modified salivarium, equipped with a piston and valves to control the direction of flow of salivary liquid. It lies beneath the food pump and between the hypopharyngeal wing (Matsuda, 1965). Embryologically the salivary syringe is produced from the venter of the maxillary and labial segments (Snodgrass, 1935; Newcomer, 1948; Parsons, 1963). The upper surface of the hypopharynx is grooved apically to form the floor of the tube which carries the food to the cibarium. The floor of the cibarium is formed by the proximal part of the anterior surface of the hypopharynx, while its

roof is the epipharyngeal wall of the ante-clypeus. The cibarium occupies a horizontal position, with the lateral and ventral walls strongly sclerotized and opens into the greatly reduced pharynx.

Weber (1928) made a comparative study of the movements of the stylets of Hemiptera in relation to their associated musculature. Miles (1958) working on Oncopeltus fasciatus has confirmed Weber's observations. He observed that during forward movement, one mandible leads either alone or with the maxillae. If the mandible is projected alone, it curves inwards and the stylets following are thereby guided to one side. If a mandible leads with the maxilla, the latter overcomes the tendency of the mandible to curve inwards and progress becomes straight forward.

Pharynx The pharynx is a tubular connecting region between the cibarium and oesophagus. Various authors such as Tower (1914) have referred to the cibarium as the pharynx, but Weber (1930) and Snodgrass (1935) made it clear that the pharynx of Hemiptera is situated posterior to the cibarium.

Oesophagus The oesophagus is a short and thin walled tube which passes between the circum oesophageal connectives in the head and posteriorly into the prothorax. It is a very narrow region joining the enlarged first ventriculus where the oesophageal valve is located. (It is difficult to determine exactly where this region ends from its gross anatomy but it seems to terminate in a valve). The histology of the oesophagus shows that the epithelium is composed of a single layer of cuboidal cells covered by a thin layer of longitudinal muscle and weak, scattered external strands of circular muscle (Fig. 4). The epithelium is provided with a continuous thin intima. At the point at which the oesophagus joins the midgut the oesophageal epithelium is invaginated into the ventriculus proper to form the oesophageal valve or invagination.

Salivary glands According to Snodgrass (1935) these glands are of ectodermal origin, arising in the embryo as paired invaginations just behind the bases of the rudiments of the second maxillary appendages. They are therefore treated here in connection with the stomodaeum.

In Oncopeltus fasciatus the salivary or labial gland consist of a pair of trilobed principal glands and a pair of tubular accessory glands with associated ducts (Fig. 1). They lie on either side of a median line through the wall of the oesophagus and extend posteriorly on the dorsal side of the anterior end of the first ventriculus. Each gland is composed of four parts, two of which form pyriform lobes. The third is bilobed while the fourth forms a convoluted tubulewith its distal end within the head cavity. Each gland has a straight duct arising at the junction of the lobes of the gland. This duct and its fellow from the other gland unite at the base of the proboscis to form a very short common salivary duct which empties into the space below the hypopharynx. The salivary duct runs into a salivary syringe equipped with a piston and valves to control the direction of flow of salivary liquid. Matsuda (1965) and Bronskill et al. (1958) studied the anatomy, histology and secretion of the salivary gland of Oncopeltus fasciatus in which tests for digestive enzymes demonstrated the presence of an amylase, proteases, invertase and lipase. It was also shown that the various lobes of the salivary glands contain different digestive enzymes. Miles (1959) also studied the salivary secretions of the milkweed bug and found that it secretes two types of saliva - sheath material which coagulates readily and forms a lining to the path of the stylets when the insect feeds on natural food material, and a watery secretion which is secreted and sucked back with the food materia. Salkeld (1959) studied the posterior lobe of the salivary gland of Oncopeltus fasciatus and found it was rich in a non specific esterase and that no true lipase was found in the salivary glands. The types of salivary secretion, its nature and enzymes were also investigated by Bongers (1969, 1970) on Oncopeltus and extensively

by Miles (1972) in Hemiptera in general.

Mesenteron (Midgut)

The midgut or mesenteron is differentiated into six well defined regions, referred to here as the 1st, 2nd, 3rd, 4th, 5th and 6th ventriculus (Fig. 2) and are described as follows. Glasgow (1914) was among the first to attempt to homologise these regions as they occur throughout the order and illustrated his work with a long series of figures.

1st. ventriculus This is a large thin walled sac-like structure, its size and shape depending very much on the amount of food or air it contains. It is capable of great distension, but it usually extends from the 1st thoracic segment to the 2nd abdominal segment. It was termed the crop by Malouf (1933), the "premier poche de ventricule chylifigue ou stomac" by Dufour (1833), the first stomach by Glasgow (1914) and by Woolley (1949), Harris (1938), Breakey (1936) and others. It has been called the first stomach of the ventriculus. The walls of the 1st ventriculus are transversely folded especially when empty, with median dorsal and ventral raphes running almost the entire length of the region. Malouf (1933) states that both the oesophagus and crop have similar histological characters in Nezara viridula, and he believed that the terms stomach and ventriculus are erroneous, but my work supports the interpretation of Dufour and Glasgow. Malouf is apparently wrong in regarding the first ventriculus as the crop; the latter (which is an ectodermal structure) is always absent in the Hemiptera. The first ventriculus is usually distended by air bubbles which eliminate the folded appearance of its walls.

2nd ventriculus The first ventriculus narrows posteriorly and passes into the 2nd. ventriculus. This is a convoluted tube forming the longest part of the mesenteron, the junction between 1st. and 2nd. ventriculus lying at the posterior end of the 2nd. abdominal segment. The 2nd. ventriculus turns

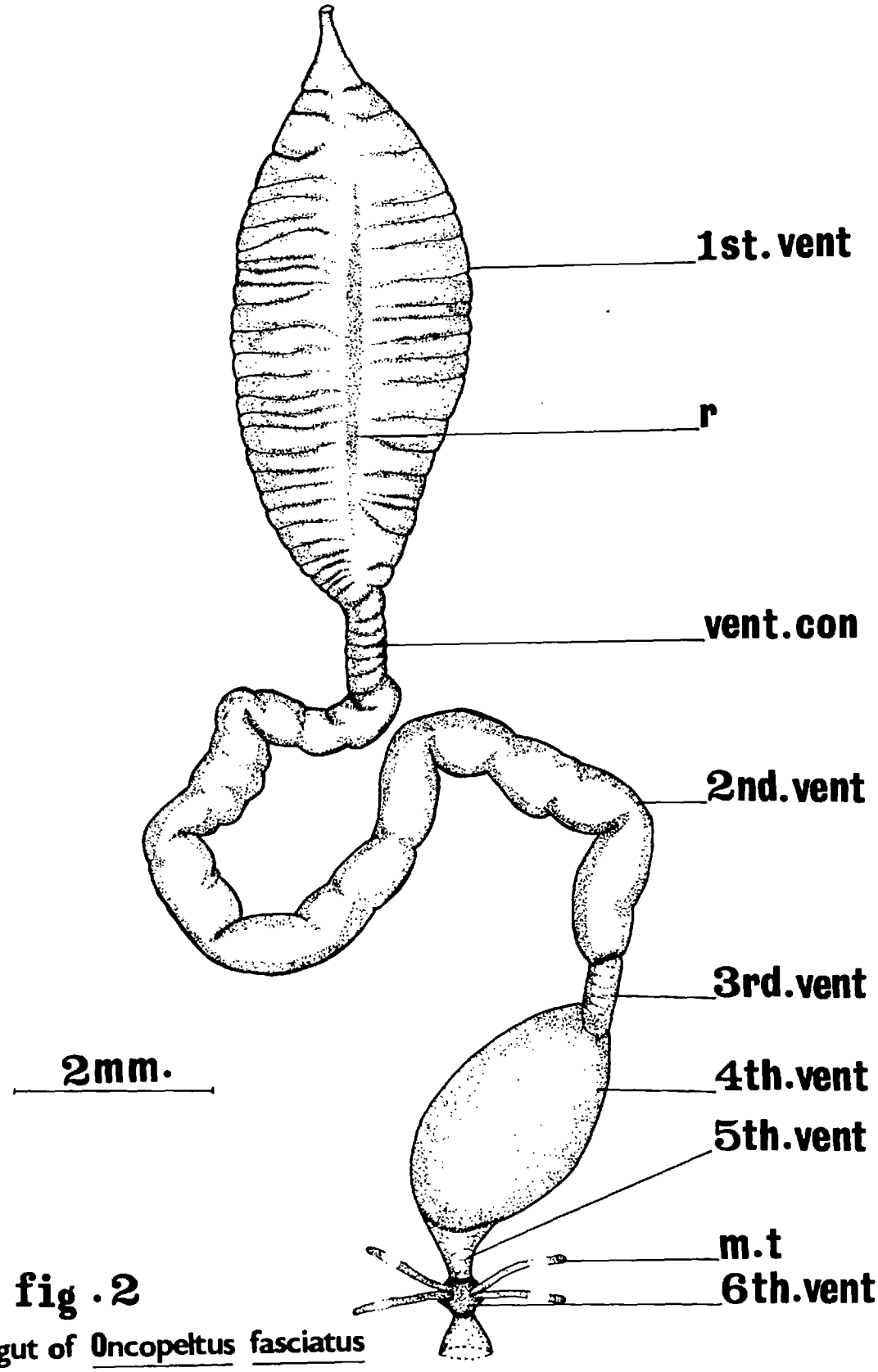


fig . 2

Midgut of Oncopeltus fasciatus

dorsally then anteriorly and ventrally with its anterior part under the 1st. ventriculus. This region has been called the "first stomach" by Malouf, the "portion filiforme" by Dufour, the "intestine posterior" by Ancona and the second stomach of the ventriculus by Glasgow and other later workers. It is very light brownish-white in colour and lies in the 2nd - 5th. abdominal segment.

3rd. ventriculus The 2nd. ventriculus joins the 3rd. by a distinct constriction. This short white region is concealed between the convolution of the second ventriculus and lies in the third abdominal segment. It seems not to have been recognized by Hood (1937) in his study of the alimentary canal of Oncopeltus fasciatus.

4th. ventriculus The third ventriculus passes posteriorly into an ovoidal 4th. ventriculus which is usually filled with food material. This organ is the "second stomach" of Malouf, the second "poche gastrique" of Dufour and the "third stomach" of Glasgow and latter authors. It is dark brownish in colour and usually contains oil droplets when it is filled with food material. Miles (1958) has found that in mature nymphal stages, this part contained only a large droplet of oily materials.

5th. ventriculus The fourth ventriculus narrows posteriorly into a short narrow smooth walled tube called here the 4th. ventriculus. This part is devoid of gastric caeca such as occur in many other Heteroptera (Glasgow, 1914; Buchner, 1953).

6th. ventriculus This is an enlargement, considered here as the most posterior section of the midgut, into which the Malpighian tubules enter when they join the alimentary canal. Hood (1937) provisionally called it the

pylorus in Oncopeltus fasciatus, but seemed doubtful.

This small part of the alimentary canal has been named by some authors working on pentatomomorph species as the ileum or pylorus and considered as the most anterior segment of the proctodaeum. It has been interpreted in this way not because of its histological features but because it received the openings of the Malpighian tubules. In this respect Snodgrass (1935) pointed out that in the majority of insects the Malpighian tubules arise from the proctodaeum. Henson (1932), however, believes that the Malpighian tubules in some insects arise from the mesenteron or from the undifferentiated zone between the endoderm and ectoderm and Srivastava and Bahadur (1961) working on the development of the Malpighian tubules in Dysdercus koenigii (Pyrrhocoridae), accepted Henson's view. They considered them as being endodermal and histologically similar to the midgut. Goodchild (1965) pointed out that the Malpighian tubules primitively open into the extreme posterior end of the midgut in Hemiptera. There is further confusion in the nomenclature of this region in different groups by many authors and the matter has been dealt with by Rastogi (1962).

What was called the pylorus by Hood (1937) and treated as part of the proctodaeum, is considered here to be the most posterior part of the midgut and is termed the 6th. ventriculus. The histological evidence on this matter will be discussed in the histological sections of the present work (pp. 69).

A pair of rather long Malpighian tubules is inserted on each side of the ventriculus. They are large and convoluted and form an entangled mass in the dorsal part of the body cavity beneath the heart and aorta. They are blind tubules with thin distal ends lying freely in the body cavity and not connected with the rectum. Each of the four tubules enters the lateral wall of the 6th. ventriculus independently. They are white in colour with longitudinal reddish streaks in the distal ends.

Proctodaeum (Hindgut)

The hindgut or proctodaeum of Oncopeltus fasciatus is short and greatly reduced, being composed of only the rectum (Fig. 1). The latter commences at the ventriculo-rectal valve which is referred to by some workers as the pyloric or the ileo-rectal valve.

Goodchild (1965) claimed that in the Pentatomomorpha the pyloric region was enlarged to accommodate the openings of the Malpighian tubules, thus forming a distinct segment of the hindgut. This is not the case in Oncopeltus fasciatus where the pylorus is histologically similar to the midgut in spite of the restricted opening of the Malpighian tubules.

The rectum is a relatively large membranous structure which extends backwards to the posterior part of the last abdominal segment. It becomes narrow in the 6th. abdominal segment and extends to the anus as a narrow tube which communicates with the exterior.

Rectal papillae The rectal glands, papillae or pads have long been regarded as absent in the order Hemiptera. This view has seemed not unreasonable as the rectal pads have an absorptive function for water and since the Hemiptera feed on liquids they might be expected not to reabsorb water very actively. Poisson (1924), however, found a well developed area in the rectum of some aquatic Hemiptera. He has suggested a respiratory function on account of numerous tracheoles. The presence of rectal gland in species such as seed sucking Hemiptera which need to conserve water is more readily understandable. In water bugs (Hydrocorisae) where these structures are also well developed a solute-absorbing function has been suggested, cf. Goodchild (1963) and Wigglesworth (1931); the latter observed a similar well developed area on the anterior dorsal side of the rectum in Rhodnius prolixus, and described it as the rectal gland. Breakey (1936) also found large cells in the rectal epithelium of Anasa tristis which he thought to have the same function as the rectal papillae in other insects.

But many other authors such as Hood (1937), Rastogi (1960) and Woolley (1949) have described the rectum as lacking rectal papillae. Harris (1938) noted two types of epithelial cells in the rectal sac in Murgantia histrionica. He tried to homologise the small cells with the rectal papillae of other insects but he could not do so and therefore concluded that the pad was absent.

The homology of the gland cells throughout several Hemiptera was claimed by Goodchild (1963b) and Bahadur (1963) but Miyamota (1961) did not recognize them in the Pentatomomorpha. Hood (1937) failed to describe the papillae in Oncopeltus fasciatus, probably because they are not in the form of well defined pads to be seen on the external surface of the rectum as in other insects.

The present work reveals that the histology of the rectum of Oncopeltus shows well defined rectal pads. The greater part of the rectal wall is formed of small cells having small nuclei measuring 4μm in diameter and with scanty cytoplasm. Another part of the rectum, however, consists of large tall cells with large nuclei measuring 16 - 18μm in diameter and with dense cytoplasm which stains deeply with PTAH and shows striations toward the inner side. Peripheral to these striations there is a thin layer of cuticle as shown in Fig. 3. The cuticle of the transporting epithelia may perhaps function as a molecular sieve, allowing only small molecules access to the apical cell-surface. Fine tracheae enter the cells in abundance. Circular and longitudinal muscles are better developed at the ventral side of the rectum than dorsally where these pads are found (Bahadur, 1963). The rectal pad cells are uninucleate though their boundaries are indistinct under the light microscope.

The rectal pads or papillae have been implicated as the major sites of rectal reabsorption. Wigglesworth (1932) has suggested that in insects where definite rectal glands are absent, the cuboidal or columnar cells

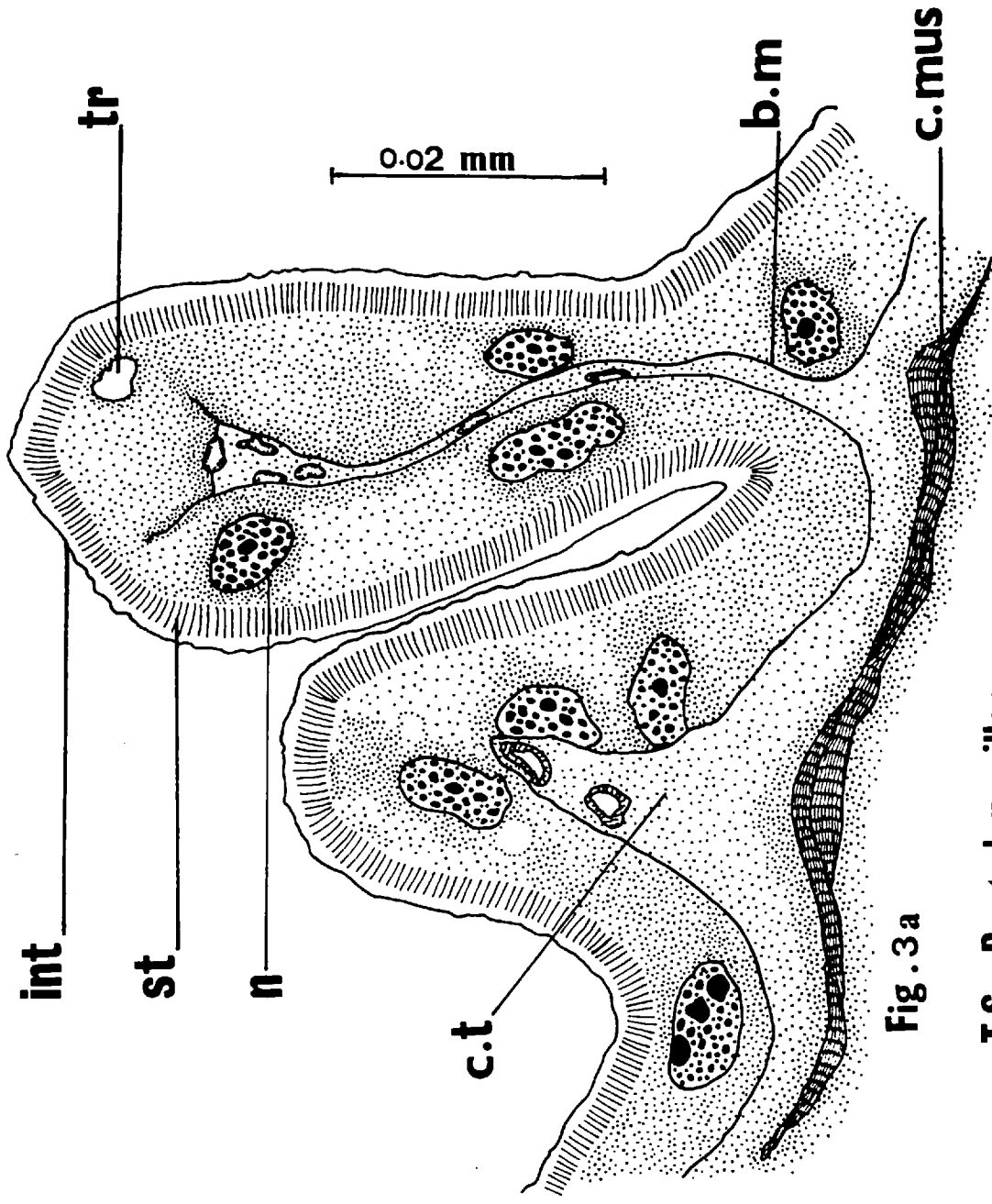


Fig. 3a T.S. Rectal papillae

which are found generally in the rectum perform the function of the rectal gland. Berridge (1970) showed the role of enclosed extracellular compartments in absorption in both the midgut and the rectum, where he demonstrated by models that the absorbed substances are not transported directly to the blood but pass through enclosed spaces which in turn communicate with the blood by restricted openings. He also believes that oxygen is normally carried to the rectal epithelia forming the pads by means of a complex network of tracheae which ramify throughout the intercellular spaces. Maddrell (1971) reviewed the mechanisms of insect excretory systems and in the light of the new findings provided by the ultrastructural studies of the rectum and rectal pads, he described the role of these pads in reabsorbing water, ions, amino acids and other small organic molecules.

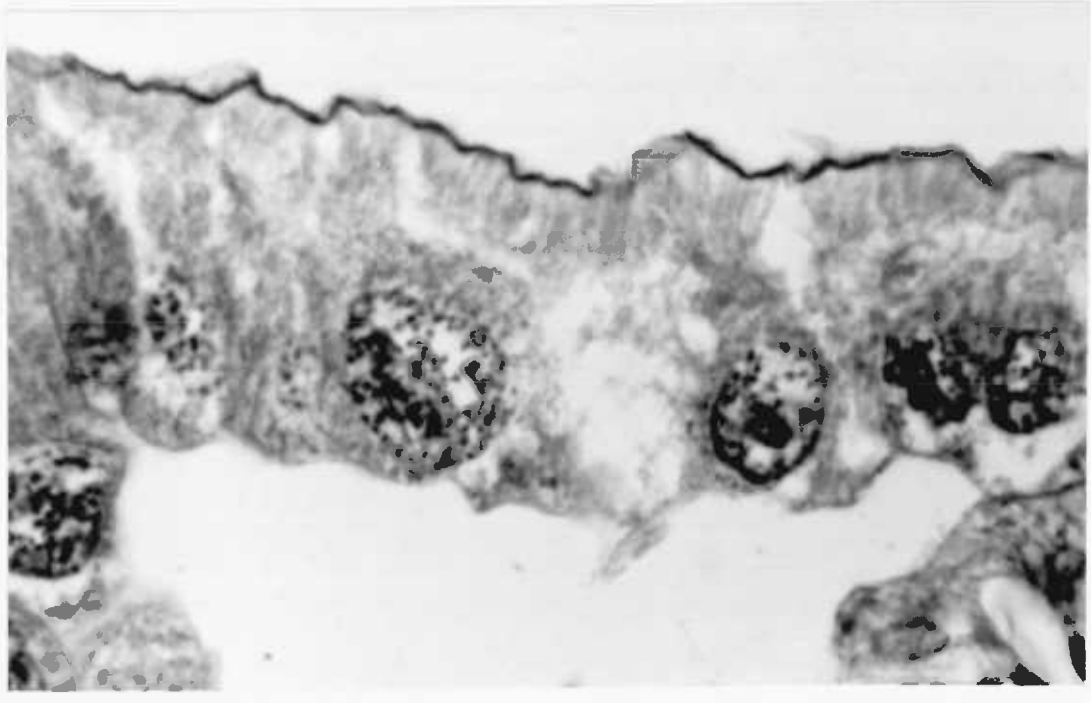


Figure 3b T.S. Rectal papillae (PTAH)

50μm

SECTION 2

HISTOLOGY OF THE MIDGUT (VENTRICULUS)

HISTOLOGY OF THE MIDGUT (VENTRICULUS)

Introduction

The midgut of Hemiptera is involved in a number of interesting and important problems ranging from investigations of the cytological changes which accompany the secretion of digestive enzymes to attempts at utilizing comparative anatomical and histological data in the classification and phylogeny of the order and its constituent suborders Heteroptera and Homoptera. Goodchild (1965) has reviewed our knowledge of the alimentary canal in the Hemiptera emphasising those structures which reflect its evolution and hence the phylogeny of the Hemiptera. He has also proposed some functional interpretations of midgut structure and histology.

Apart from the numerous studies of individual species referred to in the previous chapter, certain comparative accounts and reviews may be mentioned. The early work by Dufour (1833) dealt with the alimentary anatomy of both aquatic and terrestrial Heteroptera and the Auchenorrhynchan group of Homoptera. Licent (1912) working on Homoptera Auchenorrhyncha also provided useful histological details. Glasgow (1914) studied the gastric caeca and caecal bacteria which occur in the Heteroptera and in the course of this work he gave an account of the comparative anatomy of the Heteroptera gut based on a large number of species representing various families. The comparative studies by Elson (1937), Yanai (1952), Yanai and Iga (1956), Parsons (1959), Bahadur (1963) and Goodchild (1963a, 1963b, 1965) have revealed much interesting information relating gut structure to the classification of the suborder.

There are however still some disagreements on matters of fact and some of the earlier works are being supplanted by more recent studies. Since some hypotheses seem to be not entirely convincing and some of the existing data are hardly adequate to support the interpretations placed on them, many of these matters will appear in later discussions in the present work.

The midgut of the Hemiptera consists of columnar cells which under the light microscope bear a striated border along their apical surface, adjacent to the lumen. The epithelium rests on a basement membrane and is invested with circular and longitudinal visceral muscle fibres.

Oesophageal valve The point at which the oesophagus joins the midgut is generally called the oesophageal valve. It has been given various names by different authors such as the cardiac valve, oesophageal invagination, oesophageal lobe, etc. The function of this valve is to prevent the regurgitation of food from the first ventriculus into the oesophagus. The oesophageal valve of several species representing different families of Hemiptera has been investigated by many workers both on an individual and comparative level. Marks (1958) made a comparative study of the fore gut of several aquatic Hemiptera in which he showed that the region of the oesophageal invagination provides several variations among the species which he dealt with.

Externally the junction of the oesophagus and the midgut is not well marked in Oncopeltus fasciatus. A vertical section through the oesophageal valve shows a series of histological structure (Fig. 4a and b). The valve is well developed and clearly defined and appears funnel shaped in longitudinal section. It is composed of a posterior circular fold of the stomodaeal wall which projects into the anterior part of the cavity of the first ventriculus so forming two layers, an inner and an outer layer of stomodaeal epithelium. These two layers are continuous with each other and with the oesophageal wall. The histology of the oesophagus shows an epithelium formed of a single layer of cuboidal cells covered by a thin layer of longitudinal and circular muscle fibres. The oesophageal epithelium is covered internally by a cuticular intima.

At the anterior region of the oesophageal valve the order of muscle layers is reversed, the longitudinal muscles coming to be outside the circular layer. The junction of the stomodaeum and ventriculus is also clearly

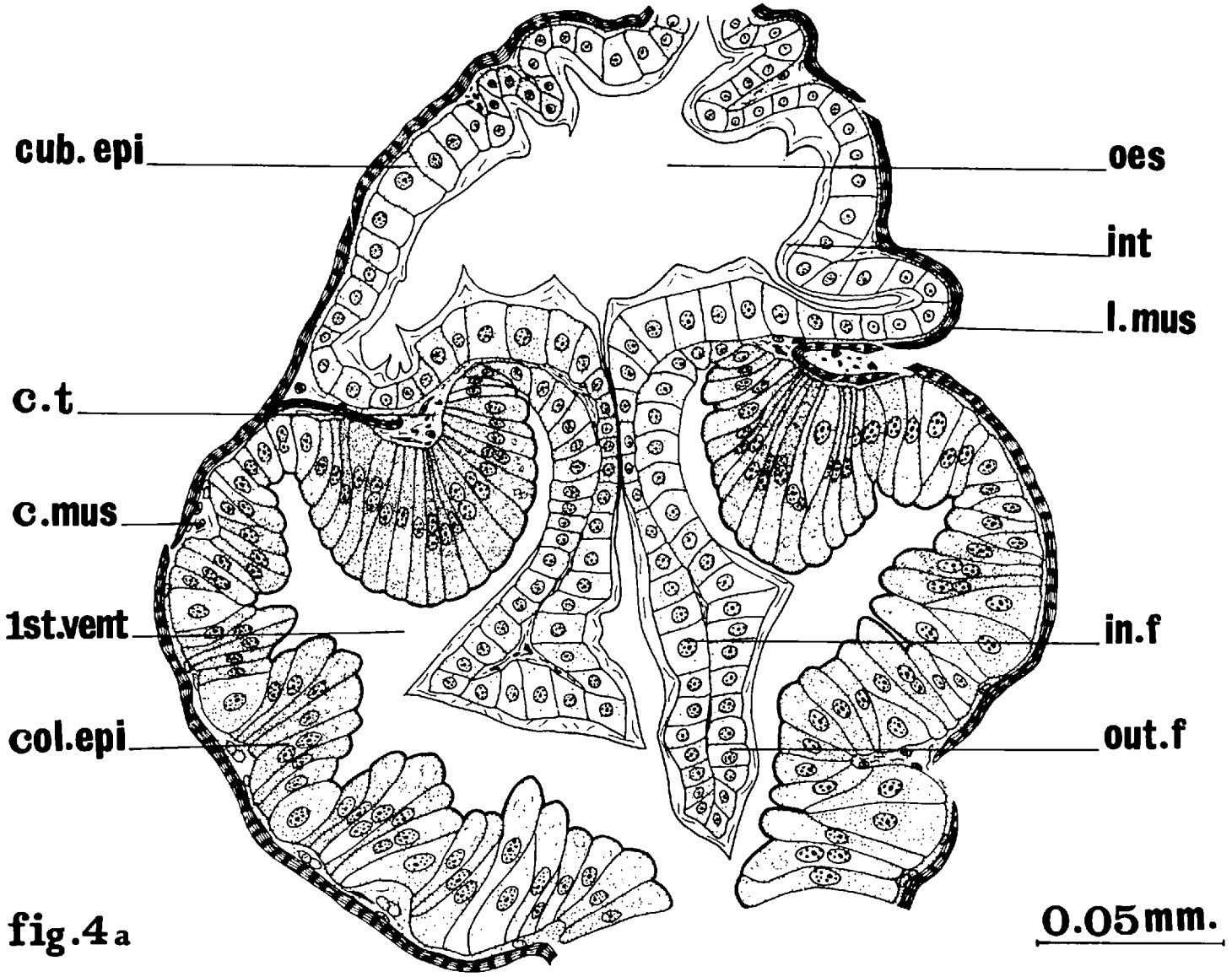


fig.4a

L.S. Oesophageal valve

marked by the abrupt change in shape of the epithelial cells from a cuboidal to a columnar type at the point where the cuticular intima terminates. Longitudinal muscles are well developed in the region of the oesophageal valve and groups of circular muscle fibres are also present. Connective tissue fills the spaces between the muscularis layers. The intima is always adherent to the oesophageal epithelium but does not project far into the midgut. The ventriculus begins at the morphologically posterior end of the outer layer or fold of the oesophageal valve. Because this valve involves a double layer of oesophageal epithelium, however, this point of transition is topographically anterior. The cells forming the epithelium of the ventriculus can be identified by the presence of a striated border. The inner epithelium of the oesophageal valve is similar to that of the rest of the oesophagus though the nuclei stain more deeply. The cells immediately behind the oesophageal valve form a compact columnar epithelium and stain more deeper with haematoxylin and other stains than the rest of the ventricular epithelium. The nuclei are ovoidal and occupy the centre of the cells.

Goodchild (1965) states that the oesophageal valve is poorly developed in seed sucking Heteroptera, but this is not the case in Oncopeltus fasciatus, where the valve appears well defined and developed. The valve is not concerned with the formation of a peritrophic membrane (as in some other orders, e.g. the Diptera) so its main function is apparently to prevent regurgitation of food and to oppose any back-pressure exerted by the distended gut wall (Wigglesworth, 1930).

The complex oesophageal invagination of the Corixidae has been studied by Sutton (1951). In both nymphal and adult stages the epithelium of the oesophagus is invaginated deeply into the ventriculus and the intima lining the oesophagus projects beyond the end of the invagination and extends deep into the lumen of the ventriculus. This membrane has been designated as the entonnoir (= funnel) by Aubertot (1934) to distinguish it from a peritrophic membrane, which it superficially resembles. The anterior portion of the midgut which surrounds the upper part of this invagination has been called

the proventriculus by Sutton and she regards this region as a functionless remnant of the proventriculus of other orders. Her claim and arguments were not supported by Marks (1958).

Parsons (1957b) also recognized an elongated oesophageal valve in species of Corixidae while Mukharji (1961) described poorly developed valves in species of Membracidae and Cercopidae. Intermediate degrees of development have been described by others such as Harris (1938), Bocharova-Mesner (1959) and Goodchild (1963b) in species of Pentatomidae. Evidently Oncopeltus resembles that of other phytophagous Heteroptera, but a further comparative study and a comparison with predacious blood sucking forms would be interesting.

Ventriculus (Midgut)

The oesophagus opens into the midgut by the oesophageal valve described above. The midgut or ventriculus is generally made up of a layer of epithelial cells, usually columnar in form and resting on a thin basement membrane. It is immediately surrounded by circular and longitudinal muscle layers embedded in connective tissue. Nowhere is there evidence of a discrete peritrophic membrane.

Cell types Before dealing with the histological character and the behaviour of the midgut epithelium throughout the different ventriculi in Oncopeltus, it is worth providing short descriptions of the various types of epithelial cells, with their diagramatic drawings. In the present study the epithelium was described by reference to these common cell types, but it must be kept in mind that the definitions cannot be clear cut and that intermediates or atypical forms may be found.

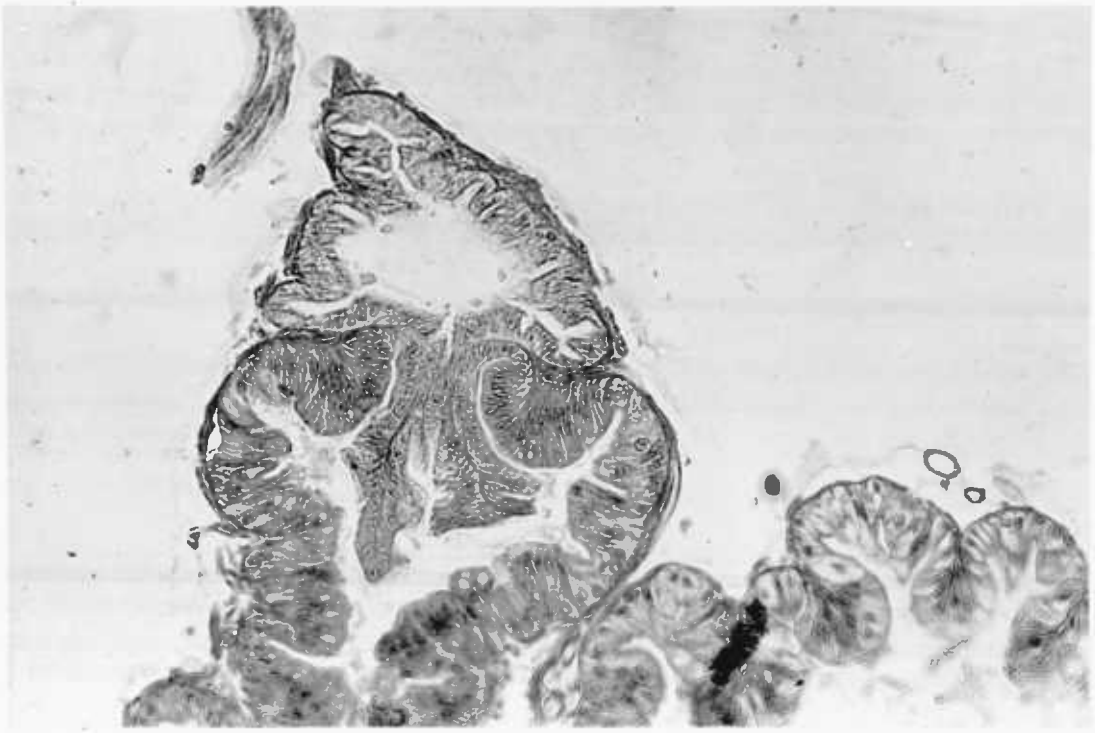


Figure 4b L.S. Oesophageal valve (MCTS)

Figure 5

Types of epithelial cells in the midgut of Oncopeltus fasciatus

a - normal columnar cell

b - extrusion cell

c - bulbous cell

d-f - young cells, various types

g - clavate cells

h - degenerate cell

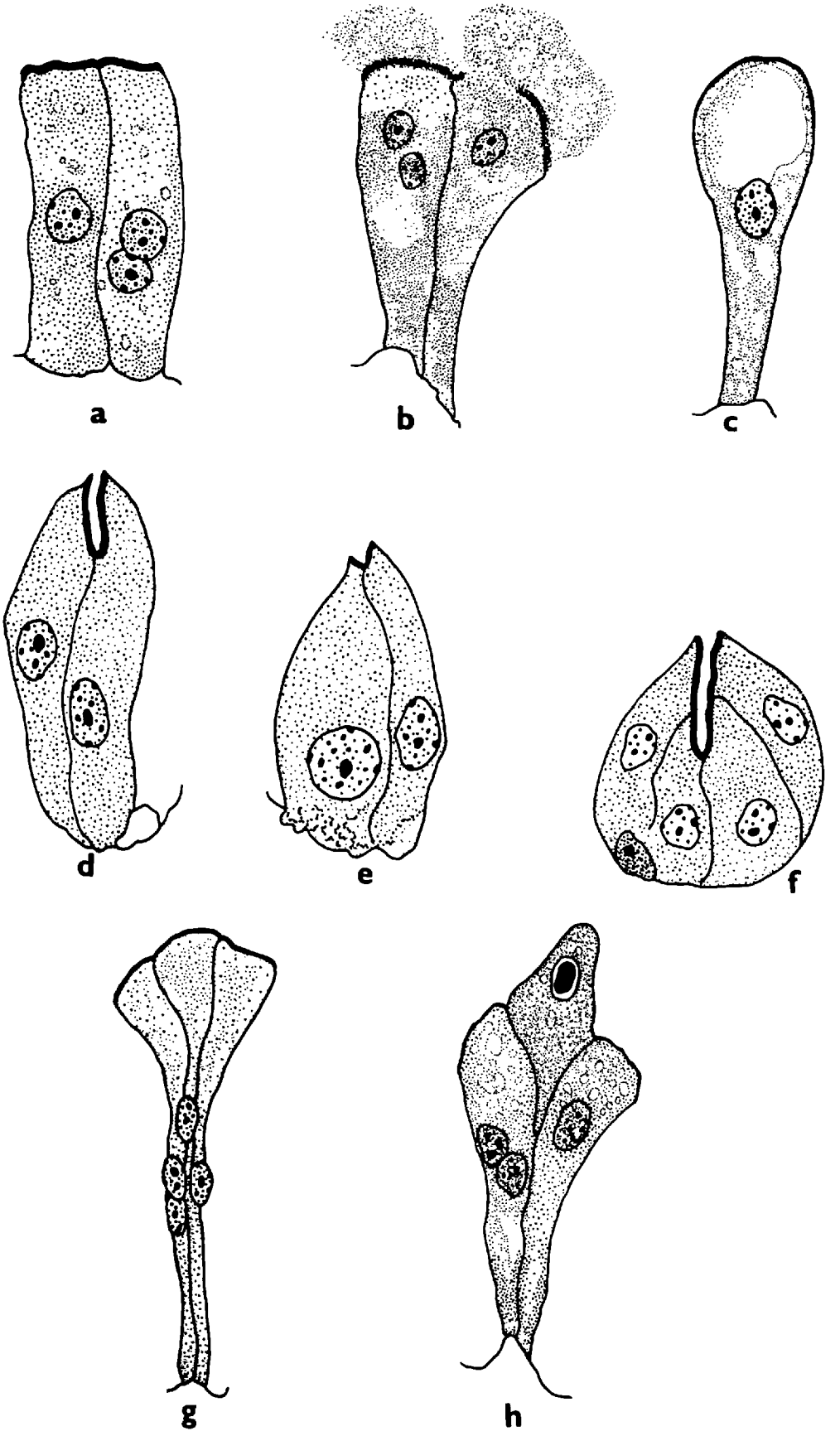


Fig. 5

a. Normal columnar cells These cells are higher than wide and almost rectangular in outline. Much variation in cell height was observed between those which are considered as high or low columnar (Fig. 5a).

g. Clavate cells The cell gradually increases in width distally giving the appearance of a club (Fig. 5g). This type of cell is a characteristic of the third and fifth ventriculus and is found predominating in the epithelium at or near the tips of the villi.

c. Bulbous cells Only the apical end of the cell is suddenly enlarged giving the apical ends of the cells facing the lumen a vesicular or blistered appearance. In such type the cell membrane at its free end bulges out into the lumen as if under pressure from within the cell. Sometimes a faint membrane or a slight constriction may be observed beneath the vesicle or the bulbous apical end separating it from the rest of the cell. The nucleus usually appears below the vesicle or the bulbous end, but in some preparations it occurs in the vesicle. Cells of this type are most frequently found some distance from the regenerative cells at or near the tips of the villi (Fig. 5c).

d-f Young cells These cells appear to be moderately low and are usually located laterally in the villi between the regenerative cells and the apical cells. The apical edges of these cells are often more or less indented or irregular in outline; this is especially the case for those nearest the regenerative cells. The young cells also exhibit some variations in shape, size and inclusions in different areas of the epithelium (Fig. 5d, e, f).

b. Extrusion cells These cells have their apical tips dissolving away. The protruding membrane appears indistinct and incomplete and the fine granular cytoplasmic inclusions of the apical end of the cell merge into the contents of the midgut lumen (Fig. 5b). The rupture of the apical cellular membrane and the subsequent release of the cytoplasmic contents apically may represent histological evidence of apocrine secretion, since most of the cells appear to remain intact despite the breakdown of the apical bulbous end. Such cells are frequently located at or near the tips of the villi.

h. Degenerating cells These cells are found squeezed between other neighbouring epithelial cells. They have intensely staining granular cytoplasm with an increased number of cytoplasmic vacuoles. Their nuclei are often clumped and show irregularity of outline (Fig. 5h). This appearance is frequently observed in the older cells furthest from the regenerative cells. These senescent cells appear to be more or less forced out of the epithelium into the lumen. Their bases first become constricted and then lose their attachment with the epithelium; by this process they are cast off into the lumen. When they are free in the lumen they undergo further degeneration. The disintegrated nuclei may often be seen in the lumen surrounded by very faint cytoplasmic contents or well mixed with the food material. It seems probable that these are secretory cells which, exhausted by their secretory activity, die during or soon after the release of their enzymatic products.

i. Regenerative cells These are very small compared with other epithelial cells. Each has a relatively large nucleus, which stains deeper than the surrounding cells. They are found embedded at the

bases of the epithelial cells, usually at the sides of the villi. Mitoses are frequently observed in the nuclei of these cells but never in the other epithelial cells. It appears therefore that these cells are regenerative centres of the epithelium; the products of their division develop into mature epithelial cells.

The midgut of Oncopeltus fasciatus was divided above into six anatomically distinct regions called here the first, second, third, fourth, fifth and sixth ventriculus. These regions have also been found to differ somewhat histologically, especially in the shape and size of the epithelial cells, the thickness of the striated border and in the muscularis layers.

The following account is based on specimens allowed free access to food and water (well fed). No differences were found in the segmentation and the epithelia of the midgut between males and females of Oncopeltus fasciatus.

First ventriculus

In spite of the fact that both longitudinal and circular muscle bands are comparatively few in this region, it is the only place where peristalis was seen to occur during dissection of living material under saline.

Malouf (1933) states that in Nezara viridula (Pentatomidae) this part is lined by a chitinous intima and he therefore considers it to be a part of the stomodaeum. No trace of intima is present in this section of the alimentary canal of Oncopeltus fasciatus and it is definitely a part of the ventriculus. Malouf is the only one to report such an intima in Hemiptera and he is probably mistaken.

Connective tissue The connective tissue takes a clear bright blue colour with Mallory's connective tissue stain and forms the matrix in which the

circular and longitudinal muscle layers lie embedded. The connective tissue becomes condensed internally round the bases of the epithelial cells where it stains reddish pink with PTAH. The basement membrane takes on a deep blue colour with MCTS. The nuclei of the connective tissue are oval or irregular in outline, stain red with MCTS and have central nucleoli with darker granules distributed centrally and peripherally. The connective tissue cell boundaries are difficult to distinguish among the matrix material.

The muscle layers Generally in this region the longitudinal muscle fibres are reduced and those of the circular muscle layer are scattered. In longitudinal section the fibres of the longitudinal muscle layer appear elongate and take up a red or orange colour with MCTS. This includes the sarcolemma whereas the connective tissue forms a more or less blue stained sheath around each fibre. The muscle fibres have clear cross striations and their width is about 3μm.

The circular muscle layer fibres appear in longitudinal section as single or in groups of two or three. They are relatively few, scattered in the matrix of connective tissue. They are oval in section and sometimes irregular in outline and are slightly punctate in appearance; they are about 3μm in diameter.

Epithelium Generally longitudinal sections through the first ventriculus show columnar epithelial cells resting on a basement membrane. These cells are apparently flexible and the epithelium varies in height according to the distension of the midgut by air and food. The boundaries between the cells are more conspicuous basally but overlap apically due to the gradual enlargement of the cells towards the lumen. The cytoplasm is granular in appearance.

Very small regenerative cells are inserted along the basal portion of the epithelium. These are undifferentiated cells arranged singly or in groups of 2 or 3 and are responsible for the maintenance of the epithelium by

replacing those lost by degeneration during adult life. This is indicated by the presence of cells intermediate in character between normal epithelial cells and small regenerative cells.

Both the columnar cells and the regenerative cells are either uni- or binucleate. Goodchild (1952) reported that all epithelial and regenerative cells are binucleate in mirid species. He stated this after examination of whole mounts since he believed that the large size of the cells renders it difficult to decide from section. It seems here that the binucleate cells are distinct in having their two nuclei in different levels which clearly appear by varying the focus of the microscope and it is evident from sections that in Oncopeltus fasciatus the epithelial cells may be binucleate or uninucleate, though the later predominate.

Detailed examination of the epithelium through the length of the first ventriculus shows a gradual change in shape of the cells, cytoplasmic granules and the presence and distribution of both the regenerative and bulbous cells. The histological character of the epithelium could generally be recognized as falling into three regions.

a. Anterior region

Sections through the tapering anterior end of the first ventriculus behind the oesophageal valve show narrow and short columnar epithelial cells uniformly arranged in the form of a thin pavement epithelium about 50 - 58μm in length. The cells have a thick striated border apically about 1μm in thickness (Fig. 6). The basement membrane in this region is undulated beneath the low villi. Cytoplasm and nuclei take a deeper stain (both with PTAH and MCTS) than in the region posterior to this point. Cytoplasmic vacuoles are found accumulating mainly toward the cell tips where the epithelial cells are slightly swollen. None of the cells was undergoing a process of dilation or discharging material into the lumen.

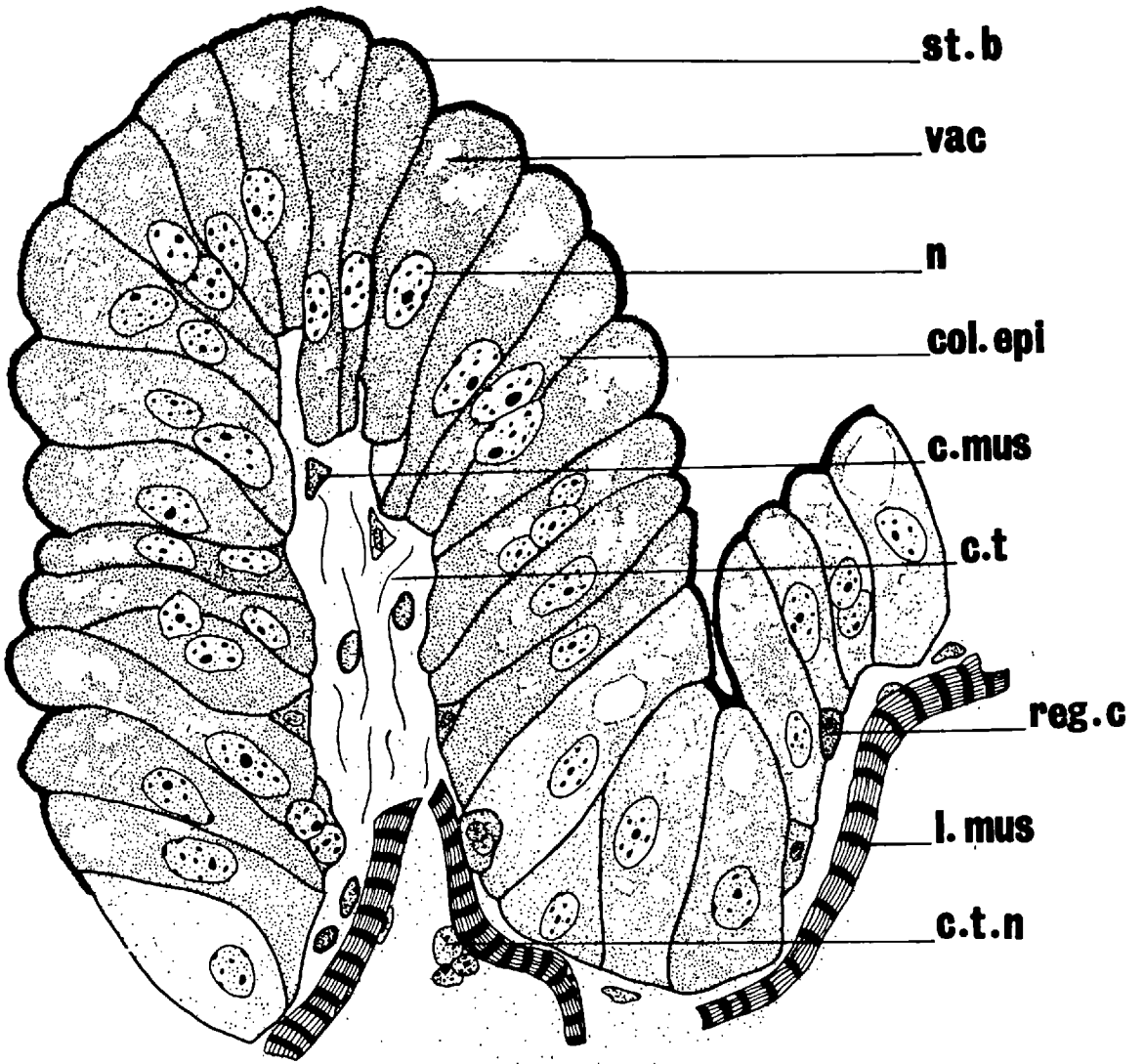


fig.6

L.S. Anterior region of the 1st ventriculus

Regenerative cells are few, scattered between the bases of the epithelial cell and are less numerous when compared with those present in sections taken from the middle region of the first ventriculus.

b. Middle region

The greater part of the first ventriculus especially the middle region shows a layer of columnar epithelial cells with irregular striated borders about 0.5 - 1μ, thick. The presence or absence of this striated border may be connected with the secretion or non-secretion of the digestive enzymes in this region and is associated with the bulbous or swollen cell tips. The cells are crowded together under lateral pressure (Fig. 7). The most conspicuous feature of the epithelial cells here is the presence of large ovoidal or spherical vacuoles sometimes aggregated together to form irregular compound structures. These vacuoles accumulate at the swollen apical region of the cells, but they may occur basally, adjacent to the nucleus or in any other region of the cell. The epithelium seemshistologically to be in its most "active" phase in this region. Food material is present appearing as a coarse granular mass. Some of the cells appear to be undergoing a process of discharging material together with cytoplasm through an indistinct and partially disorganized striated border.

The epithelium appears here to be made up of units or villi partly due to the variation of the cells length and partly associated with the folded nature of the basement membrane. Each unit is formed of the largest cells (about 60 - 65μm in height) in the middle with smaller cells, about 35 - 40μm in height on the either sides. The regenerative cells are embedded between these villi. Cells of all

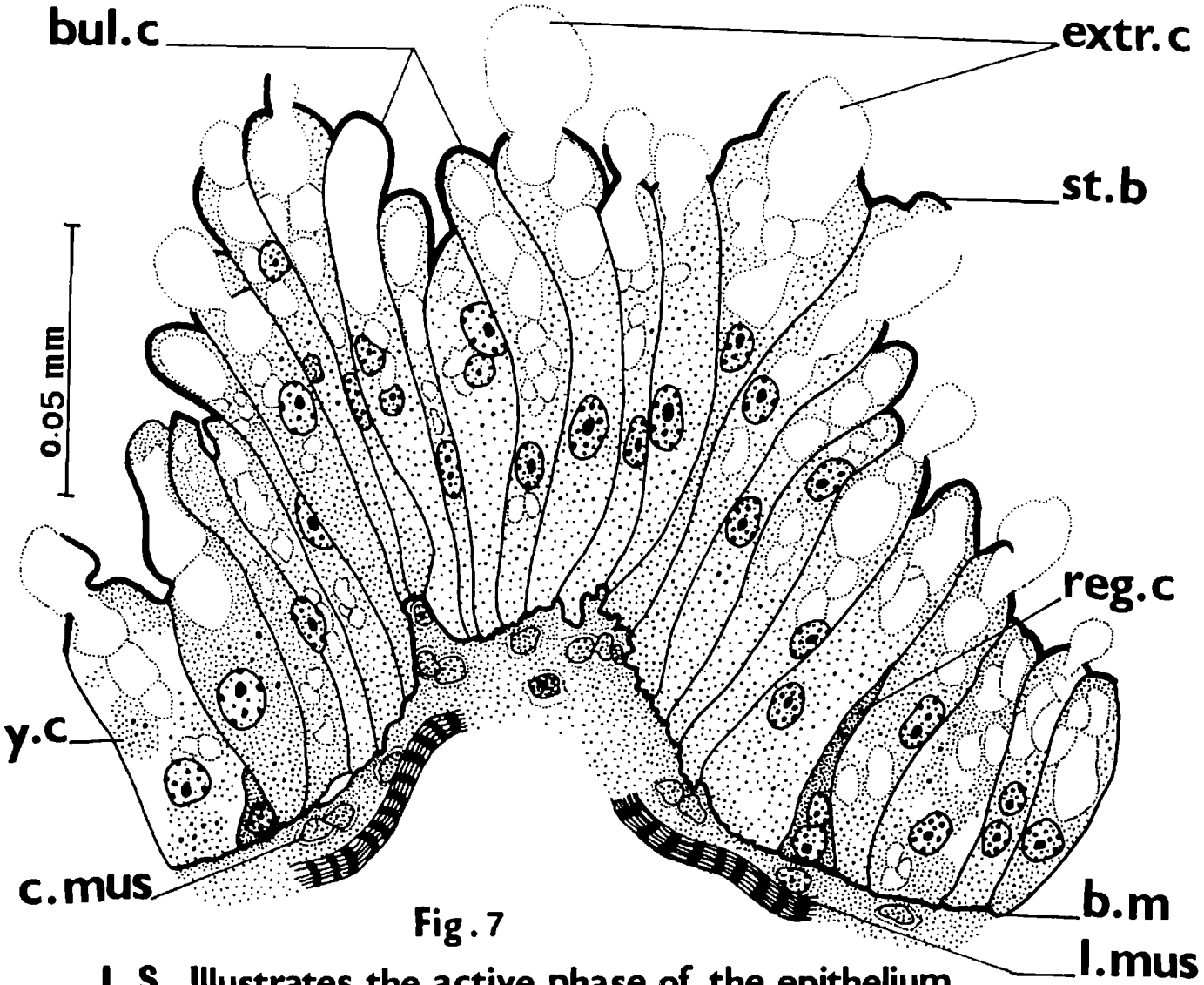


Fig. 7

L.S. Illustrates the active phase of the epithelium in middle part of 1st ventriculus

sizes between small regenerative cells and the largest columnar cells from the centre of the other are present in the epithelium. The folded condition of the basement membrane however is not regular and consequently villi are not formed at equal distances or of equal height. There are, therefore, some lower villi located between the normal high ones (Fig. 7 and 8).

The epithelial cells appear to show gradual histological evidence of secretory activity in each unit. The maximum phase is seen in the largest cells in the centre of the villus where cells are in the process of discharging or have recently discharged to judge by the histological evidence of holocrine secretion.

c. Posterior region

The first ventriculus gradually tends to have taller columnar epithelial cells which are slightly swollen toward their distal ends and are 70 - 75µm in length (Fig. 9). Their nuclei are always located centrally and the cytoplasm is denser basally with few vacuoles accumulated near the apical ends. The basement membrane is less folded than in the middle region of this segment and the villi are correspondingly prominent. No trace of extrusion cells or cytoplasmic extrusion are found in this part. The striated border is continuous and more conspicuous measuring 1.5 - 2µm in thickness.

Young cells are clearly present and easily differentiated by thin structure and shape. They are much shorter than the older cells and measure about 40 - 45µm in height. Their cytoplasm is homogenous and they have centrally located nuclei. Generally the epithelium in the posterior part of this region stains more deeply with MCTS and PTAH than that in the middle area.

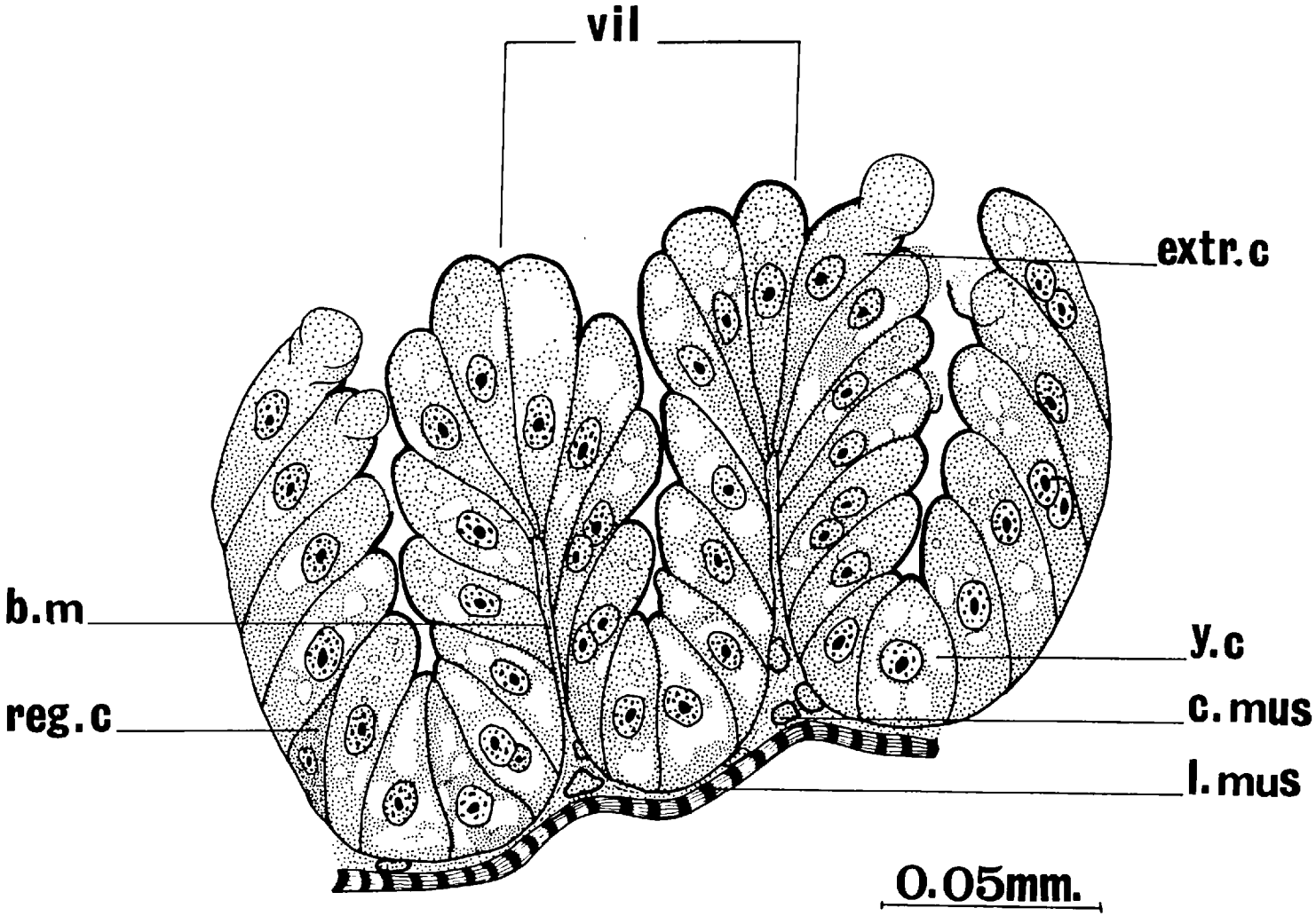


fig.8

L.S. Middle region of the 1st ventriculus

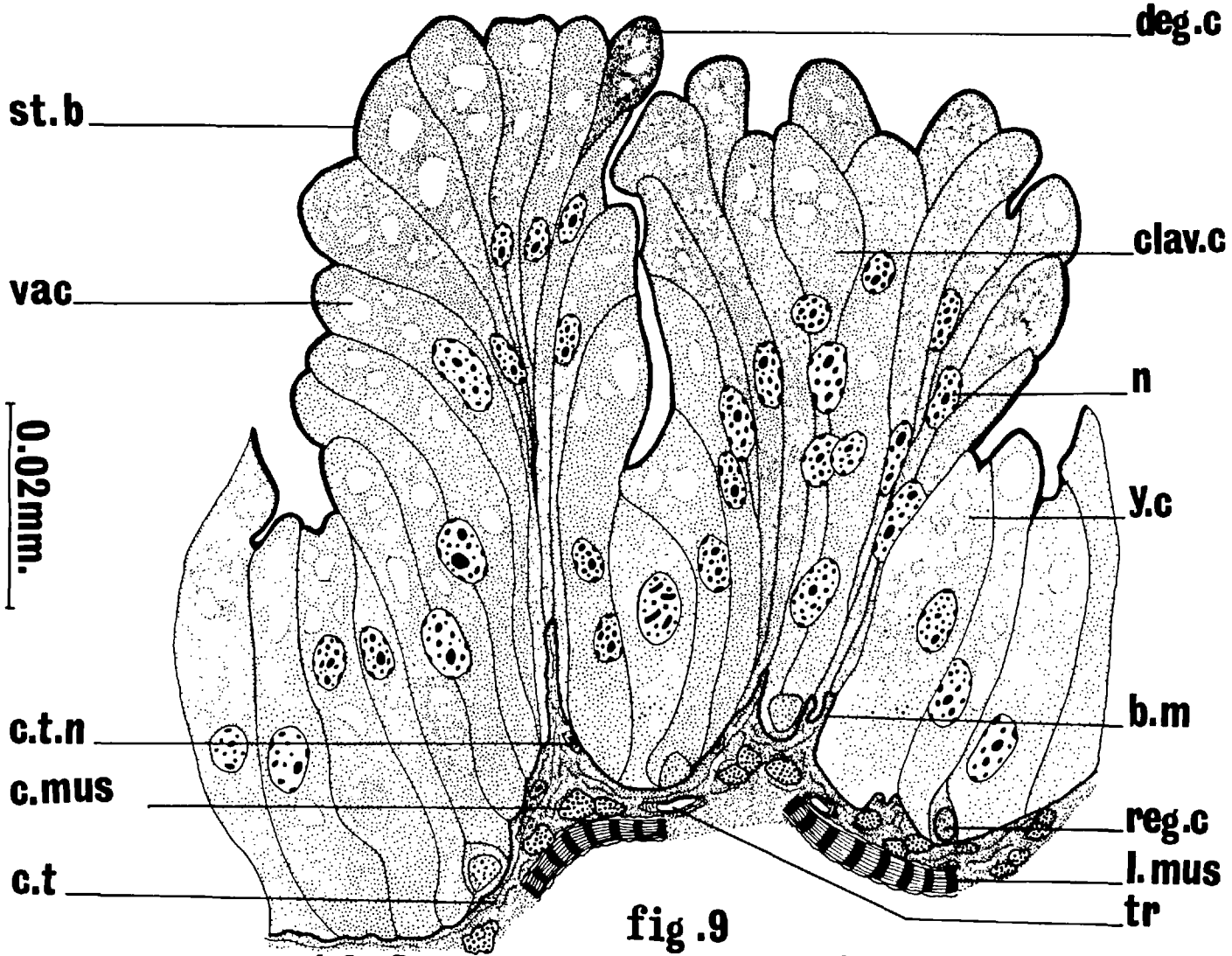


fig .9

L.S. Posterior region of 1st ventriculus near constriction with the 2nd ventriculus

Different stages of mitotic division have been observed to take place in the regenerative cells (Fig. 10). Goodchild (1952) states that no mitotic division of nuclei has been detected in the digestive system and multiplication of cells is presumed to be by amitosis. It seems that this is not the case since mitosis is clearly evident among the regenerative cells in Oncopeltus fasciatus. Goodchild also believed that multiplication of regenerative cells involves first the division of the cytoplasm into two uninucleate cells and then the division of the nuclei. This interpretation cannot be accepted since the regenerative cells are always uninucleate except when they are showing different stages of a normal mitosis.

Breakey (1936) states that no evidence of secretion was found in the first stomach of Anasa tristis and that the function of the first stomach is that of storage and absorption ;possibly his sections were taken more anteriorly in the first ventriculus so that the secretory region of the epithelium was not represented.

Ventricular constriction The first ventriculus passes into the second ventriculus by a distinctly constricted region with a very narrow lumen (Fig. 11). The epithelium of this constriction is made up of compact columnar cells that stain deeply. The connective tissue is thicker at each end of the constriction, with a well developed circular muscle layer.

Second ventriculus

This is a convoluted tubular region forming the major portion of the alimentary canal. Its wall is not as wrinkled as that of the enlarged first ventriculus. Longitudinal sections show the usual histological elements. The outermost longitudinal muscle layer is followed by the inner circular muscle fibres and basement membrane with the epithelium bounding the relatively wide lumen, containing food material mixed with microorganisms

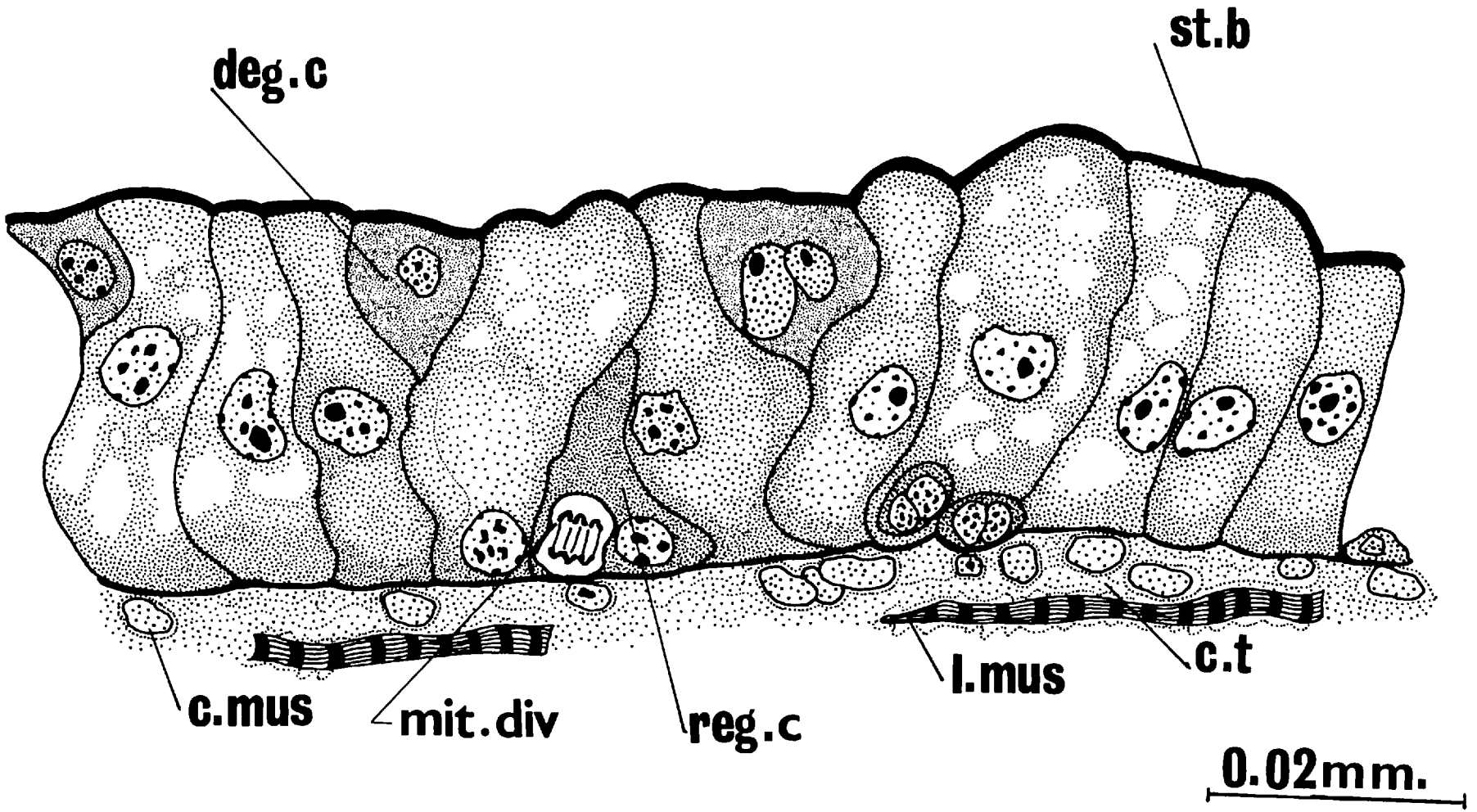


fig .10

L.S. Through 1st ventriculus showing stages of mitotic division

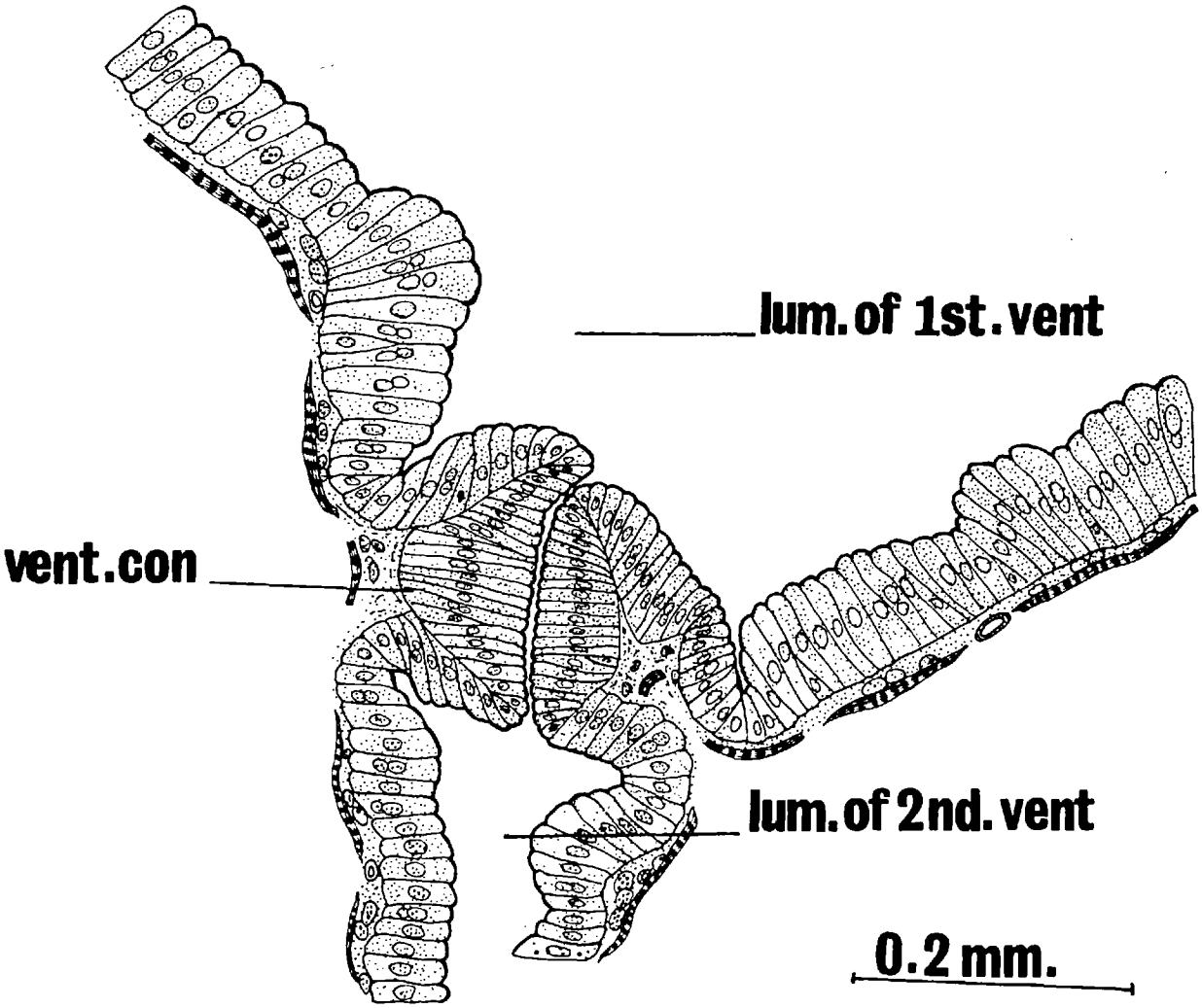


fig .11a

Constriction between 1st and 2nd ventriculus

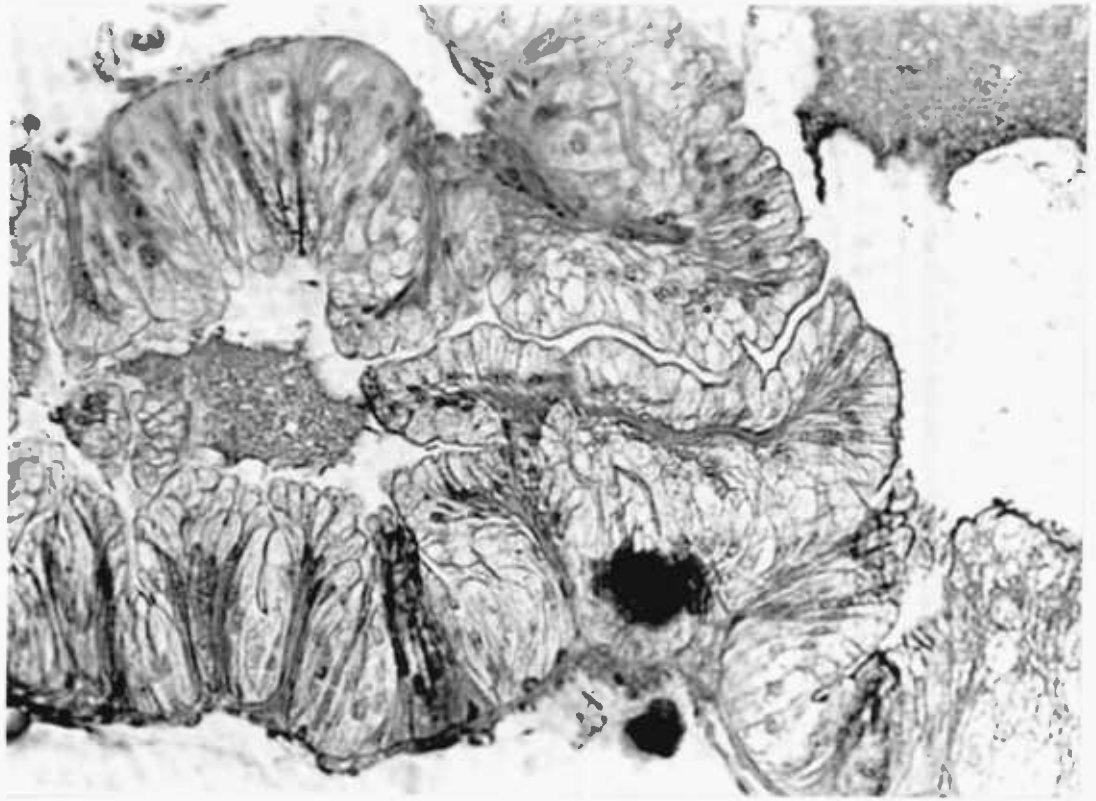


Figure 11b Constriction between 1st. and 2nd. ventriculus (MCTS)

50µm

in the normally fed specimens. The food mass appears to consist of very fine particles, presumably a fixation artefact.

Longitudinal muscle layer The longitudinal muscle layer is poorly developed and composed of delicate strands of longitudinal fibres with sarcolemma; it takes on an orange colour with MCTS and is bounded by the matrix sheath which stains deep blue around the muscle fibres; each fibre shows several cross striations within its cytoplasm. The longitudinal and circular muscle fibres may be found close to each other or widely separated depending on the extent to which the wall is stretched locally. The muscle nuclei are elliptical in outline and contain dark staining granules. They measure 4µm in length.

Circular muscle The muscle layer is relatively well developed. The circular muscle fibres stain deep red with MCTS and are found in the matrix singly or in groups of 2, 3 or even 4.

Epithelium The epithelium is generally columnar in form (Fig. 12). The basement membrane stains a bright blue colour with MCTS forming alternating straight and undulating portions and so reflecting the formation of the epithelial villi. Epithelial cells have an apical striated border about 1.5 - 2µm in thickness. No quite clear extrusion cells were found among the epithelium; large vacuoles are found, usually towards the cell apex, but these are fewer than in the first ventriculus and they are sometimes irregularly distributed or found very close to the nucleus.

As in the parts of the first ventriculus the formation of the villi leads to some epithelial cells appearing tall and narrow at the apex of each villus and shorter at the sides. Cytoplasmic granules are mainly distributed apically and take on a dark colour with PTAH; some are at the bases of the cells or around the vacuoles. Nuclei stain red in MCTS and

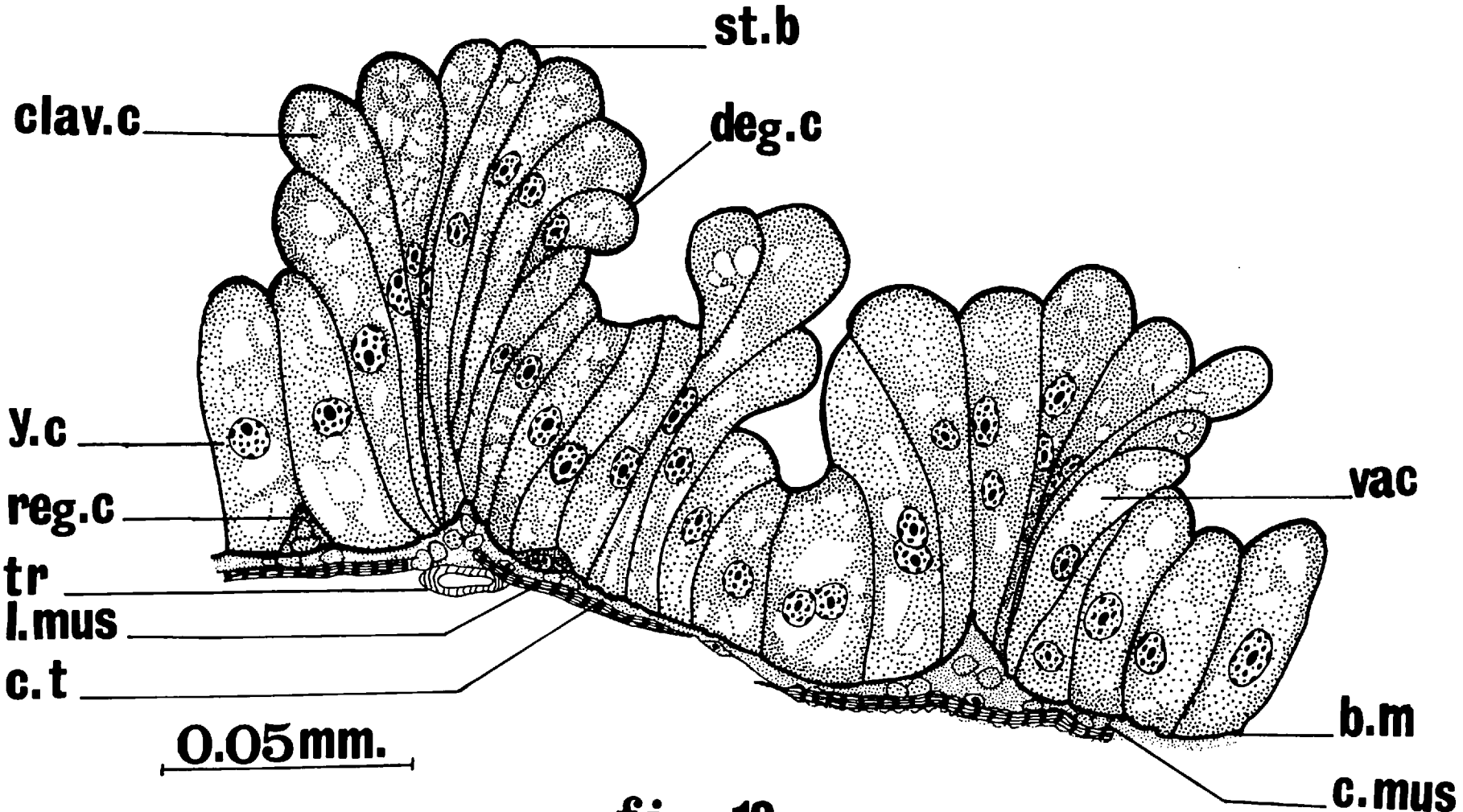


fig . 12
L.S. 2nd ventriculus

centre of the cells or sometimes occur basally. They are ovoidal in shape or more spherical with a diameter of 3µm. The nucleoli and chromatin masses are clear.

The regenerative cells or nuclei are found singly or in groups of 2 or 3 between the bases of the epithelial cells which are located at the sides of the villi. The regenerative cells are uni- or binucleate and show different stages of mitosis. Posteriorly the second ventriculus forms a constriction that leads into the third ventriculus (Fig. 13).

Third ventriculus

This segment is characterised morphologically and histologically for the first time in the present work. It was not recognized by Hood (1937) in Oncopeltus fasciatus. It is much shorter and narrower than the preceding ventriculus. The lumen is narrow and filled with food material which appears in the form of fine granules of which some stain violet blue with MCTS while the finer granules take a red colour.

In longitudinal section the external peritoneal sheath is clearly defined, staining very light blue with MCTS; it is penetrated by tracheoles which extend into and are distributed throughout the matrix of the connective tissue. Outside the epithelium lie the circular and longitudinal muscle layers with thin fibres embedded in the connective tissue.

Connective tissue The matrix sheath stains light blue with MCTS and becomes denser near the folded parts of the basement membrane. There are several nuclei distributed in the matrix. The basement membrane stains deep blue with MCTS and a pinkish colour with PTAH. It appears undulating in nature and in some regions is quite separated from the bases of the epithelial cells, though this space may be an artifact. There are fine connective fibres running in the matrix and staining bright blue with MCTS.

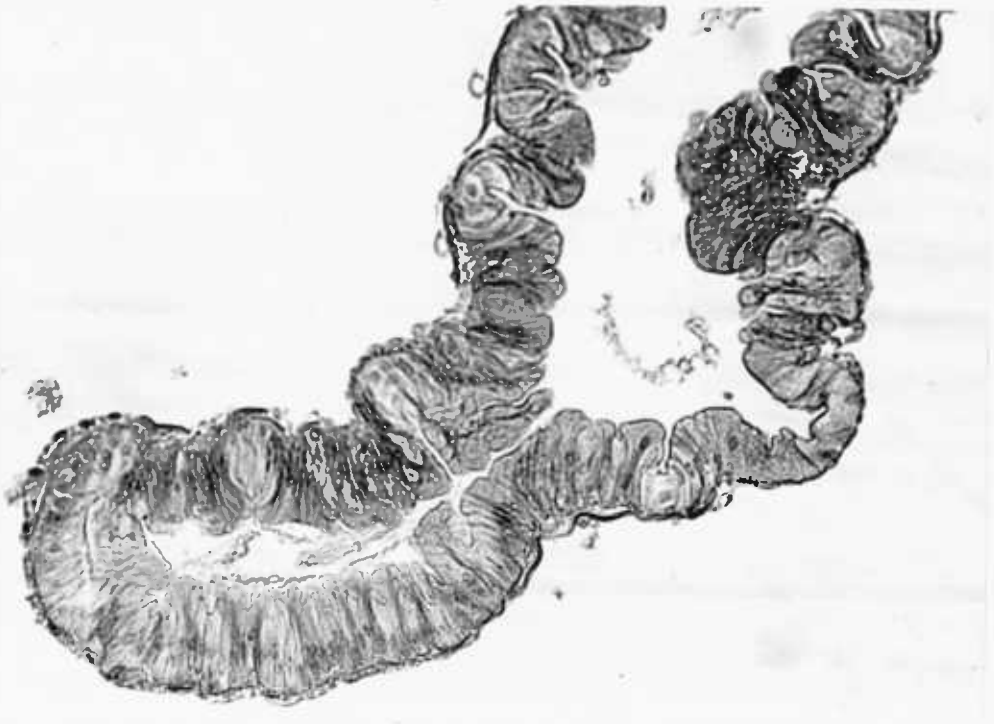


Figure 13 L.S. Constriction between 2nd. and 3rd. ventriculus (PTAH)

25µm

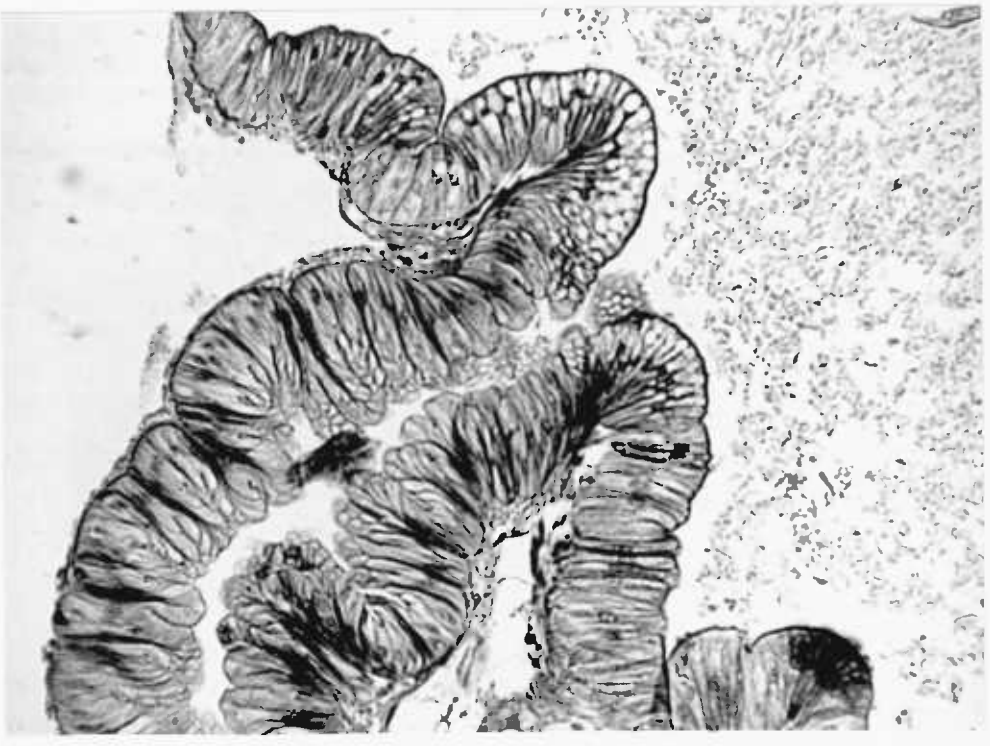


Figure 15 L.S. Constriction between 3rd. and 4th. ventriculus (MCTS)

Longitudinal muscle layer This is composed of separate longitudinal muscle fibres staining red and having an orange sarcolemma in MCTS preparations The fibres are slender and delicate, surrounded by a bright blue sheath of matrix material. The fibres sometimes run close to the basement membrane and do not form a continuous layer. The cross striations are clearly visible.

Circular muscle layer In accord with the narrow diameter of this region the muscle layers are slender and delicate. The circular fibres are situated internal to the longitudinal fibres and are also thin, being 2.5μm in diameter and scattered singly in the matrix.

Epithelium The epithelial cells of this short region are of two types (Fig. 14). Firstly there are tall columnar cells with a slender body extended apically into a bulbous end giving the cell a clavate form apparently due to the lateral pressure of the adjacent cells. Cells of the second type are shorter and normally shaped and are thought to be young cells, because of their position lateral to the regenerative nidi. The length of the cells of the first type is 70μm while those of the second type are about 50μm long. The tall clavate epithelial cells are arranged in groups separated by the normal shorter type of cells. The striated border is very thin appearing as a delicate blue margin bordering the lumen.

The cytoplasm of the epithelial cells stains more deeply for the basal three quarters becoming lighter apically. Generally there are no vacuoles though a very few small ones may be found in the light-staining apical portion of the cytoplasm.

There are two types of granules found in the cytoplasm,fine and coarse granules as well as cytoplasmic fibrillae. These organelles become more scattered and fewer in the apical portion of the cells. The coarse granules stain black in PTAH and are found scattered randomly in the cytoplasm. The

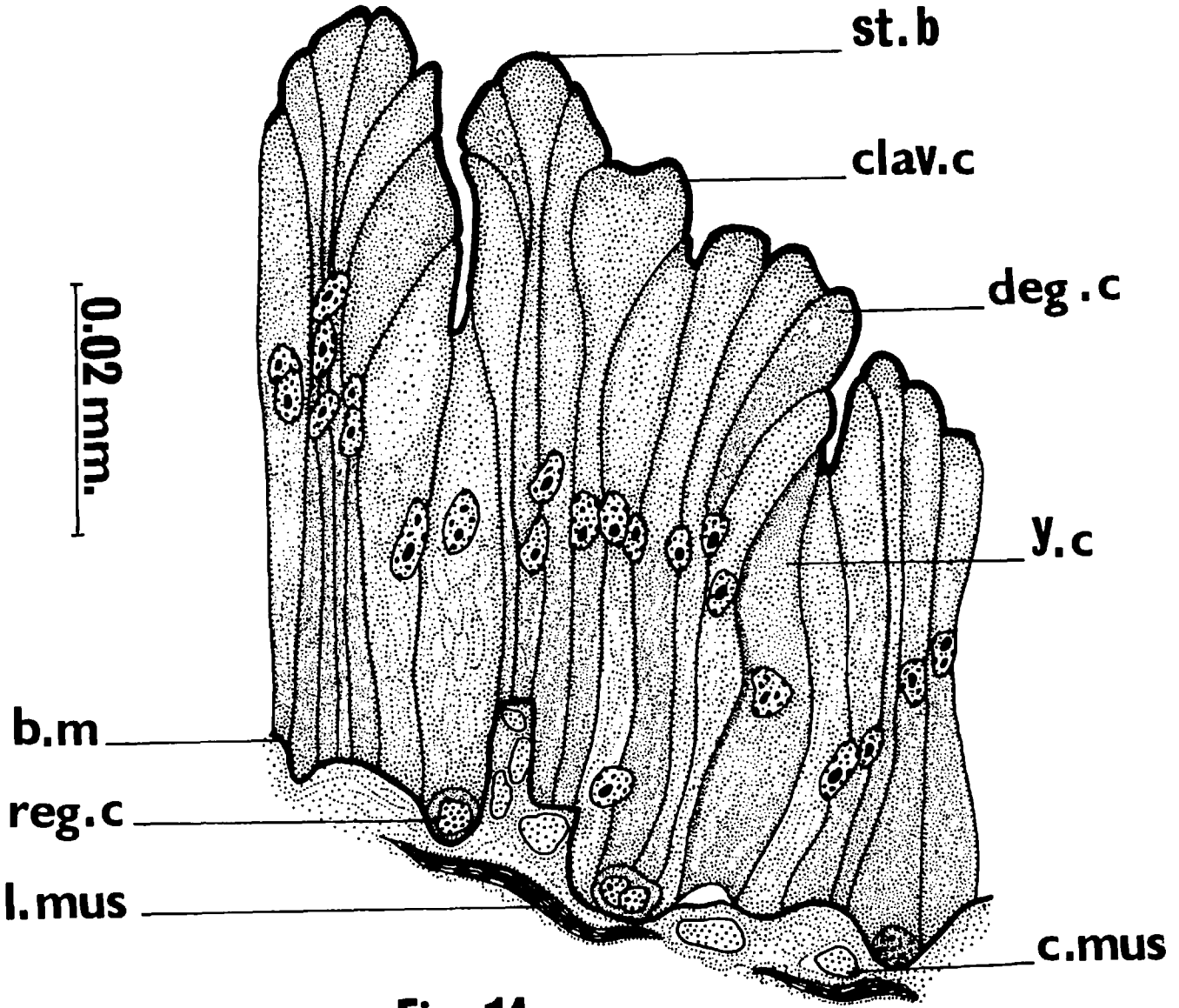


Fig .14
L.S. 3rd ventriculus

nuclei of the epithelial cells take on a red colour with MCTS and occupy the central part of the cells though some are found a little toward the base. They are oval or elliptical in outline, 10μm long and 3μm wide with the nuclei and chromatin mass is well defined.

The regenerative cells take on a deep red colour with clear chromatin granules. They are found singly or in groups of two or very rarely of three. These regenerative cells are embedded between the bases of the epithelial cells in spaces formed by the foldings of the basement membrane and have a diameter of 2μm.

There is a well developed constriction between the third and fourth ventriculi formed of columnar cells bordering a very narrow lumen. The wall of this constriction is slightly invaginated to form a valve-like structure (Fig. 15).

Fourth ventriculus

This region is shorter than the first ventriculus and wider than the preceding second and third ventriculi. Its lumen is relatively broad and it is full of food material mixed with microorganisms, which are very evident here. Occasionally there is a large mass of food material formed of homogenous granules which take on a violet-purple colour with MCTS. The wall of the fourth ventriculus is smooth externally, with a distinct constriction separating it from the preceding ventriculus.

Externally the epithelium is bounded by the circular and longitudinal muscle fibres in the usual manner with both types of muscle elements embedded in the connective tissue matrix.

Connective tissue The matrix sheath stains light blue with MCTS and black with PTAH. Fine tracheae are found running in the matrix. Connective tissue cells are also present. The basement membrane is folded, thus accompanying

the epithelium which is arranged in cell groups or villi. It takes a deep blue colour with MCTS and purple colour with PTAH.

Longitudinal muscle layer The longitudinal muscle fibres take on a red colour with MCTS and its wavy sarcolemma stains an orange colour. Cross striations in the cytoplasm of each fibre are clearly visible. The longitudinal muscle layer is formed of separate fibres which appear not to be continuous, having a diameter of 3μm. Each fibre is surrounded by a delicate sheath of matrix that stains deep blue with MCTS.

Circular muscle layer The circular muscle layer is very poorly developed in this region. The fibres are small with a diameter of 2μm and are usually found in contact with the basement membrane externally. They are always scattered singly.

Epithelium Generally the epithelium rests on the basement membrane though in some places it seems as to be separated or lifted from it. This is presumably an artefact, though it may reflect the existence of an intercellular space as is reported in other accounts of the ultrastructure of the midgut. Associated with the folded nature of the basement membrane, the epithelium falls into groups or villi (Fig. 16a). These villi are more prominent when the ventricular wall is not distended by the presence of much food material. The cells of the villi are longer at the anterior and posterior ends of this segment. When the food mass is extensive and the wall is more distended the villi in the middle part of this region disappear (Fig. 16b) though they remain distinct at each end. The length of the epithelial cells also seems to be different, those located at the ends of the ventriculus are up to 50μm long, while those in the middle are 35μm long when the wall is distended by food material. The cytoplasm has coarse and fine granules with cytoplasmic fibrillae. These elements are not evenly distributed and are more abundant apically in the cytoplasm. The staining reaction of the cytoplasm is light basally

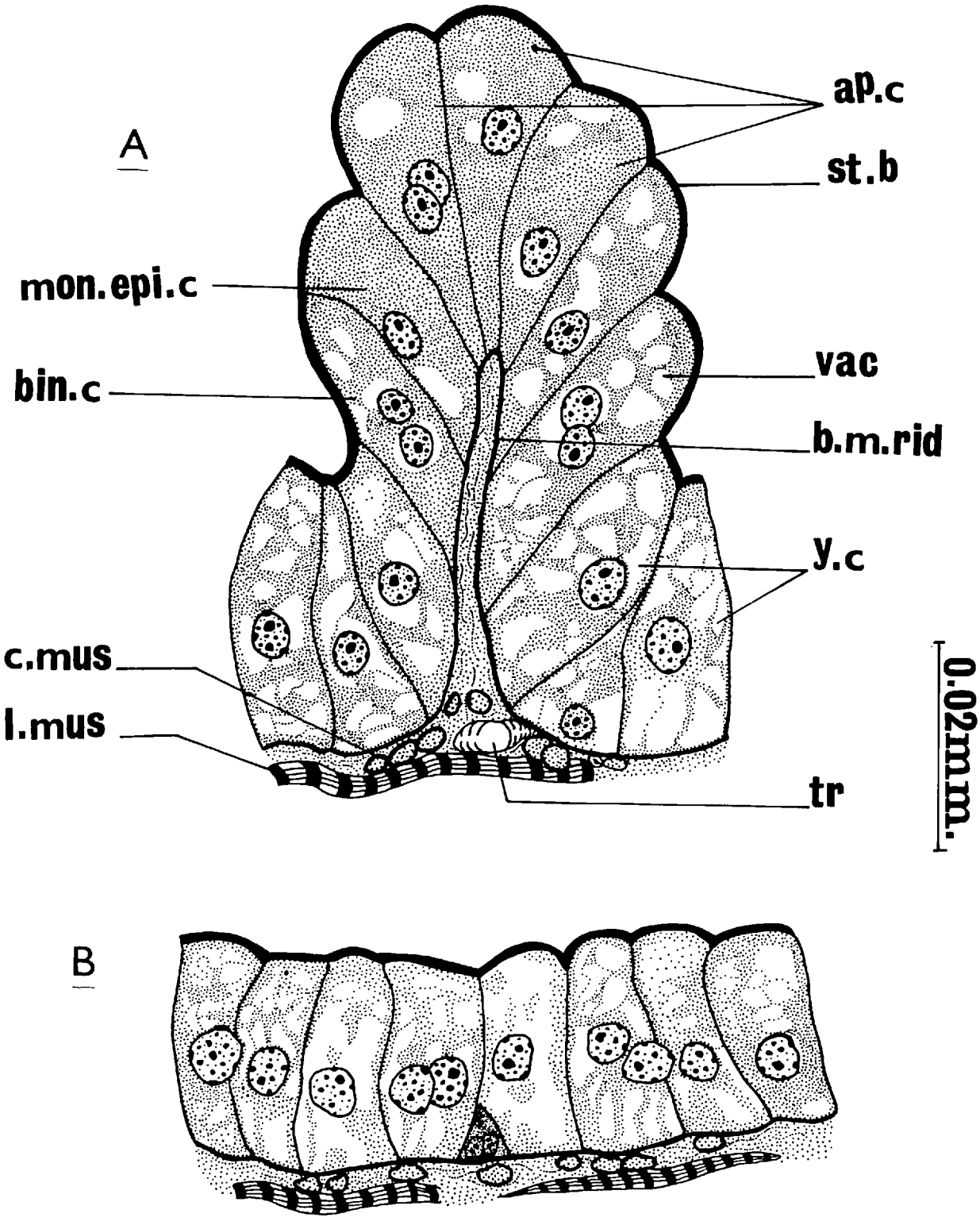


fig . 16
L.S. 4th ventriculus

and around the nucleus at the middle of the cell. Irregularly distributed small vacuoles are present but are not well differentiated from the rest of the cytoplasm, though some larger ones are well defined. The coarse spheroidal granules are usually more abundant in the apical portion of the cell. The nuclei stain black with PTAH and red with MCTS. The nuclear chromatin and nucleoli are clearly visible. The nuclei are oval or spherical in shape and 6µm in diameter. They are centrally located a little more toward the apex and very few are found basally.

The regenerative cells or nuclei are small in comparison with the epithelial nuclei and measure 3 - 4µm in diameter. They stain deep pinkish with PTAH and deep red with MCTS. They are found inserted near the bases of the epithelial cells, singly or in groups of 2 and in contact with the basement membrane. Some of them are found undergoing mitotic division.

The fourth ventriculus also joins the fifth ventriculus by a constriction similar to that at its anterior end.

Fifth ventriculus

This segment is smaller than the fourth. Its wall is smooth externally and devoid of gastric caeca such as are often present in some other Heteroptera. The lumen is more or less irregularly star-shaped in cross section. The muscle layers are poorly developed here. Microorganisms are commonly found mixed with food material as in the preceding segment. The usual arrangement of connective tissue muscle layers and epithelium is found as in other parts of the midgut.

Connective tissue The connective tissue sheat envelopes both muscle layers which are scattered in the matrix along with connective tissue fibres.

The basement membrane is not as thick as it is in the fourth ventriculus. It stains bright blue with MCTS and appears folded at approximately equal intervals, forming ridges corresponding to the villi.

Longitudinal muscle layer Longitudinal muscle fibres are developed in similar degree to those of the fourth ventriculus; they are formed of a single fibre but the cross striation here is not clearly evident.

Circular muscle layer The circular muscle layer fibres are more reduced here forming a thin layer which contrasts with that of the preceding segments. The fibres are scattered singly in longitudinal section and lie adjacent to the basement membrane.

Epithelium The epithelial cells are tall and columnar (Fig. 17) but are not of uniform height. They tend to form groups or villi and in each group the tallest cell is located centrally with those on either sides of it gradually decreasing in height. The tallest cells in the centre of each villus are about 70μm in height and 7μm in width, while the cells at the sides are about 40μm high and 9μm wide. The tall cells have bulbous apical ends, perhaps due to lateral pressure and, therefore, appear clavate in shape like those in the third ventriculus. A striated border is well developed. The cytoplasm is granular, more dense in texture basally and lighter apically. The apical bulbous ends of diametrically opposite cells were found to be in contact across the lumen and because of this the cross section of the lumen appears irregularly star-shaped as mentioned above.

The nuclei of the epithelial cells are ellipsoidal or even spherical and are situated centrally or are a little displaced toward the base or the apex. Their nucleoli and chromatin masses are very well differentiated. The nuclei measure 7μm by 4μm.

The regenerative cells are found singly or in groups of 2 and sometimes 3 at the bases of the epithelial cells. They are spheroidal or irregular in shape with the nuclei and the chromatin mass clearly differentiated. Some of the regenerative cells were found undergoing mitotic activity.

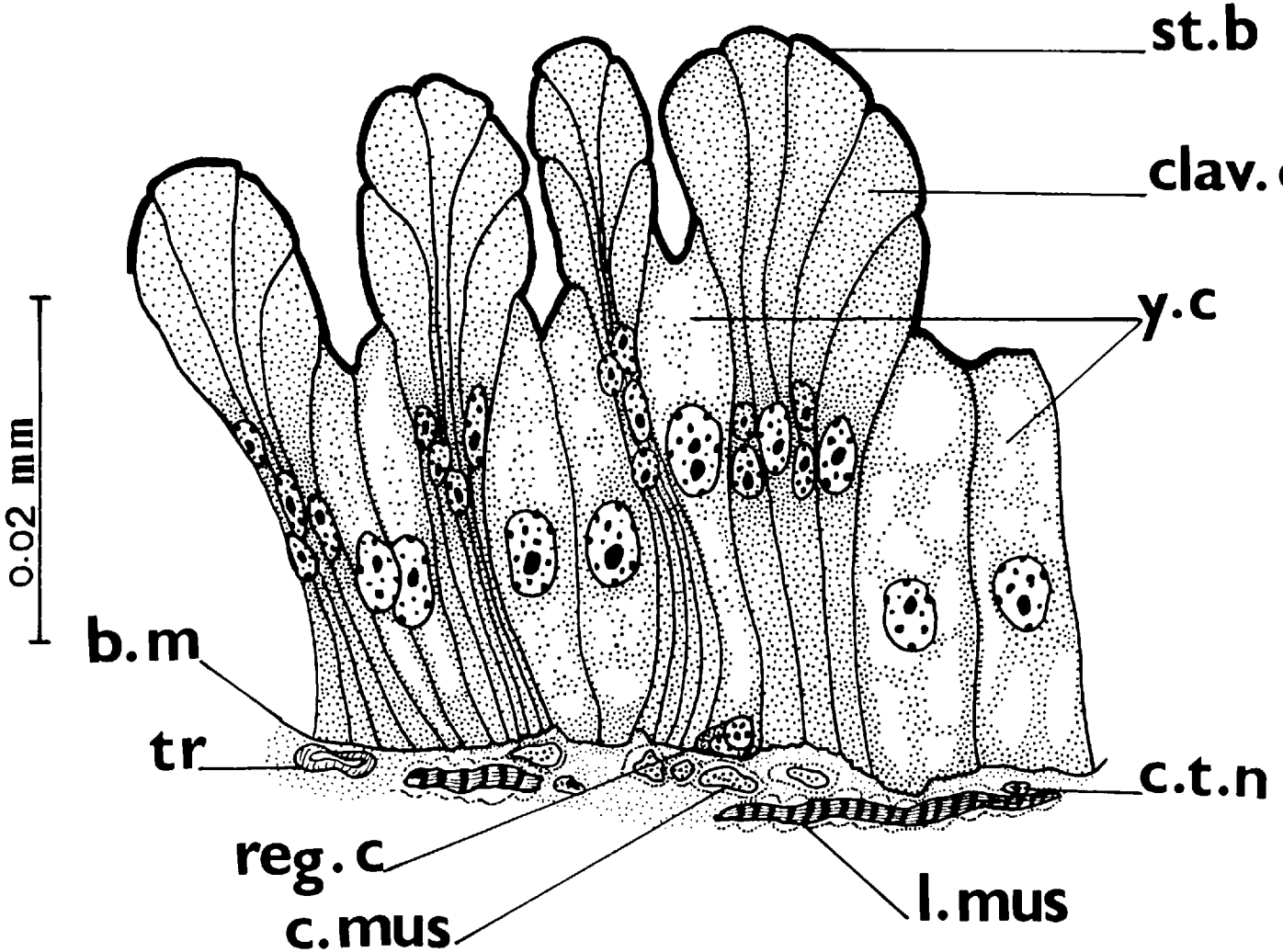


Fig.17
L.S. 5th ventriculus

Sixth ventriculus

The junction between mid and hindgut in Heteroptera has been of great interest to many workers. There has been much confusion on the exact limits of the boundary and the probable germ layer from which the structures of this region arise. The insertion of the Malpighian tubules at this region makes the situation more confusing. Hood (1937) while studying the anatomy of the digestive system of Oncopeltus fasciatus called this region the pylorus and thought the Malpighian tubules were probably part of the proctodaeum as they entered the pylorus. Breakey (1936) in Anasa tristis found that the cells of the Malpighian tubules had a striated border and stated that they and the lobes of the pylorus into which they open had a common origin. This case was also reported by Harris (1938) in Murgantia histrionica and by Srivastava and Bahadur (1961) in Dysdercus koenigii.

Hood (1937) could not decide on histological grounds whether the pylorus should be considered as part of the midgut or the proctodaeum.

Woolley (1949) also noted the pylorus and called it the ileum; he noted the similarity in histology of the ileum and midgut epithelium but for no apparent reason he then treated the ileum as part of the proctodaeum. Rastogi (1962) studying the status of the ileum in the Heteroptera recognized that it is histologically similar to the midgut in five phytophagous Gymnocerata, whereas in predaceous forms it appeared to be a proctodaeal structure; he concluded that the ileum of phytophagous Heteroptera is truly endodermal or at least a continuous of the midgut.

Goodchild (1963b) made a comparative study of a large number of Gymnocerate Hemiptera and provided much information designed to clarify this problem. He recognized the presence of an ileum which is histologically similar to the midgut in the pentatomomorpha. This he then subdivided into four types characteristic of the lygaeids, coreids, Pentatomids and pyrrhocorids.

A careful and detailed study of the histology of the midgut of Oncopeltus fasciatus carried out here shows that neither of the two terms applied to this region of the gut (pylorus and ileum) is really satisfactory and justified since in insects both terms should correctly be applied to the most anterior part of the proctodaeum which, by the definitions of Snodgrass (1935), is lined with a cuticular intima.

In the present study this structure, which had been called the pylorus by Hood (1937) in Oncopeltus fasciatus is treated as a part of the midgut and is referred to as the sixth ventriculus. It represents the most posterior region of the insect midgut and this interpretation is based on the following histological grounds :

1. The epithelial cells of this region are composed of columnar cells 70µm in length, with dense cytoplasm and prominent nuclei similar to the more anterior midgut epithelium (Fig. 18).

2. The ileo-rectal valve which has been referred to by many workers such as Goodchild (1963b) is here called the ventriculo-rectal valve and is situated posterior to the sixth ventriculus (Fig. 19). This valve is the most anterior part of the hind gut in which a cuticular intima can be detected.

3. The proctodaeum is characterized by the presence of an intima lining but such an intima is absent from the sixth ventriculus.

4. The sixth ventriculus is interposed between the fifth ventriculus of the midgut and the rectum in Oncopeltus fasciatus. It is separated from the former by the usual ventricular constriction as occurs between any other two segments of the midgut (Fig. 20). This constriction is formed of long columnar epithelium reducing the lumen to a narrow tube. No more elaborate valve or valve-like

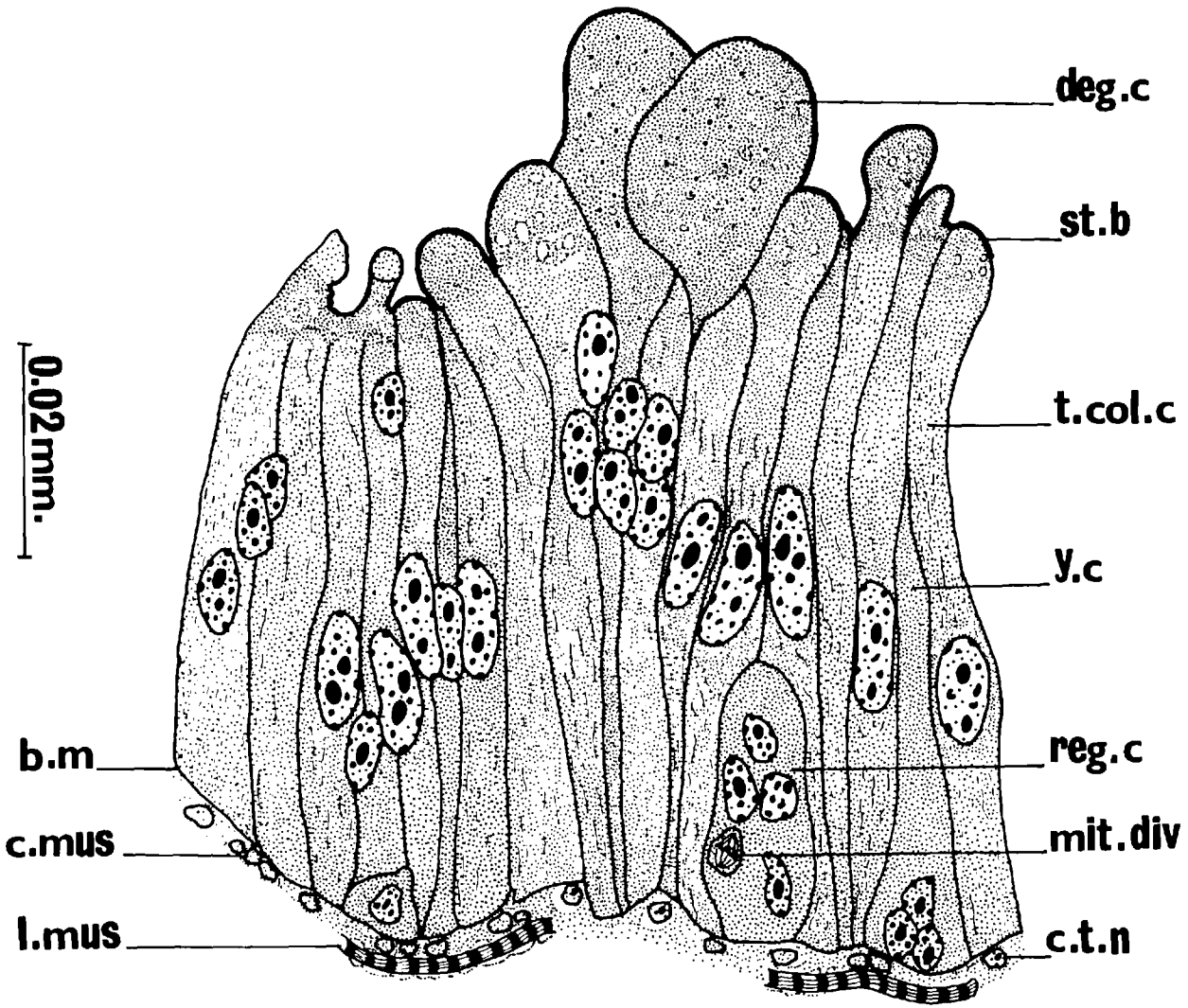


fig. 18a
L.S. 6th ventriculus

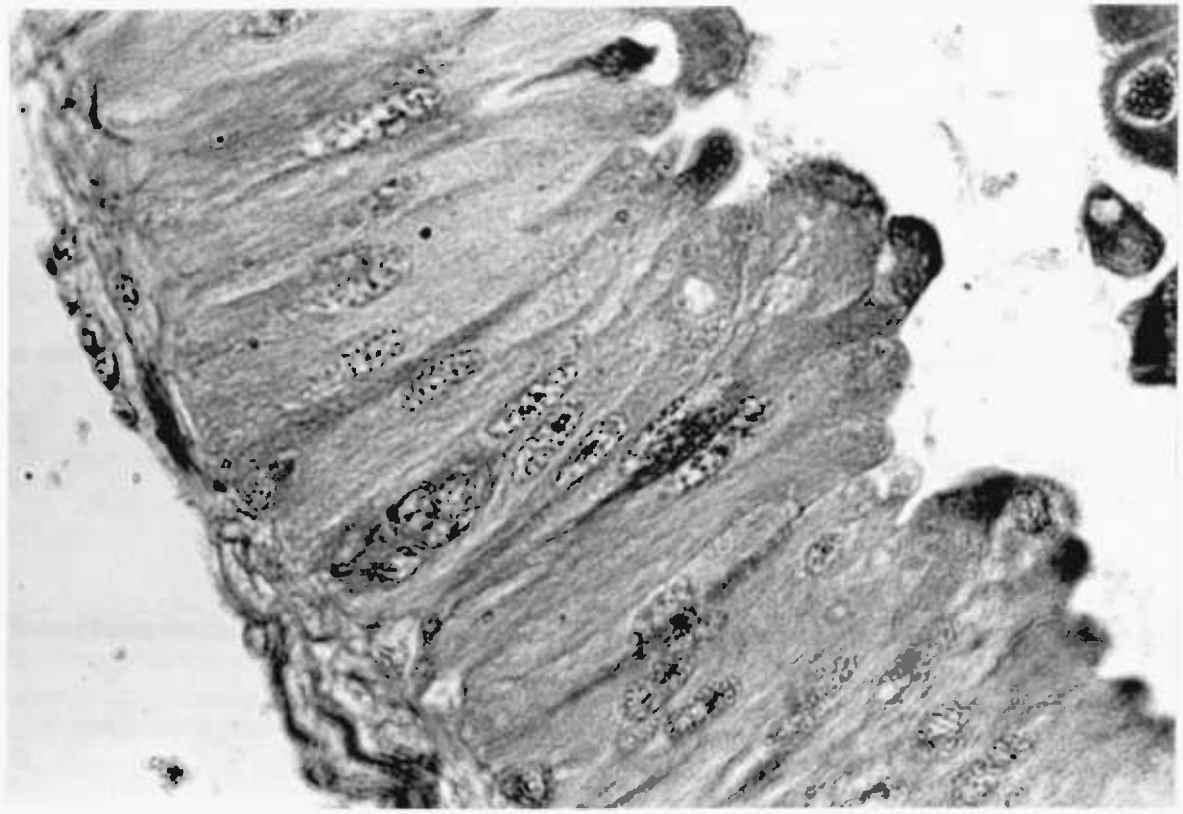


Figure 18b L.S. 6th. ventriculus (PTAH)

25µm

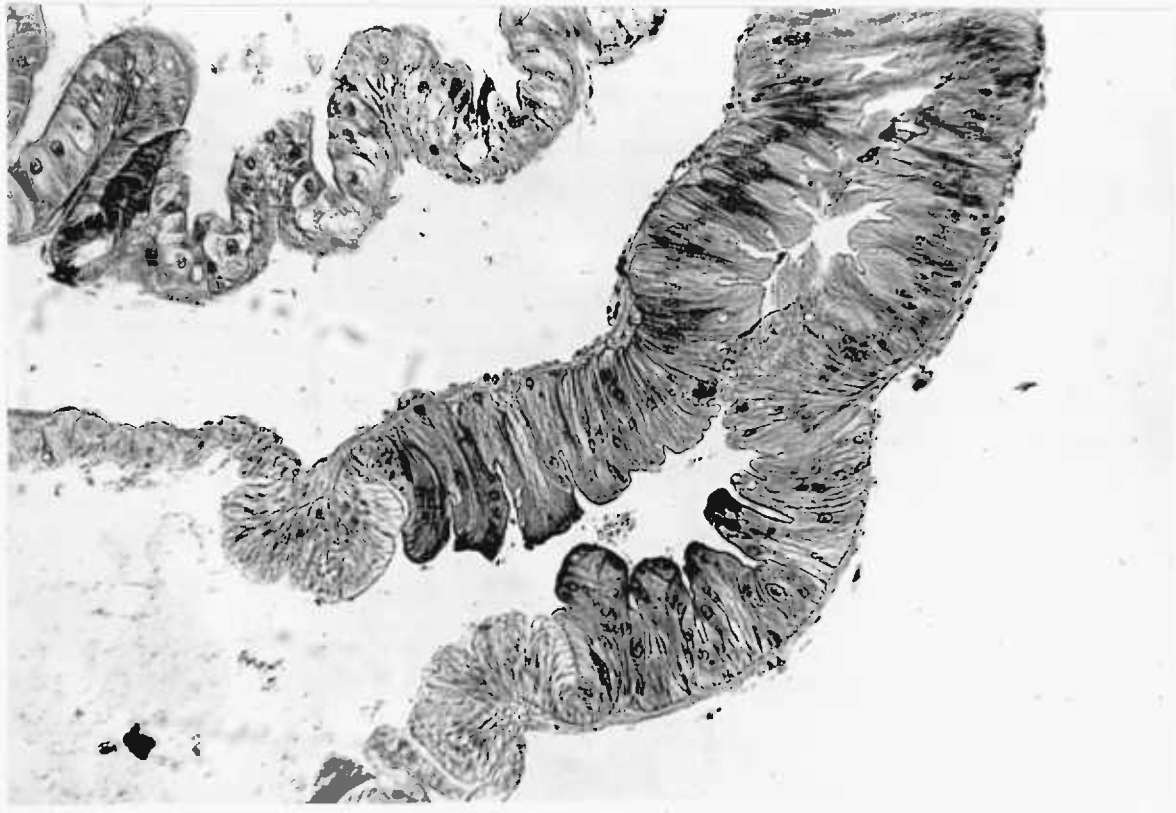


Figure 20 L.S. Connection between 5th. and 6th. ventriculus with ventriculo-rectal valve posteriorly (PTAH)

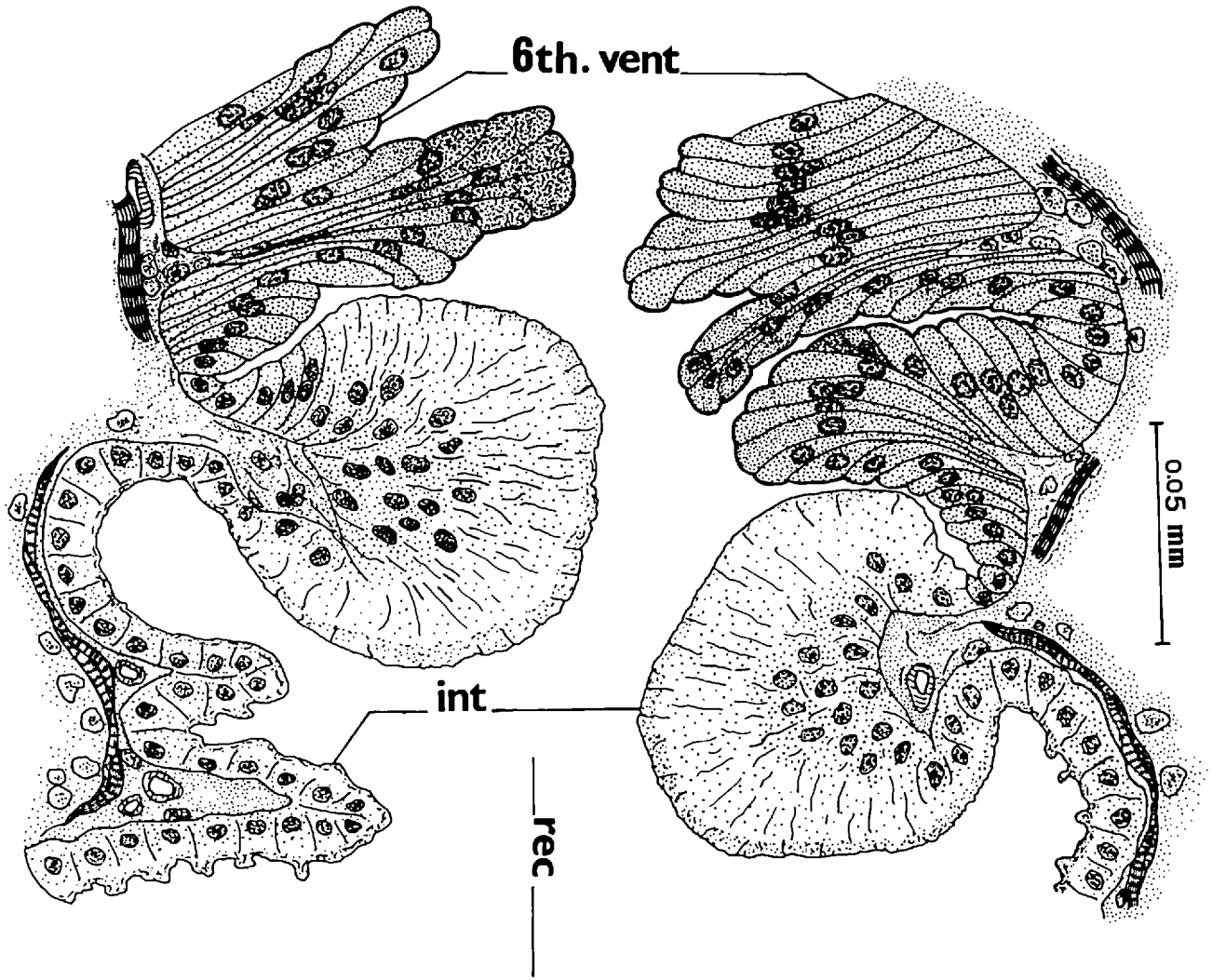


Fig. 19

L.S. Through ventriculo-rectal valve

structure as Goodchild (1963b) had suggested, is present here.

5. The musculature of this region is poorly developed and similar to that of the preceding ventriculus in the arrangement of an inner circular and an outer longitudinal muscle layer.

6. The cuticular intima starts lining the epithelium from the ventriculo-rectal valve (ileo-rectal valve of others) and continues back into the rectum (Fig. 19).

7. The epithelial cells of this region have a striated border as do so those of the more anterior midgut epithelium and they appear to be secretory in nature (Fig. 18).

8. Regenerative cells have been seen at the bases of the epithelium. Some of them were undergoing mitotic division as in the more anterior ventriculi.

All the above facts lead to the main conclusion that the so called ileum or pylorus of Oncopeltus fasciatus, for long treated erroneously as a part of the hindgut (proctodaeum) is really a part of the midgut (ventriculus). It seems likely that such a region is endodermal in origin as Butt (1949) who has studied the embryology of Oncopeltus suggests. Both Butt and also Savage (1956) who worked on Carausius morosus support Henson's interpretation of the Malpighian tubules and their origin. The sixth ventriculus is comparable to the lygaeid type of ileum described by Goodchild (1963b). In general Goodchild's subdivisions of ileal structures require modification as more material is studied. His Lygaeid type, in which Oncopeltus fasciatus falls, does not agree entirely with the conditions found here where the 5th ventriculus appears to enter this region anteriorly and is joined to it by a valve shaped constriction as described above. Moreover the epithelial

cells of the 6th ventriculus (the ileum of Goodchild) are cylindrical in shape with a well developed striated border, not thin and without striations as stated by Goodchild for his Lygaeid type. There is no obvious explanation of the discrepancy between my account of Oncopeltus and that of Goodchild for other species of Lygaeidae.

The points of entry of the Malpighian tubules are simple, not involving any special cell type and are associated in pairs on each side; the openings lie in a plane parallel to the axis of the intestine (Fig. 21a and b).

Henson (1932) established that the Malpighian tubules of Pieris brassicae were endodermal in origin and then his view was subsequently extended to other insects (Henson 1937 - 1946), but they have not been adequately reflected in the opinions of those working on the Heteroptera apart from a brief discussion made by Srivastava and Bahadur (1961).

In Oncopeltus fasciatus the basal part of each Malpighian tubule where it joins the wall of the alimentary canal is formed of columnar cells with a well developed striated border and they appear continuous with the rest of the sixth ventricular epithelium (Fig. 21a and b).

In transverse sections, each Malpighian tubules (Fig. 22) has a narrow lumen and the cells contain relatively large nuclei centrally located in the cytoplasm, with very prominent nucleoli and a mass of chromatin granules. The cytoplasm is granular and the cell borders show a striated appearance towards the lumen; no muscle layers are present. The Malpighian tubules are histologically comparable with the sixth ventriculus and, therefore, probably also of endodermal origin.

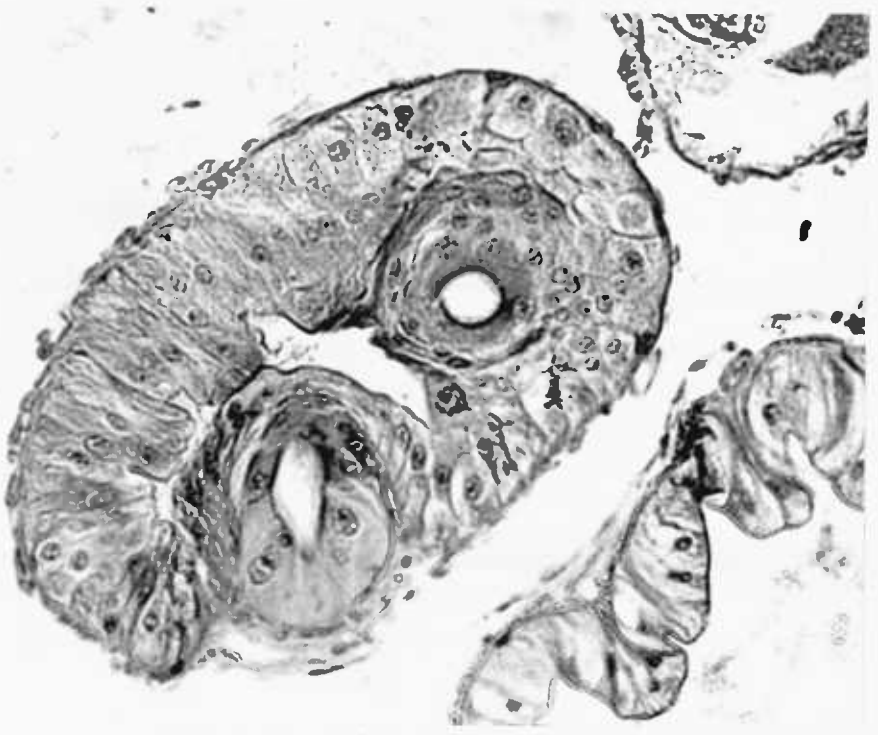


Figure 21a 6th. ventriculus and Malpighian tubules, oblique section near their junction (PTAH)

25µm

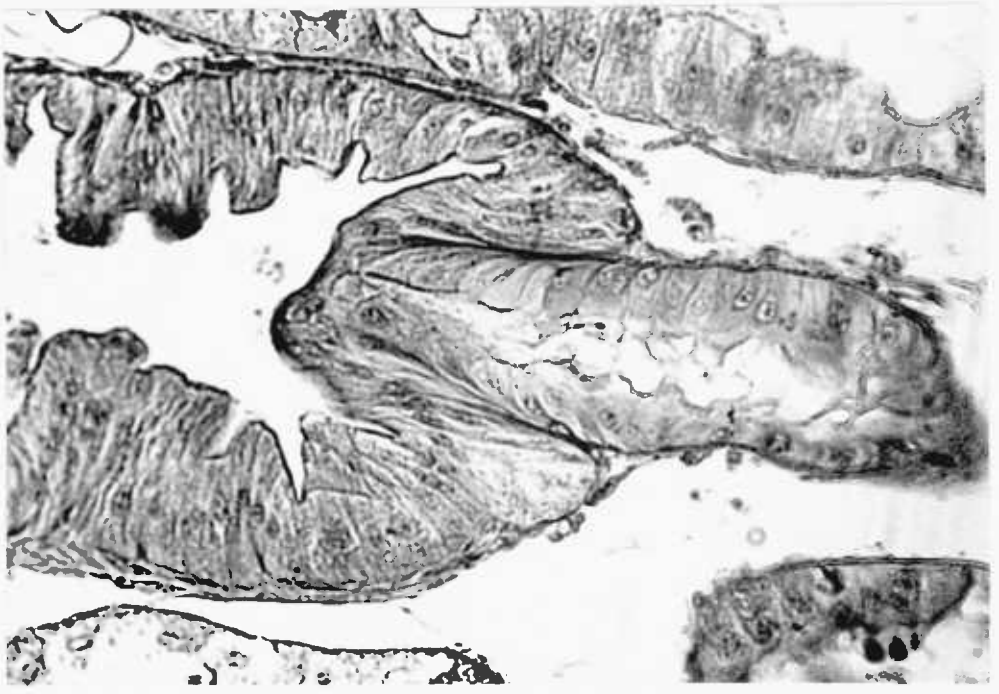


Figure 21b L.S. Malpighian tubules 6th. ventriculus (PTAH)

Figure 22. T.S. Malpighian tube (PTAH)

25µm

SECTION 3

HISTOLOGICAL CHANGES IN THE MIDGUT EPITHELIUM DURING POST-METAMORPHIC DEVELOPMENT

HISTOLOGICAL CHANGES IN THE MIDGUT EPITHELIUM DURING POST-METAMORPHIC DEVELOPMENT

Introduction

The present work is based on the study of histological changes, especially on the changes undergone by various features of the epithelial cells, such as vacuolation and granulation. It is my aim to throw some light on the histological and cytological character of the epithelium in order to understand the cyclic changes in cell structure, that might be related to the stages of digestion at progressive ages. These histological changes might well be correlated with the output of digestive enzymes (Roeder, 1953). Attention has also been paid to the capacity of the epithelial cells to regenerate at progressive ages and quantitative estimates of the activity of regeneration cells (nidi) were made and are described in this chapter. The occurrence of mononucleated and binucleate epithelium cells in the mid-intestine was also observed. It is of interest that the frequency of either bi-or mono-nucleate cells in the epithelium appears to be related to the structure of the mid intestine and is, therefore, perhaps of phylogenetic significance (Yanai, 1952).

Information on the histological changes in midgut epithelium of different groups of insects during metamorphosis are available from Folsom and Welles (1906) on Collembola, Haseman (1910) on Psychoda alternata Say (Diptera : Psychodidae), Mansour (1927) on Sitophilus oryzae L. (Coleoptera : Curculionidae), Pradham (1939) on Coccinella septempunctata (Coleoptera : Coccinellidae) Muralirangon and Ananthakrishnan (1972) on Eyprepocnemis alacris alacris (Orthoptera : Acrididae), and many others.

Studies on the metamorphic changes in the midgut of Hemiptera, however, are relatively few. Srivastava and Sinha (1965) have described changes in the "larval" and "pupal" midgut of Centrococcus insolitus (Homoptera : Coccidae) and Sinha (1968) investigated the histological changes

in the midgut epithelium during postembryonic development in Chrysocoris stollii (Wolff)(Heteroptera : Pentatomidae). The histological changes and the behaviour of the midgut epithelium through the life span of an adult insect has never been the subject of study. Studies on the histological changes in the midgut of adult insects have only been made during short periods of starvation and normal feeding or before and after meals while looking for cytological evidences of secretion. Duspiva (1939), working on the carnivorous adult water beetle, Dytiscus marginalis, investigated well-marked changes in the cells of the midgut epithelium. The "resting cells" decrease from about 90% to 50% within 45 minutes after feeding, whereas the "secretory cells" increase from 0.0 to about 60%. Somewhat similar findings on the cytological changes in the midgut epithelium were made by Buchman (1929) on Diptera, Parsons (1959) on aquatic Heteroptera, Goodchild (1952) on some species of Miridae and others.

The present study is a first attempt to study the histological changes and behaviour of the midgut epithelium in an adult Heteropteran insect during progressively older adult stages, which are therefore referred to as postmetamorphic changes. Some knowledge of these is clearly desirable if one is to make comparisons among specimens which are treated differently but happen also to be of various ages.

A stock culture of Oncopeltus fasciatus was reared in the laboratory at 26°C = 70F and a relative humidity of about 75%. As soon as the insects reached the adult stage, they were sorted out from the main culture cage and kept under similar conditions of temperature and humidity, where they were allowed to remain alive for an appropriate period and were supplied with seeds and water every day. The old seeds were then removed and when the insect reached the appropriate age (newly emerged, 5, 10, 15, 20, 25 or 30 days) they were killed and dissected in the usual way (see Materials and Methods). The data were collected from a sufficiently large number of individuals, usually six individuals of each age. Ten readings were made

from each preparation and the average was obtained as mentioned in Tables below each item. On account of the variations in experimental data the standard error of the mean value was calculated by using the following formulae :

$$S.D. = \sqrt{(SX^2 - \frac{(SX)^2}{n})/(n-1)}$$

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

S.D. = Standard Deviation

S.E. = Standard error of mean

SX = total of readings

SX^2 = total of squares of readings

n = number of readings

$\sqrt{n}$ = square root of number of readings

Since the data was mostly recorded in whole numbers, all data from mean values and standard errors were rounded up to the second decimal place. Each reading was made at random in the section and examined with a X100 objective (oil immersion lens). Tables were provided to illustrate the measurements and dimension of epithelium cells, nuclei and thickness of their striated border. All measurements were obtained with the aid of an eye piece micrometer. Subsequent references to age are based on the definition that zero age counted from the day on which the emerged adult was first noted.

1st. ventriculus

External features and food contents In newly emerged specimens the first ventriculus is very delicate, often containing air bubbles, and white or transparent in colour, with the posterior part slightly yellowish and

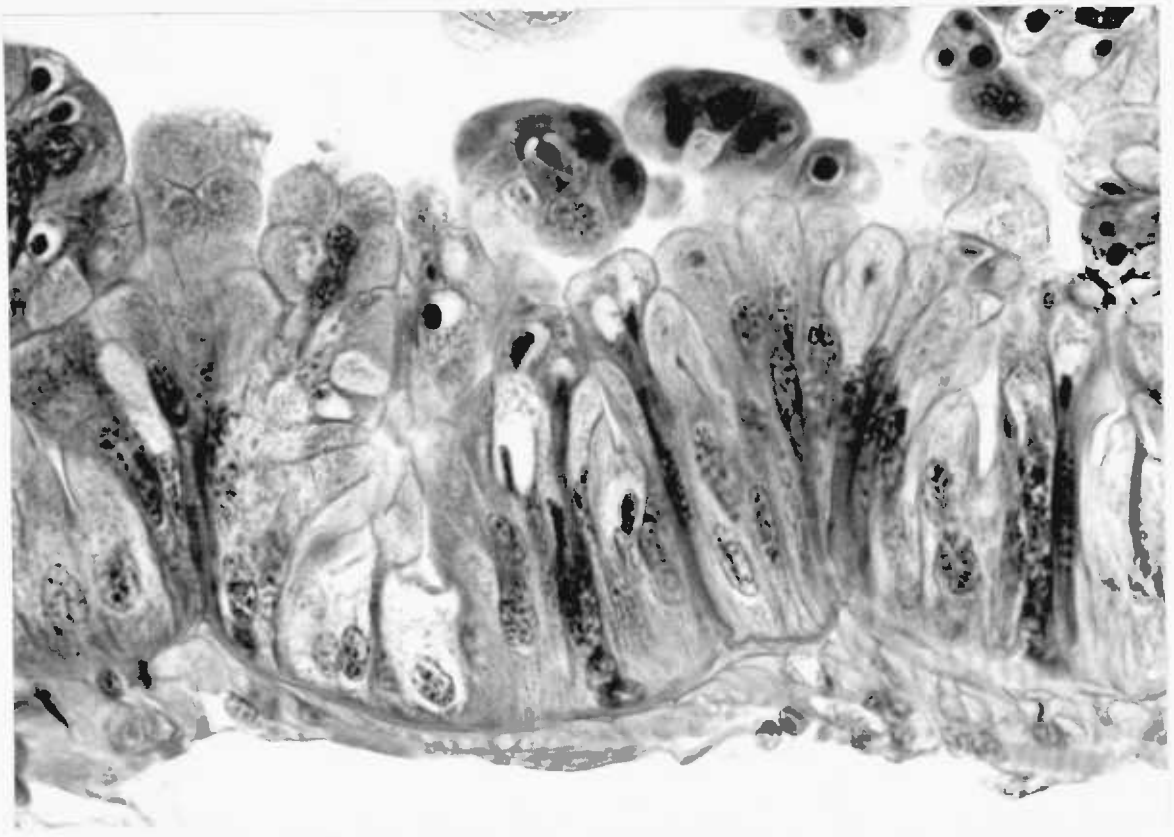
containing a semipasty food mass. As the specimens get older the wall of this segment becomes more wrinkled and less transparent and a brownish orange food mass is formed.

Epithelium The first ventricular epithelium can be arranged in three intergrading subregions, anterior, middle and posterior as described on pp. . Data for the Tables given in this chapter were chosen randomly from any part of the whole segment and not confined to a special region.

In newly emerged specimens the cells of the epithelium are uniform in appearance though they may already show some degenerative changes and are composed of a normal columnar type with a thin striated border about 0.9µm thickness (Fig. 23a and b). During the different ages of the adult changes take place in the epithelium arrangement and cell proportions. Fig. 24 represents the epithelium of the anterior, middle and posterior region of the first ventriculus respectively in 10 day old specimens where vacuoles are found apically and nuclei located in the distal half of the cell body. The middle and posterior regions show some extrusion cells as well as those with apical disruption involving the emergence of material through the striated border. The gradient in percentages of resting cells, vacuolated cells, extrusion cells and degenerating cells throughout the different ages in this ventriculus are given in Table 2 and illustrated in Fig. 25. The histogram shows that the percentage of resting cells decreases from 61.07% to 11.84% through progressive ages in well fed Oncopeltus fasciatus while that of both the degenerating and vacuolated cells increases from 8.4% to 72.4% and 47.3% to 89.5% respectively when the insect becomes older. Figure 26a, b, c represents the epithelium of the anterior, middle and posterior regions of this ventriculus respectively in 30 day old specimens.

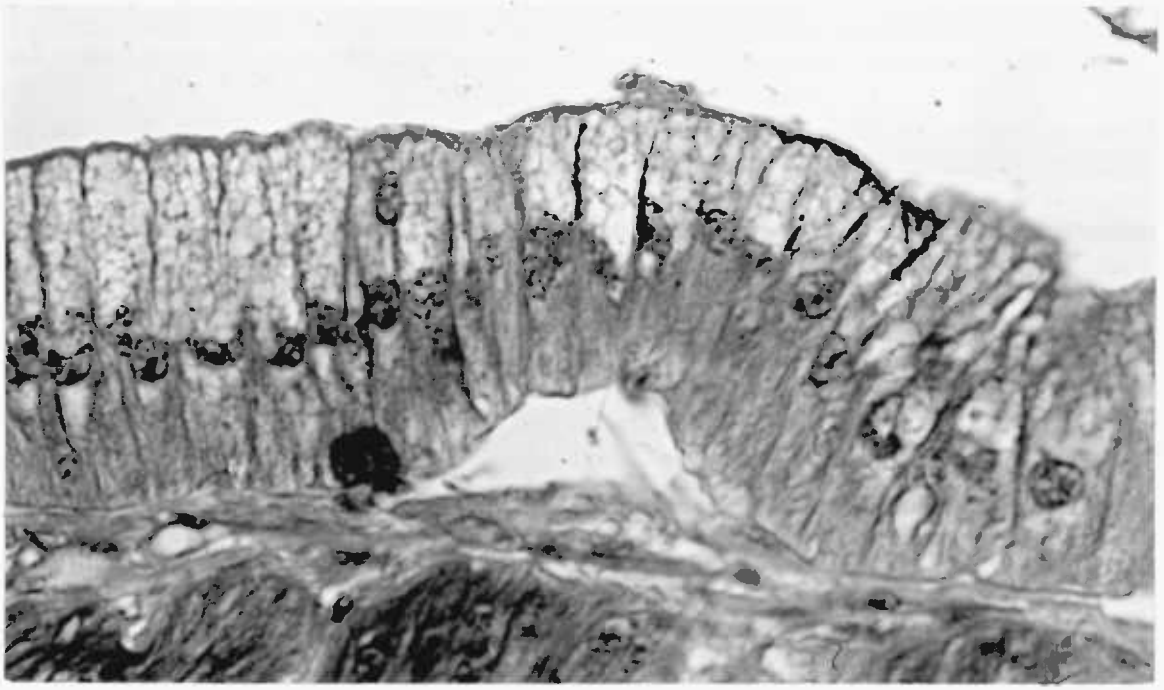
A - Anterior region

25µm



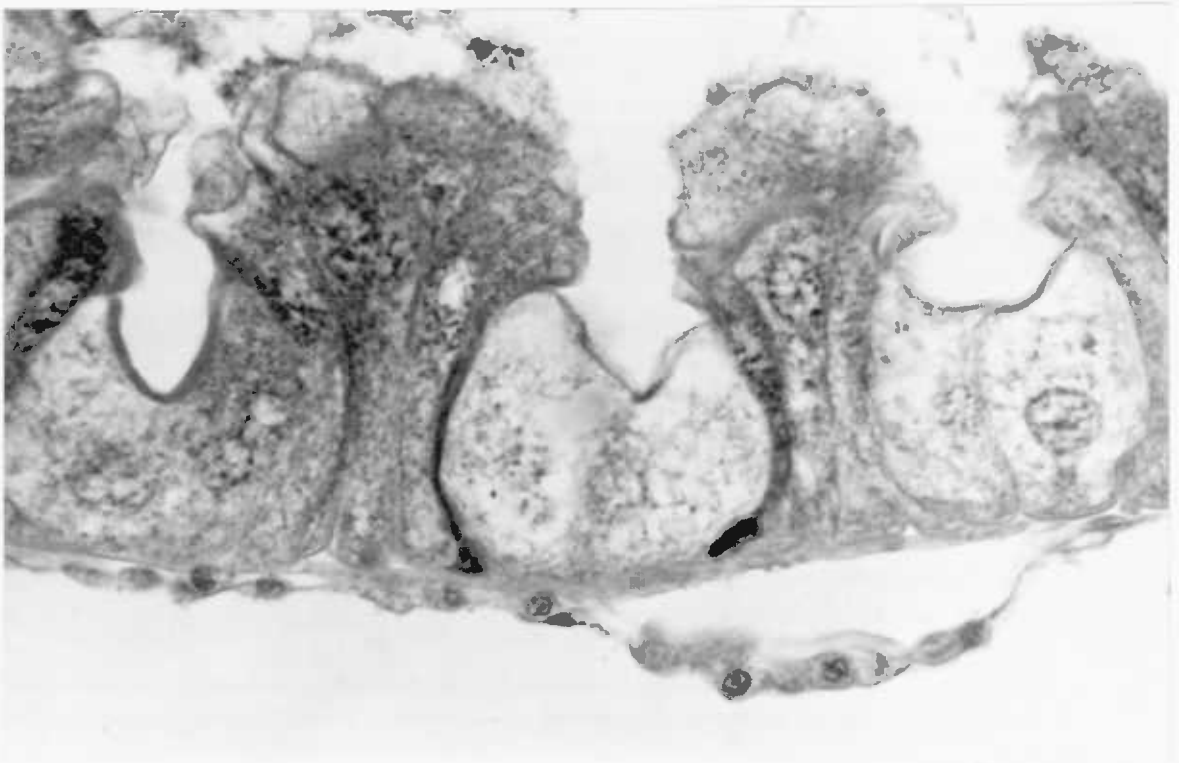
B - Posterior region

Figure 23A and B, L.S. 1st. ventriculus in newly emerged to adult



A - Anterior region

25µm



B - Middle region

Figure 24A and B, L.S. 1st. ventriculus, 10 days old

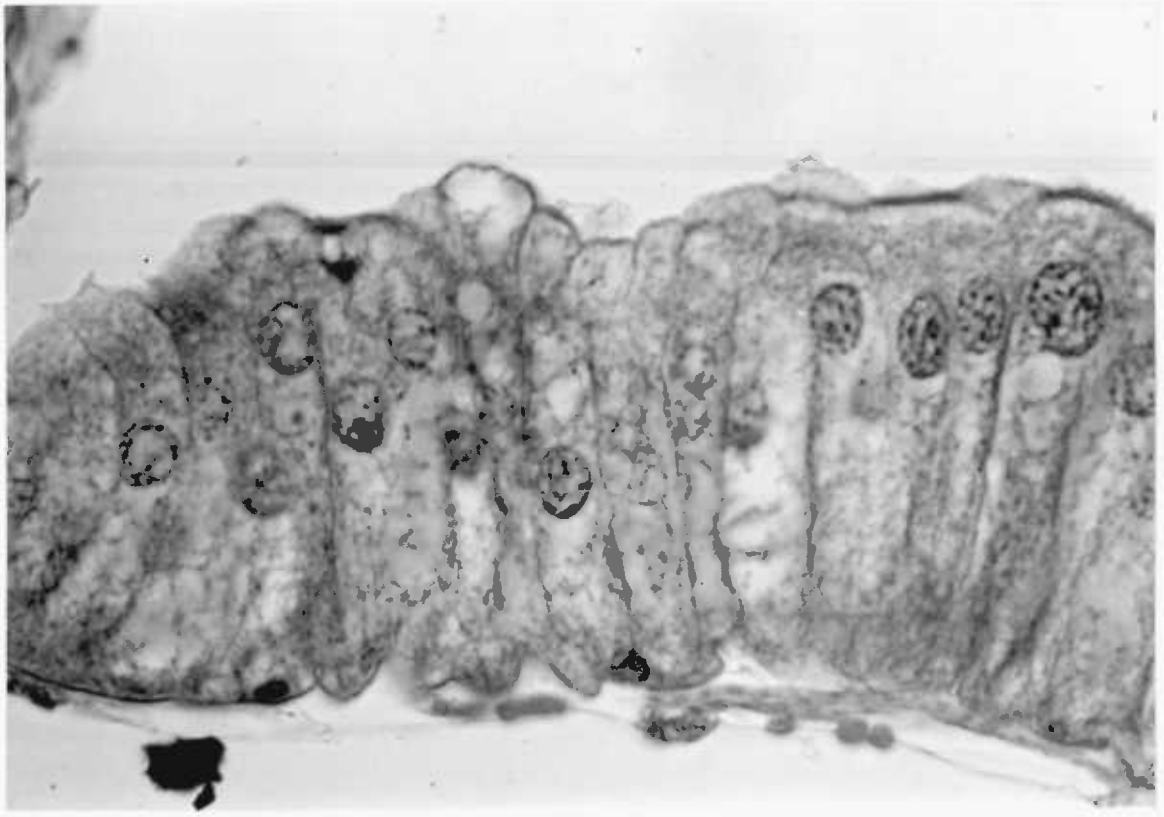


Figure 24C

Posterior region of 1st. ventriculus, 10 days old

50µm

Table 2 Comparative data and percentage ratio of cell types in midgut epithelium in different ages of 1st. ventriculus

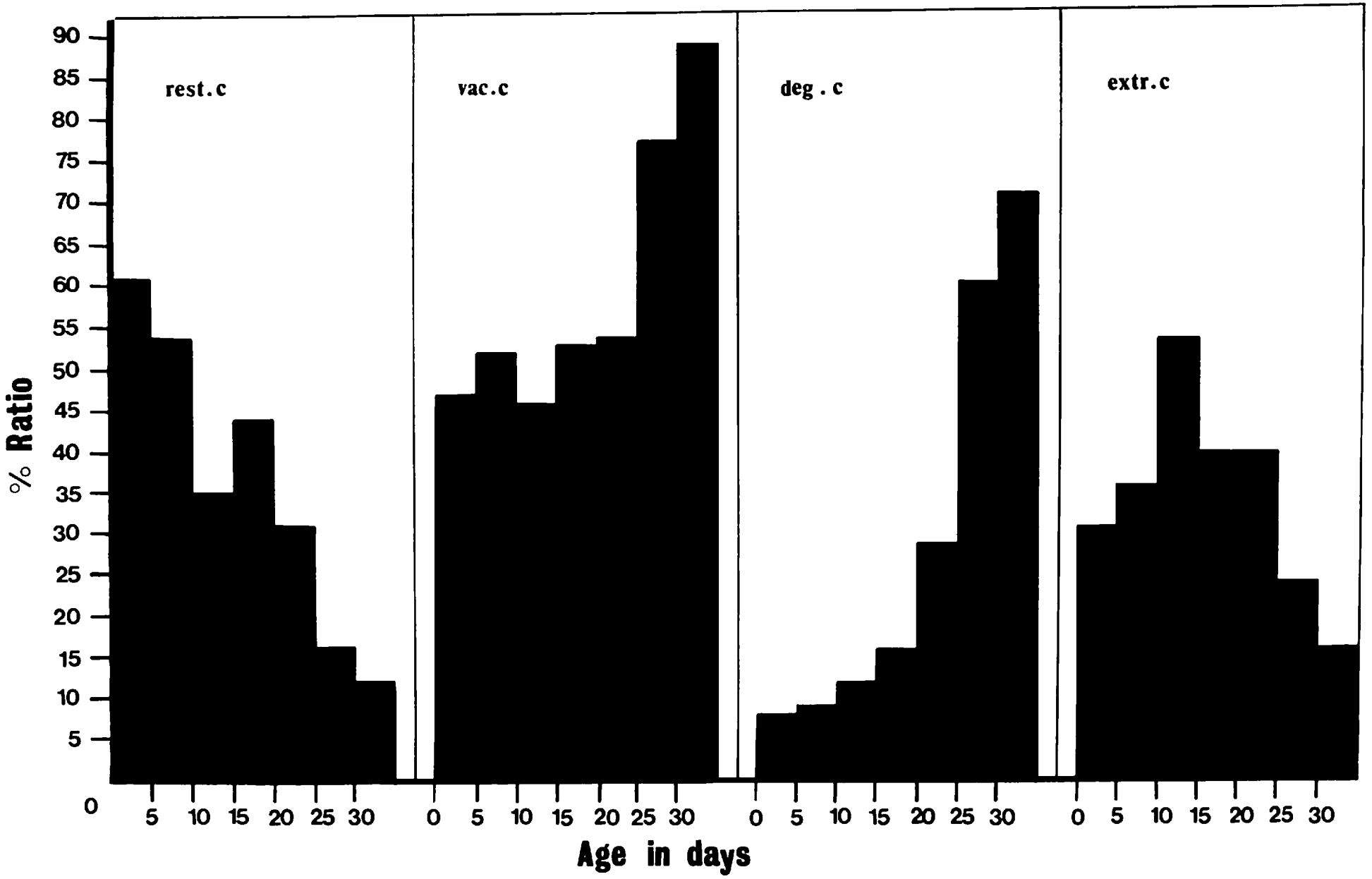
Age in days	Cell epithelium per section	Extrusion cells	% Ratio	Degeneration cells	% Ratio	Vacuolation cells	% Ratio	Resting cells	% Ratio
0	26.2	8	30.5	2.2	8.4	12.4	47.3	16	61.1
5	15.8	5.8	36.7	1.4	8.9	8.2	51.9	8.6	54.4
10	17.4	9.4	54	2	11.5	8	46	6	34.5
15	16	6.4	40	2.6	16.3	8.4	52.5	7	43.8
20	14.4	5.8	40.3	4.2	29.2	7.8	54.2	4.4	30.6
25	14.2	3.4	24	8.6	60.6	11	77.5	2.2	15.5
30	15.2	2.4	15.8	11	72.4	13.6	89.5	1.8	11.9

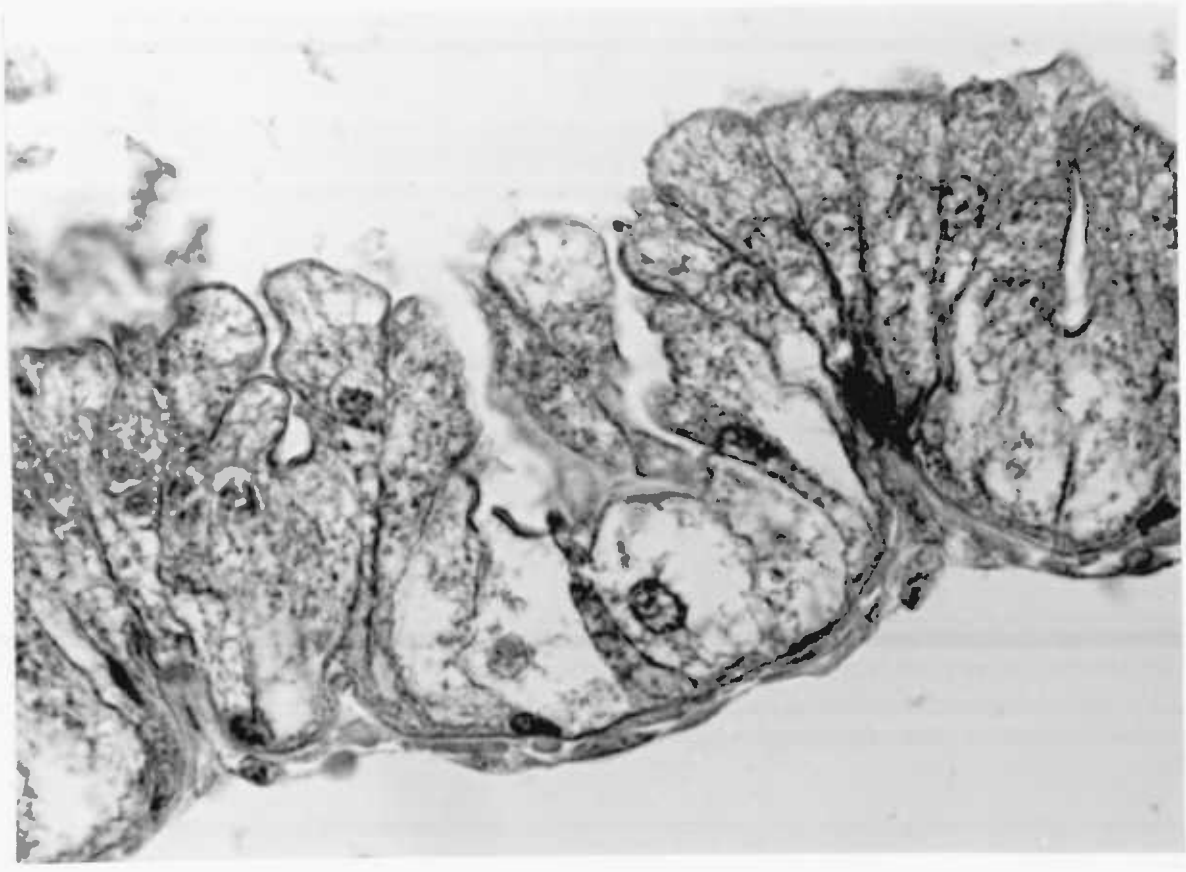
In this Table and following similar tables the total percentages exceed 100 since a given cell may, for example, show both degeneration and vacuolation.

Figure 25

Histograms of the first ventriculus to illustrate the percentage ratio of cell types (resting, vacuolation, degeneration extrusion cells) at different ages.

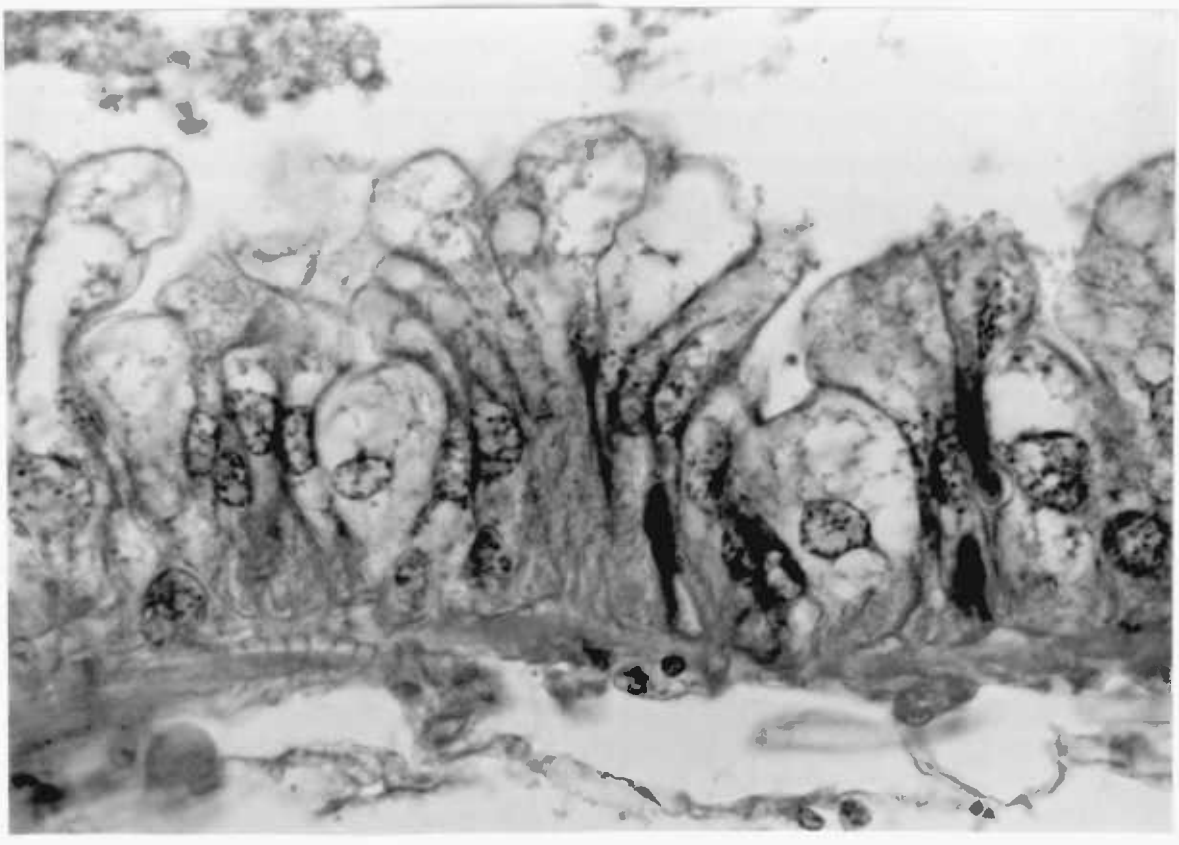
Fig. 25


rest.c
vac.c
deg . c
extr.c
% Ratio
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0 5 10 15 20 25 30
Age in days



A - Anterior region

25µm



B - Middle region

Figure 26 A and B, L.S. 1st. ventriculus, 30 days old

Figure 26C

L.S. 1st. ventriculus, 30 days old, Posterior region

50µm

The percentage of the mono and binucleate cells in the epithelium is calculated in different ages, and given in Table 3. This shows that the epithelium is dominated by mononucleate cells. The abundance of nidi or regenerative cells in the epithelium of the differently ages specimens show a gradual decrease through progressive ages in all segments as indicated in Table 4 and Fig. 27. Measurements for the dimensions of cell types and their nuclei and thickness of the striated border are given in Table 5.

Table 3 Average frequency of mononucleate and binucleate of epithelial cell in 1st. ventriculus in different ages

Age in days	Cells per section	Mononucleate frequency (A)	%	Binucleate frequency (B)	%	B/A Ratio
0	26.2	19.9	75.9	6.4	24.3	1:3.1
5	14.8	12	80.8	2.8	19.2	1:4.2
10	15.4	12.2	79.2	3.2	20.8	1:3.8
15	15.4	12.6	82	2.8	18	1:4.5
20	14.2	11.8	83.1	2.4	16.9	1:4.9
25	15.2	13.1	86.4	2.1	13.6	1:6.4
30	15.2	12.9	85.1	2.3	14.8	1:5.8

Table 4 Mean number of epithelial cells and nidi per section with percentage of nidi to epithelial cells in 1st. ventriculus at different ages (± standard error)

Age in days	Mean number of epithelial cells per section (A)	S.E. (±)	Mean number of nidi per section (B)	S.E. (±)	B/A Ratio in %
0	21.5	± 1.8	6	± 0.7	27.9
5	21.3	± 1.1	4.5	± 0.6	21.2
10	18.8	± 0.5	3.8	± 0.25	20
15	19.8	± 1.2	3.8	± 0.25	19
20	30	± 1.4	5	± 1	16.7
25	36.3	± 1.2	6	± 0.6	16.6
30	33.8	± 1.5	4.8	± 0.5	14.1

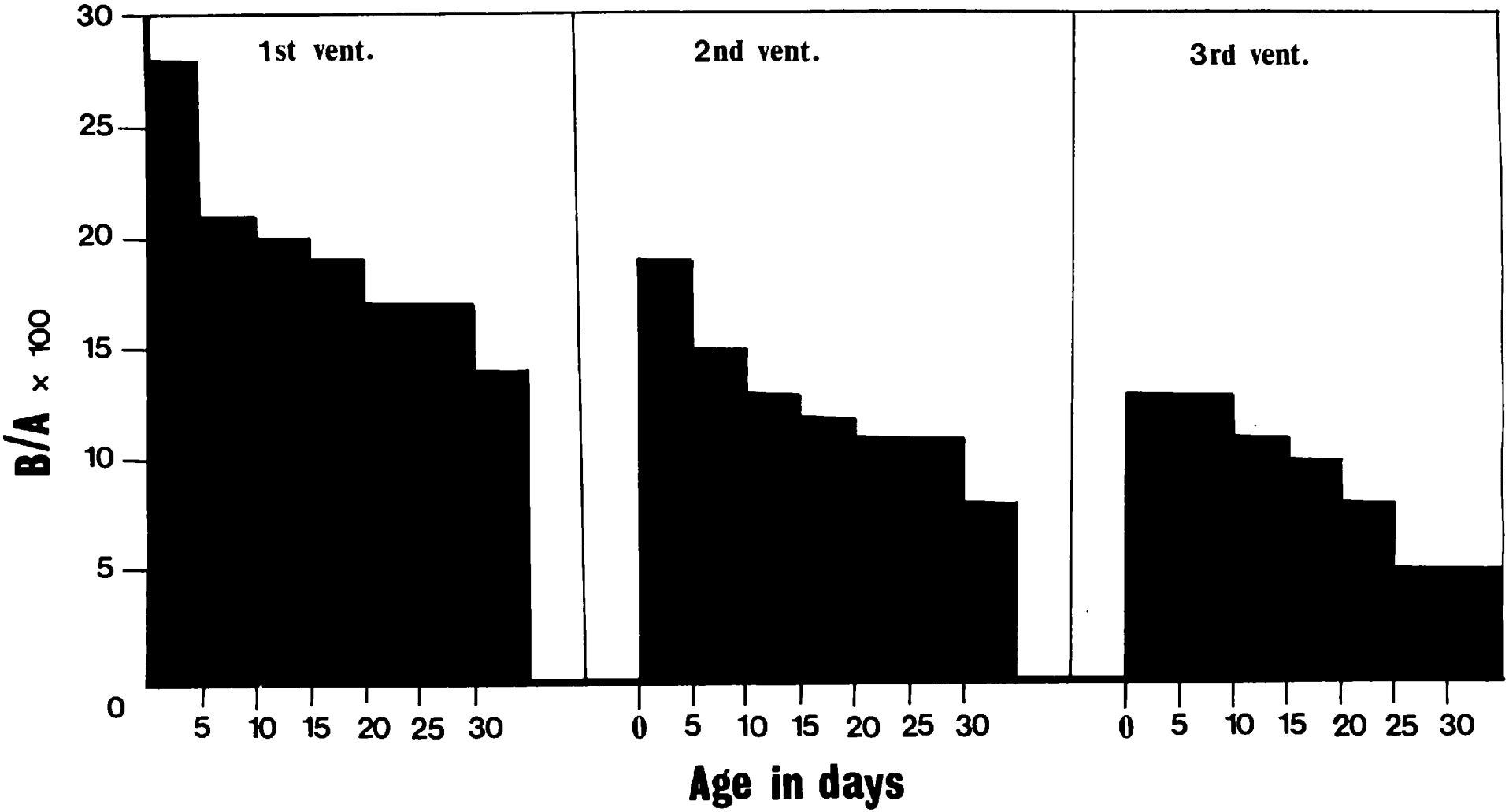
Table 5 Comparative data on the histology of the 1st. ventriculus in different ages (in µm)

Age in days	Height of villar cells	Height of young cells	Nucleus of villar cells		Nucleus of young cells		Thickness of striated border
			Length	Maximum diameter	Length	Maximum diameter	
0	31.7 - 39.3	19.7 - 26.7	8	5	6.9	4.9	0.9
5	58.7 - 70.7	30 - 46.7	9.1	5.7	8.5	7	1.6
10	62 - 72	28.3 - 46.7	10.5	4.5	5.6	6.3	1.6
15	61.7 - 70.2	28.3 - 45.7	12.2	4.4	9.2	6.8	0.7
20	55 - 70	26.7 - 40	11.3	7	10.3	7.7	0.9
25	54 - 65.3	23 - 35	7.8	5	8.2	7	1.1
30	38 - 55	18.7 - 34	7.4	4.7	7.2	5.6	0.6

Figure 27

Histograms to represent the percentage of the mean number of nidi per section to the mean number of epithelial cells per section at progressive ages in the 1st, 2nd and 3rd ventriculus.

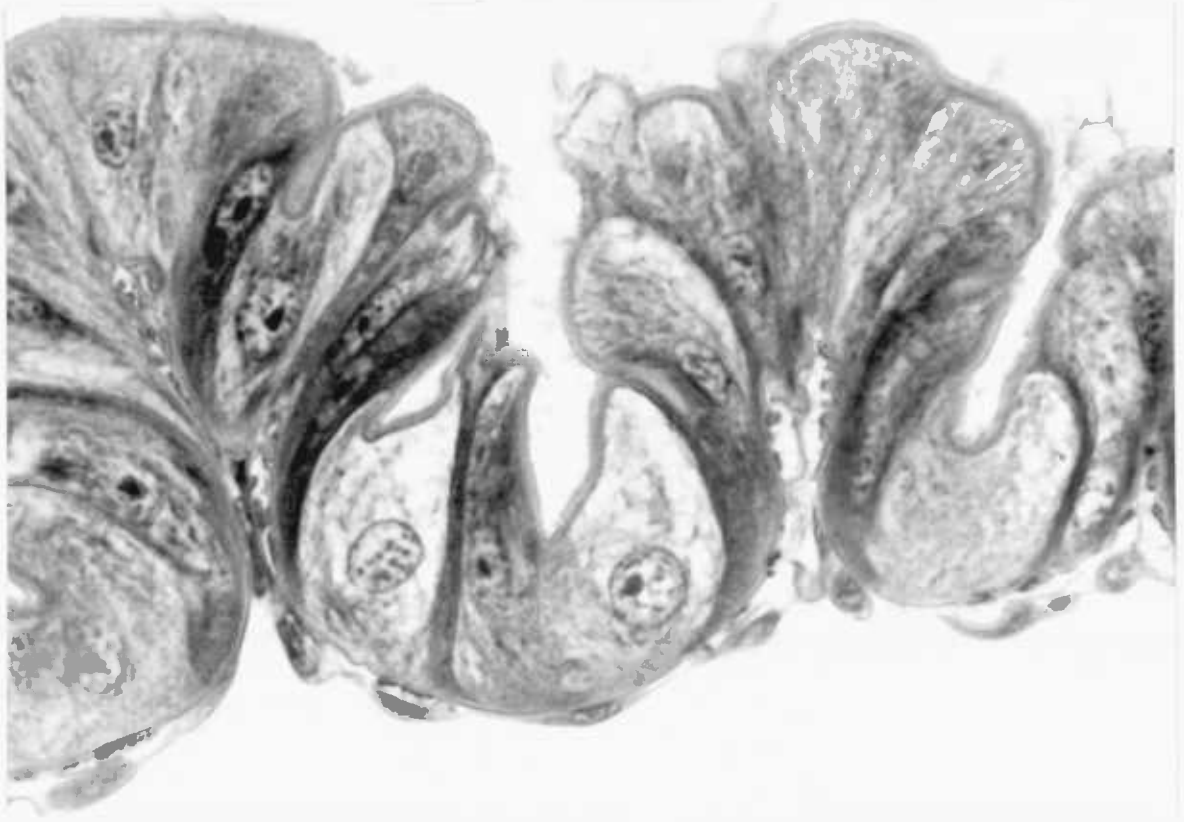
Fig. 27


1st vent.
2nd vent.
3rd vent.
B/A x 100
0
5
10
15
20
25
30
0 5 10 15 20 25 30
0 5 10 15 20 25 30
0 5 10 15 20 25 30
Age in days

2nd ventriculus

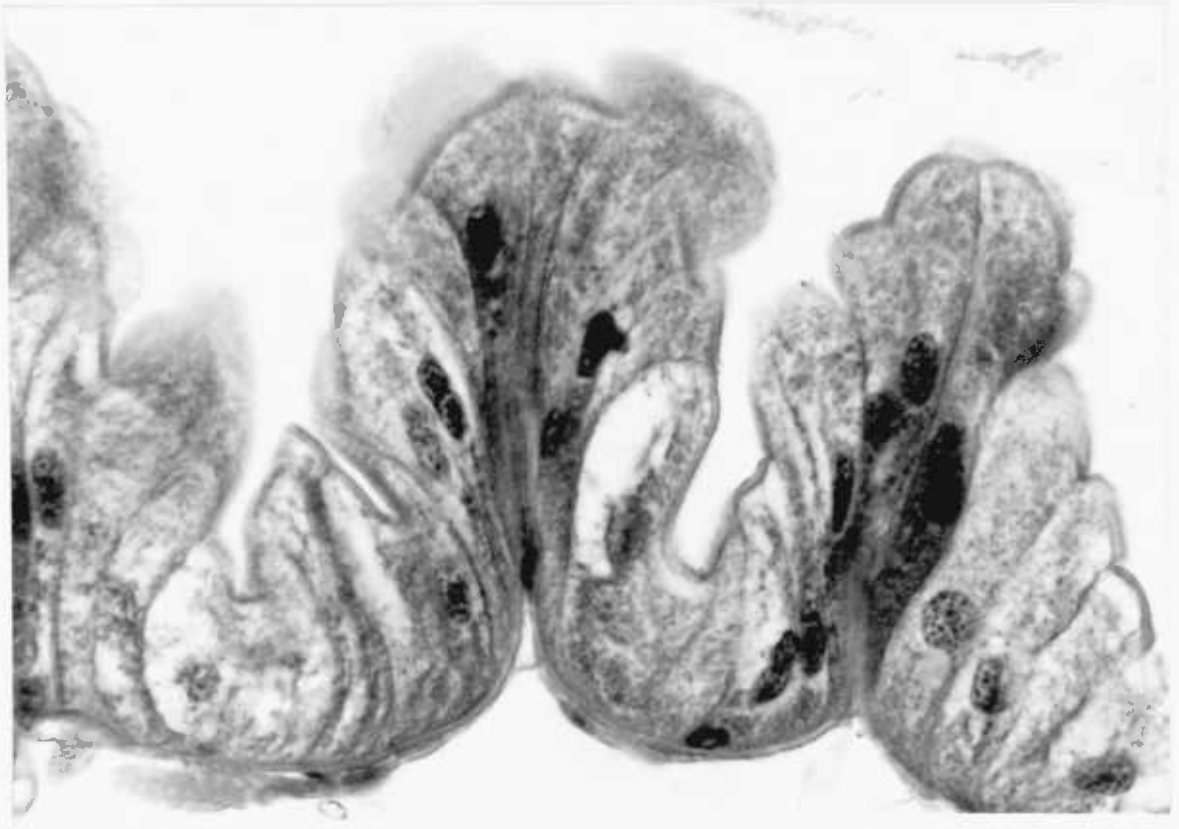
External features and food content In newly emerged specimens this region appears as a delicate transparent tubular structure containing droplets of food material. In older specimens its walls become thicker, less transparent and yellowish in colour posteriorly, and the contents become more concentrated.

Epithelium In newly emerged specimens the epithelium forms low villi with dense cytoplasm basally. The apical cells of the villi have homogenous cytoplasm with a thin striated border about 0.75μm deep, few vacuoles and coarse granules accumulated apically. Young cells are more prominent with lighter cytoplasm apically and centrally located nuclei (Fig. 13). In five day old specimens the epithelium shows no material diffusing through the striated border. The villar cells are slightly vacuolated and strongly basiphil (Fig. 28a). In this stage the young cells appear remarkably distinct, having relatively large and centrally located nuclei, while in 15 days old the apical region of the cells show diffused material (Fig. 28b). Figure 29 represents the epithelium of this ventriculus in specimens that are 20, 25 and 30 days old respectively. It shows that in older specimens vacuolated cells increase from 18.3% to 75.8% in the epithelium while resting cells tend to decline from 72.5% to 19.8%. Comparative data of the types of cells forming the epithelium of this region with their percentage through different ages are given in Table 6 and Fig. 30.



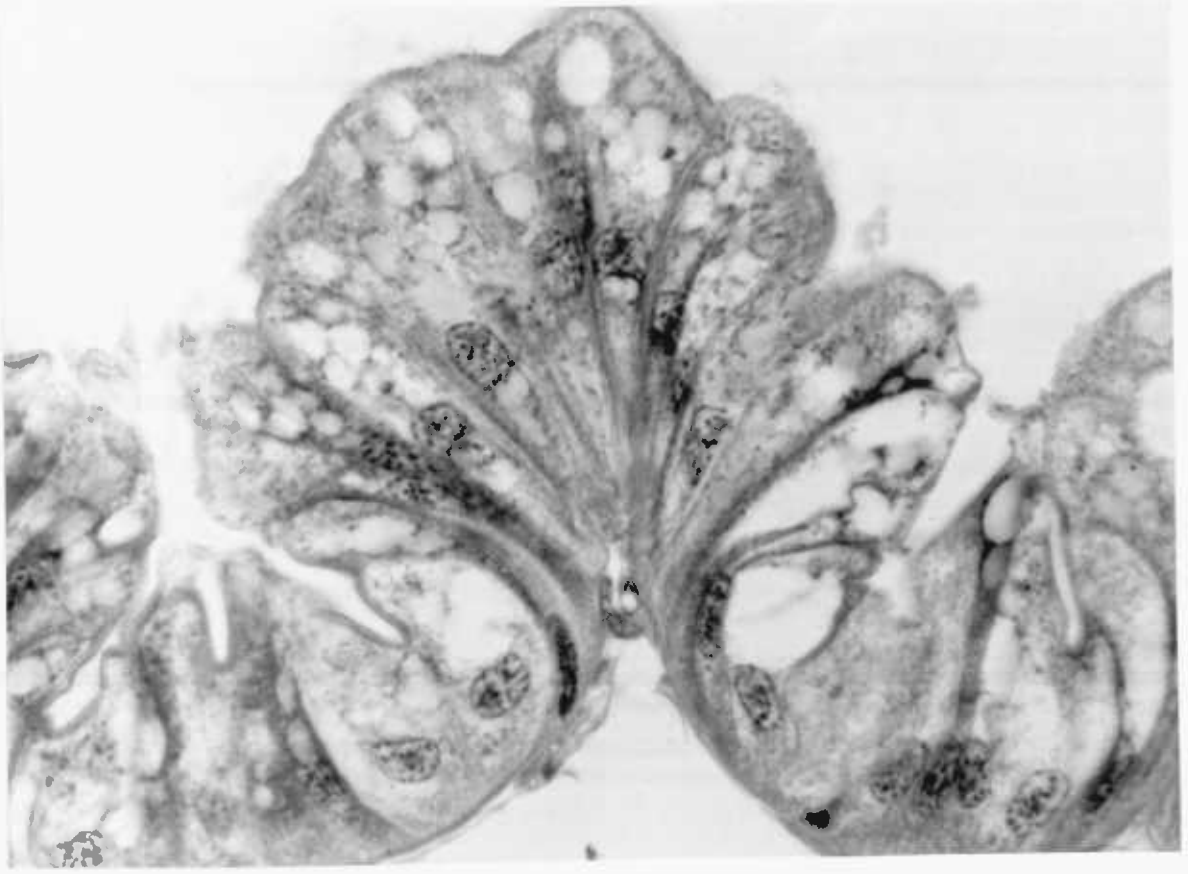
A - 5 days old

25µm



B - 15 days old

Figure 28 A and B, L.S. 2nd. ventriculus



(A)

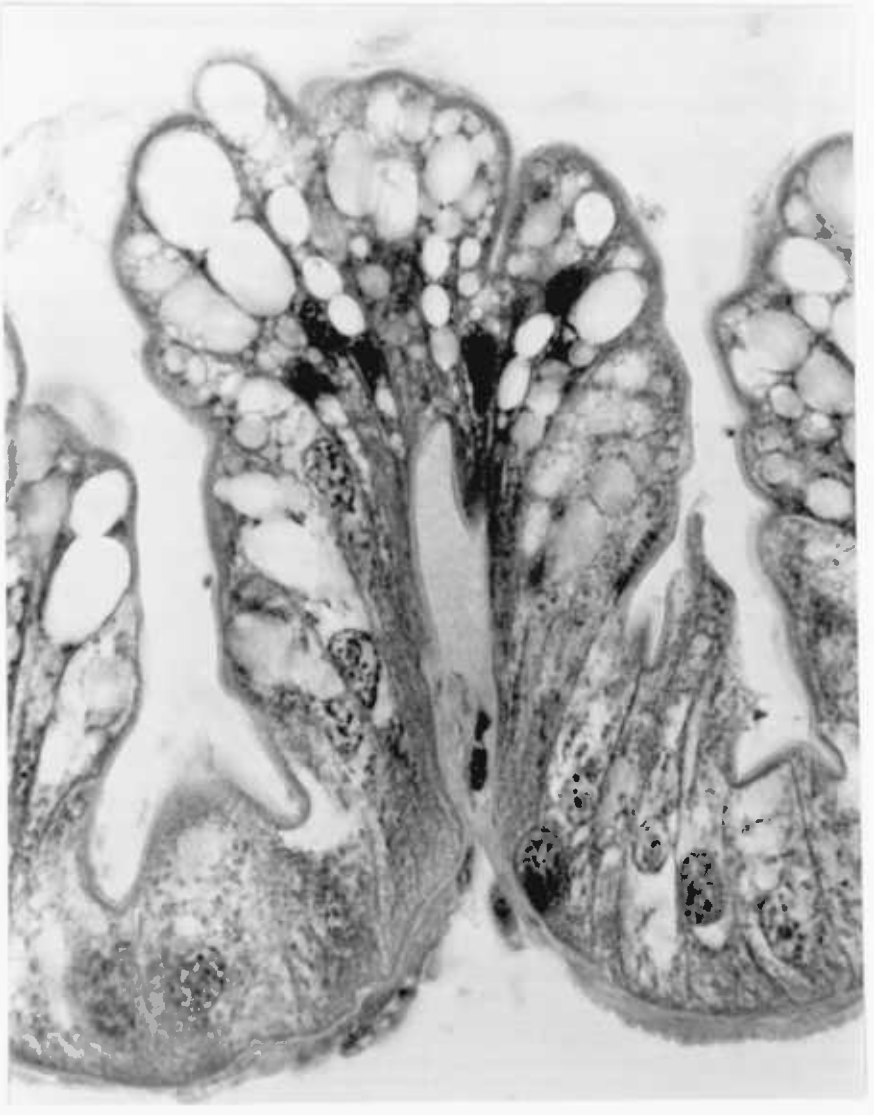
Figure 29

2nd. ventriculus

a - 20 days old

b - 25 days old

25µm



(B)

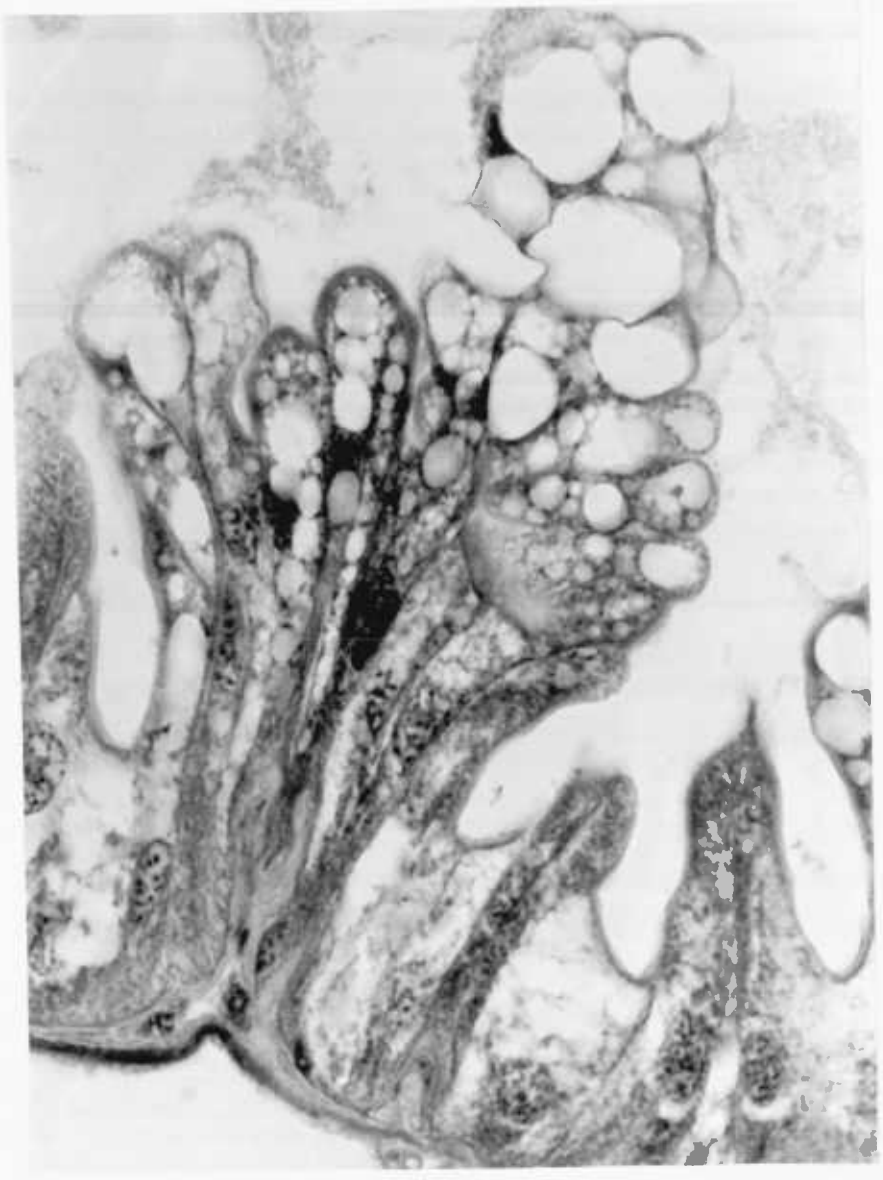


Figure 29 C, 2nd. ventriculus, 30 days old

50µm

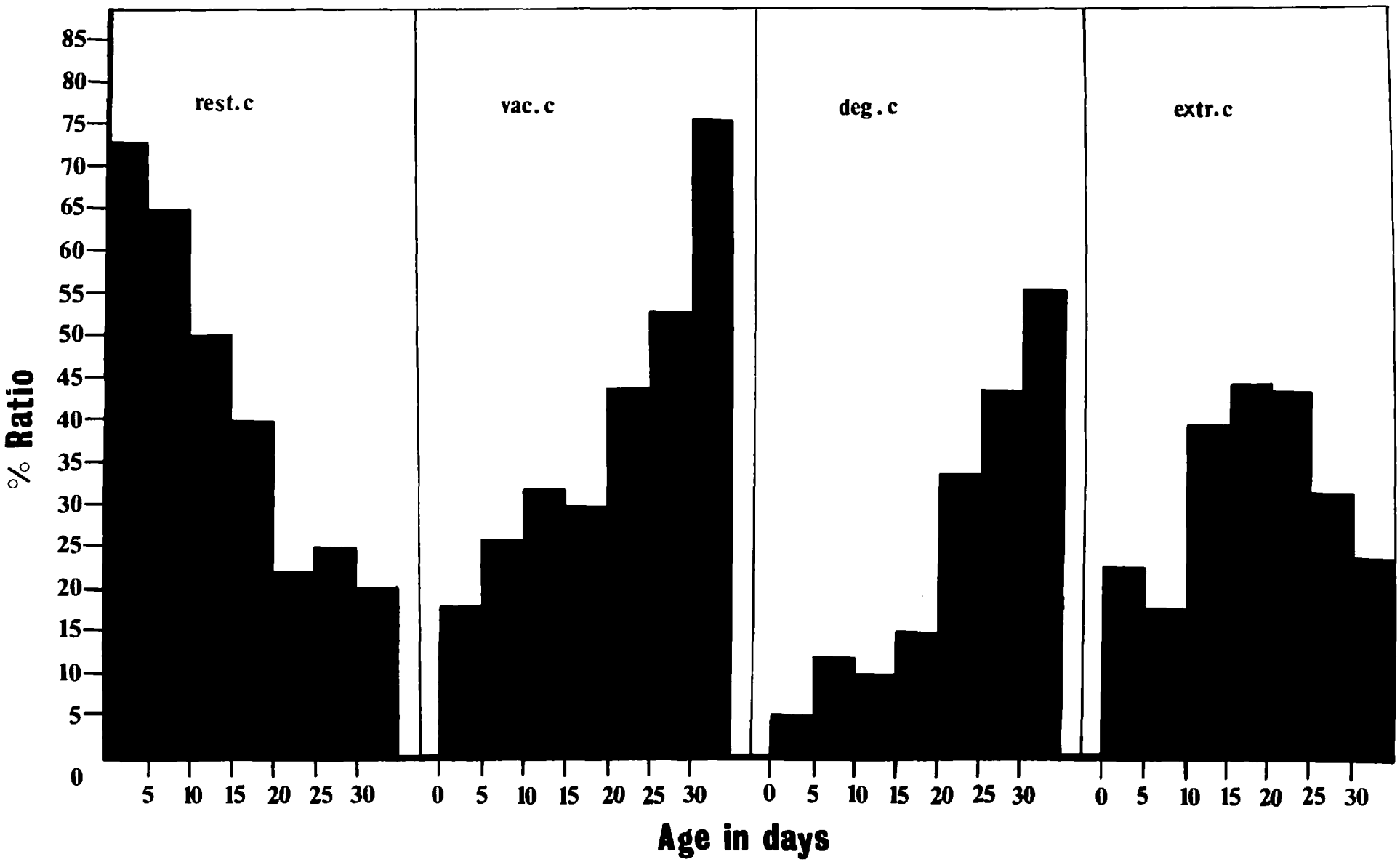
Table 6 Comparative data and percentage ratio of cell types in midgut epithelium in different ages of 2nd. ventriculus

Age in days	Cells epithelium per section	Extrusion cells	% Ratio	Degeneration cells	% Ratio	Vacuolation cells	% Ratio	Resting cells	% Ratio
0	24	5.4	22.5	1.2	5	4.4	18.3	17.4	72.5
5	21.8	5	17.9	2.6	11.9	5.6	25.7	14.2	65.1
10	19.6	7.8	39.8	2	10.2	6.2	31.6	9.8	50
15	24	10.8	45	3.6	15	7.2	30	9.6	40
20	16.4	7.2	43.9	5.6	34.2	7.2	43.9	3.6	22
25	22	7	31.8	9.5	43.2	11.6	52.7	5.5	25
30	18.2	4.4	24.2	10.2	56.1	13.8	75.8	3.6	19.8

Figure 30

Histograms to illustrate the percentage ratio of cell types of the second ventriculus (resting, vacuolation, degeneration, extrusion) at different ages.

Fig. 30


rest.c
vac.c
deg.c
extr.c
% Ratio
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
0 5 10 15 20 25 30
Age in days

Mononucleate cells appear more frequently in the epithelium of this ventriculus with slight changes in the percentage as shown in Table 7, while Table 8 and Fig. 27 show the decline in the abundance of the nidi or regenerative cells as the insect becomes older. Measurements for the dimensions of cell types and their nuclei and thickness of the striated border are given in Table 9.

Table 7 Average frequency of mononucleate and binucleate of epithelial cells in 2nd. ventriculus in different ages

Age in days	Cells per section	Mononucleate frequency (A)	%	Binucleate frequency (B)	%	B/A Ratio
0	22	19.2	87.3	2.8	12.7	1:6.9
5	14.8	11.9	80.6	2.9	19.4	1:4.2
10	18.6	14.6	78.5	4	21.5	1:3.7
15	20	16.6	83	3.4	17	1:4.9
20	16	13	79.3	3.2	19.5	1:4.1
25	22	18.3	83.3	3.7	16.7	1:5
30	18.2	16	87.9	2.2	12.1	1:7.3

Table 8 Mean number of epithelial cells and nidi per section with percentage of nidi to epithelial cells of different ages in 2nd. ventriculus (± standard error)

Age in days	Mean number of epithelial cells per section (A)	S.E. (±)	Mean number of nidi per section (B)	S.E. (±)	B/A Ratio in %
0	25	± 1.3	4.8	± 0.6	19.2
5	26.5	± 1.9	4	± 0.7	15.1
10	24.8	± 1.1	3.25	± 0.3	13
15	27	± 1.1	3.2	± 0.4	11.9
20	22.5	± 2.3	2.5	± 1	11.1
25	25.5	± 1	2.8	± 0.7	11
30	32.5	± 0.7	2.8	± 0.5	8.5

Table 9 Comparative data on the histology of 2nd. ventriculus in different ages (in µm)

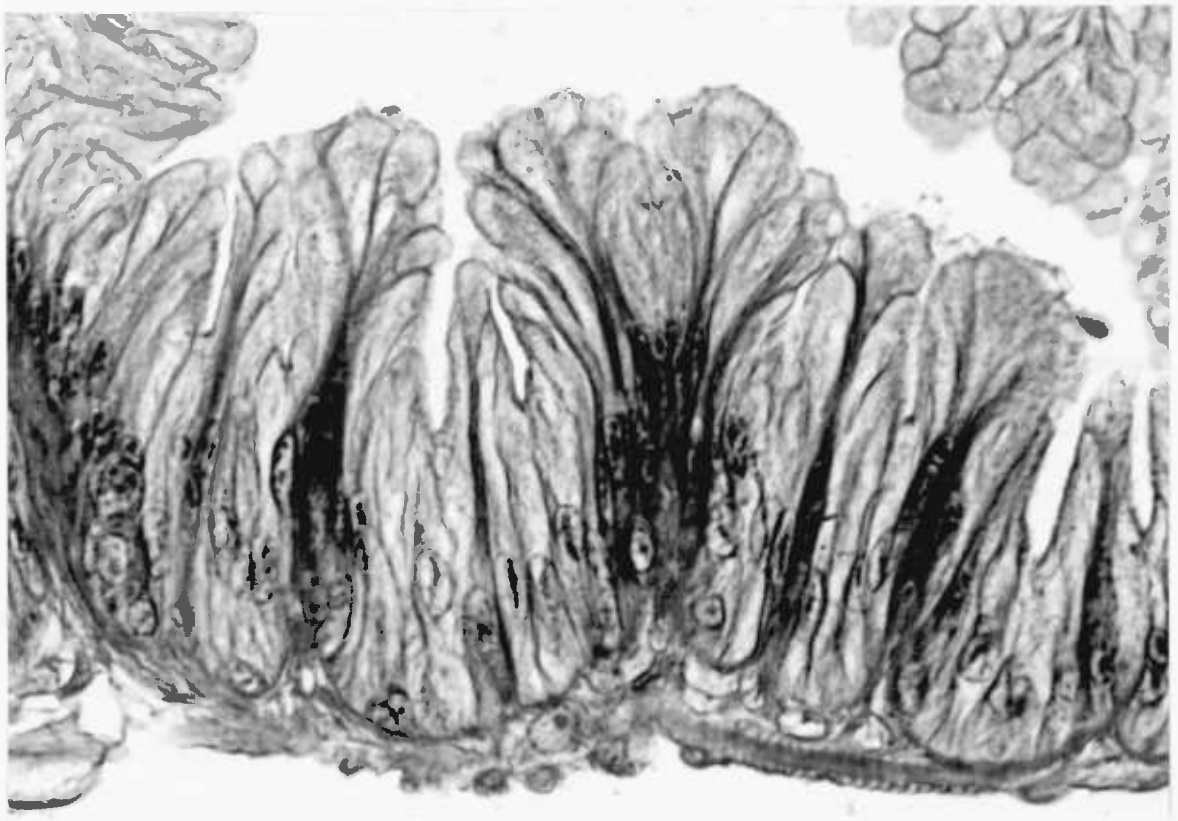
Age in days	Height of villar cells	Height of young cells	Nucleus of villar cells		Nucleus of young cells		Thickness of striated border µm
			Length	Maximum diameter	Length	Maximum diameter	
0	40 - 60	24 - 30	10.3	4.3	6.5	6.3	0.8
5	53 - 66	24 - 30	9.8	4.8	8.6	6.6	0.8
10	43 - 70	28 - 35	9.5	4.6	8.3	6.3	1.6
15	40 - 57	20 - 35	9.7	5.5	7.9	6.3	1.4
20	45 - 58	30 - 40	8.5	4	7.5	5.5	1.3
25	49 - 55	20 - 35	7.7	4	7.5	6	1.3
30	40 - 50	18 - 26	7.5	4.8	7.7	5.8	1.3

3rd. and 5th. ventriculus

These two regions of the midgut are very similar in the types and arrangement of their epithelial cells; they have, therefore, been treated together here.

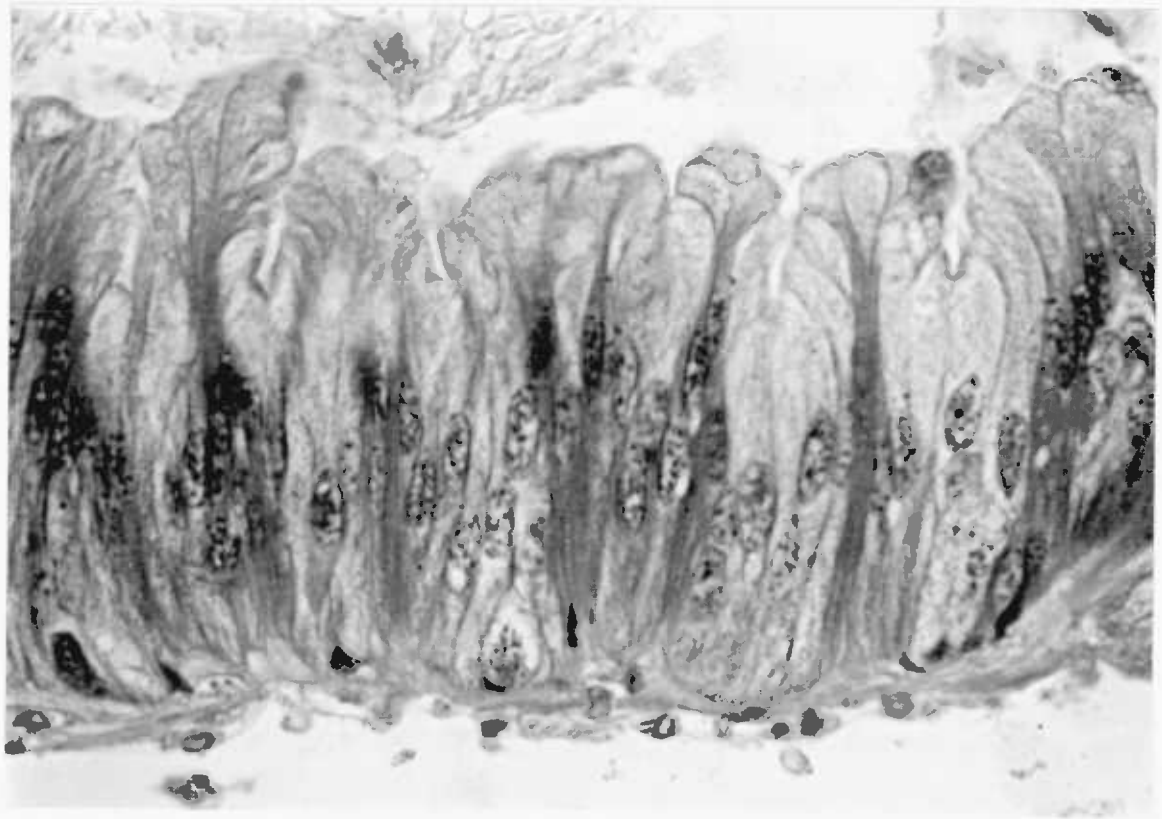
External feature and food content In newly emerged specimens these regions appear as empty, white or transparent parts. In older specimens they become less transparent in appearance having food material in the form of droplets in the 3rd. ventriculus and yellowish-brown particles in the fifth.

Epithelium In newly emerged specimens the epithelium in both regions is dominated by clavate cells and elongate ovate young cells. Due to the folded pattern of the basement membrane the epithelium falls into villi, Fig. 31a and b. The measurements of the cell types of the epithelium and their nuclei with the thickness of the striated border throughout different ages are shown in Tables 10 and 11. The most striking similarity in the epithelium of these two ventriculi is the relatively high proportion of binucleate cells compared with previous two regions of the midgut as shown in Tables 12 and 13. The percentage of nidi found in the epithelium also gradually decreases in older specimens. Comparative data on the nidi are given in Tables 14 and 15 with Fig. 27 and 32 illustrating the mean number of nidi over the mean number of epithelial cells per section. Another characteristic feature of the epithelium here is the common diffusion of materials apically through the striated border of the clavate cells which differs completely from the apical extrusion or blistered appearance usually found in the first ventriculus. The former may represent merocrine secretion, and the latter holocrine secretion. It is, however, difficult to be sure whether these two effects are not different forms of merocrine secretion



A - 3rd. ventriculus

25µm



B - 5th. ventriculus

Figure 31 L.S. newly emerged to adult

Table 10 Comparative data on the histology of the 3rd. ventriculus in different ages (in µm)

Age in days	Height of villar cells µm	Height of young cells µm	Nucleus of villar cells µm		Nucleus of young cells µm		Thickness of striated border µm
			Length	Maximum diameter	Length	Maximum diameter	
0	48 - 55	31 - 40	10	3.4	7.8	6.5	0.4
5	53 - 66	35 - 50	11	5.3	9.5	8	0.5
10	65 - 72	40 - 60	8.6	3.4	10	5	0.5
15	60 - 75	40 - 45	10	4	9.5	5	0.6
20	70 - 76	30 - 45	8.7	2.7	7	5	0.8
25	60 - 67	34 - 47	10	4	7.8	4.6	0.8
30	56 - 64	35 - 45	9	3	7.7	4.3	1

Table 11 Comparative data on the histology of the 5th. ventriculus in different ages (in µm)

Age in days	Height of villar cells µm	Height of young cells µm	Nucleus of villar cells µm		Nucleus of young cells µm		Thickness of striated border µm
			Length	Maximum diameter	Length	Maximum diameter	
0	65 - 70	45 - 50	11.8	2.8	10.2	4.4	0.5
5	60 - 72	40 - 45	9.2	4.2	9.6	5.5	0.4
10	57 - 65	36 - 45	10.6	2.7	9.4	5	0.8
15	45 - 68	39 - 44	11	3	10	6	0.8
20	48 - 66	35 - 40	9.3	3.7	8.5	4.8	0.8
25	58 - 65	33 - 38	9.3	4	9	5	0.8
30	50 - 63	28 - 35	10	4	9	5	1

Table 12 Average frequency of mononucleate and binucleate epithelial cells in 3rd. ventriculus in different ages

Age in days	Cells per section	Mononucleate frequency (A)	%	Binucleate frequency (B)	%	B/A Ratio
0	34.6	27.9	80.7	6.7	19.3	1:4.2
5	23.4	15.2	65	8.2	35	1:1.9
10	40.8	26.4	64.7	14.4	35.3	1:1.8
15	39	25.4	65.13	5.6	34.4	1:4.5
20	19.4	15.2	78.4	4.2	21.6	1:3.6
25	42	32.7	78	9.5	22.5	1:3.5
30	36	28.7	79.8	7.5	20.9	1:3.8

Table 13 Average frequency of mononucleate and binucleate of epithelial cells in 5th. ventriculus in different ages

Age in days	Cells per section	Mononucleate frequency (A)	%	Binucleate frequency (B)	%	B/A Ratio
0	33.6	23.1	68.8	10.5	31.2	1:2.2
5	23.4	15.2	65	8.2	35	1:1.9
10	26.6	19.14	72	7.5	28.1	1:2.6
15	34.4	22.8	66.3	9.6	27.9	1:2.4
20	20.2	15.2	75.3	5	24.8	1:3
25	27	20.1	74.4	6.9	25.6	1:3
30	34.8	26.8	77	8	23	1:3.4

Table 14 Mean number of epithelial cells and nidi per section with percentage of nidi to epithelial cells of different ages in 3rd. ventriculus (± standard error)

Age in days	Mean number of epithelial cells per section (A)	S.E. (±)	Mean number of nidi per section (B)	S.E. (±)	B/A Ratio in %
0	36	± 1.9	4.8	± 0.5	13.3
5	37.5	± 1.9	4.8	± 0.5	12.8
10	38.3	± 0.9	4.3	± 0.2	11.2
15	41.3	± 0.9	4.3	± 0.2	10.3
20	42	± 1.4	3.5	± 0.2	8.3
25	42.5	± 1.6	2.3	± 0.3	5.3
30	41.7	± 1.2	2	± 0.3	4.8

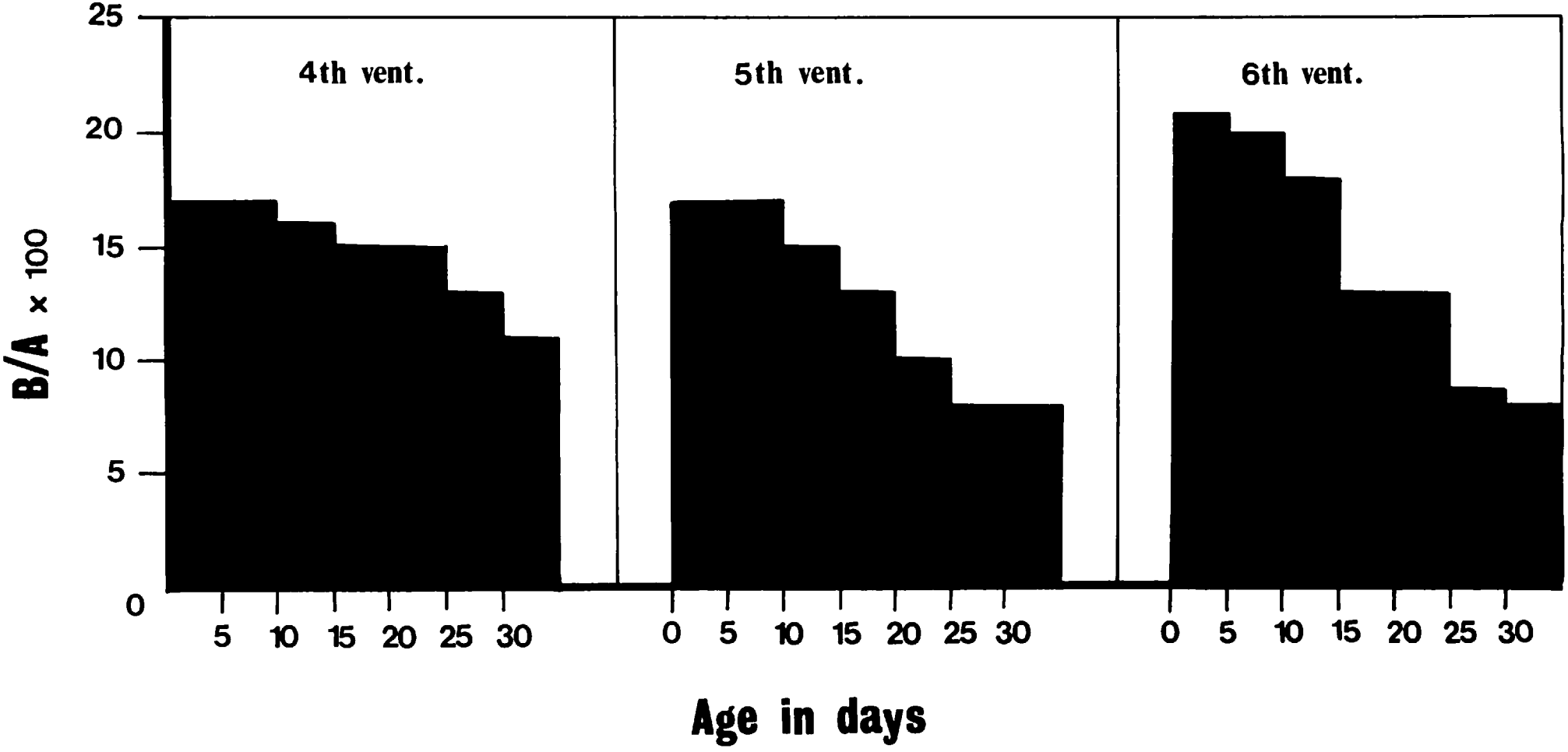
Table 15 Mean number of epithelial cells and nidi per section with percentage of nidi to epithelial cells of different ages in 5th. ventriculus (± standard error)

Age in days	Mean number of epithelial cells per section (A)	S.E. (±)	Mean number of nidi per section (B)	S.E. (±)	B/A Ratio in %
0	24.2	± 1.1	4.2	± 0.5	17.4
5	28.3	± 2	4.7	± 1.4	16.6
10	34.2	± 1.3	5	± 0.5	14.6
15	37.5	± 1.1	5	± 0.5	13.3
20	48.3	± 2.1	5	± 0.5	10.4
25	31.7	± 1.3	2.6	± 0.4	8.2
30	21	± 0.9	1.8	± 0.4	8.3

Figure 32

Histograms to represent the percentage of the mean number of nidi per section to the mean number of epithelial cells per section at progressive ages in the 4th, 5th and 6th ventriculus.

Fig. 32

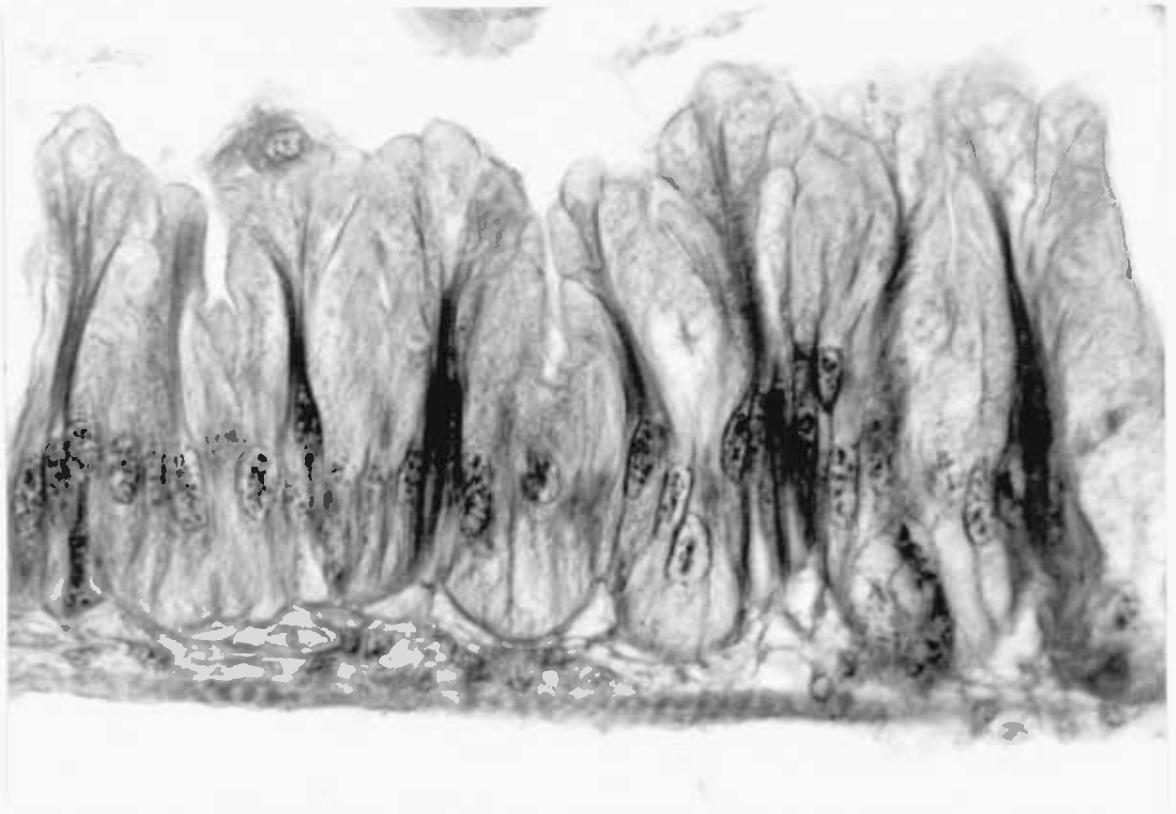

4th vent.
5th vent.
6th vent.
B/A x 100
25
20
15
10
5
0
0 5 10 15 20 25 30
Age in days

since whether the second represent holocrine secretion depends on whether there is eventual disruption of the whole cell. Generally speaking the epithelia of the 3rd. and 5th. ventriculi are similar in their cellular types and arrangement and seem to undergo similar histological changes during the life span of the adult. The changes are, however, very slight compared with other parts of the ventriculus. Figure 33a and b represents the epithelium of 10 day old specimens of the 3rd. and 5th. ventriculi respectively. The epithelium at this age appears to be made up of crowded villi composed of laterally compressed clavate cells with elongate young cells at the sides of the villi. As the insect grows older the epithelium shows rather fewer resting cells and more degenerating cells. In 30 day old specimens the epithelium appears to consist of almost collapsed villi with degenerating cells(which are more prominent in the 3rd. ventriculus), and clavate cells which are clumped on each other with relatively large numbers of scattered small vacuoles throughout their cytoplasm. Figure 34a and b and Tables 16 and 17 illustrate the different types of cells, and see also Figs. 35 and 36.

4th. ventriculus

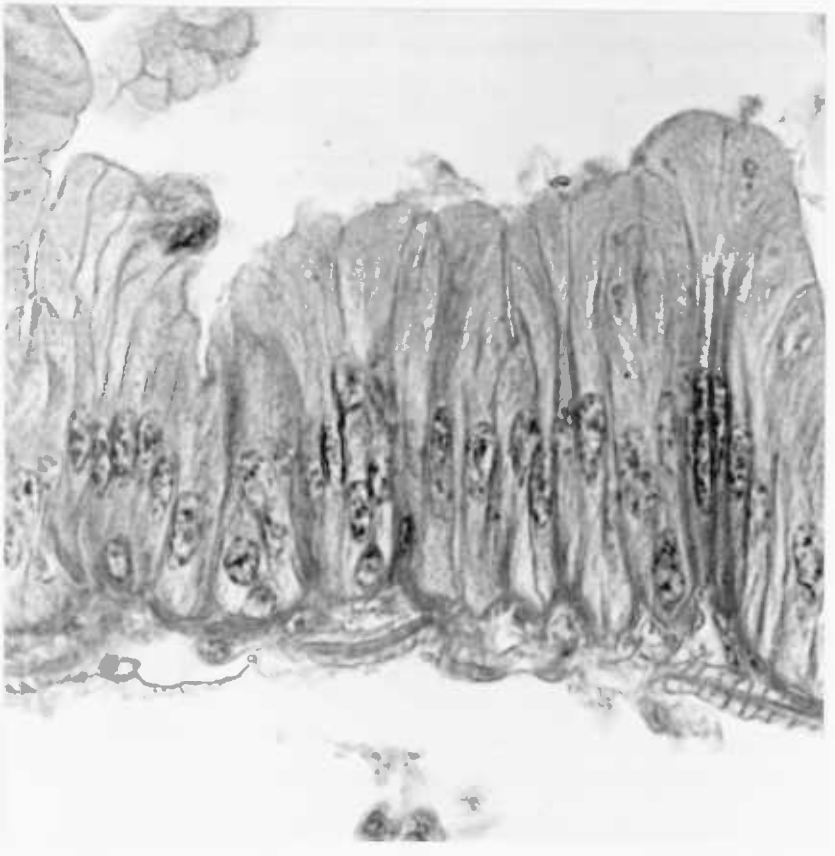
External features and food content This region appears as a transparent structure filled with yellowish pasty food material mixed with oily droplets in newly emerged specimens. In older specimens the oily substance disappears and the food mass is a yellowish and less concentrated material.

Epithelium The epithelium of the fourth ventriculus was found to undergo the least amount of change during the different ages studied here. In specimens 0 - 15 days old the epithelium is almost unaltered having the basement membrane slightly folded to form low villi which are taller at either ends of this region (Fig. 37). All measurements of cellular types, lengths and nuclei with thickness of striated border are shown in Table 18,



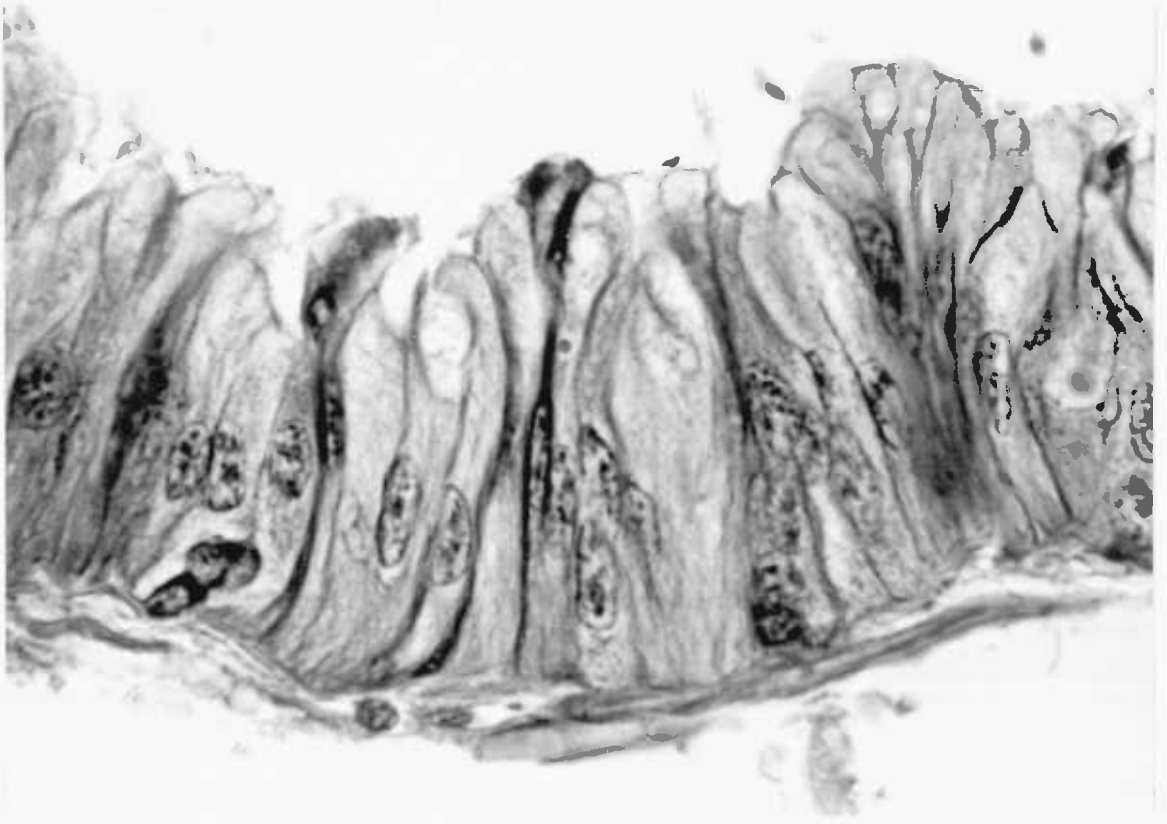
A - 3rd ventriculus

25µm



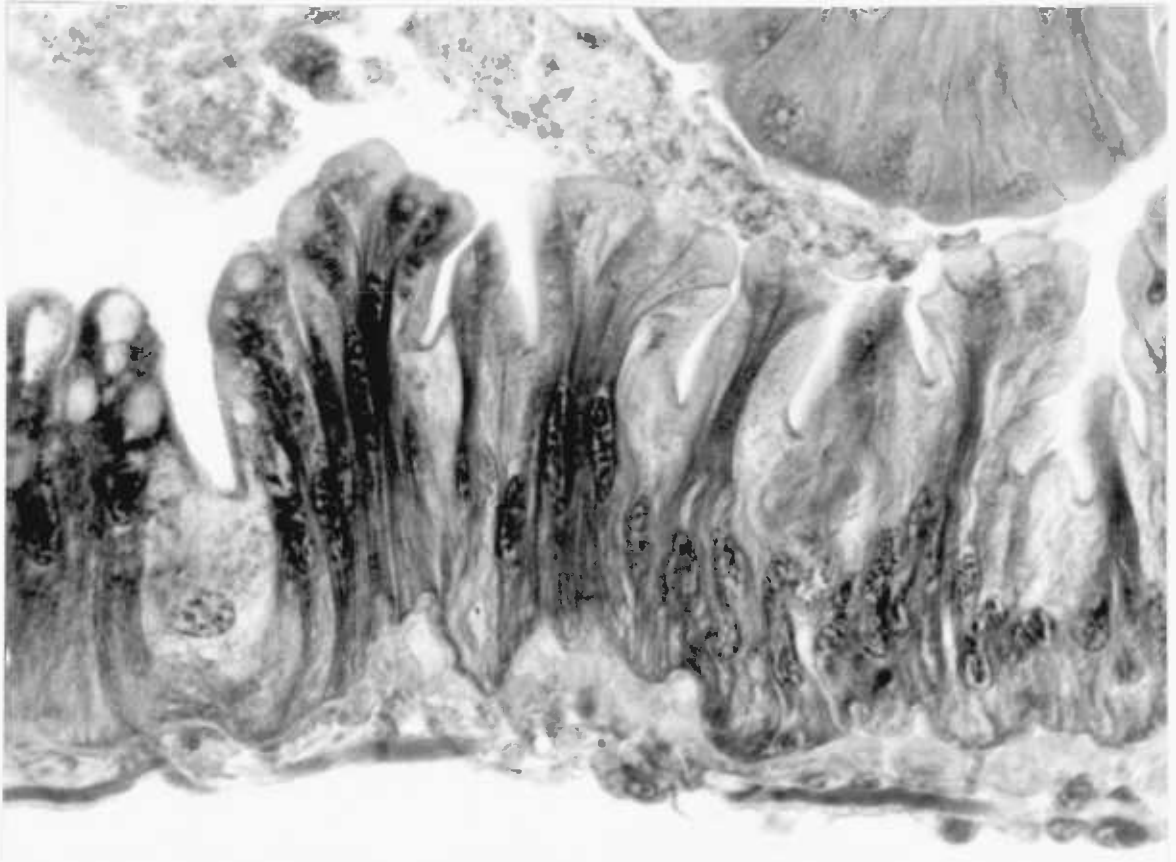
B - 5th ventriculus

Figure 33 A and B L.S., 10 days old



A - 3rd. ventriculus

25µm



B - 5th. ventriculus

Figure 34 A and B, 30 days old

Table 16 Comparative data and percentage ratio of cell types in midgut epithelium of 3rd. ventriculus in different ages

Age in days	Epithelial cells per section	Extrusion cells	% Ratio	Degeneration cells	% Ratio	Vacuolation cells	% Ratio	Resting cells	% Ratio
0	34.6	3.4	9.8	2.8	8.1	1.6	4.6	28.4	82.1
5	23.4	2.4	10.3	1.3	5.6	1.2	5.1	19.7	84.2
10	40.8	4.8	11.8	3.8	9.3	2	4.9	32.2	78.9
15	39	3	7.7	4.6	11.8	3	7.7	31.4	80.5
20	19.4	2.2	11.3	3.5	18.0	2.6	13.4	13.7	70.6
25	42	2.8	6.7	5.2	12.4	4.4	10.5	34	80.9
30	36	3.2	8.9	8.8	24.4	3.4	9.4	24	66.7

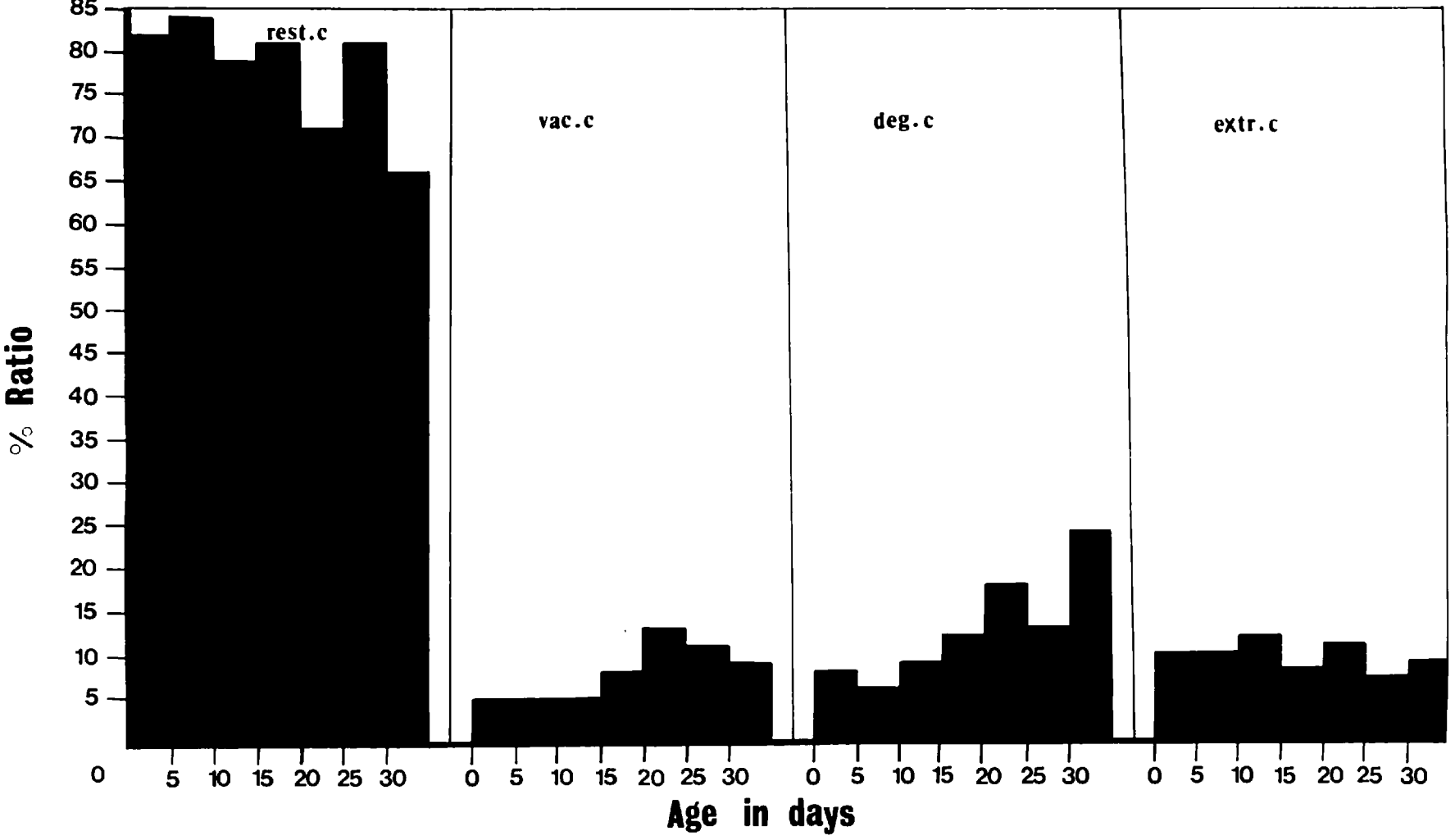
Table 17 Comparative data and their percentage ratio of cell types in midgut epithelium in different ages of 5th. ventriculus

Age in days	Epithelial cells per section	Extrusion cells	% Ratio	Degeneration cells	% Ratio	Vacuolation cells	% Ratio	Resting cells	% Ratio
0	33.6	4.2	12.5	1.8	5.4	1.6	4.8	7.6	82.1
5	23.4	2.4	10.3	2.4	10.3	1.2	5.1	18.6	79.5
10	26.6	2	7.5	2.8	10.5	1.4	5.3	21.8	82
15	34.6	5	14.5	4	11.6	2.6	7.5	25.6	74
20	20.2	4.2	20.8	2.3	11.4	2.2	10.9	13.7	67.8
25	27.6	6	21.7	3.8	11.8	3.5	12.7	17.8	64.5
30	34.8	6.4	18.4	5.6	16.1	3.4	9.8	22.8	65.5

Figure 35

Histograms to illustrate the percentage ratio of cell types of the third ventriculus at different ages.

Fig. 35


rest.c
vac.c
deg.c
extr.c
% Ratio
85
80
75
70
65
60
55
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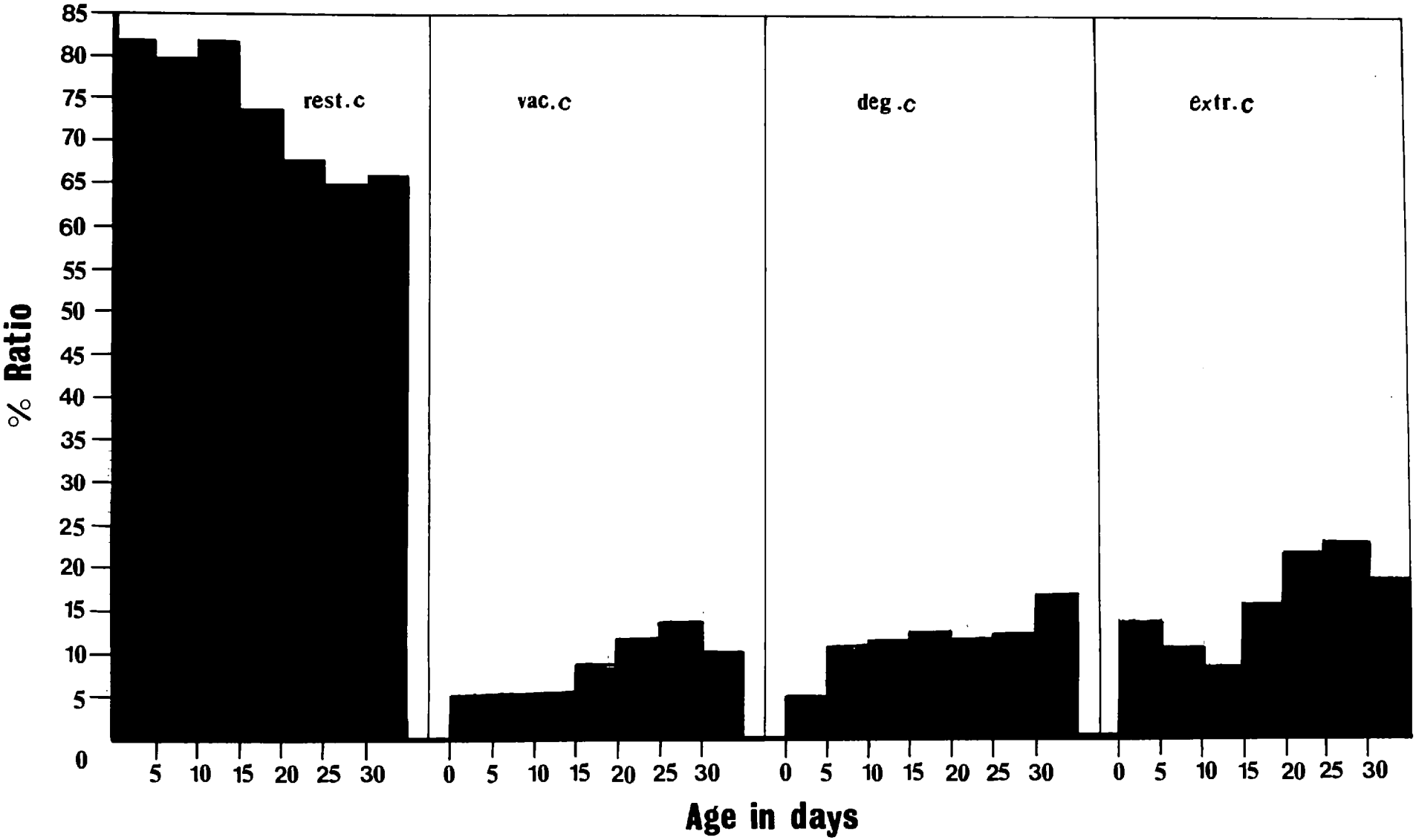
45
40
35
30
25
20
15
10
5
0
0 5 10 15 20 25 30
Age in days

Figure 36

Histograms to illustrate the percentage ratio of cell types of the fifth ventriculus at different ages.

Fig. 36


% Ratio
85
80
75
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rest.c
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5 10 15 20 25 30
0 5 10 15 20 25 30
0 5 10 15 20 25 30
0 5 10 15 20 25 30
Age in days

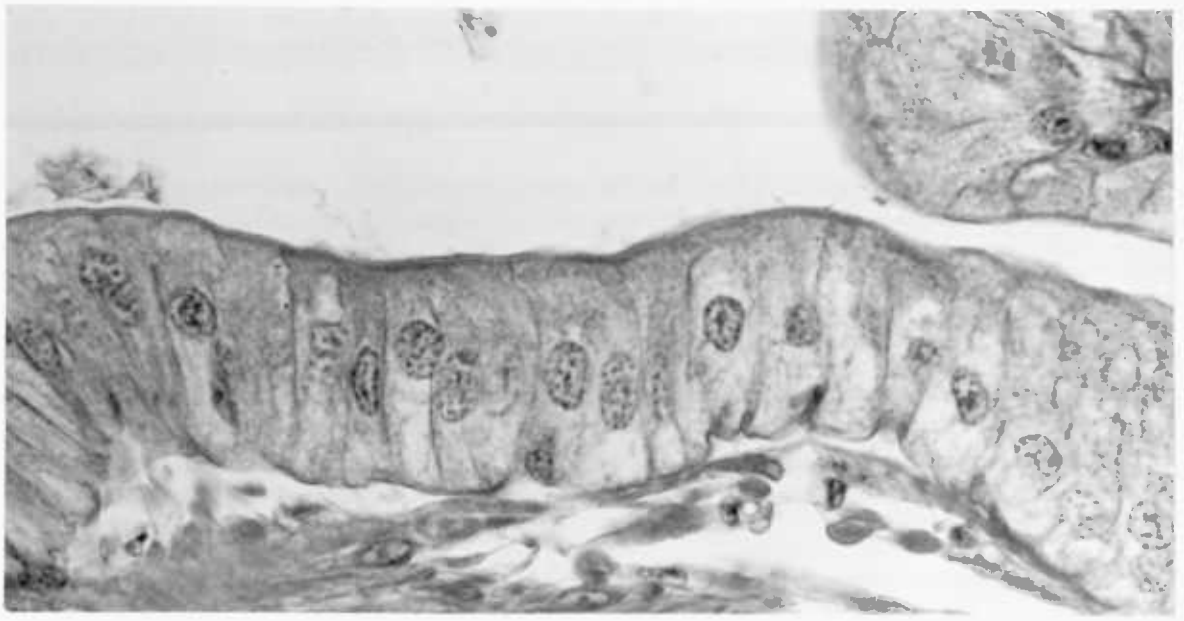


Figure 37 4th. ventriculus, 10 days old

25 µm

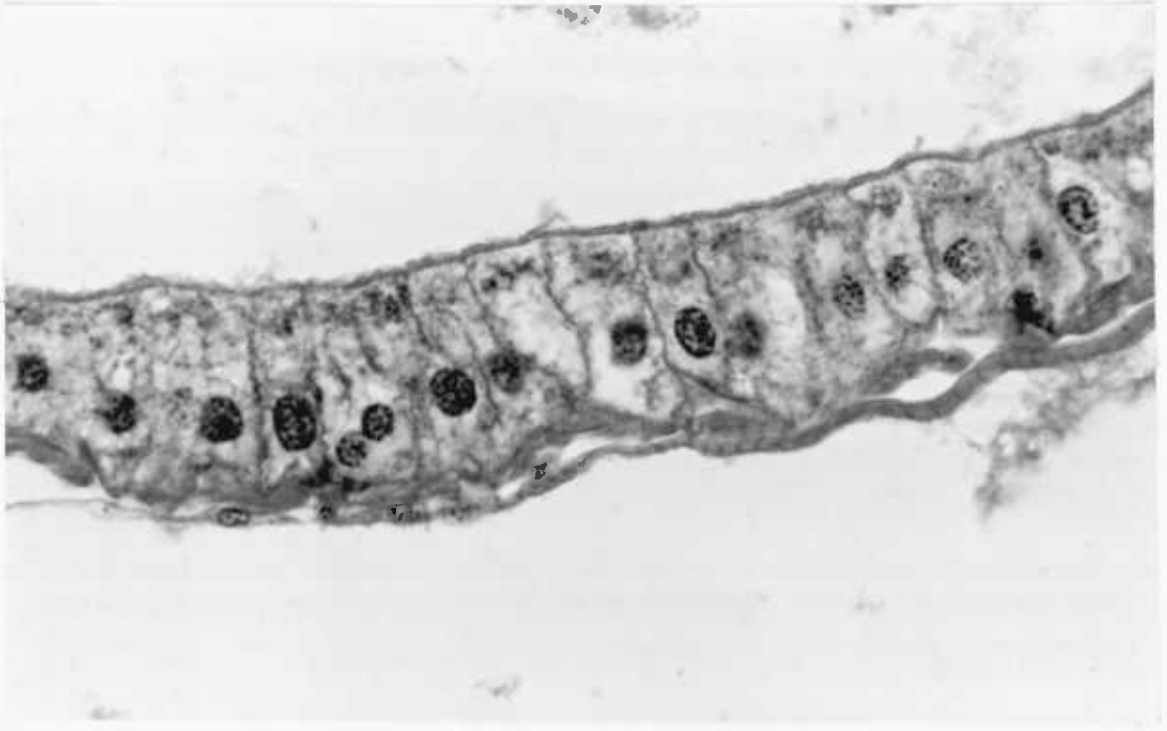


Figure 39 4th. ventriculus, 30 days old

Table 18 Comparative data on the histology of the 4th. ventriculus at different ages (in µm)

Age in days	Height of villar cells µm	Height of young cells µm	Nucleus of villar cell µm		Nucleus of villar cell µm		Thickness of striated border µm
			Length	Maximum diameter	Length	Maximum diameter	
0	33 - 40	20 - 27	10	4.7	8.2	5.6	1.8
5	47 - 52	30 - 35	10	5	7.9	5.3	1.1
10	45 - 57	35 - 47	9.3	6.4	6.9	5.8	1.5
15	45 - 50	22 - 34	8.8	5.5	12	6	1.5
20	45 - 49	32	7	3	14	11.3	2
25	40 - 45	18 - 29	9	4	8	6	1
30	33 - 35	20 - 25	7	5	7	6.5	1.6

while comparative data of the frequency of both mononucleate and binucleate cells in the epithelium throughout the life span are given in Table 19. The ratio of cell types in the epithelium does not vary greatly during the older stages as shown in Table 20 and Fig. 38. Figure 39 represents the epithelium of this segment in 30 day old specimens and shows that young cells are less differentiated and the epithelium is generally more vacuolated. The percentage of nidi in the epithelia decreases as shown in Table 21 and Fig. 32.

Table 19 Average frequency of mononucleate and binucleate epithelial cells in 4th. ventriculus at different ages

Age in days	Cells per section	Mononucleate frequency (A)	%	Binucleate frequency (B)	%	B/A Ratio
0	15	11.8	78.3	3.3	21.7	1:3.6
5	13	10.4	80.2	2.6	19.9	1:4
10	16.4	12.8	78	3.6	22	1:3.6
15	14.8	12	81	2.8	18.8	1:4.3
20	14.4	11.5	79.5	3	20.5	1:3.9
25	27	21	77.4	6.1	22.6	1:3.4
30	15.8	12.7	80.2	3.1	19.8	1:4

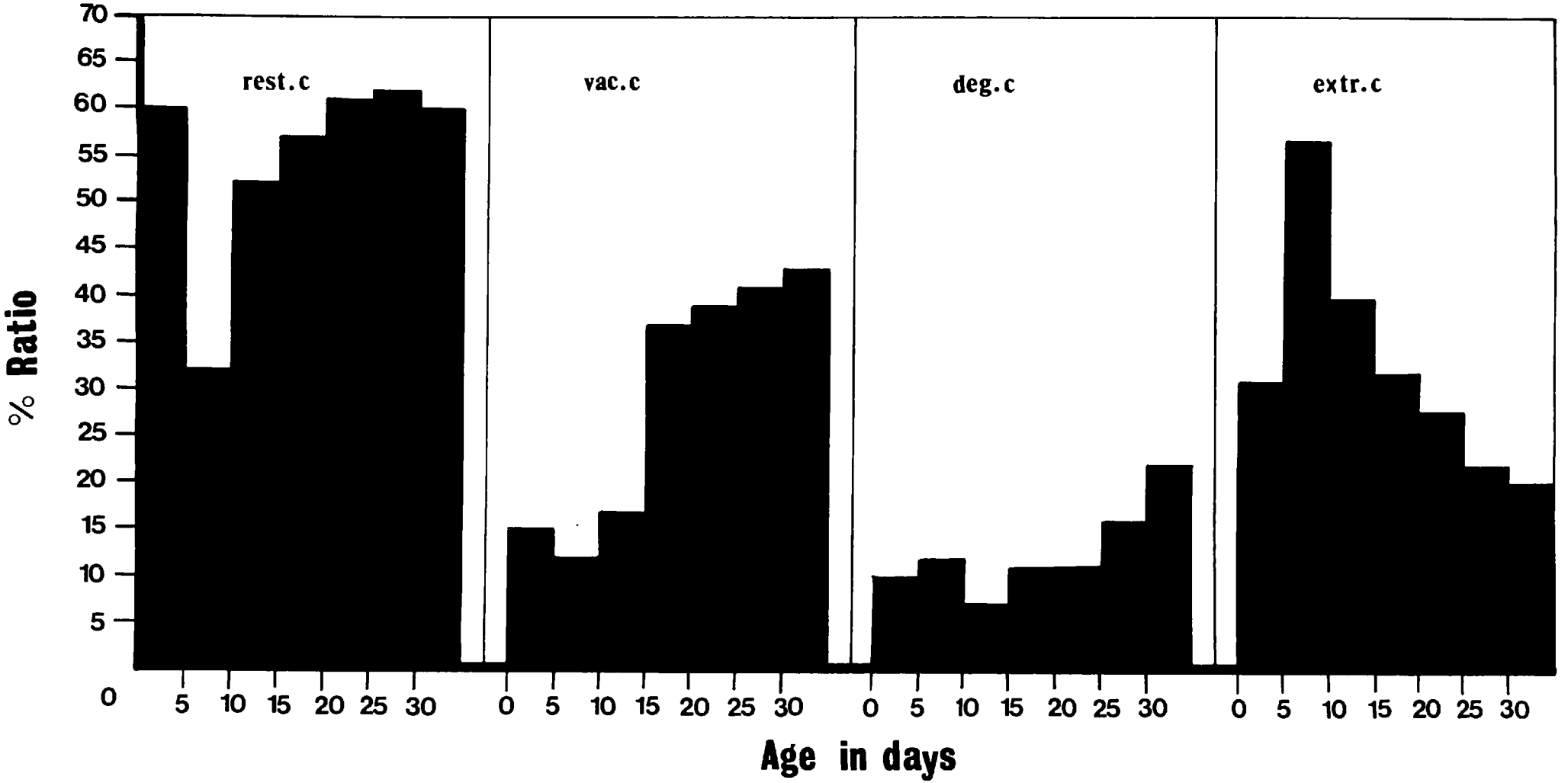
Table 20 Comparative data and percentage ratio of cell types in midgut epithelium of 4th. ventriculus at different ages

Age in days	Epithelial cells per section	Extrusion cells	% Ratio	Degeneration cells	% Ratio	Vacuolation cells	% Ratio	Resting cells	% Ratio
0	16.4	5	30.5	1.6	9.8	2.4	14.6	9.8	59.8
5	13	7.4	57	1.5	11.5	1.5	11.5	4.1	31.5
10	16.4	6.6	40	1.2	7.3	2.7	16.5	8.6	52.4
15	15.8	5	31.7	1.8	11.4	5.8	36.7	9	57
20	14.4	4	27.9	1.6	11.1	5.6	38.9	8.8	61.1
25	27	6	22.2	4.4	16.3	11	40.7	16.6	61.5
30	15.8	3	19	3.4	21.5	6.8	43	9.4	59.5

Figure 38

Histograms to illustrate the percentage ratio of cell types of the fourth ventriculus at different ages.

Fig. 38


rest.c
vac.c
deg.c
extr.c
% Ratio
70
65
60
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15
10
5
0
0 5 10 15 20 25 30
Age in days

Table 21 Mean number of epithelial cells and nidi per section with percentage of nidi to epithelial cells in 4th. ventriculus at different ages (± standard error)

Age in days	Mean number of epithelial cells per section (A)	S.E. (±)	Mean number of nidi per section (B)	S.E. (±)	B/A Ratio in %
0	17.3	± 1	3	± 0.2	17.4
5	14	± 0.5	2.4	± 0.2	17.1
10	20.5	± 0.8	3.3	± 0.4	15.9
15	15.3	± 0.8	2.3	± 0.3	14.7
20	17.3	± 0.6	2.5	± 0.5	14.5
25	16.4	± 0.8	2.2	± 0.4	13.4
30	26.6	± 1.3	3	± 0.5	11.3

6th ventriculus

This region has been considered by previous workers as the pylorus or ileum, but is clearly recognisable in the present work as the posterior region of the midgut on the basis of the similarity of the structure and behaviour of its epithelium with that of the rest of the midgut. This region is more or less trapezoidal in shape and white or transparent in colour becoming creamy in older specimens containing particles of food material.

Epithelium The epithelium is dominated by tall columnar cells with a thin striated border in newly emerged specimens (Fig. 40). Some of the cells appear to be undergoing a degenerative process while regenerative cells are frequently present between the bases of the epithelial cells singly or in groups of 2 or 3. Their percentage seems to decline through the life span of the adult insect as shown in Table 22. Figure 32 represent the histograms through progressive ages of the 6th. ventriculus. The epithelium is more compact in older insects and due to lateral pressure the cells have their apical ends rounded with a thin striated border. Young cells stain less basally and have centrally located nuclei (Fig. 41). The frequency of binucleate cells throughout the different ages is relatively higher than in the previous region (Table 23) but the epithelium is still dominated by mononucleate cells generally. Figure 42 represents the epithelium of this segment in 25 and 30 day old specimens respectively, where the tall columnar cells appear with more protruding apical ends. The young cells are not well differentiated. Epithelial cells with few vacuoles and degenerating cells are more frequently present. Comparative data on

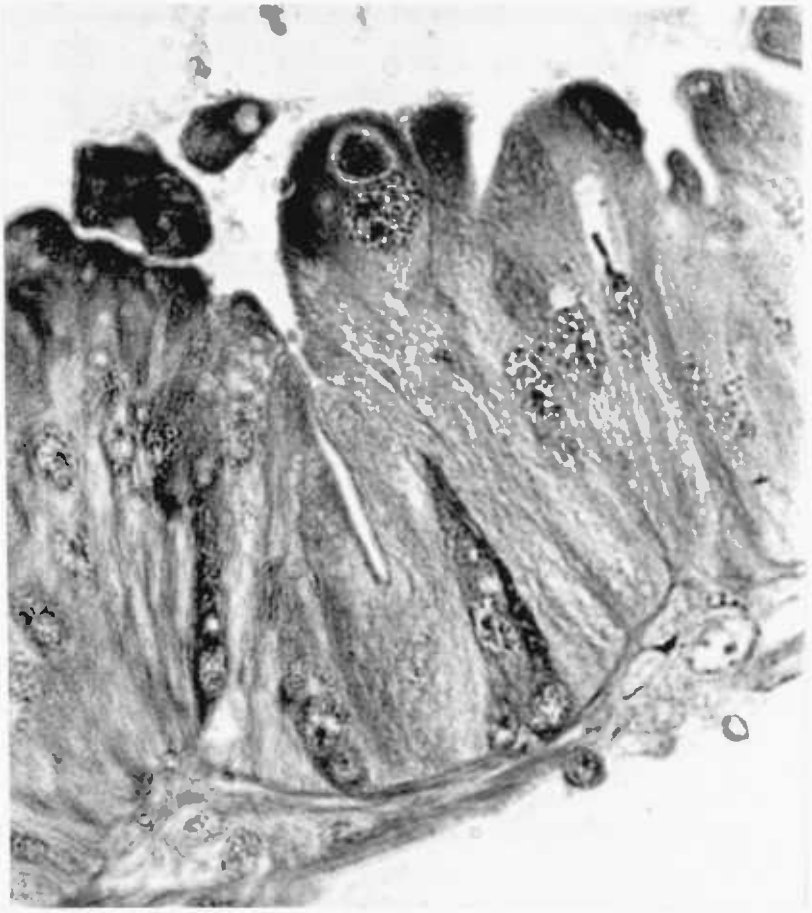


Figure 40 L.S. 6th. ventriculus, newly emerged to adult

25µm

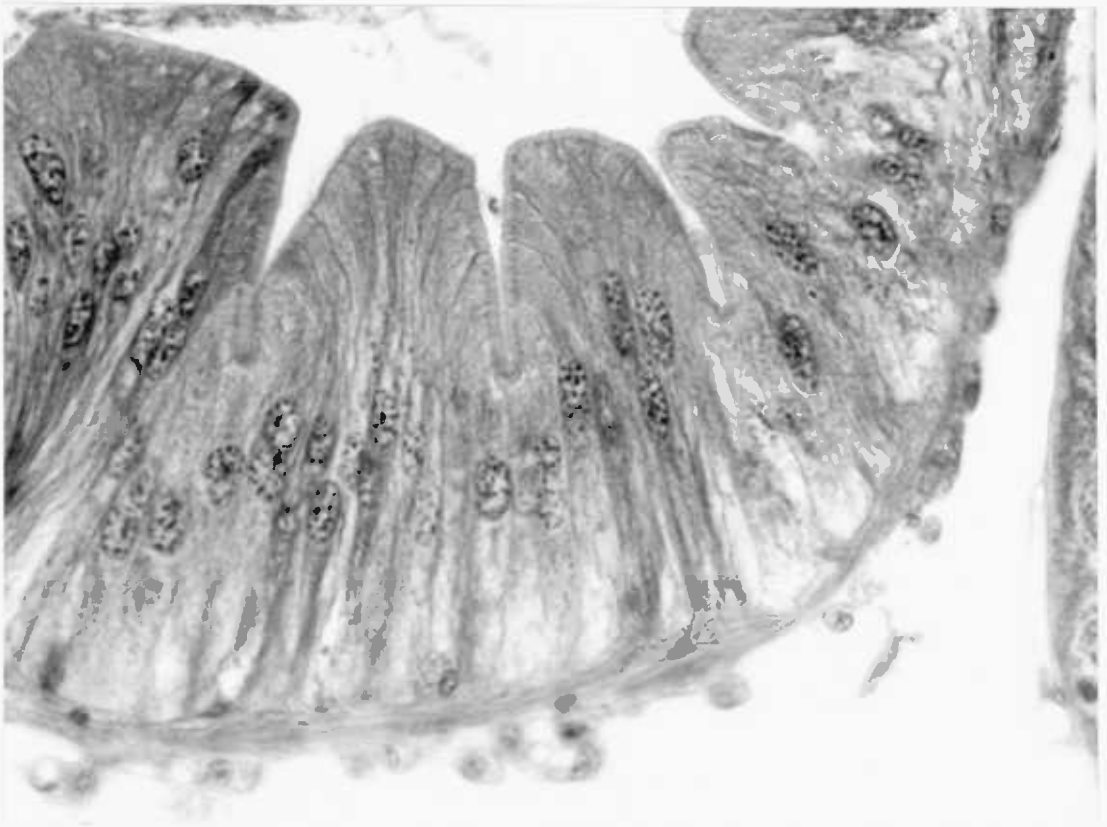


Figure 41 T.S. 6th. ventriculus, 15 days old

Table 22 Mean number of epithelial cells and nidi per section with percentage of nidi to epithelial cells in 6th. ventriculus at different ages (± standard error)

Age in days	Mean number of epithelial cells per section (A)	S.E. (±)	Mean number of nidi per section (B)	S.E. (±)	B/A Ratio in %
0	18.8	± 0.6	4	± 0.3	21.3
5	19.4	± 0.5	4.2	± 0.3	19.8
10	22.5	± 1.3	4	± 0.2	17.8
15	33.3	± 1.8	4.3	± 0.1	12.9
20	48	± 2.3	6	± 0.5	12.6
25	32.4	± 1.5	3	± 0.2	9.3
30	21	± 0.3	1.8	± 0.4	8.3

Table 23 Average frequency of mononucleate and binucleate of epithelial cells in 6th. ventriculus in different ages

Age in days	Cells per section	Mononucleate frequency (A)	%	Binucleate frequency (B)	%	B/A Ratio
0	21	12	57	9	42.9	1:1.3
5	18.4	11	59.8	7.4	40.2	1:1.5
10	22.6	14.8	65.5	7.8	34.5	1:2
15	28	18.6	66.4	9.4	33.6	1:2
20	18.8	12.30	65.4	6.5	34.7	1:1.9
25	15.8	10.4	65.8	5.62	35.4	1:1.9
30	18	12.1	67	5.94	33	1:2

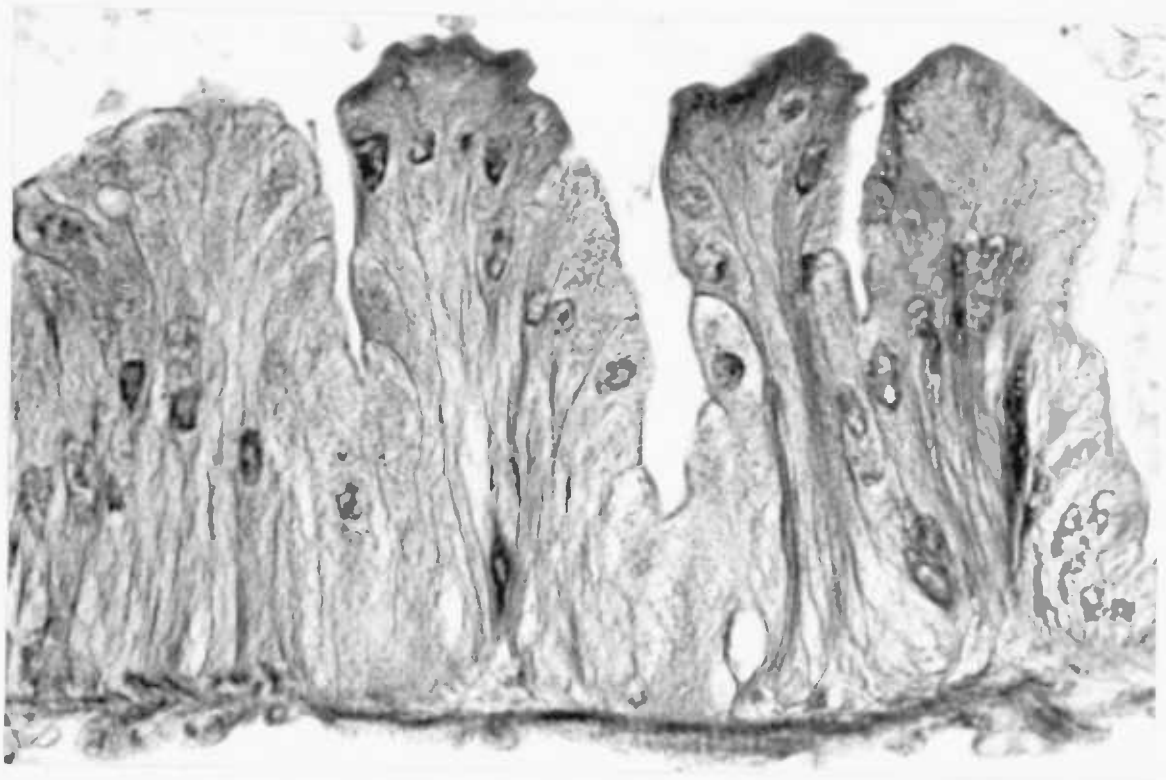
Table 24 Comparative data and their percentage ratio of cell types in midgut epithelium of 6th. ventriculus at different ages

Age in days	Epithelial cells per section	Extrusion cells	% Ratio	Degeneration cells	% Ratio	Vacuolation cells	% Ratio	Resting cells	% Ratio
0	21	1.8	8.6	1.2	5.7	1.4	6.7	18	85.7
5	18.4	1.4	7.6	2	10.9	1.6	8.7	15	81.5
10	22.6	2.2	10.6	1.4	6.2	1.6	7.1	18.8	83.2
15	28	4.2	15	1.8	6.4	1.9	6.8	22	78.6
20	18.8	5.4	28.7	2.4	12.8	2.4	12.8	11	58.5
25	15.8	5.4	34.2	3.4	21.5	2.4	15.2	7	44.3
30	18	6	33.3	3.6	20	2.4	13.3	8.4	46.7

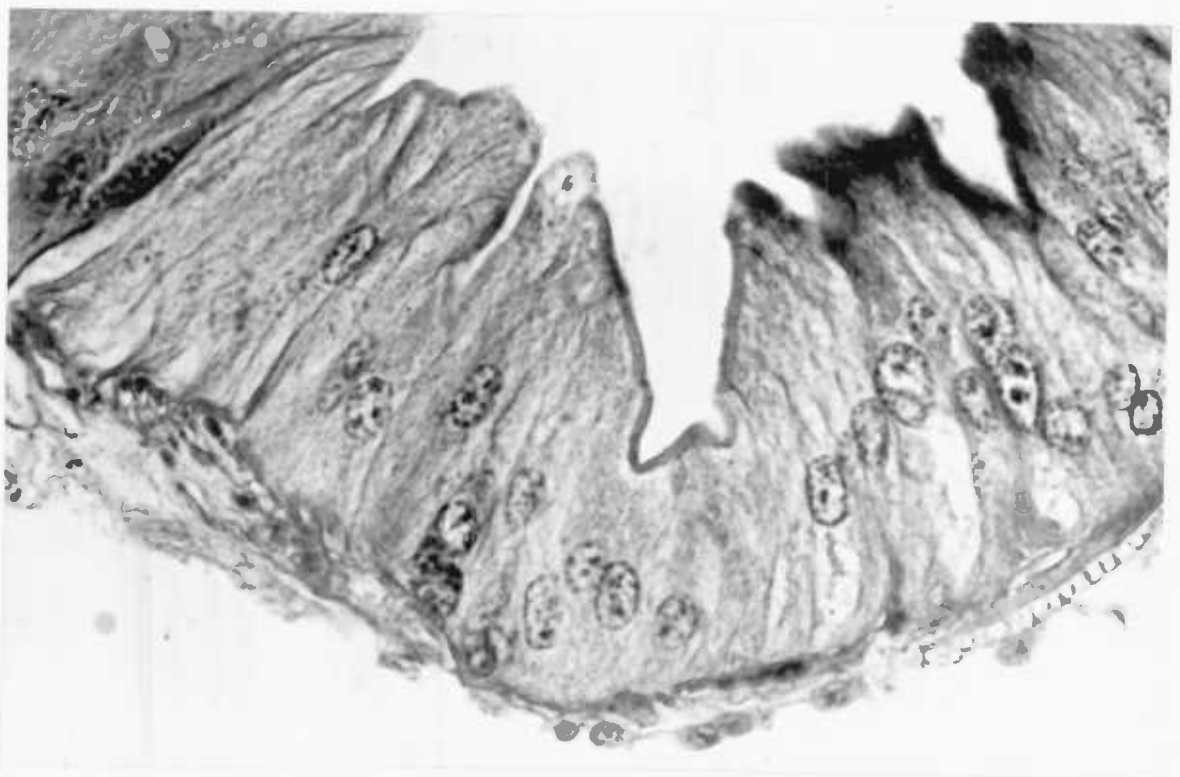
Table 25 Comparative data on the histology of 6th. ventriculus at different ages (in µm)

Age in days	Height of villar cells µm	Height of young cells µm	Nucleus of villar cells µm		Nucleus of young cells µm		Thickness of striated border µm
			Length	Maximum diameter	Length	Maximum diameter	
0	40 - 50	45 - 50	6.5	4.5	6.8	5	0.4
5	53 - 60	40 - 45	9.4	4.8	6.7	5.7	0.8
10	55 - 70	36 - 45	11.5	6.5	7.7	5.7	1
15	50 - 60	39 - 44	9	4	6	5.3	0.5
20	40 - 60	35 - 40	9	2	8.6	6	1
25	50 - 60	33 - 38	8.5	4.5	8	5.5	1.3
30	34 - 57	28 - 35	8	4	8.9	6	1.3

the cell types and their percentages are shown in Table 24 and the histograms in Fig. 43 illustrate the position of cell types through progressive ages. All measurements of the cells and their nuclei, with the thickness of the striated border, are given in Table 25.



A. 25 days old 25µm



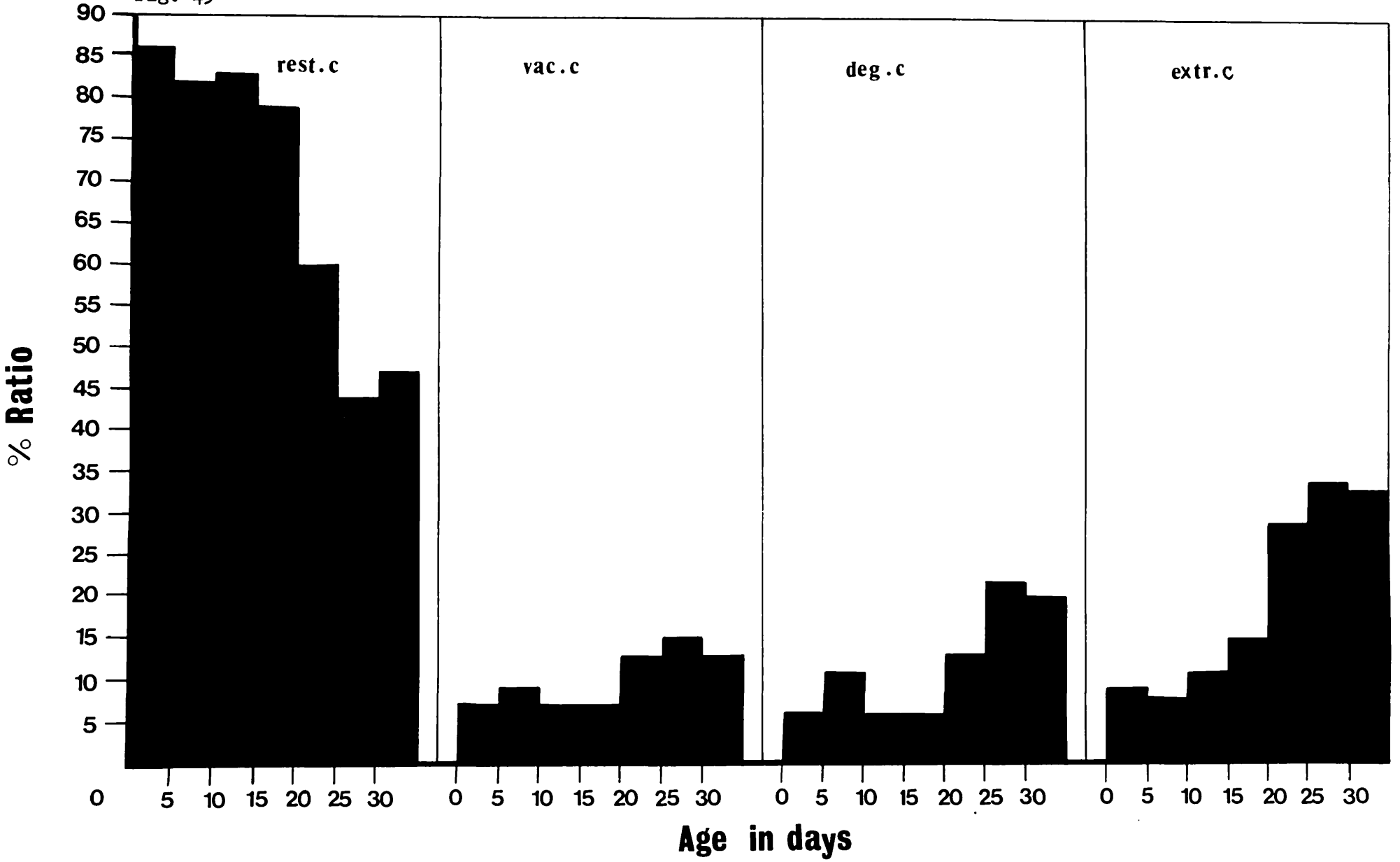
B. 30 days old

Figure 42 A and B L.S. 6th. ventriculus

Figure 43

Histograms to illustrate the percentage ratio of cell types (resting, vacuolation, degeneration, extrusion) of the sixth ventriculus at different ages.

Fig. 43


rest.c
vac.c
deg.c
extr.c
% Ratio
90
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35
30
25
20
15
10
5
0 5 10 15 20 25 30
Age in days

GENERAL CONCLUSION

When the data of this investigation are compared the following remarks may be derived.

1. There is no line of sharp demarcation between the different segments of the midgut in the shape, arrangement and behaviour of the epithelium at various postmetamorphic stages.

2. The epithelia of the segments of the midgut differ in the variation and proportions of different cell types and their behaviour throughout the life span of the adult specimens.

3. The first ventriculus exhibits large differences in shape of the cell types and behaviour of the epithelium throughout the life of adult specimens and this may suggest that this segment plays the most important role of secretion and digestion.

4. Generally, the 3rd, 5th and 6th ventriculus are similar in many aspects of their cell types and the behaviour of the epithelium throughout different ages. This may as well suggest that these 3-segments perform similar functions.

5. One of the striking points is that vacuolation rises rapidly with age in the epithelium of the first and second segments (Fig. 44). This phenomena is parallel to the relatively high percentage of degenerating cells (Fig. 45). Very little alteration occurs in the percentage of vacuolated and degenerating cells in the 3rd, 5th and 6th ventriculus.

Figure 44

The percentage of vacuolation cells to epithelial cells per section in midgut segments at different ages.

Fig. 44

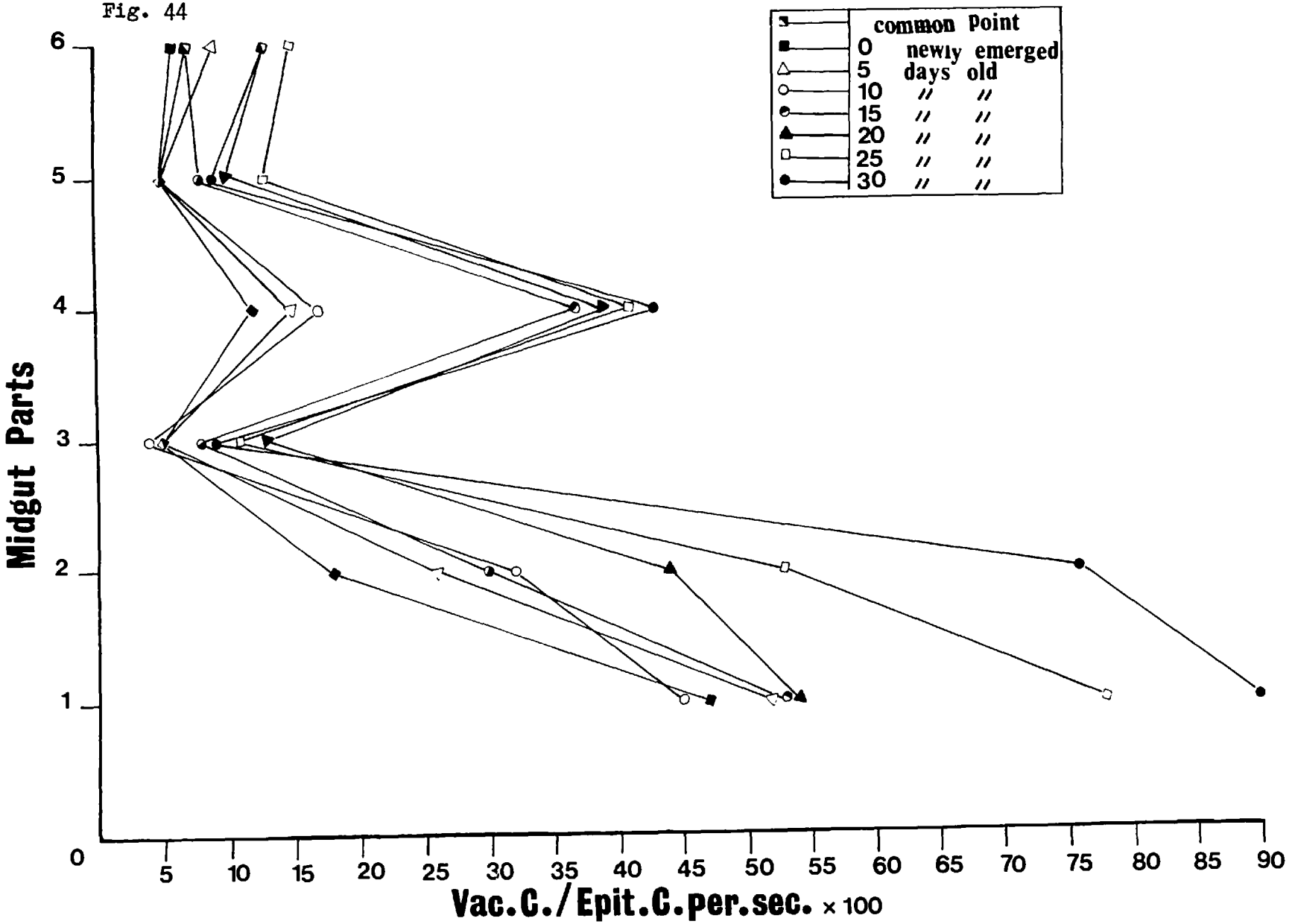
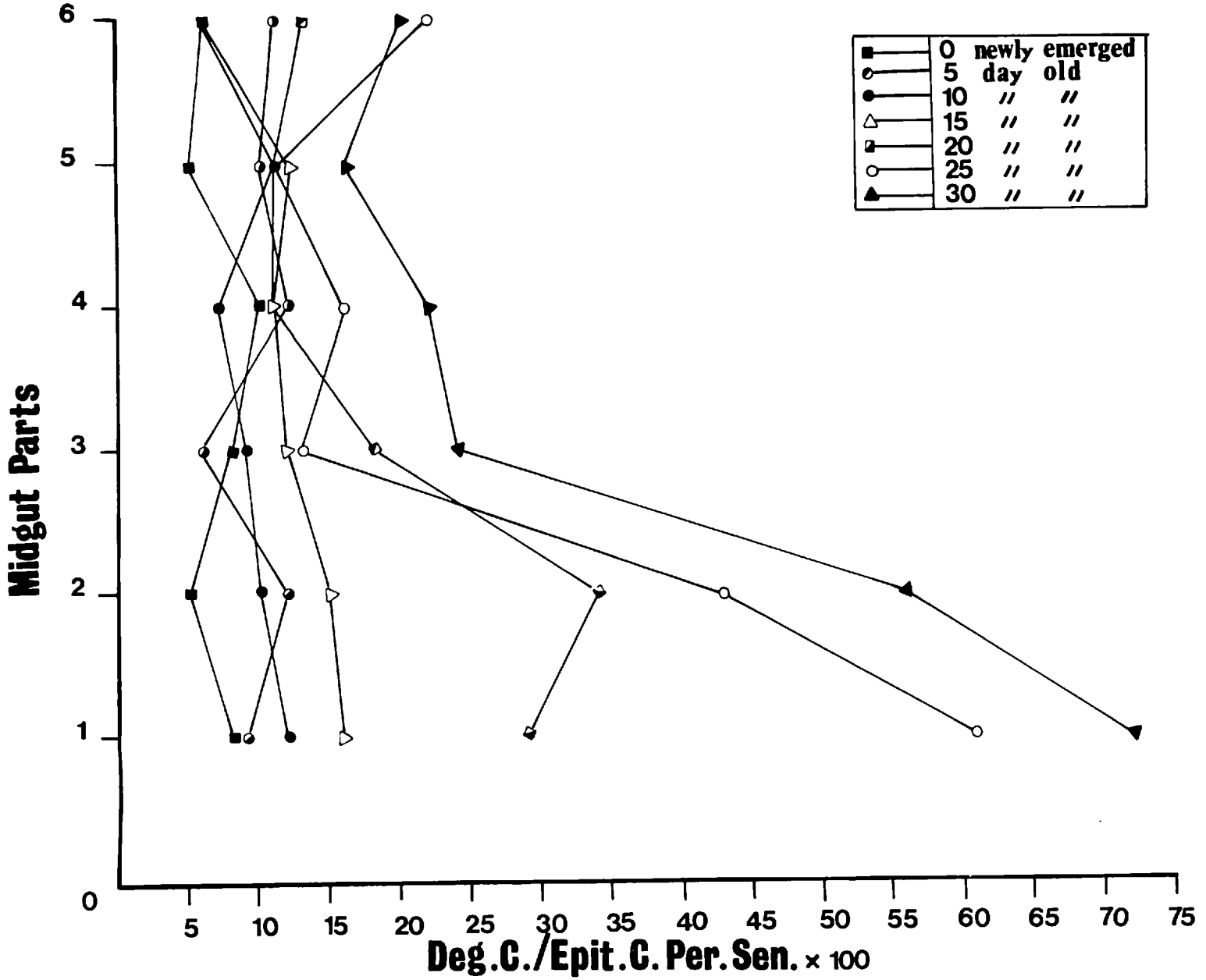

common Point
0 newly emerged
5 days old
10 // //
15 // //
20 // //
25 // //
30 // //
Midgut Parts
0
1
2
3
4
5
6
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Vac.C./Epit.C.per.sec. x 100

Figure 45

The percentage of degeneration cells to epithelial cells per section in midgut segments at different ages.

Fig. 45


Midgut Parts
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40
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50
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70
75
Deg.C./Epit.C. Per. Sen. x 100
0 newly emerged
5 day old
10 // //
15 // //
20 // //
25 // //
30 // //

6. When the percentage ratios of the extrusion cells of all six segments are compared throughout life this shows that the 1st, 2nd and 4th segments are almost alike while the 3rd segment has the least amount of change (Fig. 46). Those of both of 5th and 6th segments are intermediate in this respect.

7. The percentage of resting cells in the different segments of the midgut shows that resting cells decline rapidly with age in the 1st and 2nd segments; those of the 3rd and 5th ventriculus are almost alike while the percentage of resting cells in both 4th and 6th are intermediate between the two extremes (Fig. 47).

8. The percentage of nidi to epithelial cells in different segments of the midgut is higher during progressive ages in the first ventriculus; this may be because a higher proportion of activity takes place in the first ventriculus (Fig. 48).

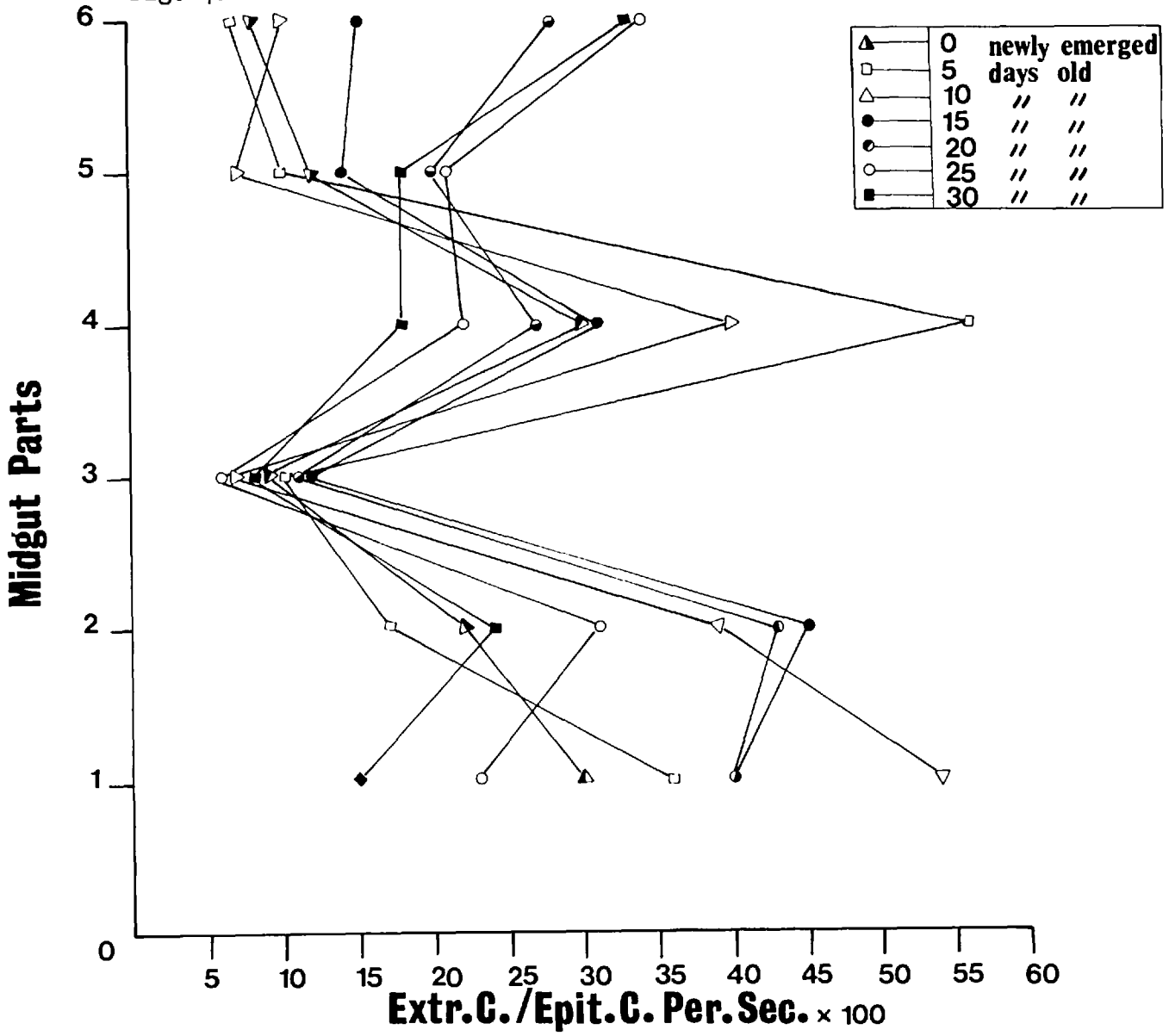
9. It appears that the epithelium is dominated by uninucleate cells throughout the life span of the adult in different segments. This fact is illustrated in the present work by many photographs.

10. The amount of vacuolation in the epithelial cells increases progressively with age and this is correlated with proportions of both the extrusion and the degenerating cells, while the number of nidi declines. This latter change may be regarded as a sign of senescence.

Figure 46

The percentage of extrusion cells to epithelial cells per section in midgut segments at different ages.

Fig. 46


Midgut Parts
0
1
2
3
4
5
6
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15
20
25
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35
40
45
50
55
60
Extr.C./Epit.C. Per.Sec. × 100
0 newly emerged
5 days old
10 // //
15 // //
20 // //
25 // //
30 // //

Figure 47

The percentage of resting cells to epithelial cell per section in midgut segments at different ages.

Fig. 47

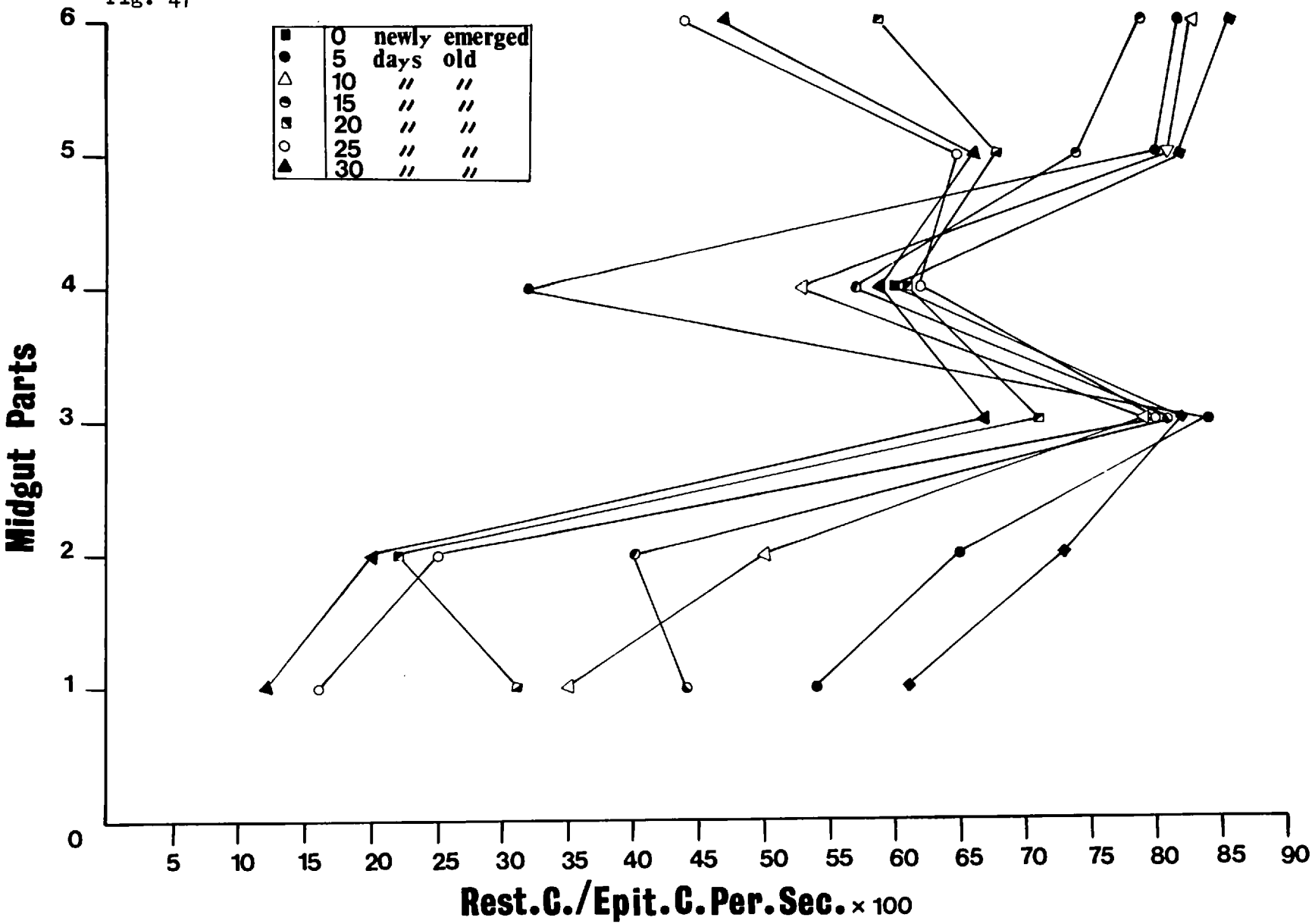
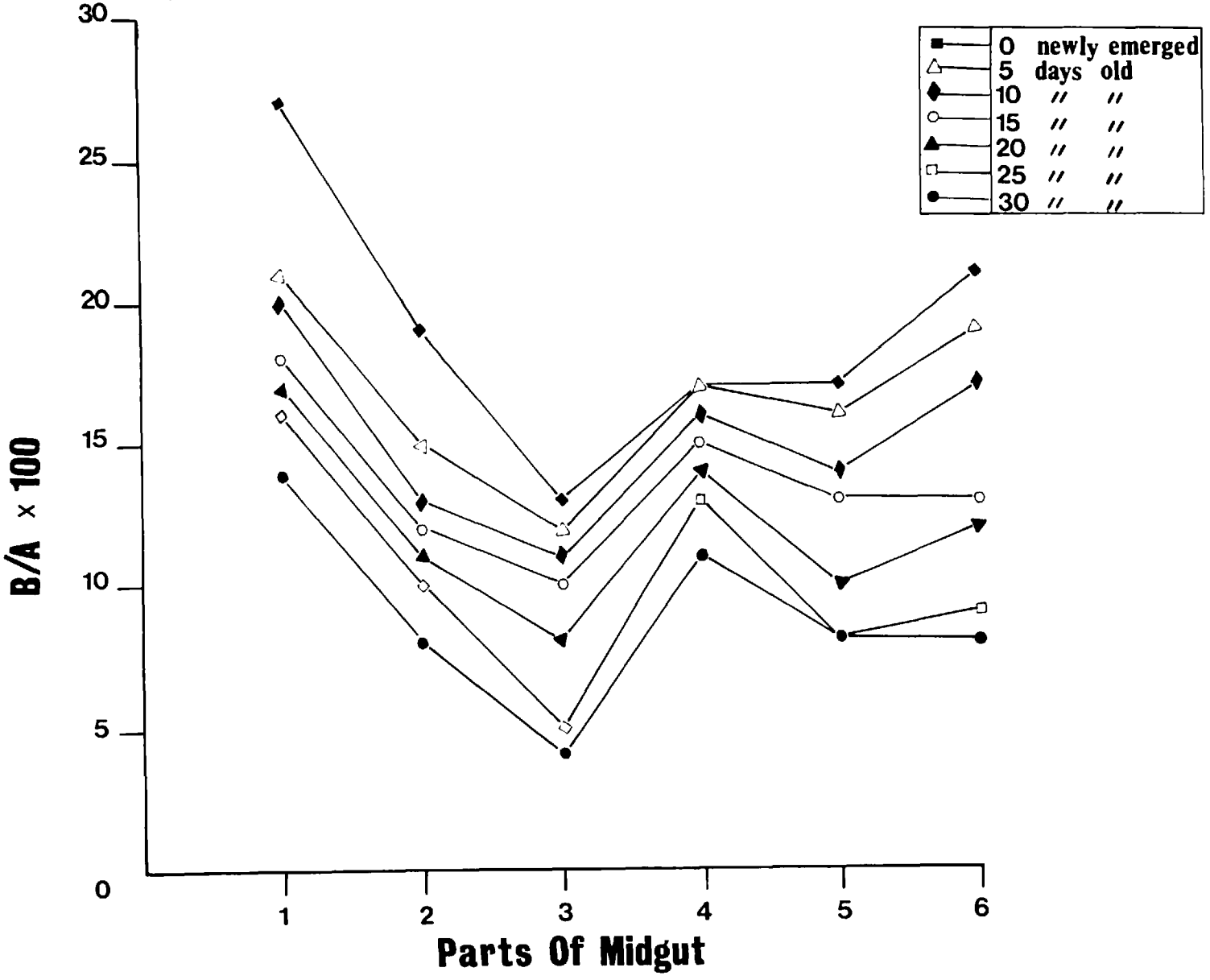

0 newly emerged
5 days old
10 // //
15 // //
20 // //
25 // //
30 // //
Midgut Parts
0
1
2
3
4
5
6
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
Rest.C./Epit.C.Per.Sec. × 100

Figure 48

The percentage of nidi (mean number) to epithelium cells (mean number) per section of the segments of midgut at different ages.

Fig. 48


30
25
20
15
10
5
0
B/A x 100
1
2
3
4
5
6
Parts Of Midgut
0 newly emerged
5 days old
10 // //
15 // //
20 // //
25 // //
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SECTION 4

HISTOLOGY OF MIDGUT IN STARVED AND DESICCATED ADULTS

HISTOLOGY OF MIDGUT IN STARVED AND DESICCATED ADULTS

Introduction

Detailed studies on the secretion of digestive enzymes in relation to feeding have been undertaken on insects by various workers. Schlottke (1937) found that the effect of feeding starved insects was always an initial increase of enzyme concentration in the gut but as the digestion proceeded further the enzyme activity decreased.

A correlation between enzyme secretion and feeding was carried out on a histological and chemical basis by Duspiva (1939) in Dytiscus marginalis. According to her account, feeding starved adults leads in one minute to the midgut epithelium cells discharging trypsin-like enzyme in the form of cytoplasmic globules which pass forward into the crop where maximal enzyme concentrations are recorded. Duspiva's observations suggest that the histological changes in the midgut epithelium are related directly to changes in the midgut epithelium secretion.

Day and Powning (1949) investigated a number of factors involved in the process of digestive in insects in which the significance of the cytological appearance of epithelial cells was discussed. Their work on the cockroach Blattella germanica (L.) suggested that the cytoplasmic extrusions apparently represent various stages in cellular degeneration. Dadd (1954) working on Tenebrio molitor found that newly emerged adults had practically no protease activity in the total midgut extracts. On subsequent starvation some protease activity built up and showed maximal activity on the 5th. or 6th. postemergence day, thereafter decreasing until death. M.A. Khan (1961) working on the secretion of some digestive enzymes in Locusta migratoria and Dysdercus fasicatus, stated that the midgut epithelium was histologically uniform without traces of cytoplasmic extrusions at the time of high enzyme activity.

Comparatively little work has been carried out on the secretory process in Heteroptera; Breakey (1936) described secretion in the midgut

epithelium of Anasa tristis De Geer as of the holocrine type. Goodchild (1952) described a merocrine type of secretion in some mirid bugs. M.A. Khan (1961) demonstrated that invertase was mainly produced in the 1st. ventriculus of the midgut of Dysdercus fasciatus whereas amylase was mostly secreted from the salivary glands. Khan and Ford (1962) studied digestive enzyme production and its relationship to cytological changes in the midgut epithelium in Dysdercus fasciatus Sign. They tried to extend some of Day and Powning's results and described differences between the cytological appearance of the midgut epithelium in starved and fully fed specimens. They concluded that the production of cytoplasmic extrusion in the epithelium is directly related to the presence or absence of food in a particular gut region and that the cytological changes were therefore a response to starvation. They believed that cytoplasmic vacuolation, shedding of globules of cytoplasm and loss of nucleus were degenerative changes that affect the midgut cells during starvation in Dysdercus and that this breakdown was rapidly checked when the insect was fed.

It seemed therefore worth comparing the histology of epithelium in fully fed and watered adult specimens of Oncopeltus fasciatus with starved and desiccated specimens. In the following account the cytological differences in the epithelium of the midgut in starved, desiccated and starved and desiccated insects were compared with normal ones. The experiment was carried out in three cases as in Table 26.

Starvation

This experiment was conducted on groups of adult specimens of different ages and each group was starved for a known period of time as shown in Table 26. The insects were supplied only with water during the period of starvation. All measurements of the cells and their nuclei, with thickness of striated border are given in Table 27, 28 and 29.

Table 26 Experimental feeding regimes of Oncopeltus fasciatus adult and their duration in days

Types of experiments	Adult age at start of experiment	Normal feeding prior to experiment	Duration of experiment in days	Experimental feeding	Final age in days
Starvation (water only)	3	Seed+Water	2	Water only	5
	0	Nil	5	"	5
	3	Seed+Water	17	"	20
Desiccation (seed only)	3	Seed+Water	2	Seed only	5
	0	Nil	5	"	5
	10	Seed+Water	10	"	20
Starvation and Desiccation	0	Nil	3	Nil	3

Starvation Experiments (Water only)

Table 27 3 days old, starved for 48 hours (5 days old)

Parts of midgut	Striated border thickness in µm	Epithelial cells height		Nuclear measurement	
		Young cell	Villar cell	Length	Breadth
1	0.8	26	65	8.2	5.9
2	1.8	30	70	8.8	5.8
3	0.4	36	75	9.8	5.2
4	0.9	27	62	8.8	4.8
5	0.8	35	53	9	5.2
6	0.9	28	65	9.3	5.8

Table 28 Newly emerged, starved for five days

Parts of midgut	Striated border thickness in µm	Epithelial cells height		Nuclear measurement	
		Young cell	Villar cell	Length	Breadth
1	0.6	25	55	8	5.6
2	1.3	20	65	8	5.6
3	0.8	34	62	9.3	5
4	0.8	27	43	8.3	5
5	0.75	32	52	8.7	4.5
6	0.7	23	50	7.7	4.8

Table 29 3 days old, adult specimens, starved for 17 days

(20 days old)

Parts of midgut	Striated border thickness in µm	Epithelial cells height		Nuclear measurement	
		Young cell	Villar cell	Length	Breadth
1	0.2	15	46	7.5	4.7
2	1.6	16	49	7.8	4.4
3	0.2	33	53	7.7	3.3
4	0.5	24	40	7.3	4.2
5	0.8	22	50	8	3.2
6	0.4	20	47	7.4	4.3

First ventriculus In specimens starved for 48 hours there were slight traces of food material and air bubbles while in those starved for longer periods the lumen appeared completely empty of food but filled with air bubbles.

In 3 day old specimens, starved for 48 hours, sections taken in the middle of the first ventriculus where the activity of the segment might be highest, show a thin basement membrane, epithelial cells with bulbous apical ends, and well differentiated young cells. Some of the epithelial cells show apical extrusions. The cytoplasm is reticulate, with few vacuoles and prominent coarse granules are irregularly distributed (Fig. 49). The nuclei are placed almost centrally or a little towards the base of the cell. Chromatin granules accumulate peripherally in the nuclei.

In newly emerged specimens starved for five days (Fig. 50), the epithelium is composed of cells with their apical ends highly bulbous and some show cytoplasmic extrusion through their bulbous tips. The cytoplasm is reticulate and lighter staining apically; the nuclei are not well outlined; the young cell takes on a rounded shape.

In specimens starved for longer periods (17 days), the epithelium falls into more closely associated villi. The brush border is irregular and disrupted in many places throughout the whole surface. The cytoplasm is more deeply basiphil with prominent coarse granules accumulated more apically. The few small vacuoles appear throughout the cytoplasm. The nuclei are centrally placed or a little towards the base and are more basiphil (Fig. 51). Young cells are almost rounded in shape and their cytoplasm stain only faintly.

Generally the epithelium in starved specimens shows the feature of apically rounded or protruding forms much less prominently than in fed specimens. The epithelium hardly ever shows shedding of cells during any

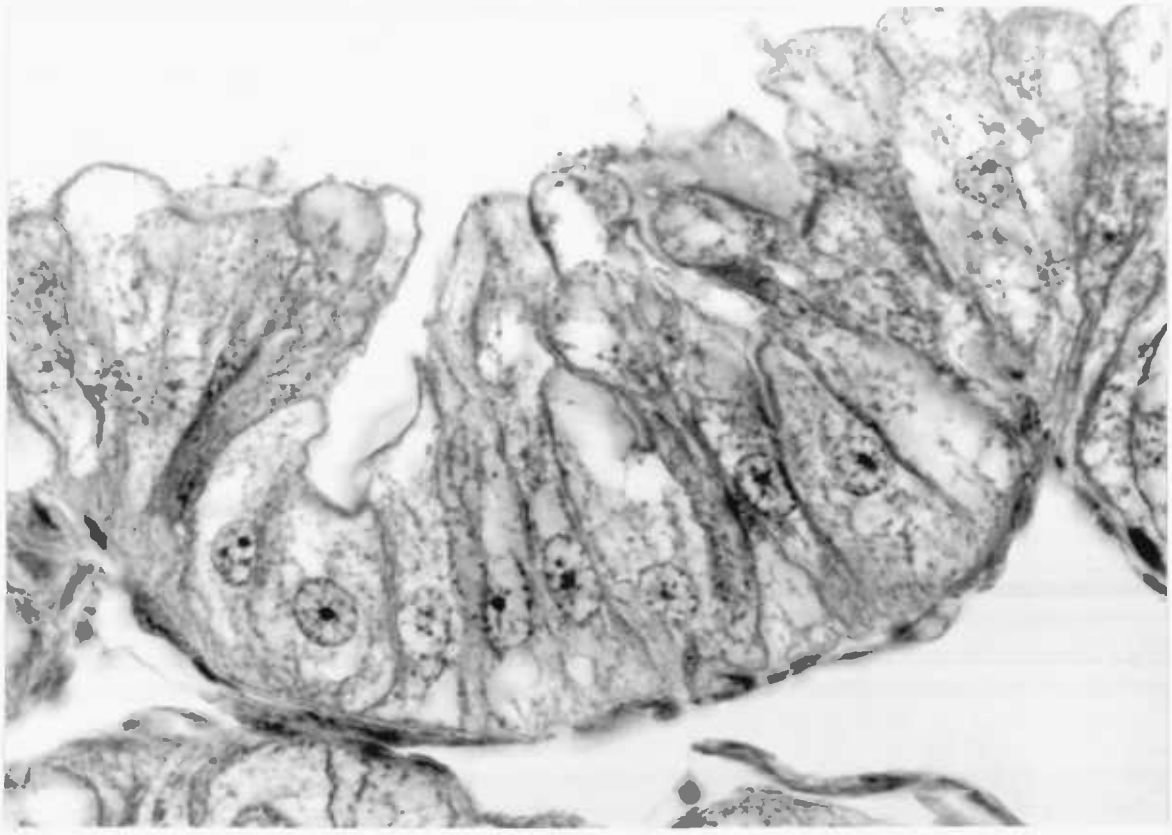


Figure 49 L.S. 1st. ventriculus, 3 days old starved for 48 hours

25µm

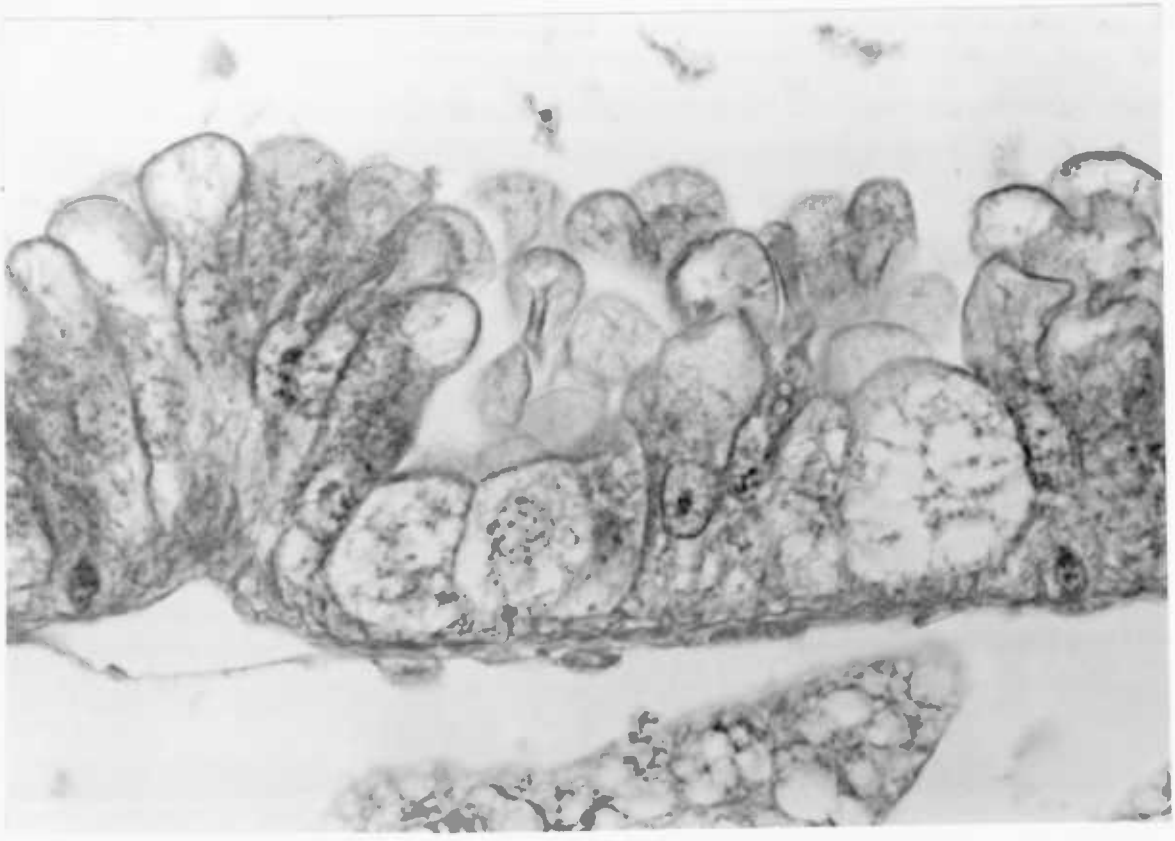


Figure 50 L.S. 1st. ventriculus, newly emerged starved for five days

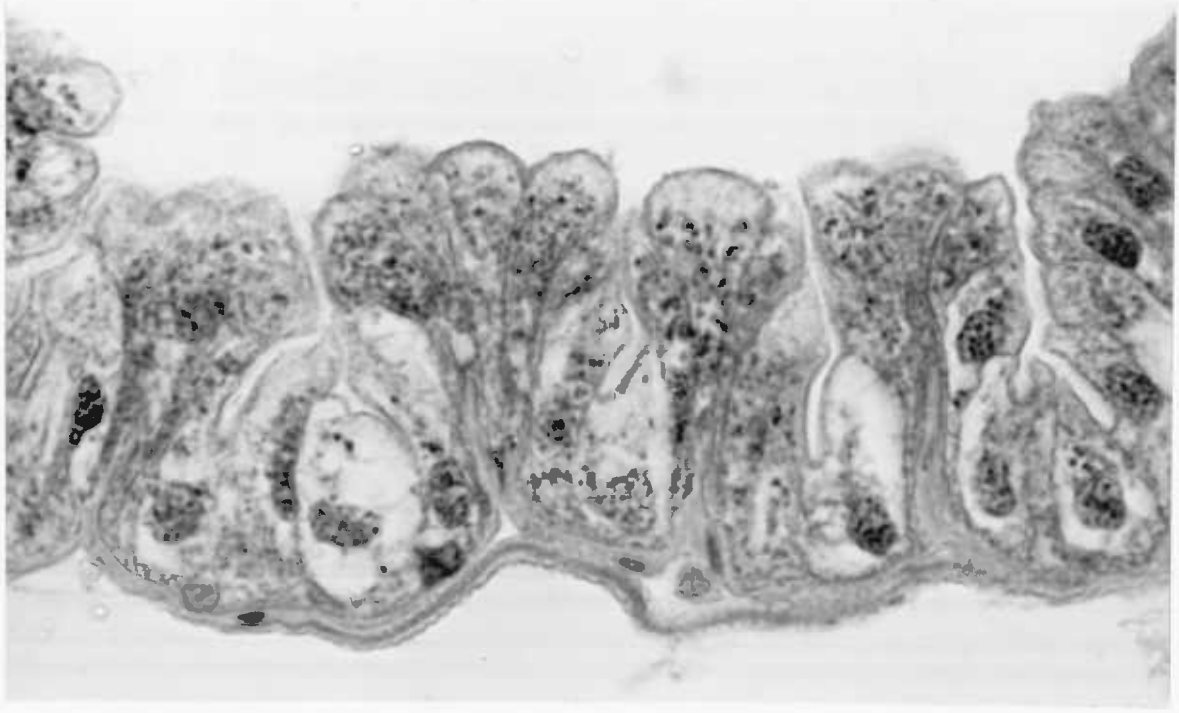


Figure 51 L.S. 1st. ventriculus starved for 17 days

50µm

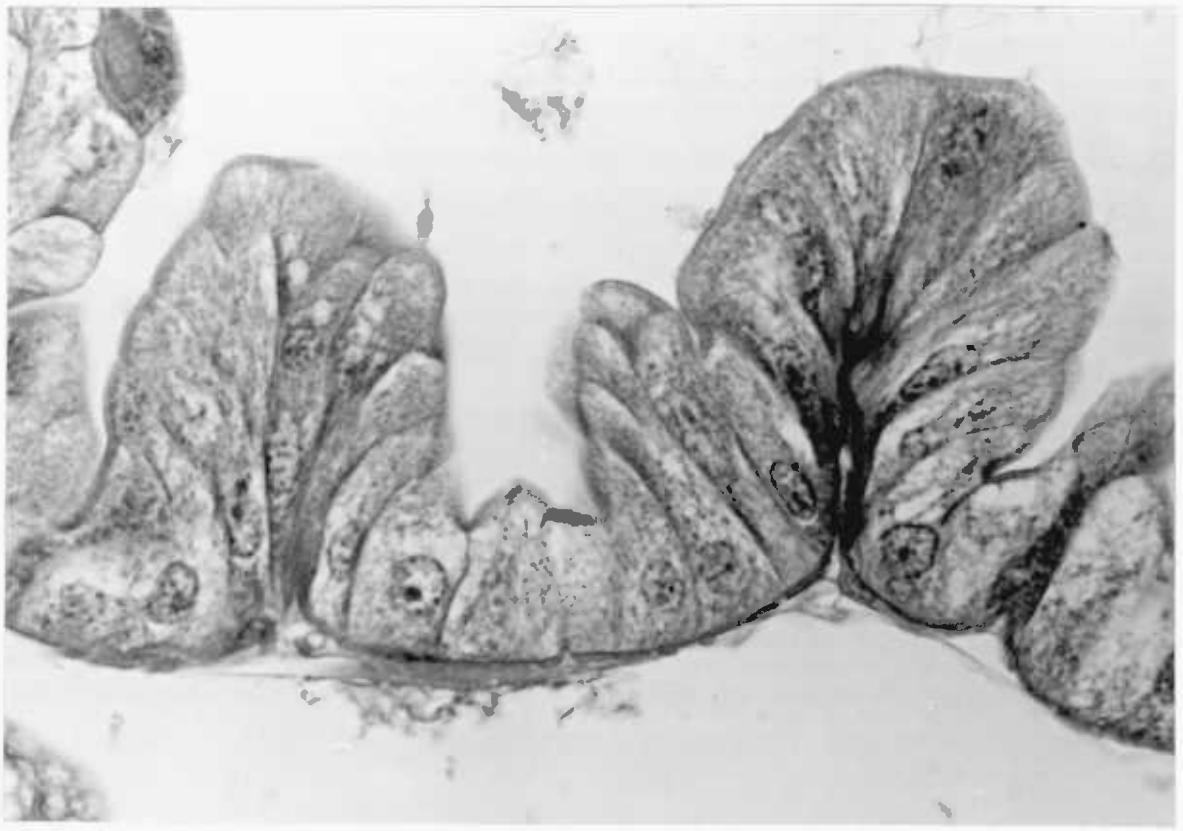


Figure 52 L.S. 2nd. ventriculus, starved for 48 hours

period of starvation in this ventriculus.

Second ventriculus This segment appears empty, with a few air bubbles inside it.

In specimens starved for 48 hours (Fig. 52) the epithelium falls into irregular villi with an entirely columner appearance. The striated border is relatively thick, and the cytoplasm homogenous. The nuclei are rounded in outline and tend to lie basally with clearly defined nucleoli and chromatin masses. The young cells are relatively small with irregular apical ends. There are no traces of bulbous cells but there are slight signs of extrusion through the tips of some cells.

In specimens starved for five days (Fig. 53) the epithelium is mainly formed of bulbous cells. The cytoplasm shows well defined coarse granules accumulated towards the apical halves of the cells. The nuclei are not well defined. The young cells tend to show clumping. Some of the bulbous cells show cytoplasmic material extruding through their apical tips. In specimens starved for 17 days (Fig. 54), the epithelium is mainly composed of bulbous cells, whose cytoplasm shows well defined, apically accumulated, coarse granules. The striated border is thicker with some extrusions occurring through it from the cells into the lumen. The nuclei are more regularly apical. No sign of cell shedding has been observed in the epithelium of this ventriculus during starvation.

Third and Fifth ventriculus The effect of starvation on the epithelia of the 3rd. and 5th. ventriculi appears similar in both segments and they are, therefore, described together here. Both ventriculi appear quite empty and contain no air bubbles.

Specimens starved for 48 hours have a very uniform epithelium forming closely associated villi built up of clavate cells. The cytoplasm

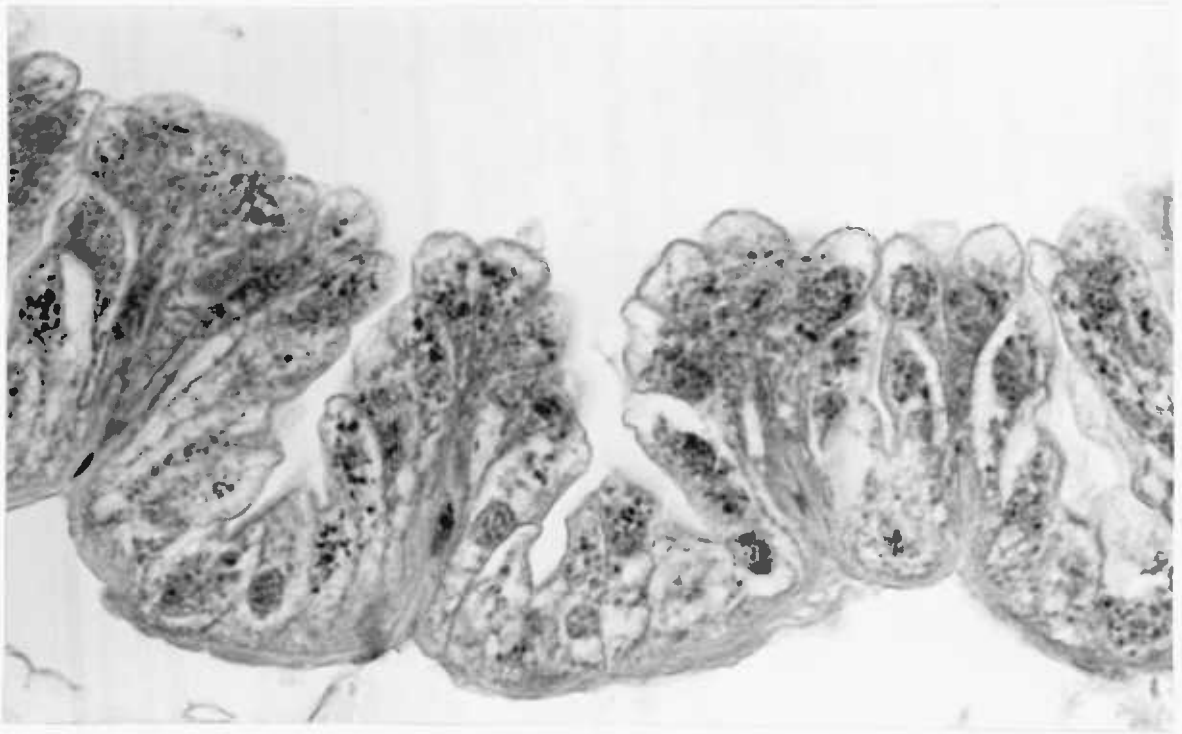


Figure 53 L.S. 2nd. ventriculus, starved for five days

25µm

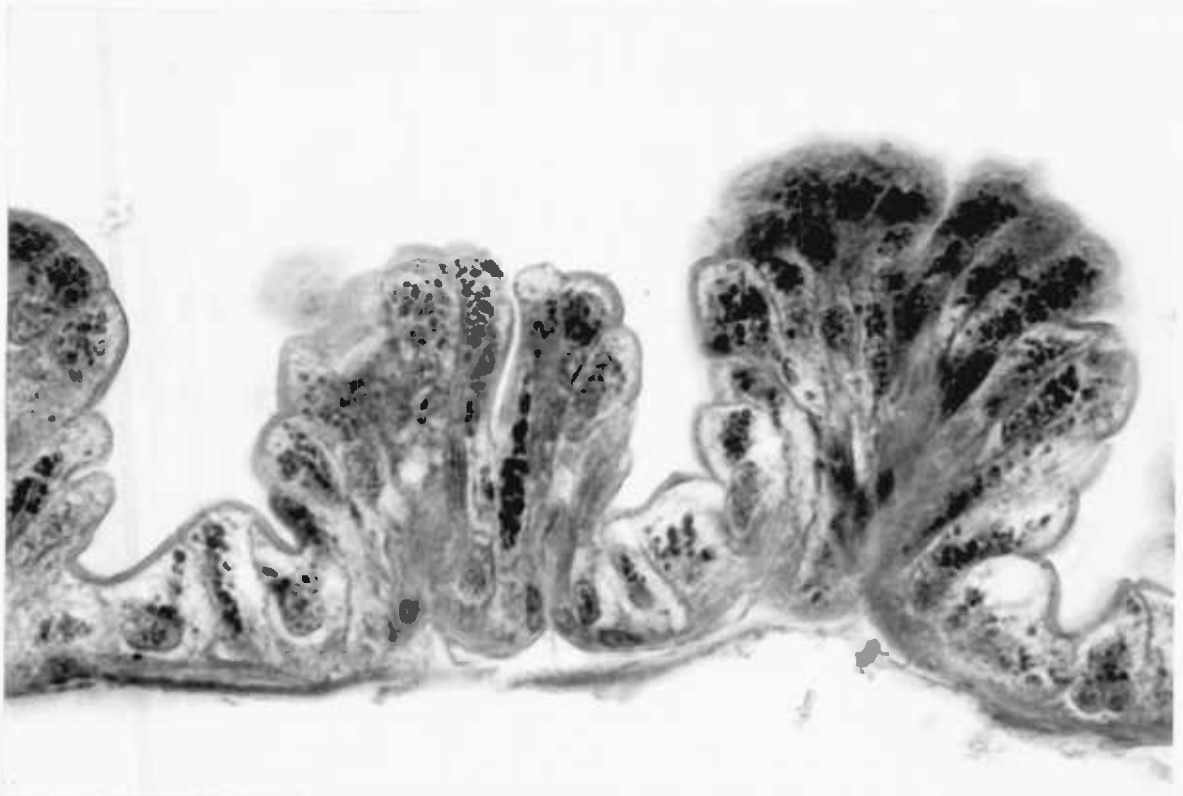


Figure 54 L.S. 2nd. ventriculus, starved for 17 days

is lightly staining and homogenous in appearance, with basally located nuclei. The striated border is completely uniform and extends over the whole surface. No coarse granules are present in the cytoplasm. No signs of extrusion are ever seen at the apical ends of the epithelial cells. Figure 55 represents the epithelium of 5th. ventriculus.

Specimens starved for five days have almost the same appearance in the epithelia of the two ventriculi. Figure 56 and 57 represent sections taken from the 3rd. and 5th. segments respectively. The epithelium of specimens starved for a longer period (17 days) is built up of more compact cells forming narrow, closely associated villi. The striated border is thinner and the cytoplasm stains lightly having no coarse granules. The nuclei are more regularly basal in position. Again there is no sign of extrusion after this prolonged period of starvation (Fig. 58).

Fourth ventriculus No food material or air bubbles were found in the lumen of the 4th. ventriculus of starved individuals.

The midgut epithelia of specimens starved for 48 hours show very slight change from those fed normally. The epithelium is uniform, made up of low columnar cells not arranged in villi. The striated border is uniformly thick and extends over the whole surface. The cytoplasm is reticulate and stains deeply in PTAH. In specimens starved for five days the epithelium is almost the same (Fig. 59) with relatively large, well differentiated nuclei, occupying central positions in the cells.

In specimens starved for 17 days (Fig. 60) the epithelium also shows very slight change. There are low columnar cells with a thick, irregular striated border. The cytoplasm is rich in fine granules, giving a reticulate appearance. The nuclei are relatively large and are more regularly central in position. No signs of extrusion were observed in the epithelium of this ventriculus during starvation.

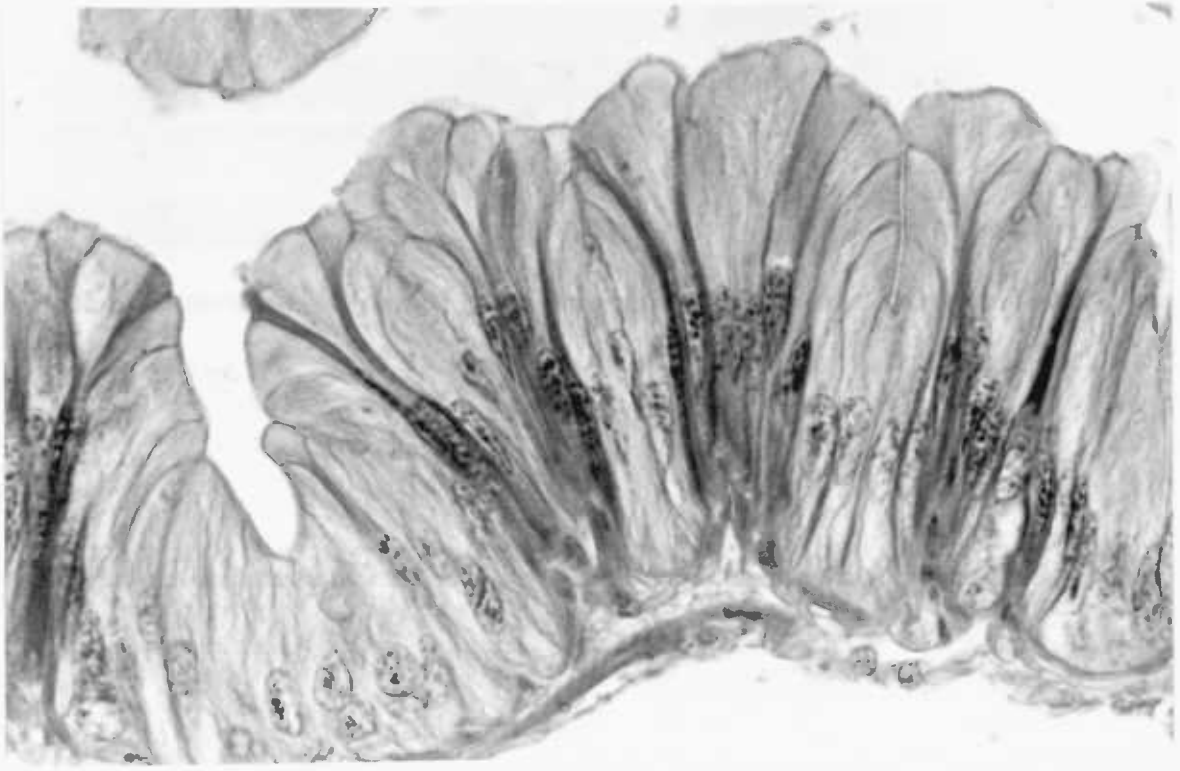


Figure 55 L.S. 5th. ventriculus, starved for 48 hours

50µm

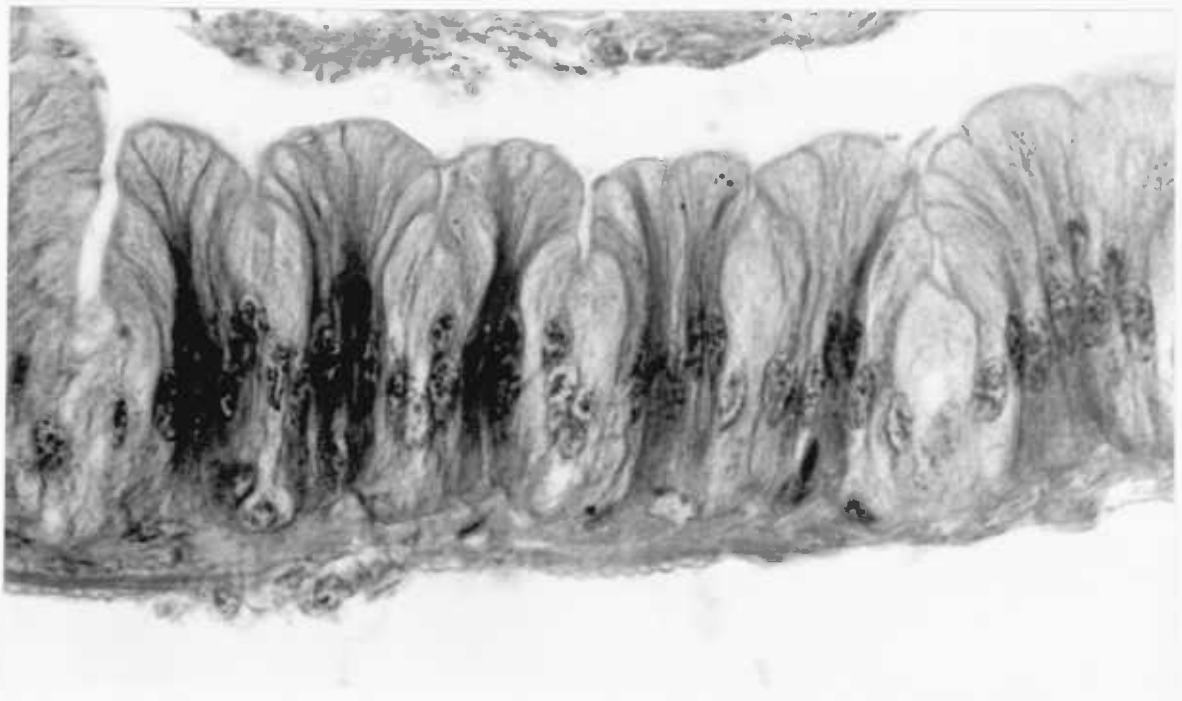


Figure 56 L.S. 3rd. ventriculus, starved for 5 days

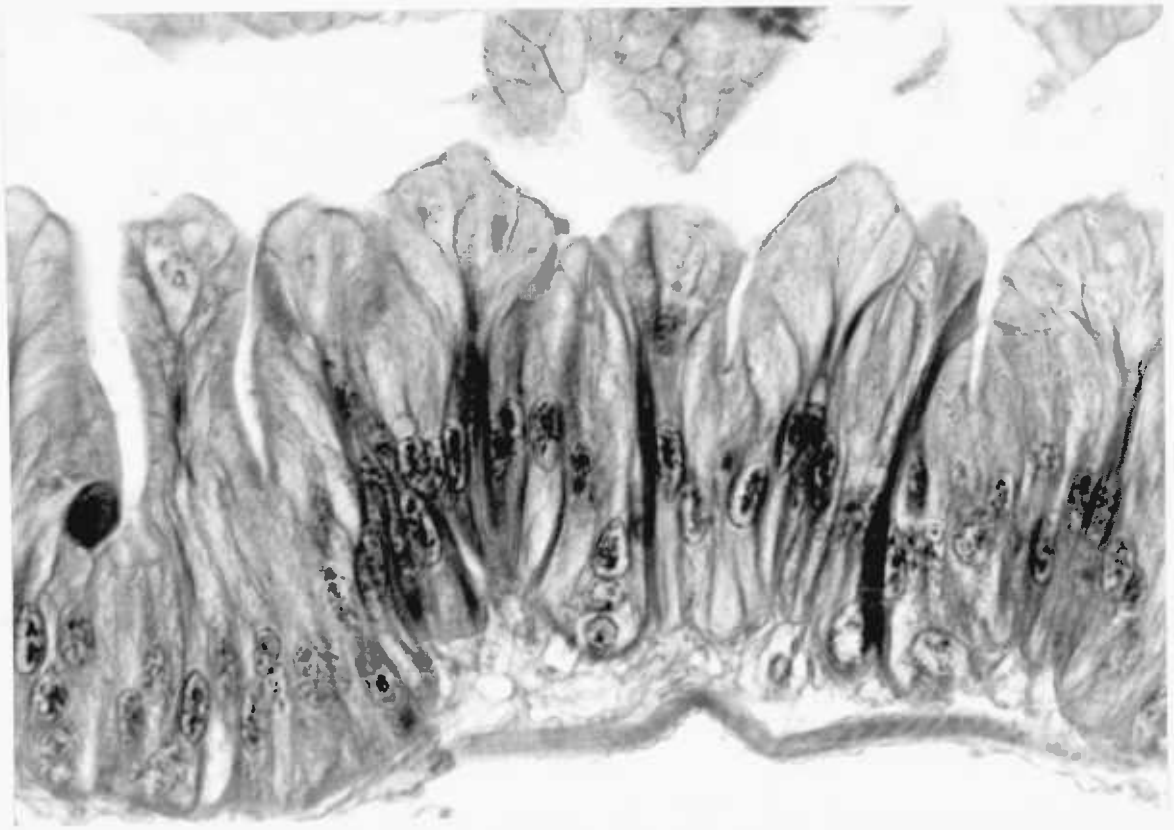


Figure 57 L.S. 5th. ventriculus, starved for 5 days

25µm

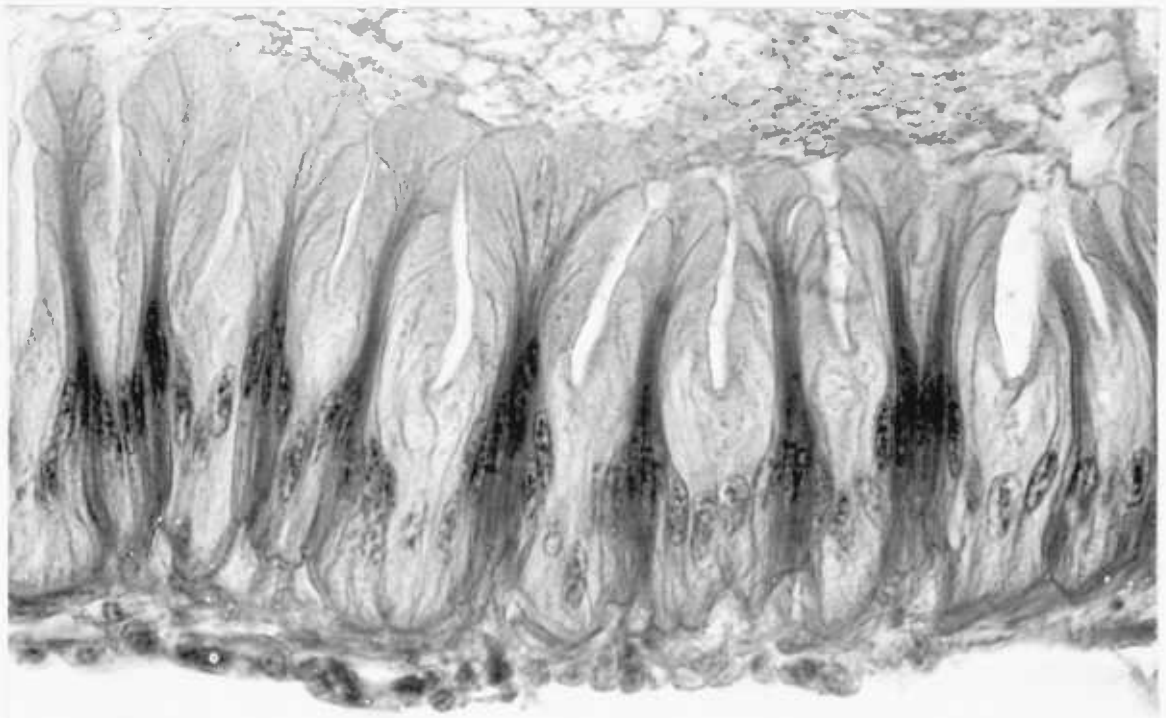


Figure 58 L.S. 3rd. ventriculus, starved for 17 days

Figure 59 L.S. 4th. ventriculus, starved for 5 days

50µm

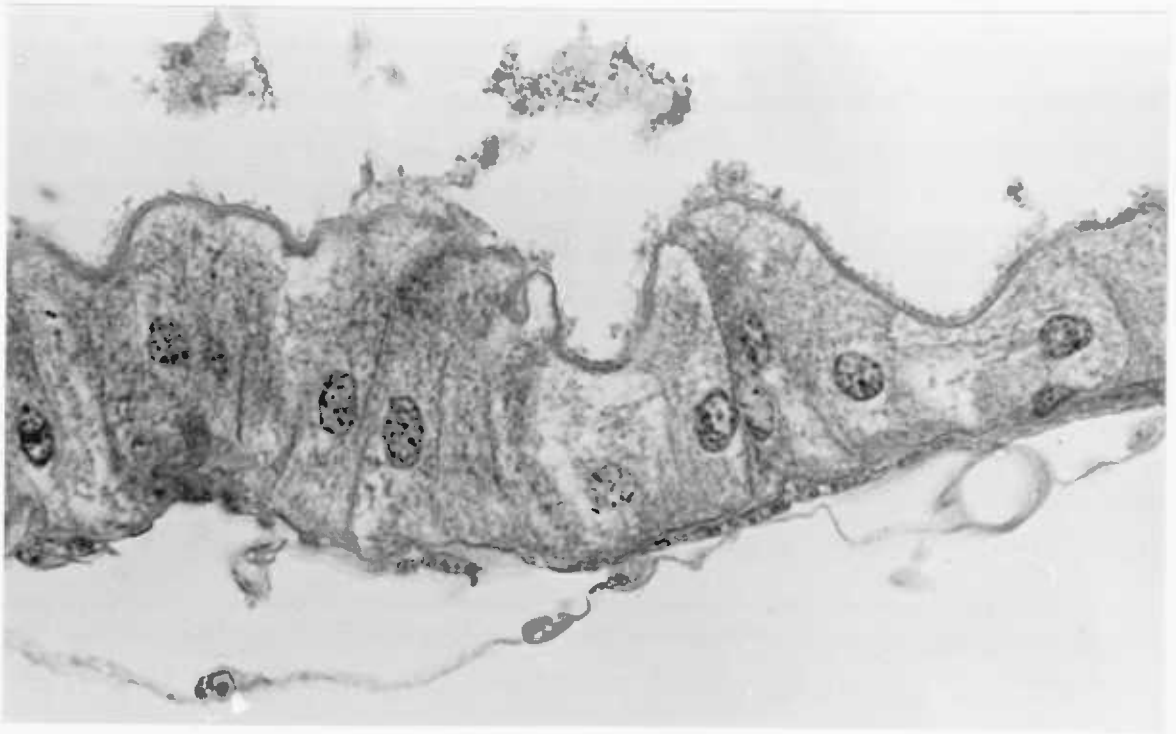


Figure 60 L.S. 4th. ventriculus, starved for 17 days

Sixth ventriculus No trace of food material was found in any of the starved individuals in this ventriculus.

In specimens starved for 48 hours the epithelium falls into villi built up of long columnar cells. Their cytoplasm is provided with fine granules distributed throughout the cell and so giving a reticulate appearance. The nuclei are relatively small and almost centrally located (Fig. 61).

In specimens starved for 5 days, the epithelium contains cells with bulbous apical ends (Fig. 62) with only slight signs of extrusion through their striated border. The cytoplasm has faintly staining granules and appears lighter around the nuclei and toward the apical ends. The nuclei are centrally located or lie a little towards the base. In specimens starved for a longer period (17 days) the epithelium shows one cell of a bulbous type; the nuclei are not well defined but no sign of casting off or extrusion takes place in the epithelium even in this stage of prolonged starvation (Fig. 63).

Desiccation

The desiccation experiment was also conducted on three different groups of adult specimens and each group was desiccated for a certain period as shown in Table 26. Only seeds were provided for the insects during the period of desiccation. All measurements of the cells and their nuclei, with thickness of striated border are given in Table 30, 31 and 32.

First ventriculus This segment has highly folded walls and an empty lumen during desiccation. Specimens desiccated for 48 hours have a compact low columnar epithelium with an irregular striated border. The cytoplasm is reticulate and takes on a deep colour with PTAH. The nuclei are centrally

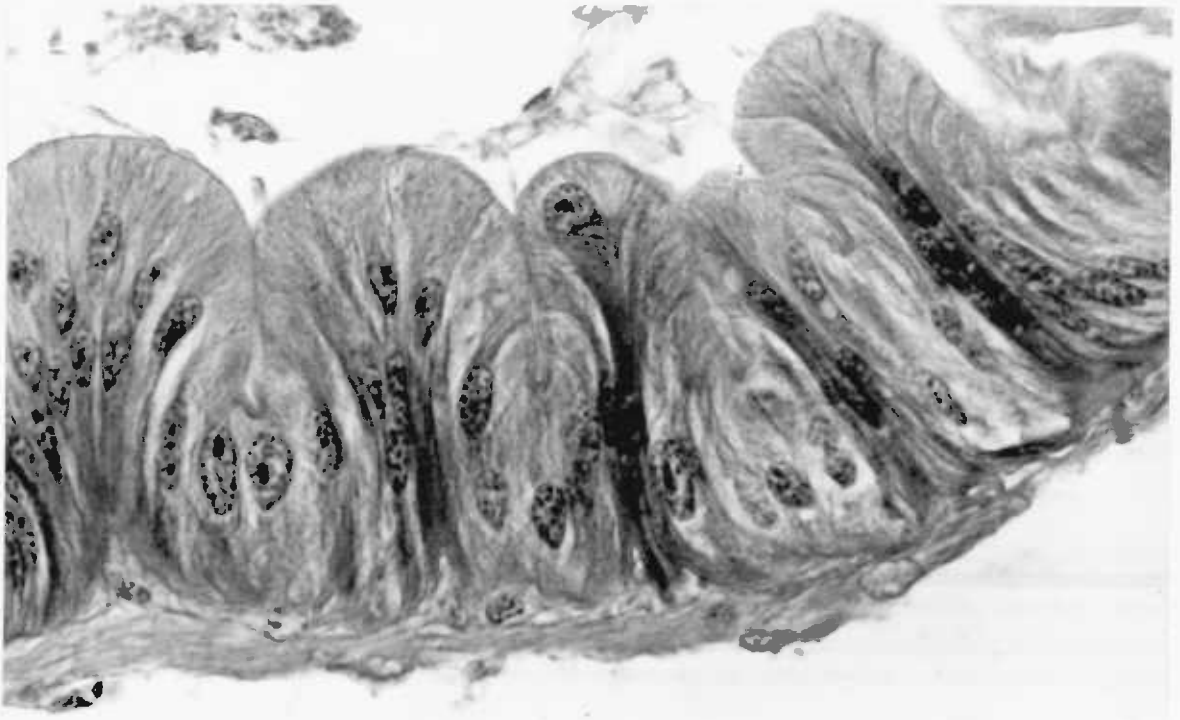


Figure 61 L.S. 6th. ventriculus, starved for 48 hours

25µm

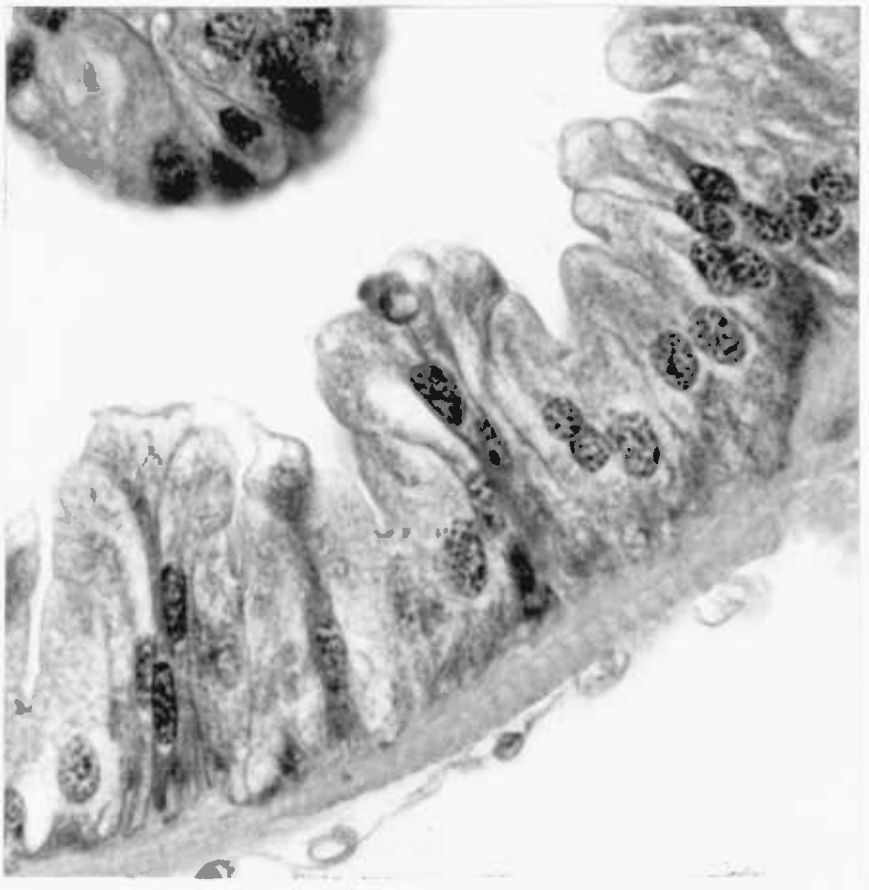


Figure 62 T.S. 6th. ventriculus, starved for 5 days

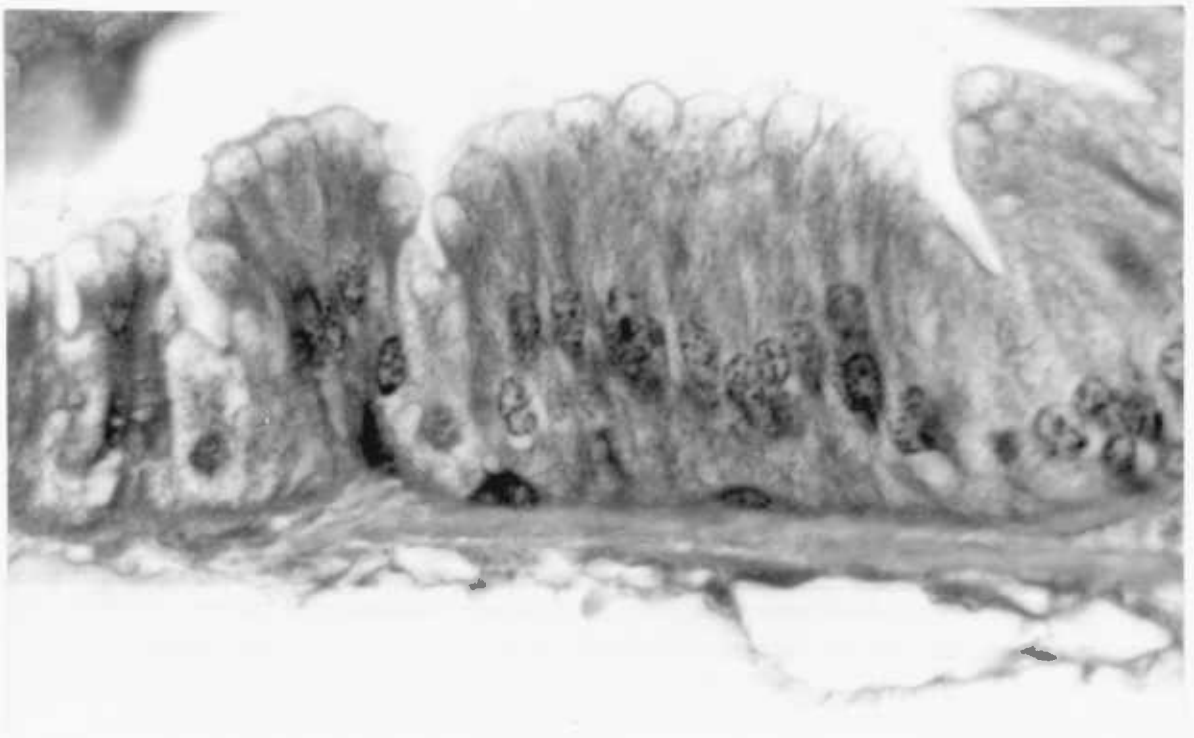


Figure 63 L.S. 6th. ventriculus, starved for 17 days

25µm

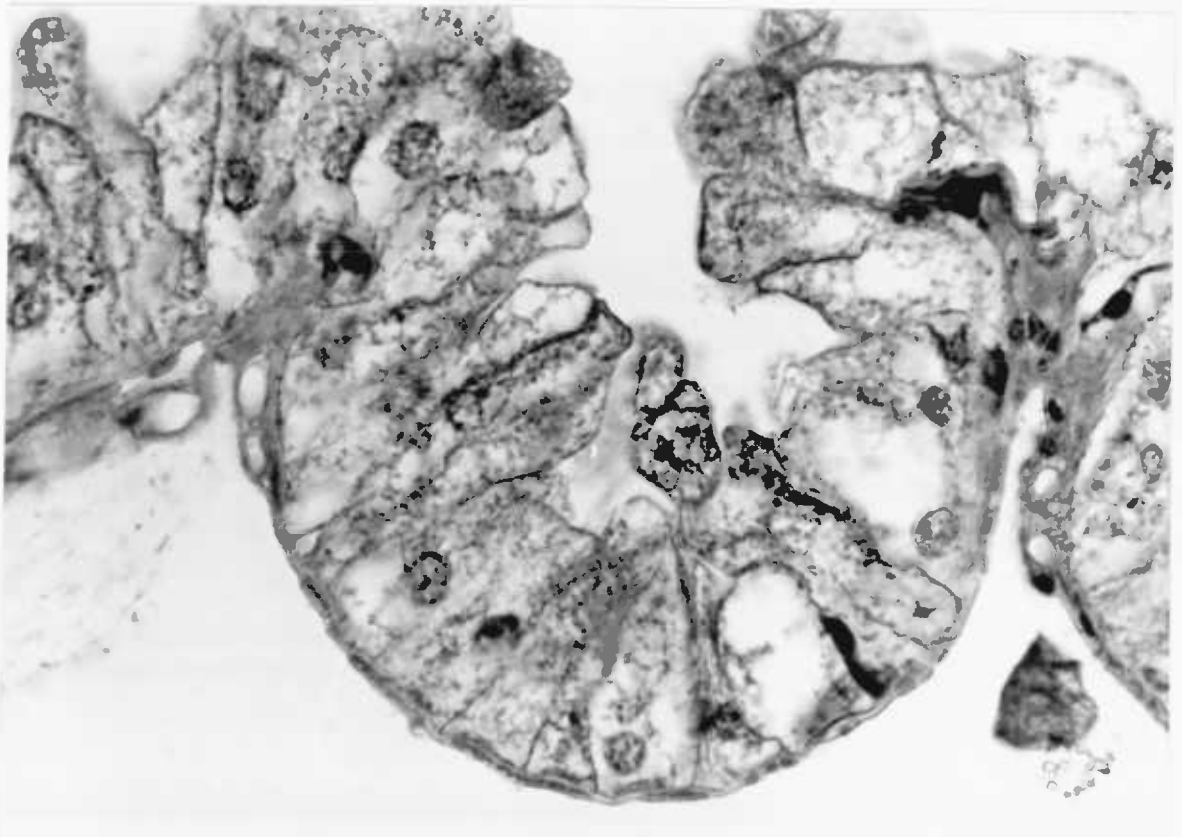


Figure 65 L.S. 1st. ventriculus, desiccated for 5 days

Desiccation experiment (Seeds only)

Table 30 3 days old, desiccated for 48 hours (5 days old)

Parts of midgut	Striated border thickness in µm	Epithelial cells height		Nuclear measurement	
		Young cell	Villar cell	Length	Breadth
1	0.9	48.6	62	8.8	5.7
2	0.9	32	57	7.6	5.8
3	0.8	44	68	7.6	4.4
4	0.8	32	50	7.3	6.1
5	0.8	46	65	8.6	4.6
6	0.3	28	50.5	9	6.6

Table 31 Newly emerged, desiccated for five days

Parts of midgut	Striated border thickness in µm	Epithelial cells height		Nuclear measurement	
		Young cell	Villar cell	Length	Breadth
1	0.7	26	55	8	7.2
2	0.8	27	45	7	5
3	0.7	37	63	7.5	4
4	0.2	23	40	7	5.9
5	0.5	22.3	54.2	7.8	3.4
6	0.7	35	46	8.4	5.7

Table 32 10 days old, adult specimens, desiccated for 10 days

(20 days old)

Parts of midgut	Striated border thickness in µm	Epithelial cells height		Nuclear measurement	
		Young cell	Villar cell	Length	Breadth
1	0.3	15	46.8	7.9	6
2	0.7	25	42	6.4	5.3
3	0.1	28	48	7.1	4.2
4	0.3	26	39	6.4	6.2
5	0.2	35	40	5	4.8
6	0.9	23	44	7.3	5.5

Starvation and Desiccation experiments

Table 33 Newly emerged, starved and desiccated for three days

Parts of midgut	Striated border thickness in µm	Epithelial cell height		Nuclear measurement	
		Young cell	Villar cell	Length	Breadth
1	0.5	12	44	8.8	4.2
2	1	15	36	9.4	5
3	0.4	29	58	8.6	5.6
4	0.6	15	20	8.3	5
5	0.8	27	49	9.3	4.3
6	0.6	9	31	8	3

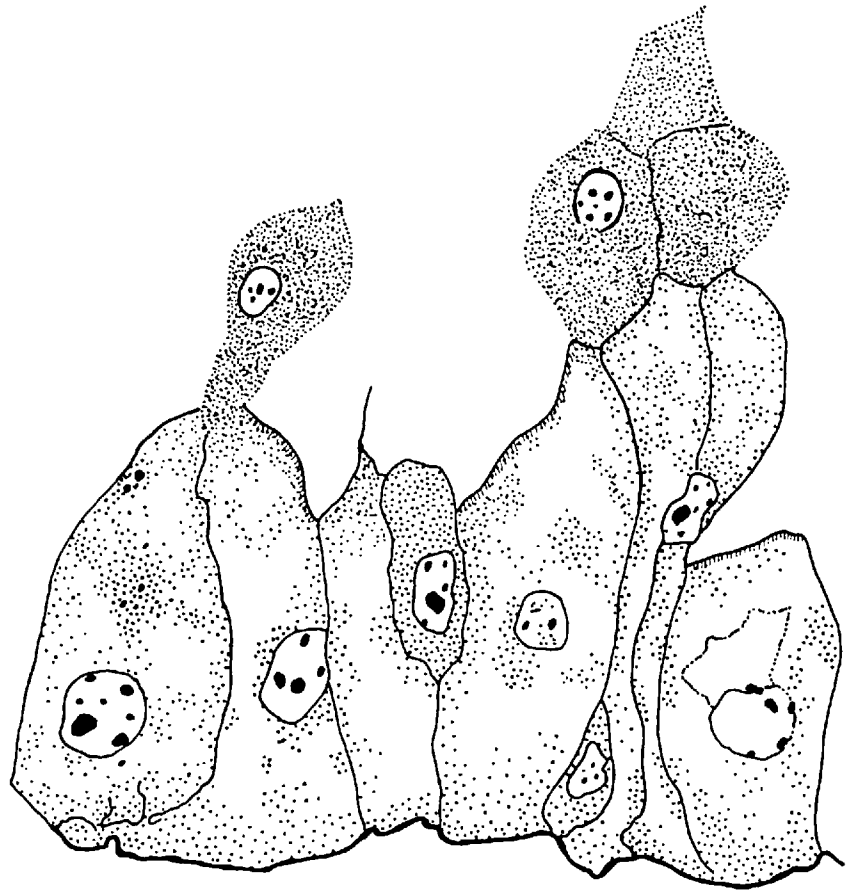
located and generally smaller than usual in size. The apical ends of the epithelial cells show a bulbous appearance with extrusion (Fig. 64a).

Specimens desiccated for five days (Fig. 65) are similar to the above while those desiccated for 10 days have the epithelium more clearly affected. The striated border is very thin (about 0.3μm) and the cytoplasm contains irregularly distributed vacuoles of different sizes. The nuclei are relatively small and not well defined (Fig. 66). Bulbous and protruding apical regions are common among the epithelial cells.

Second ventriculus This ventriculus appears narrow and yellowish in colour having no food material or air bubbles in desiccated specimens. In specimens desiccated for 48 hours, the epithelium is columnar with a uniform thick striated border. The cytoplasm is vacuolated with nuclei placed centrally or a little towards the apex (Fig. 64b). In specimens desiccated for five days the epithelium tends to be lower with a relatively thin striated border. The cytoplasm is reticulate and some cells show trace of extrusion.

In specimens desiccated for 10 days the epithelium shows less numerous villi, some formed of columnar cells with more bulbous ends and with poorly defined nuclei (Fig. 67). No sign of cell shedding was ever observed in the epithelium of this ventriculus even during prolonged periods of desiccation.

Third and Fifth ventriculus Both the 3rd. and 5th. ventriculi are empty and their epithelia respond similarly to desiccation. In specimens desiccated for 48 hours the epithelium is similar to that of normally fed specimens. The cells are normally clavate in shape, the cytoplasm stains lightly with fine granules evenly distributed and with some cytoplasmic material extruded through the apical ends of few cells (Fig. 68a and b).



a_1st vent.

b_2nd vent.

fig. 64 a and b, 3 days old desiccated for 48 hours

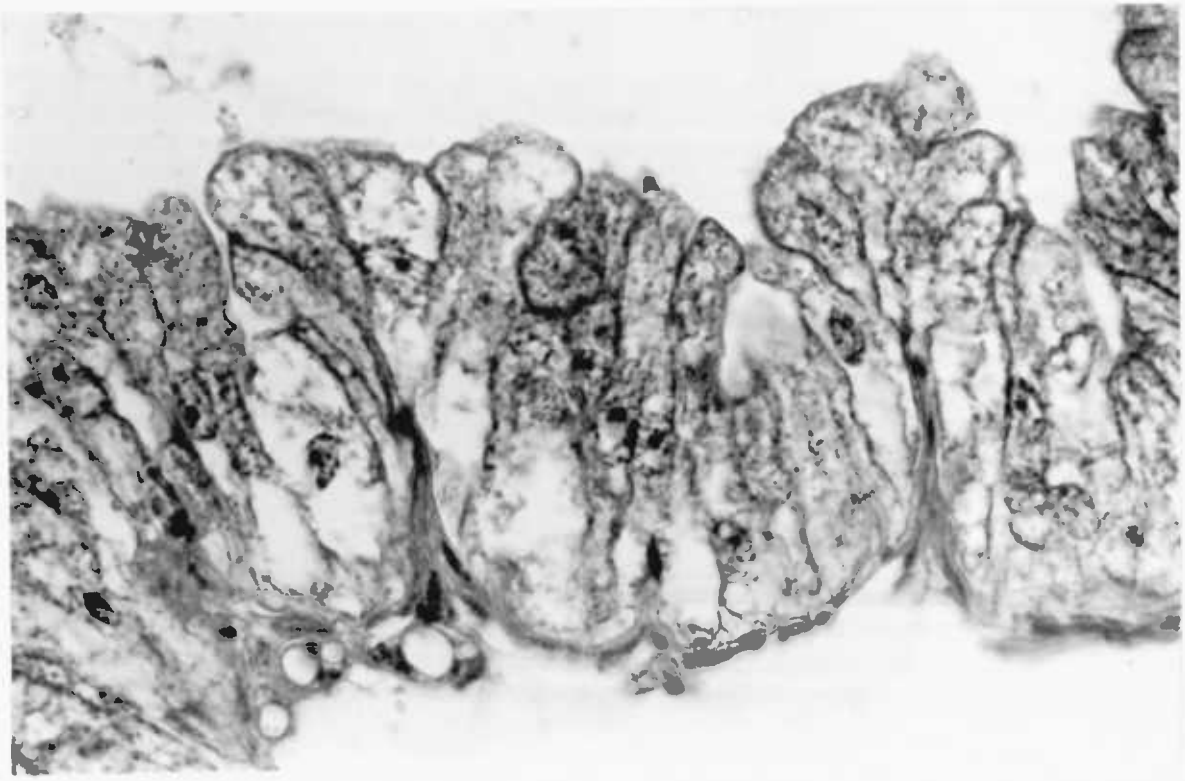


Figure 66 L.S. 1st. ventriculus, desiccated for 10 days

25µm

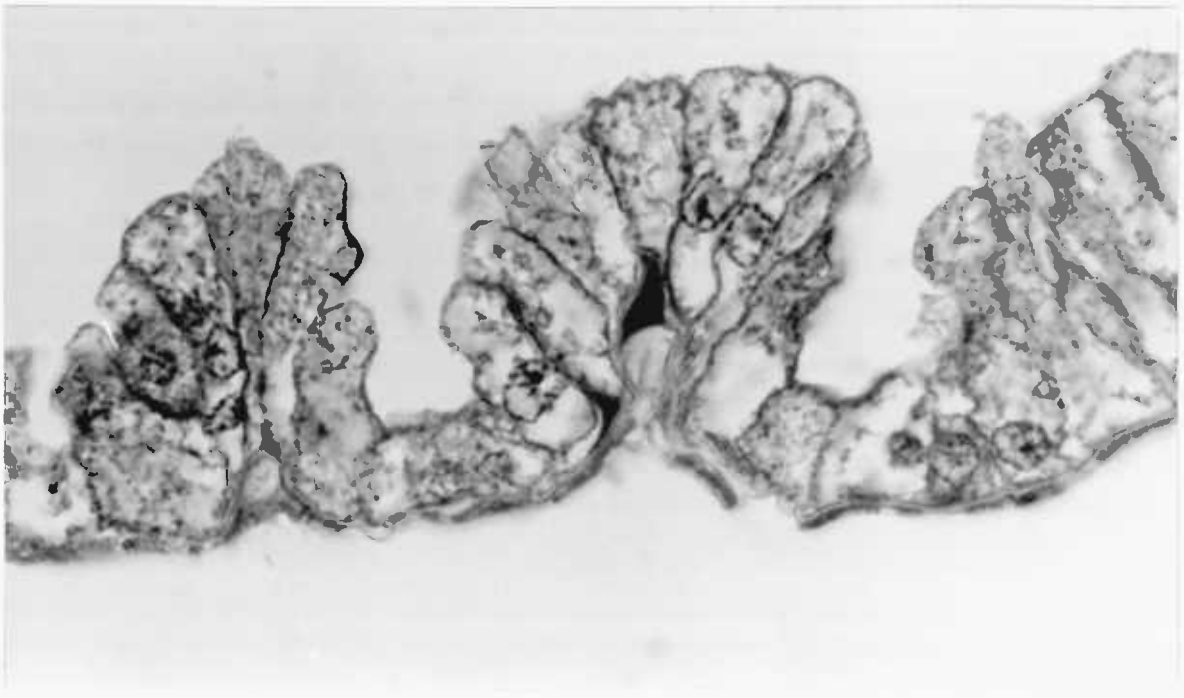


Figure 67 L.S. 2nd. ventriculus, desiccated for 10 days

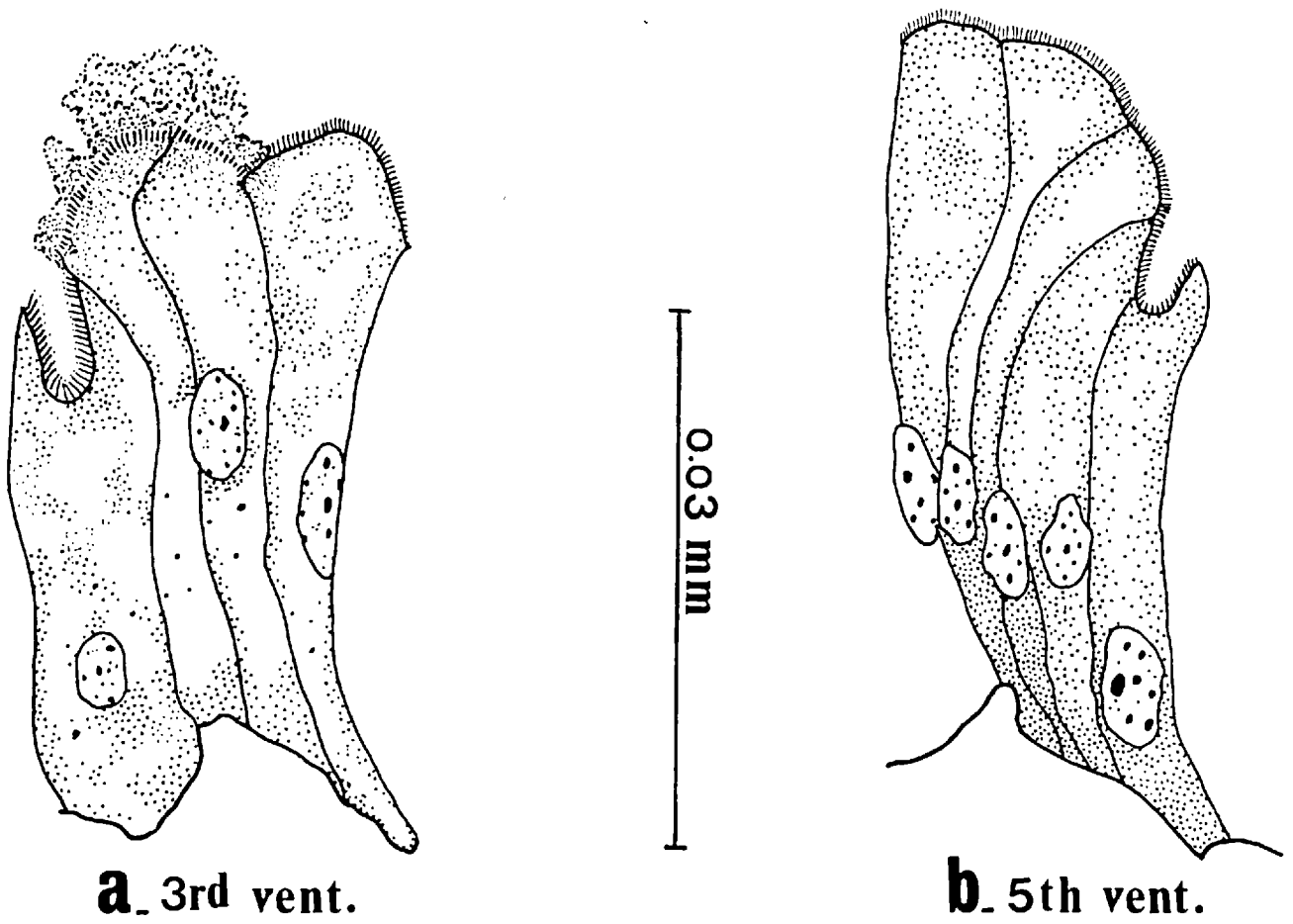


fig. 68 a and b , 3 days old desiccated for 48 hours, 5days old

Specimens desiccated for 5 and 10 days have clavate cells with a thin striated border. The villi are not well differentiated and the cytoplasm takes on a deep colour with PTAH. The nuclei are more basiphil and are more regularly basal in position. Cellular boundaries are not well defined. Figure 69 and Fig. 70a represents the epithelium of the 5th. and 3rd. ventriculus respectively. Again no cast-off apical region of epithelial cells were found after this period of desiccation.

Fourth ventriculus This small yellowish segment has no food material or air bubbles during desiccation. The epithelium in specimens desiccated for 48 hours is normally columnar with a uniformly thick striated border. The cytoplasm is reticulate with relatively large nuclei having well defined nucleoli. No signs of blistering or extrusion were found at the apical ends of the cells (Fig. 71). In those desiccated for five days the epithelium is almost the same but some extrusion appears at the apical ends of some cells (Fig. 70b), while specimens desiccated for 10 days have the epithelium collapsed. It takes a deep colour with PTAH and the cytoplasm is highly vacuolated. The apical ends of some cells are protruding (Fig. 70c).

Sixth ventriculus The lumen of this ventriculus contain a small amount of fine particulate matter similar to that found at the bases of the Malpighian tubules.

In specimens desiccated for 48 hours the epithelium appears tall and columnar with a thick striated border. The cytoplasm has small scattered vacuoles. The nuclei are almost central. In those desiccated for 5 and 10 days the epithelium has responded more to desiccation. The cellular membranes are not differentiated, the striated border is thin and broken throughout its surface and the nuclei are small, and vary in position (Fig. 72). Bulbous and extruded apical ends are evident in many cells.

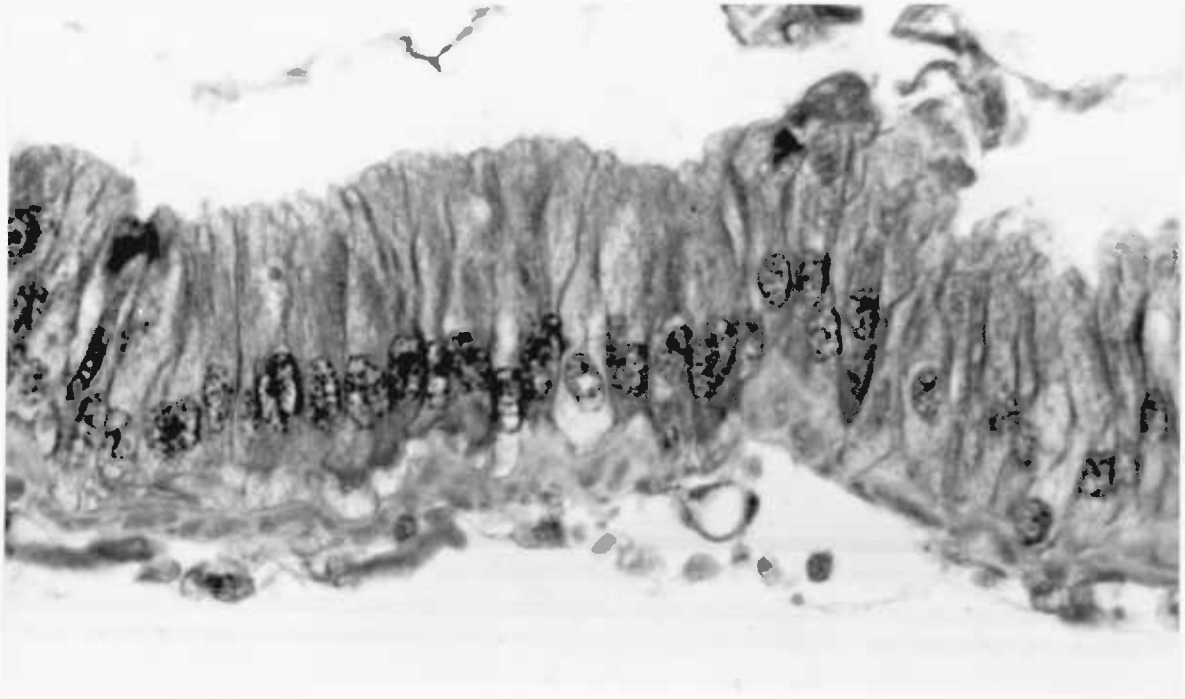


Figure 69 L.S. 5th. ventriculus, desiccated for 10 days

25µm

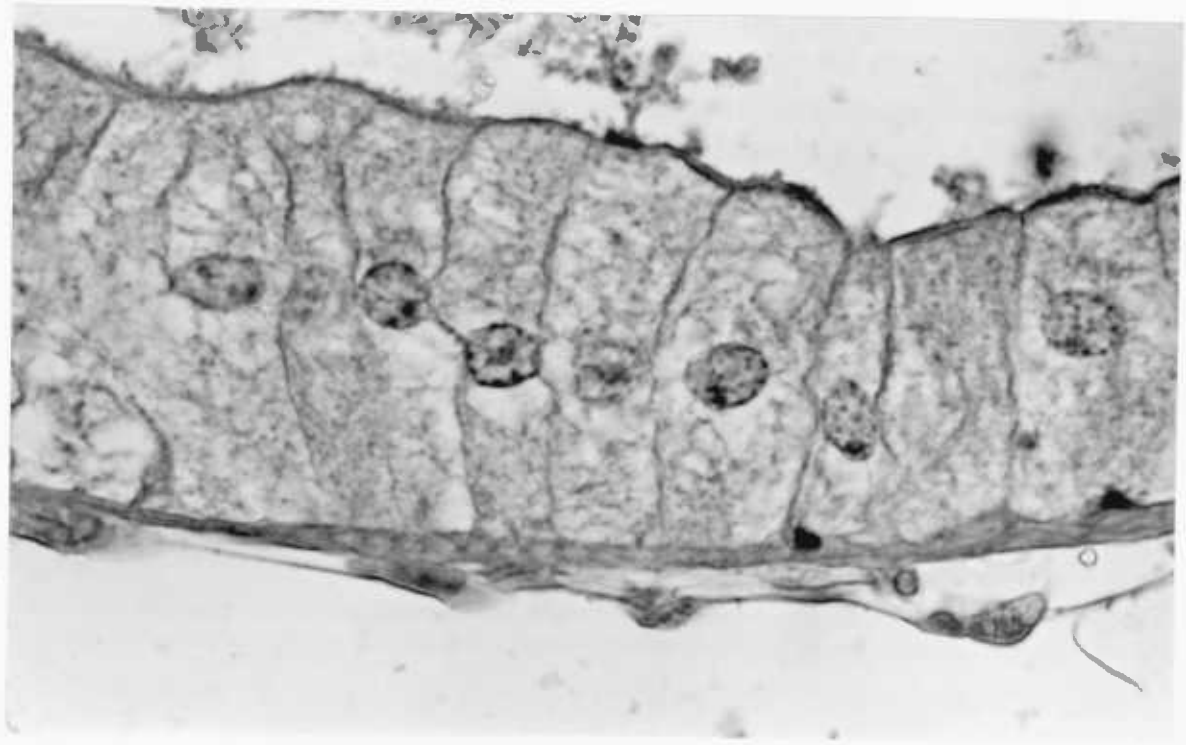
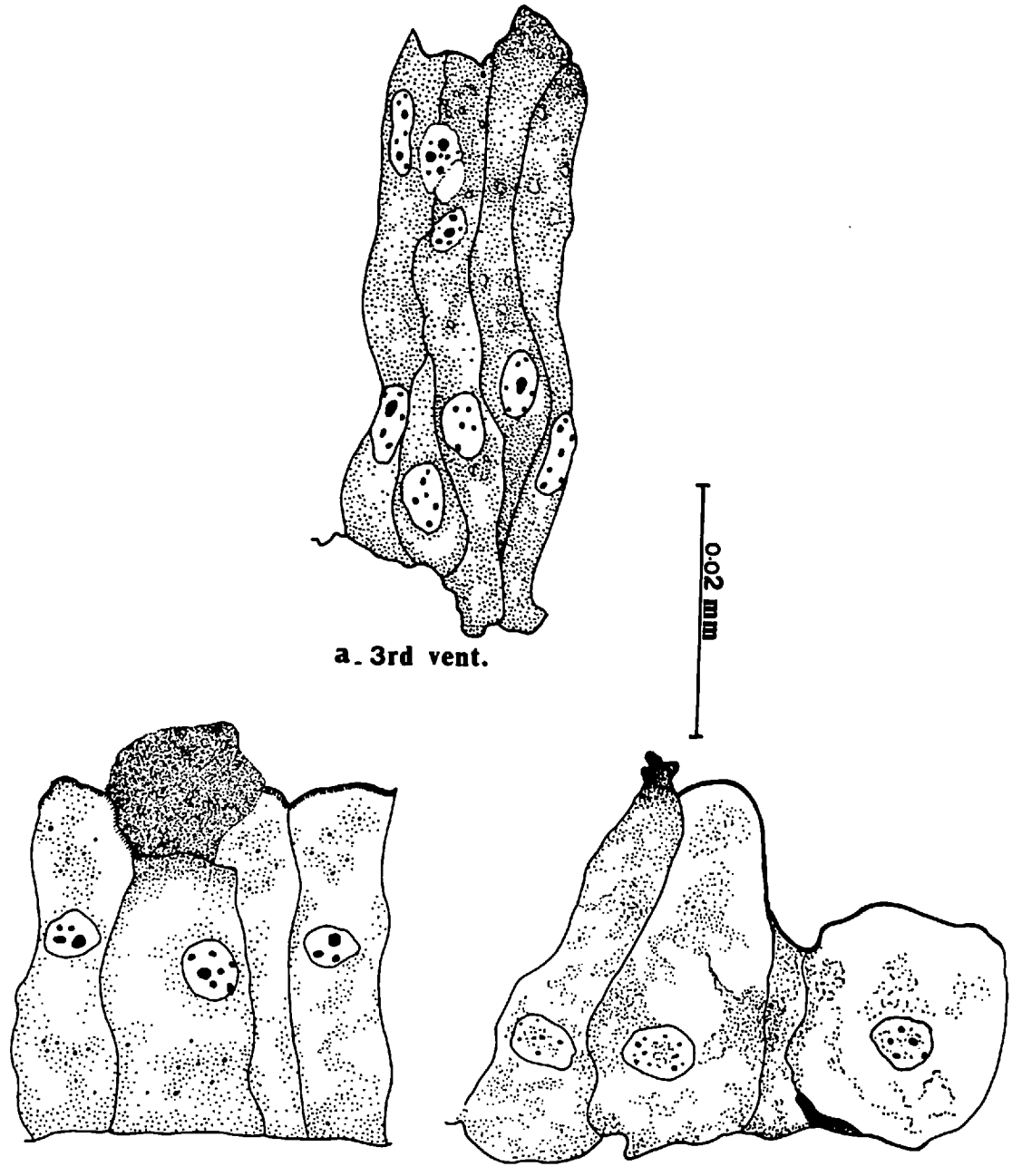


Figure 71 L.S. 4th. ventriculus, desiccated for 48 hours



a. 3rd vent.

b. 4th vent.

c. 4th vent.

fig. 70— a. 1o days old, desiccated for 1o days, 2o days old.

b. newly emerged, desiccated for 5 days.

c. 1o days old, desiccated for 1o days.

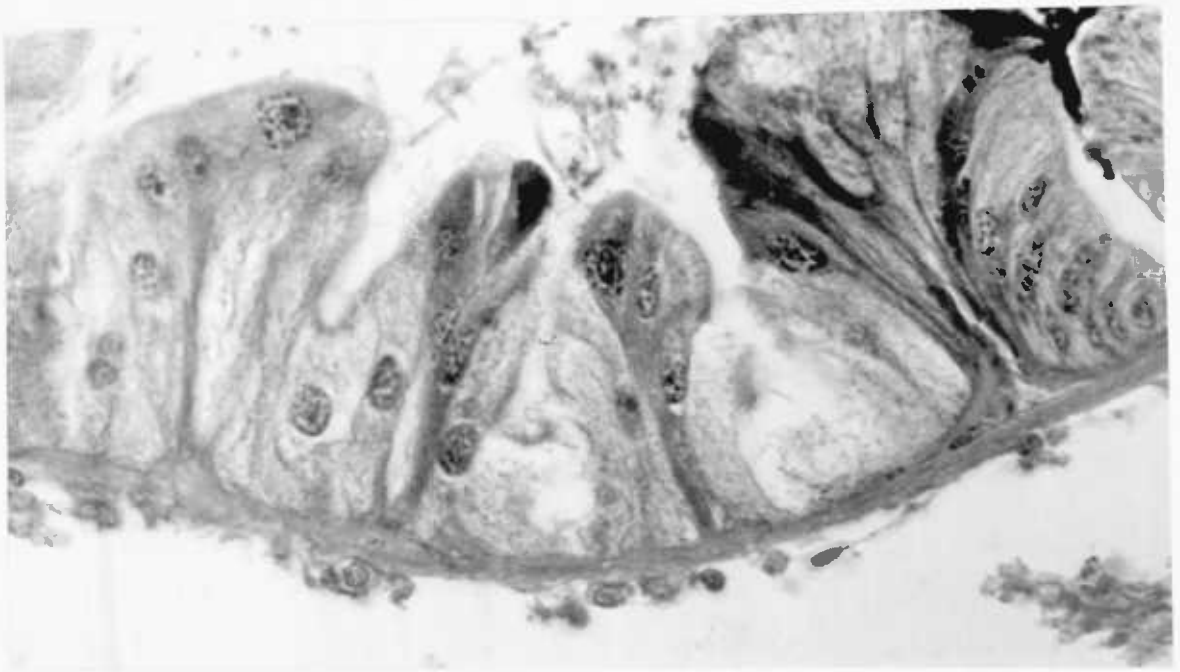


Figure 72 L.S. 6th. ventriculus, desiccated for 5 days

25µm

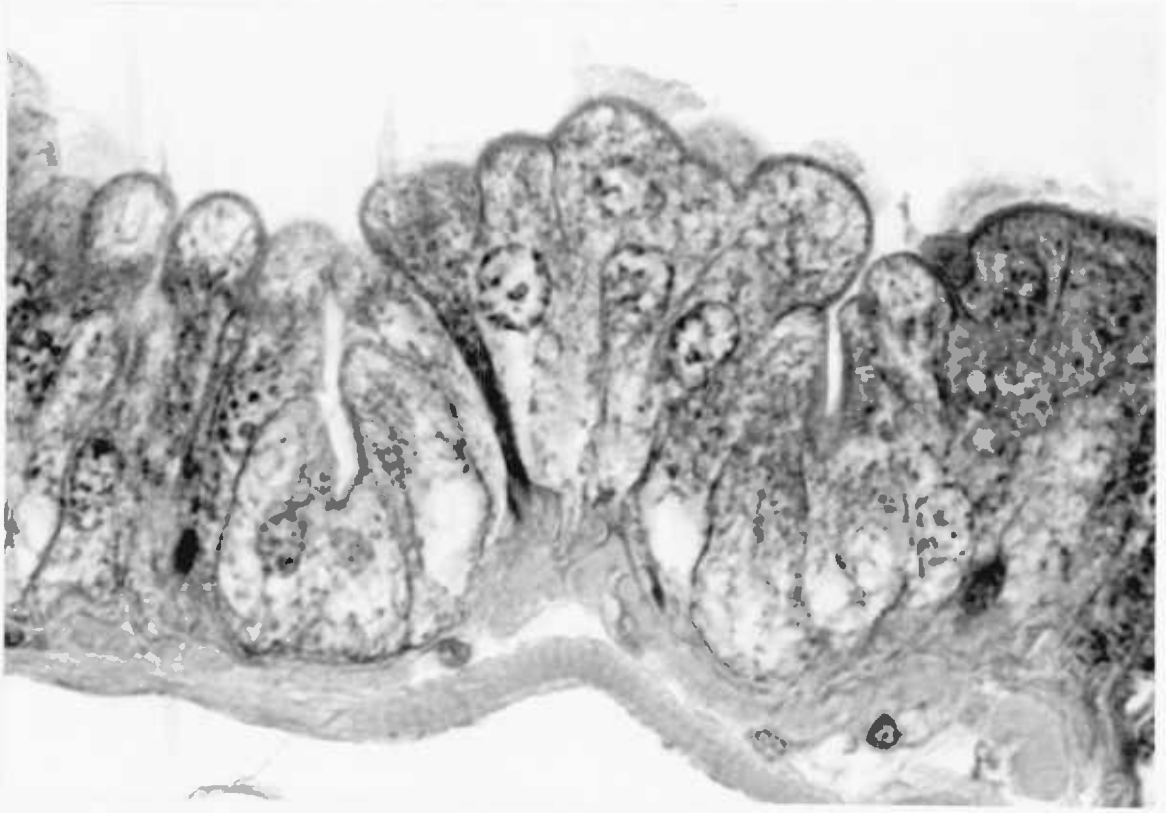


Figure 73 L.S. 1st. ventriculus, starved and desiccated

Starvation and Desiccation

In order to observe how starvation and desiccation affect the epithelium and in order to evaluate the previous results of other authors in this respect, this experiment was conducted on specimens which were not supplied with either seeds or water. In this condition the insects could not survive more than three days. Sections from the midgut were examined after specimens had been starved and desiccated for three days and the following observations were recorded. All measurement of the cells and their nuclei, with thickness of striated border are given in Table 33.

First ventriculus The epithelium falls into villi. The striated border is irregular, the cellular boundaries are not well differentiated and the cytoplasm has coarse granules, irregularly distributed. The apical ends of the epithelial cells show blistering and extrusion. The nuclei have no well defined nuclear membranes. Figure 73 and 74 represent the epithelium of the anterior and middle regions of this ventriculus.

Second ventriculus The epithelium appears collapsed with a very irregular striated border that varies in thickness and is broken in many places throughout its surface. The cytoplasm has coarse granules accumulated apically. The nuclei are irregular in shape and centrally located and have no well defined nuclear membrane (Fig. 75a).

Third and Fifth ventriculus The epithelium is much affected by starvation and desiccation. The cells have almost lost their clavate shape, the cytoplasm is highly vacuolated and the striated border is irregular in thickness and broken throughout its surface. Some of the cells show blistering and extruded ends. The nuclei are more basiphil than usual. Cellular and nuclear membranes are not well differentiated. The cytoplasm is more lightly

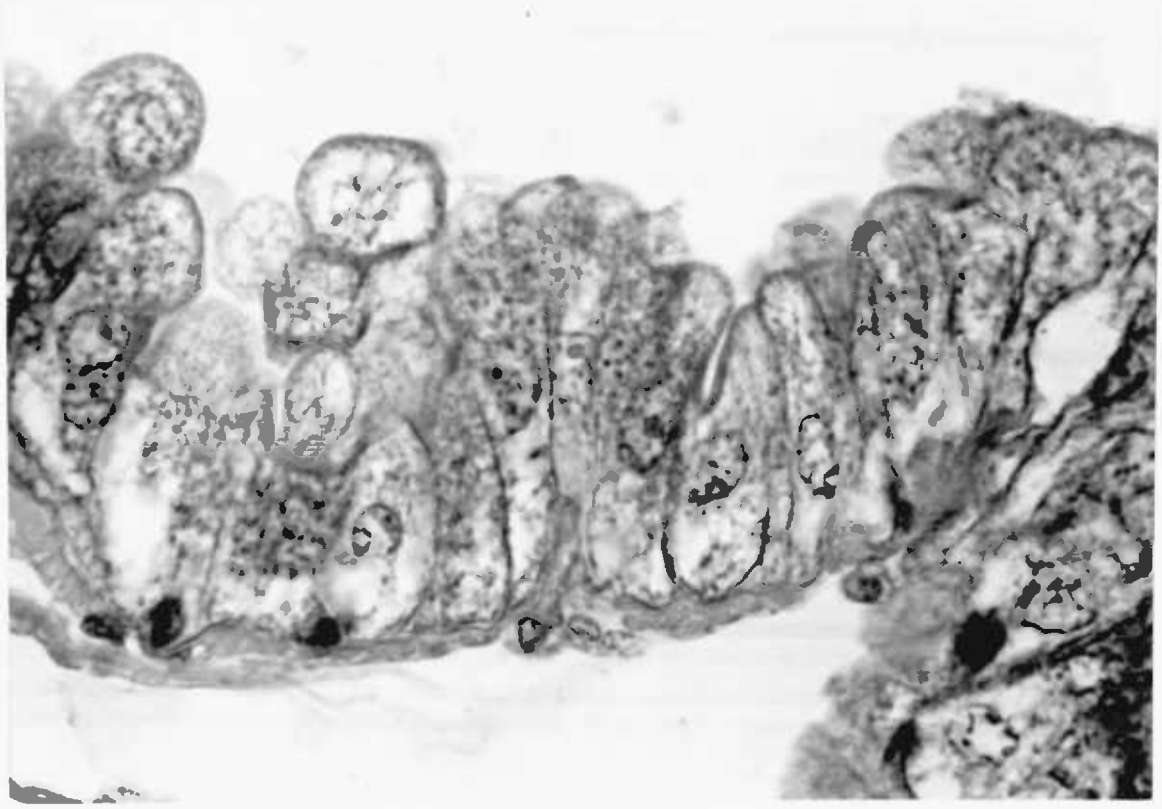


Figure 74 L.S. 1st. ventriculus, starved and desiccated

25µm

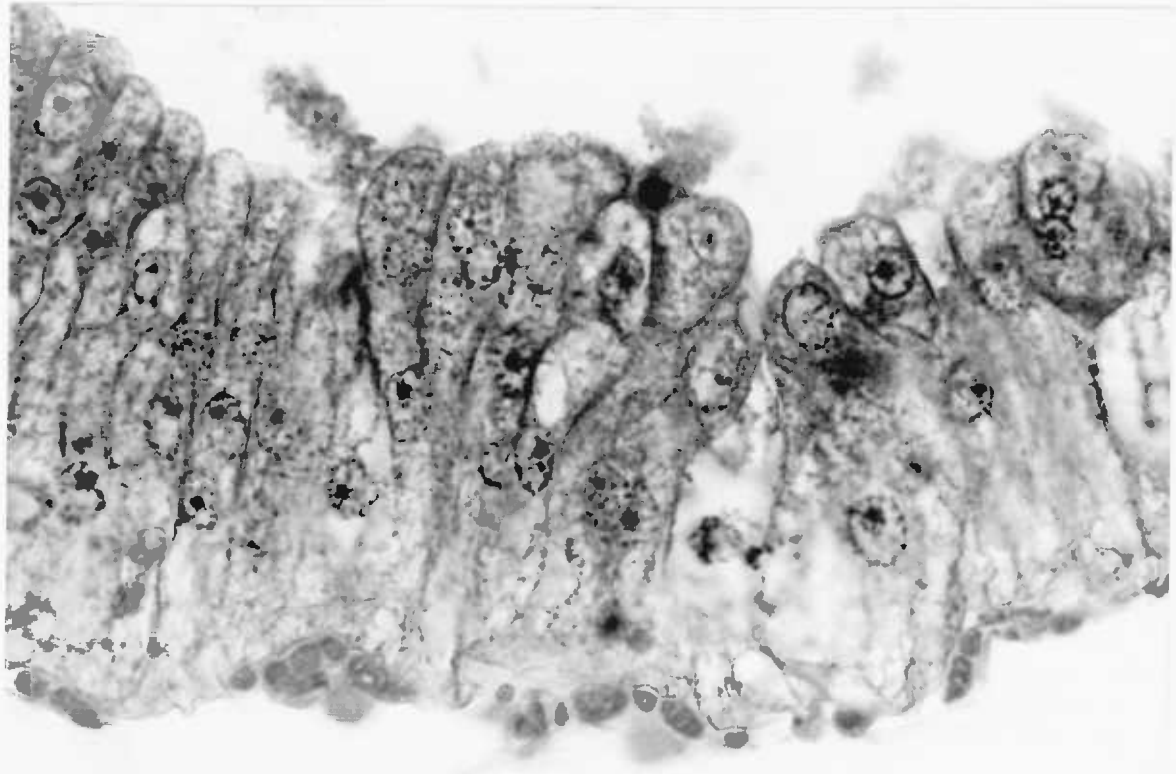
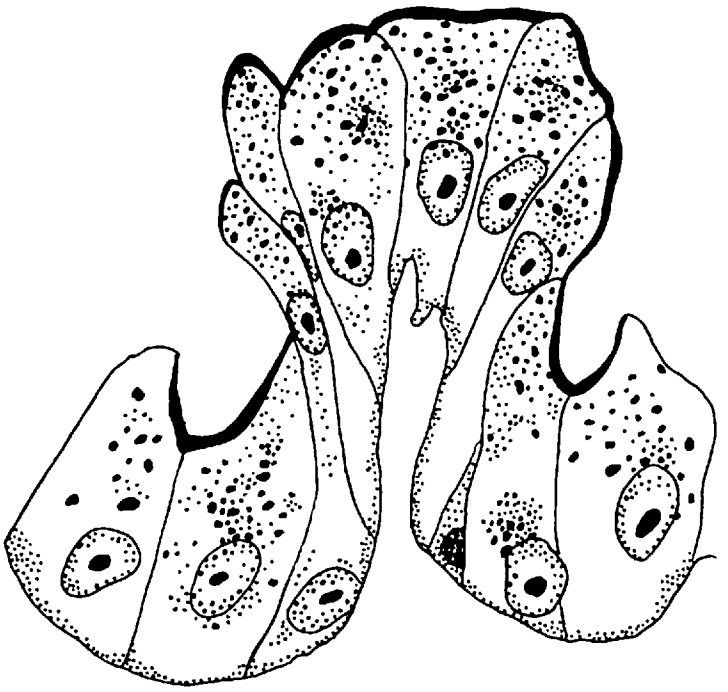
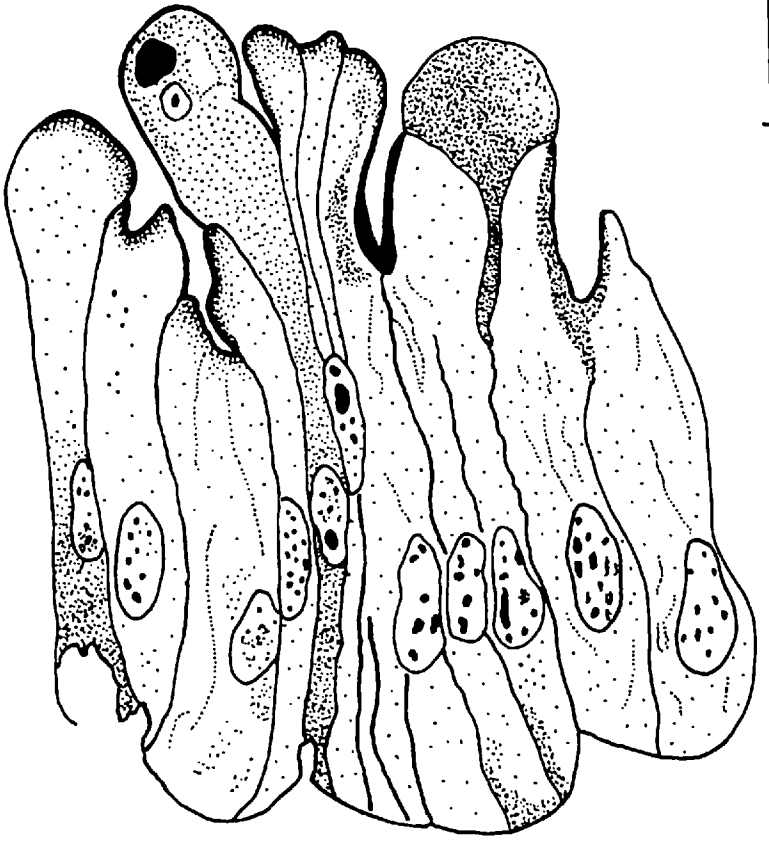


Figure 76 L.S. 5th. ventriculus, starved and desiccated



a- 2nd vent.



b- 3rd vent.

fig. 75 a and b newly emerged, starved and desiccated for 3 days

stained towards the basal halves of the cells. Many cells show a degenerative appearance. Figure 75b represents the epithelium of the 3rd. ventriculus while Fig. 76 represents that of the fifth ventriculus.

Fourth ventriculus The epithelium is formed of high columnar cells not arranged in villi. The striated border is irregular, the cytoplasm vacuolated and the nuclei are more basiphil and almost centrally located. Some of the epithelial cells show blistering and extrusion (Fig. 77).

Sixth ventriculus The epithelium is also built up of high columnar cells with an irregular striated border. The cytoplasm takes on a deep colour with PTAH and its structure appears highly disturbed. Neither the cellular nor the nuclear membrane are well defined. The cytoplasm appears more vacuolated basally (Fig. 78).

General conclusions The following facts could be concluded from the observations made in the above experiments.

1. Starved specimens can survive for a longer period than desiccated, while specimens which were deprived of both seeds and water could not survive longer than 3 days.

2. The effect of starvation and desiccation were greatest in the first ventriculus epithelium. Next to this the second ventriculus epithelium was most affected.

3. The midgut epithelium generally showsbulbous apical ends with some extrusions especially in the first and second ventriculi during starvation and desiccation. This phenomena was also observed in normally fed specimens but in these it occurs in a higher degree.

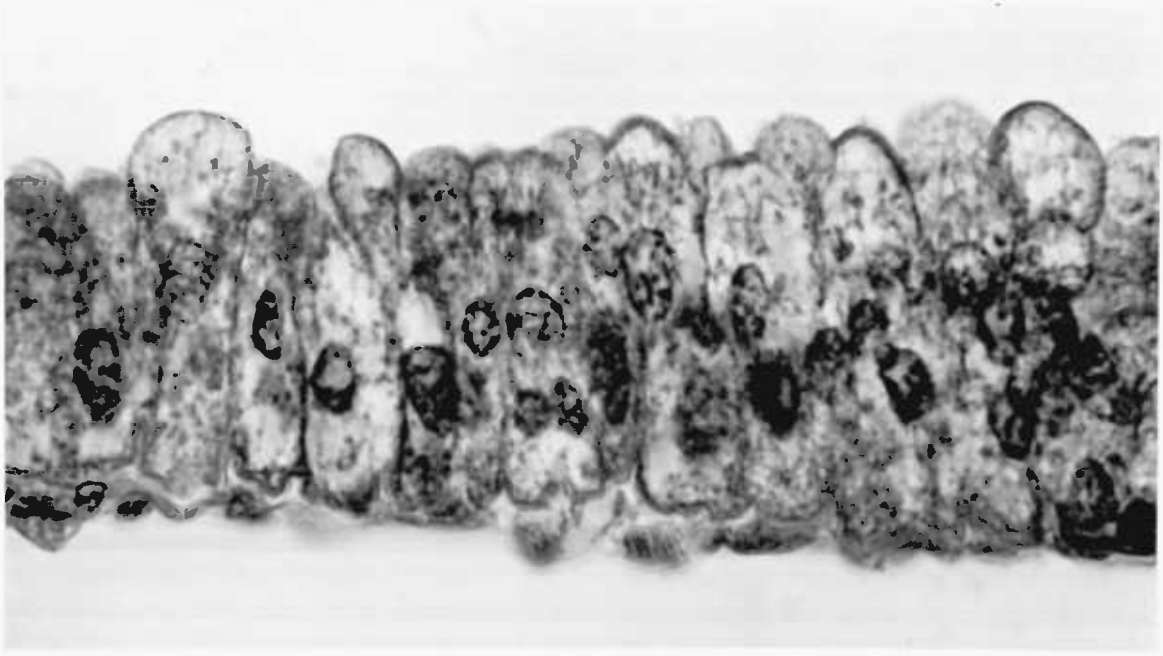


Figure 77 L.S. 4th. ventriculus, starved and desiccated

50µm

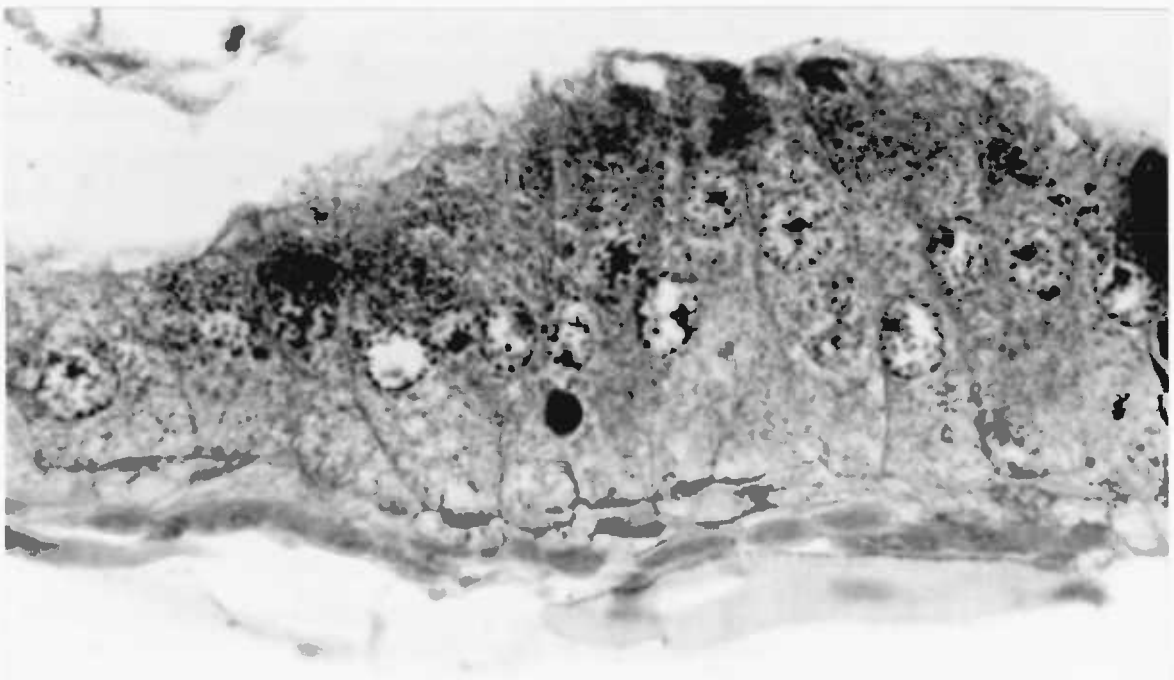


Figure 78 L.S. 6th. ventriculus, starved and desiccated

4. The midgut epithelium never show shedding of the apical ends of the cells, a phenomena that can usually be observed in the midgut epithelium of normally fed specimens, especially in the first segment.

5. The epithelia of the 3rd. and 5th. ventriculi respond similarly to starvation and desiccation; the cells lose their clavate shape, becoming more elongated. Such deformation was never observed in normally fed specimens. The similarity in the behaviour of the epithelia of these two ventriculi was also previously observed during the study of postmetamorphic changes in the midgut epithelium of Oncopeltus fasciatus (p. 78).

6. The epithelium of the sixth ventriculus shows some bulbous cells with the same apical extrusions during starvation and desiccation as those found in more anterior segments of the midgut. This provides further evidence that this segment is a part of the midgut.

SECTION 5

ULTRASTRUCTURE OF THE MIDGUT EPITHELIUM

ULTRASTRUCTURE OF THE MIDGUT EPITHELIUM

Introduction

The structure and physiology of the midgut epithelium of insects has been studied in great detail and is thoroughly documented (Roesder, 1953; Wigglesworth, 1972; Rockstein, 1965). With the advent of more precise cytological methods and high-resolution microscopy, many differences in fine structure have been demonstrated, providing valuable information on different aspects of the subject. Reviews of the light- and electron-microscopical morphology have been given by McGee - Russell and Ross (1968), Smith (1969) and Threadgold (1976). Very little work has been done on the cytophysiology of the Hemiptera, nearly all of it on Homoptera : Auchenorrhyncha.

The electron microscope has proved very valuable in revealing some structural features in the midgut of Oncopeltus fasciatus in the present work. One of the purposes of the present study was to investigate the ultra-structure of the midgut epithelium to determine whether a morphological expression of its functional differentiation could be demonstrated. For this purpose the midgut epithelium of the 1st., 2nd., 3rd., 4th. and 6th. ventriculi of Oncopeltus were studied partially at 5 and 15 days only; all the insects used were well fed specimens. The main features of the ultra-structure appearance can be summarized as follows.

Apical cell border The apical border is composed of microvilli, each of which is an elongate cylindrical projection whose length varies, depending on its location. Its cytoplasm is of uniform texture and lacks organelles. In the first ventriculus, the lumen adjacent to the apical border, contains considerable, irregular more or less flocculent material. The microvilli are constant in length, diameter, direction and shape and lie parallel to

each other, with rounded ends. In cross section they appear as clusters of small, circular or oval profiles and they are not confined to the apical region of each epithelial villus but are also found on its basal portions (Fig. 79). The material covering the microvilli varies in extent, depending on the gut segments (Fig. 80). In the 2nd. ventriculus, the microvilli are closely packed and the flocculent material is accompanied by long microvillar projections emerging from the cell surface as shown in Fig. 81. This rather indistinct material is thicker here than in the 1st. ventriculus and seems to be composed of a network of membranes in close contact with microvilli. Figure 82 illustrates the cell border of the 4th. ventriculus. As in the other regions, this shows quite remarkable finger-like microvilli with similar material at the apex. The 6th. ventriculus also has very prominent microvilli, with similar material covering their outer surface, thus confirming that this segment is a part of the midgut (Fig. 83). Inside each microvillus there are indications of an axial filament which extends basally as an intracytoplasmic rootlet (Fig. 84f). A true terminal web is lacking but the apical cytoplasm, where the rootlets are situated, is devoid of some cellular organelles (mitochondria; Golgi bodies) and is also clearly different from the underlying cytoplasm.

Intercellular membrane The plasma membranes of adjacent cells are in close contact (Fig. 85). At intervals small groups of irregularly shaped projections are present on the lateral margin of a cell. These interdigitate with a neighbouring cell to form a locking mechanism (Figs. 86, 87). Where cells adjoin, the cell membrane appears as an electron-opaque layer, clearly separated from the membrane of the adjacent cells by a less dense region of fairly constant width, at one or two placed in some sections. Figure 86 also illustrates the extension of the intercellular membrane and the arrow indicates its ends; the intercellular material has disappeared gradually.

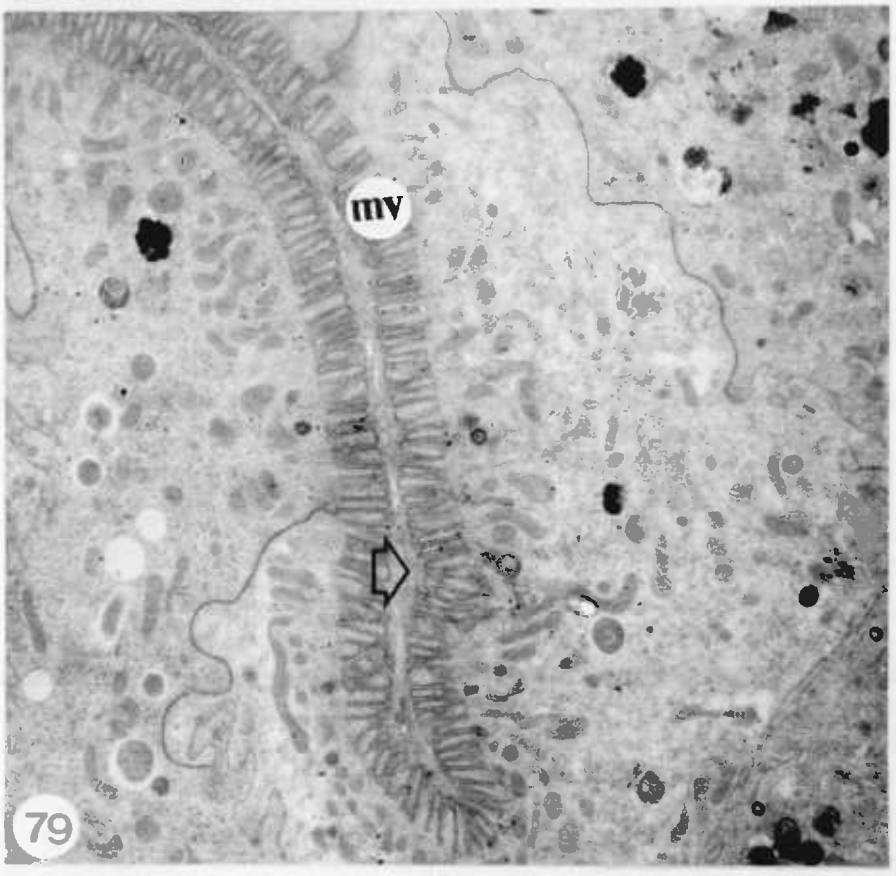


Figure 79 Electron micrograph L.S. of 1st. ventriculus apical border, Microvilli (mv) restrict the lumen (arrow) filled with presumed secretory material. x 3000

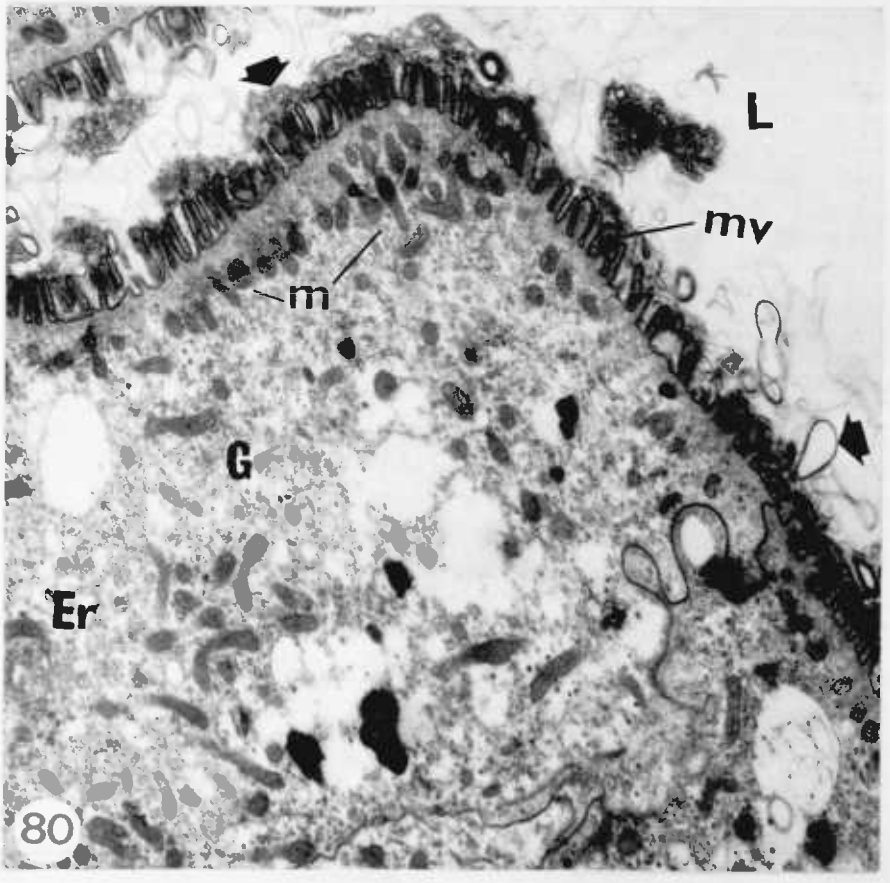


Figure 80 T.S. 1st. ventriculus 15 days old apical region of epithelial cells. Region indicated by arrows shows adherent flocculent material. x 6000

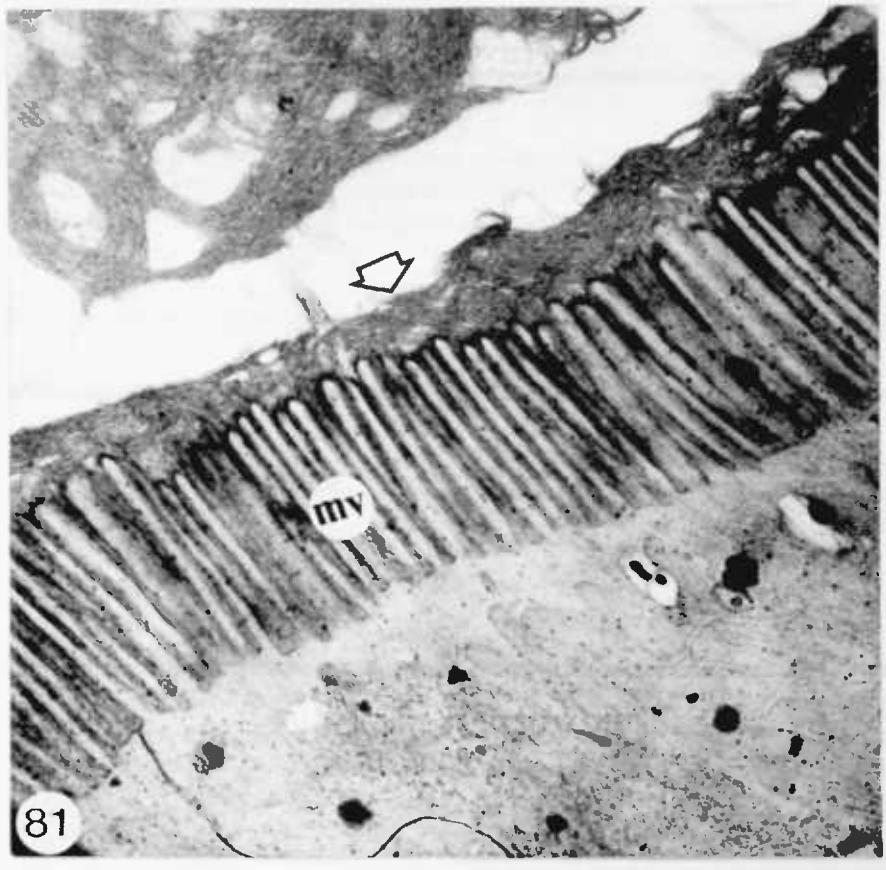


Figure 81 L.S. 2nd. ventriculus, apical region of epithelial cells showing flocculent material (arrow) x 8500

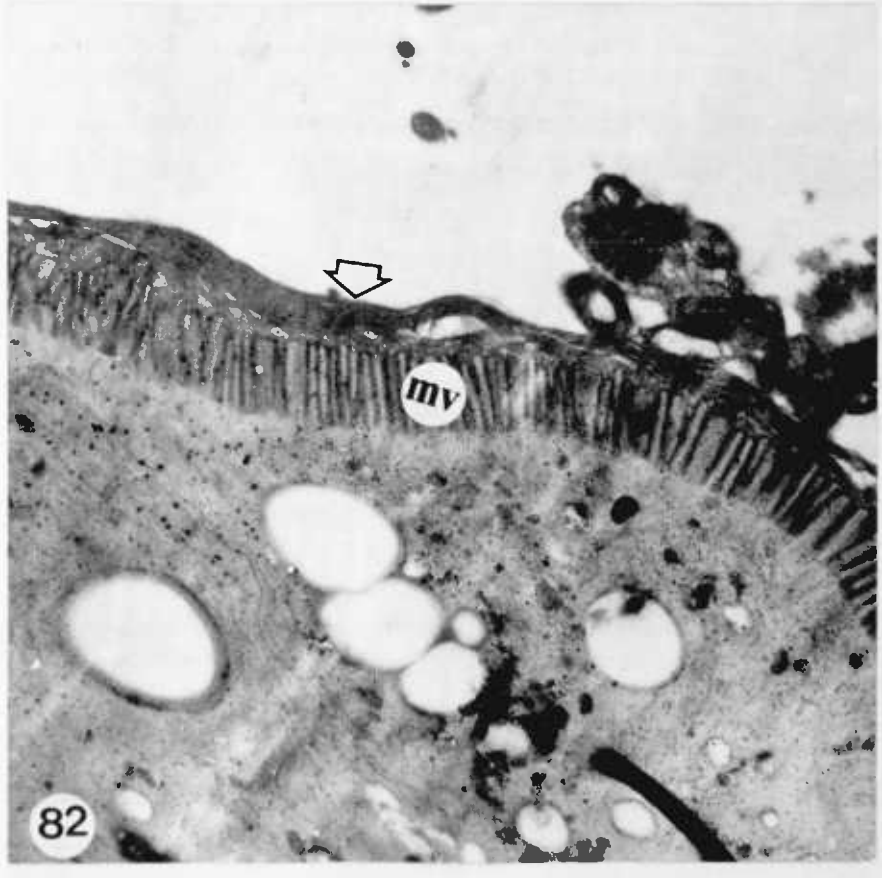


Figure 82 L.S. 4th. ventriculus, apical region of epithelial cells showing flocculent material (arrow) x 8000

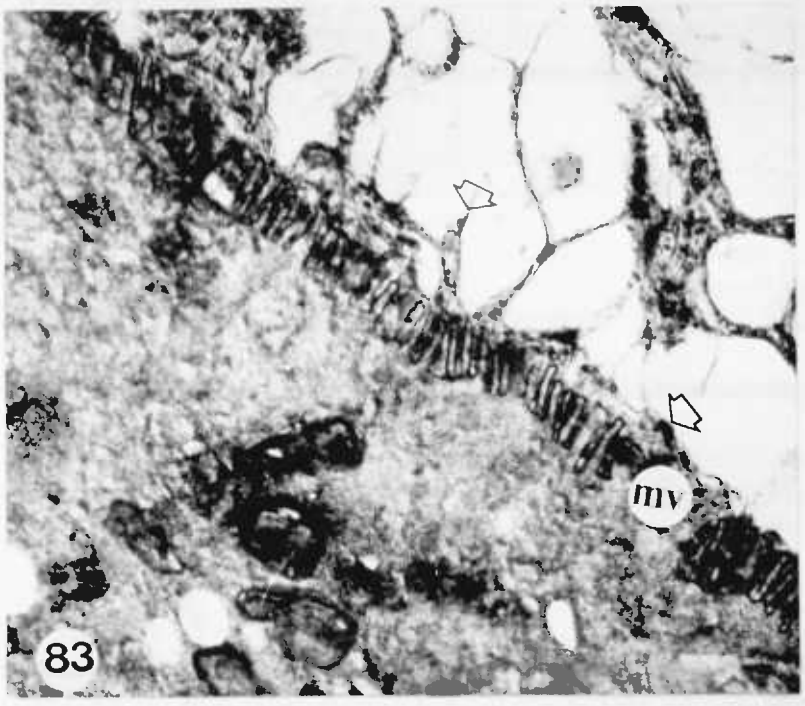


Figure 83 **L.S.** 6th. ventriculus, apical region of epithelial cell showing floccular network of material (arrows) x 5500

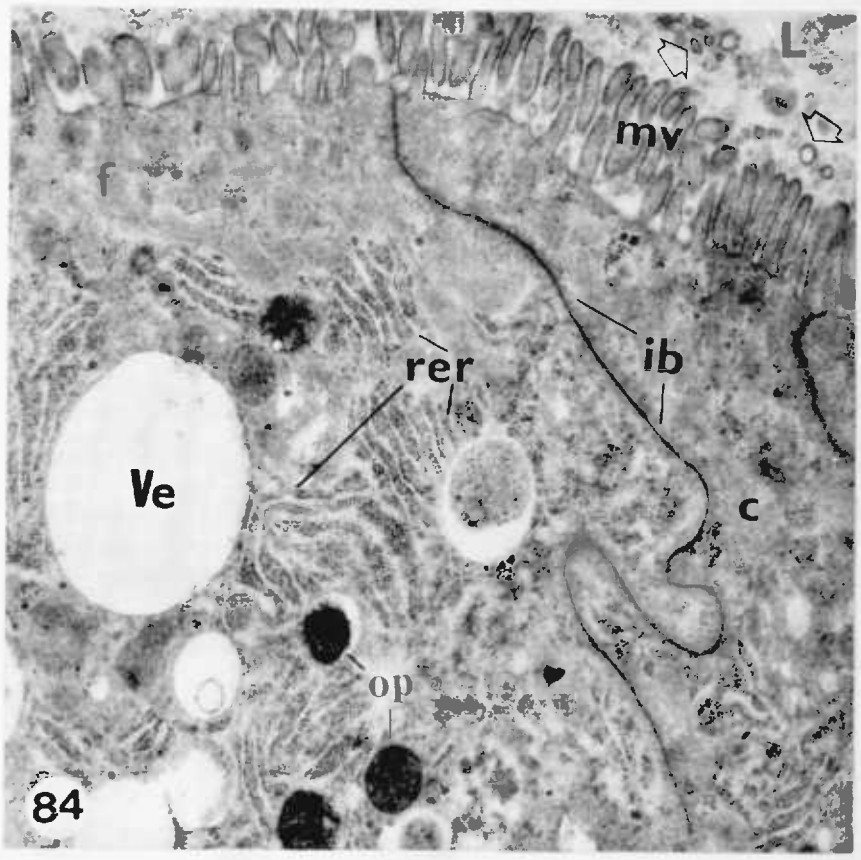


Figure 84 **L.S.** 1st. ventriculus, 5 days old. Portion of apical region at their surface to show microvilli (mv) projecting into the lumen (L); electron-dense profiles (arrows); rough endoplasmic reticulum (rer); inter-cellular boundary (ib); vesicles (ve); opaque granules (op) x 8000

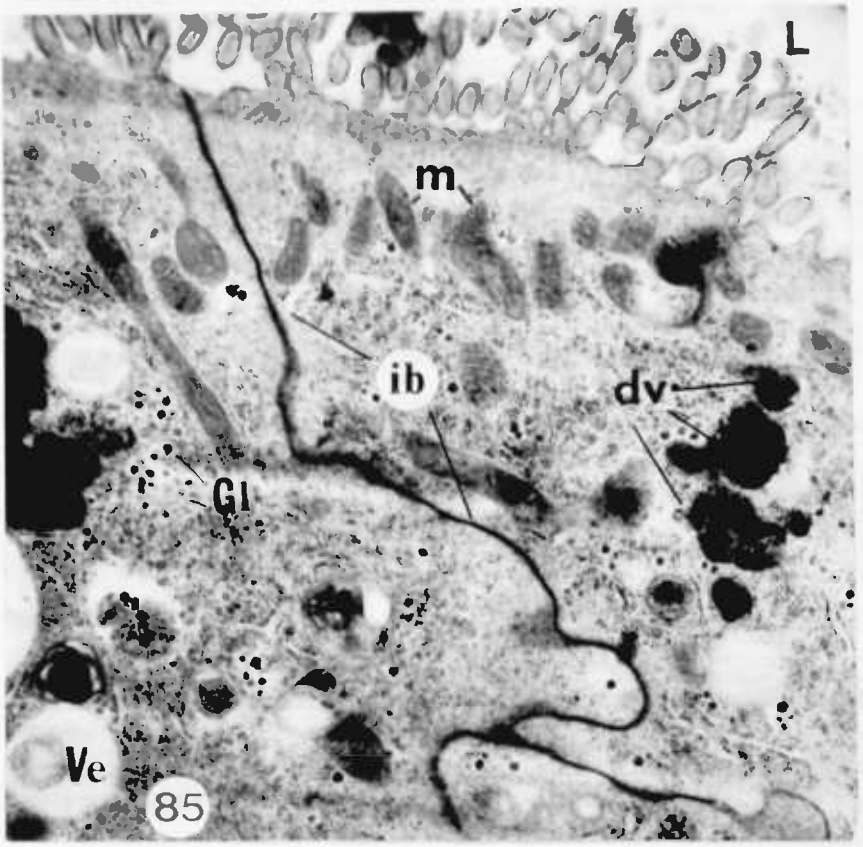


Figure 85 L.S. 1st. ventriculus apical region showing intercellular boundary (ib); mitochondria (m); dense vesicles (dv); vesicles (ve); Glycogen (Gl) x 8500

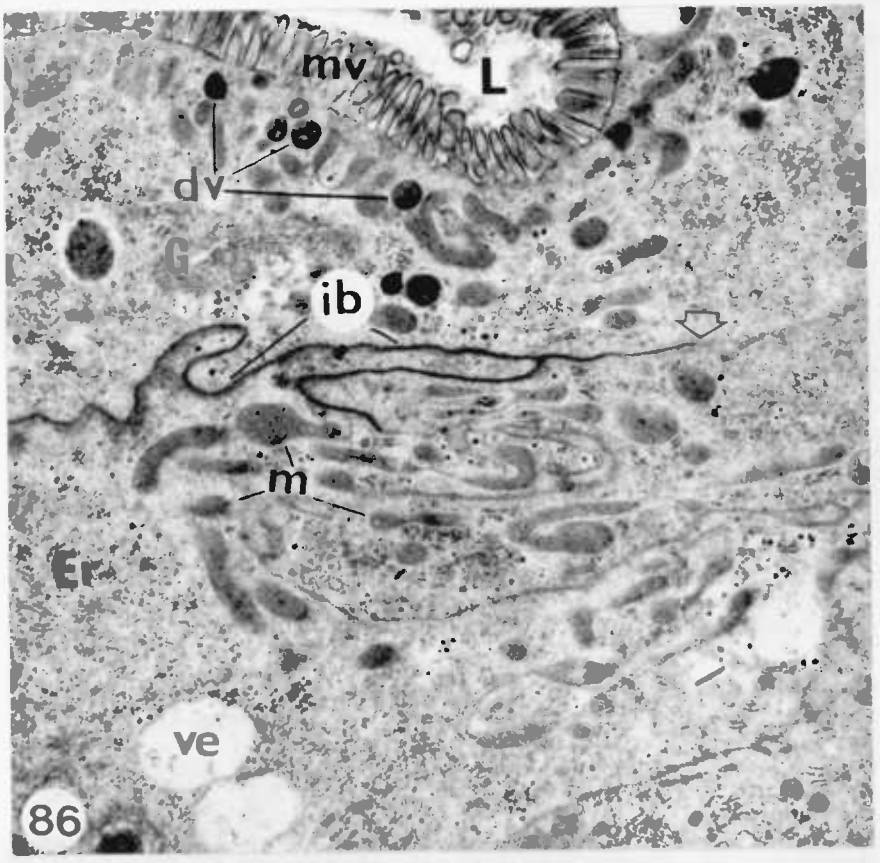


Figure 86 L.S. 1st. ventriculus; apical region showing the extent of intercellular membrane (ib) and arrow indicating gradual disappearance of electron-dense boundary x 7000

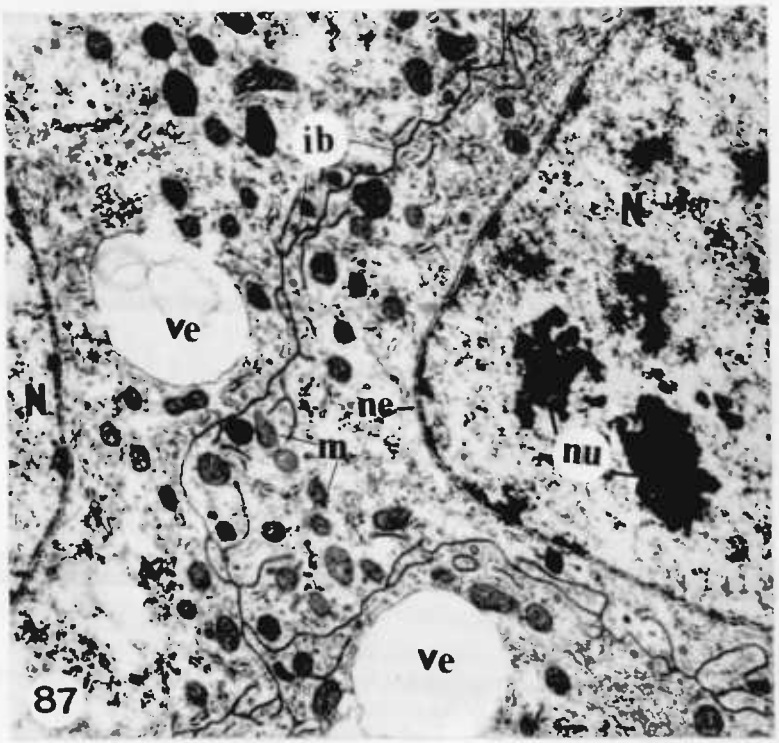


Figure 87 L.S. 1st. ventriculus; illustrating intercellular boundary (ib) enclosing mitochondria (m) and nuclei (N) of two cells x 8500

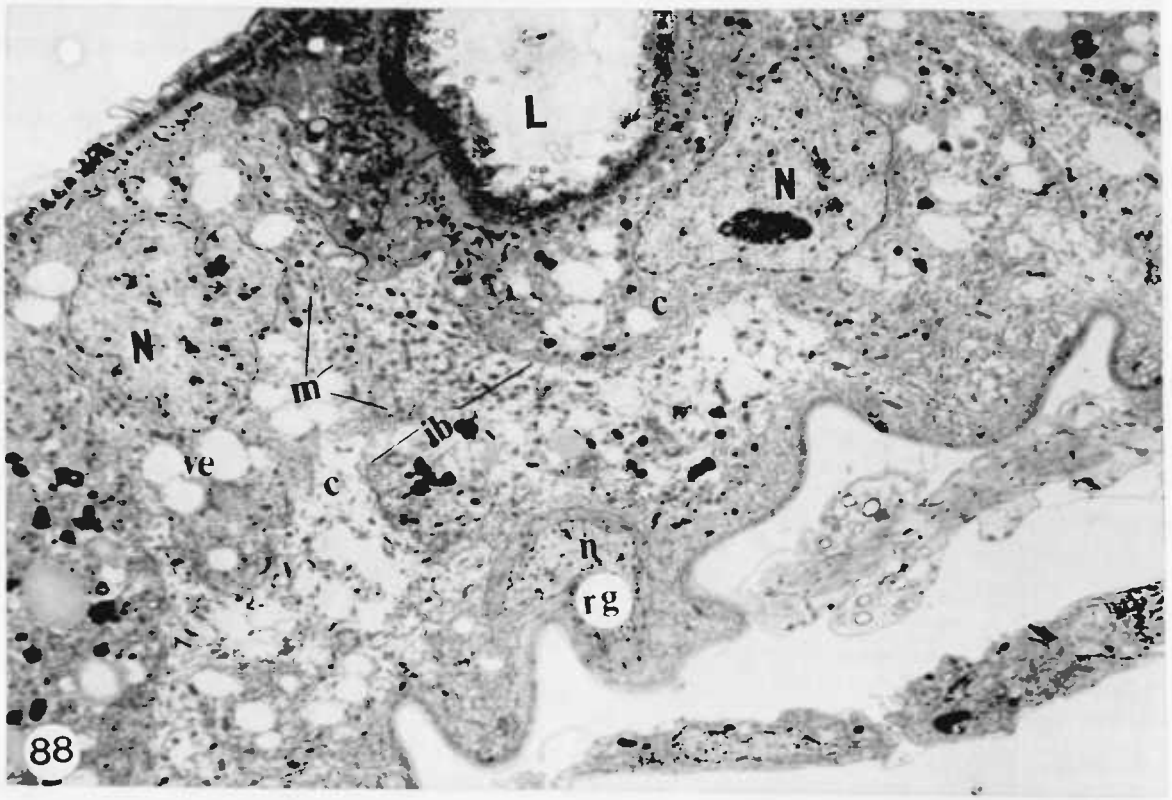


Figure 88 T.S. 1st. ventriculus; 15 days old; illustrating cytoplasm (c) losing its rough endoplasmic reticulum, with cluster of vesicles (ve) x 2000

Cytoplasm The cytoplasm immediately below the striated border or microvilli (Figs. 84, 85, 89) lacks the endoplasmic reticulum which elsewhere consists of rough surfaced membrane surrounding vesicles (Fig. 84). The cytoplasm looks dense, with much rough endoplasmic reticulum at an early age and then at 15 days old it becomes slightly less dense with scattered endoplasmic reticulum and numerous free ribosomes (Figs. 80, 87, 88). Some cells contain large numbers of vesicles containing clear, electron-lucent material or with membranous contents; other cells contain abundant bodies similar to lysosomes, cytolysosomes, phagolysosomes and residual bodies (Figs. 84, 85, 86, 89). The mitochondria are more numerous near the apical border (Figs. 80, 89). A large vacuole surrounded by small dense masses was sometimes found near the luminal border (Figs. 84, 89). Mitochondria, possessing the usual external membrane and internal cristae, are numerous, particularly in the region of the cell adjacent to the lumen; elsewhere they are scattered through the cytoplasm including that lying basal to the nucleus near to the basement membrane (Fig. 90). They are elongate in form and for the most part are arranged more or less parallel to the long axis of the cell (Figs. 87, 89). Glycogen micro granules occur in these cells but no areas were found at present that can definitely be recognised as always containing the glycogen. It is probably distributed generally throughout the cytoplasm.

A Golgi complex may occur in all regions of the midgut cells, but is never well defined. Vesicles containing low density material and surrounded by smooth membranes are fairly numerous (Figs. 84, 86*). Similar structures are sometimes seen in groups associated with the spherical vesicles and they may represent the Golgi complex, if indeed it is present in these cells. The number and size of the vacuoles vary in different cells; single vacuoles are often present, each surrounded by a distinct membrane (Figs. 84, 87). A large number of heterogenous structures were observed, all having

* G = Golgi complex

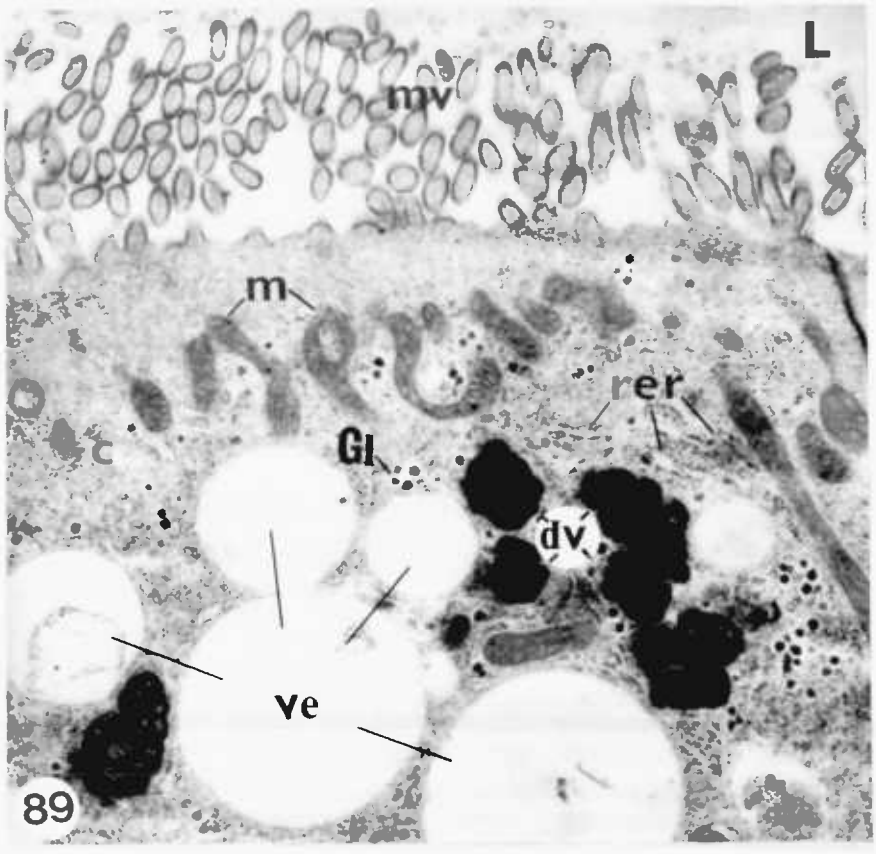


Figure 89 L.S. 1st. ventriculus; apical region 5 days old; showing Mitochondria (m) accumulated apically with dense vesicles (dv) and vesicles (ve) x 8500

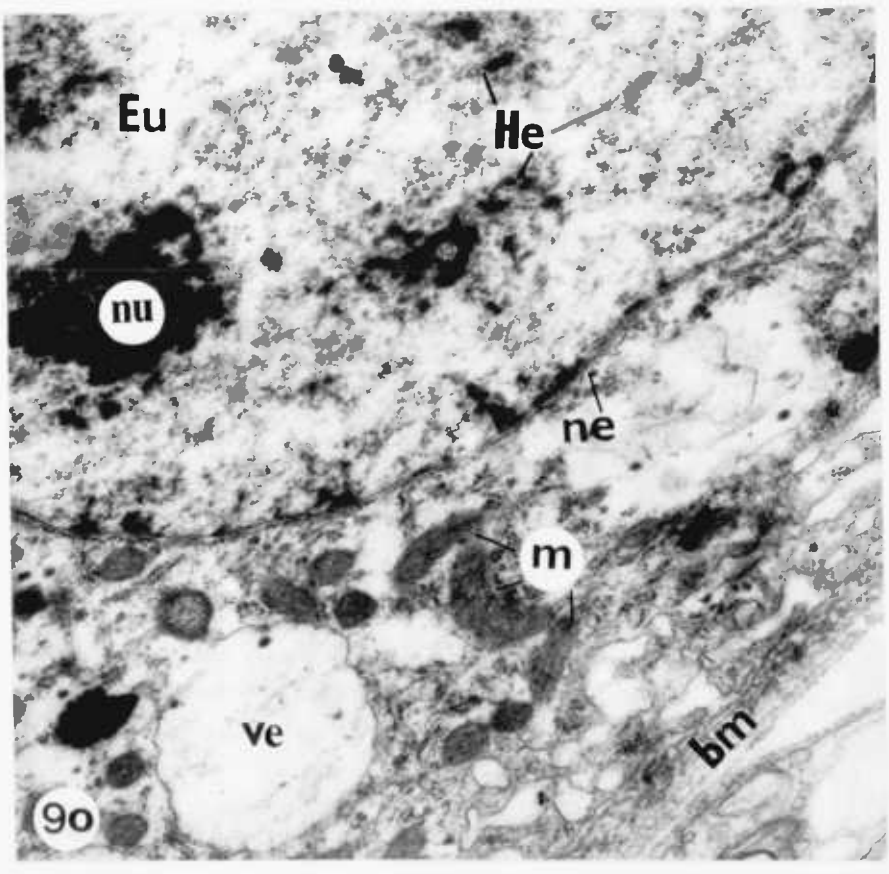


Figure 90 L.S. 1st. ventriculus, illustrating Mitochondria (m) enclosed between nucleus (N) and basement membrane (bm) x 13 500

a single limiting membrane which suggests that they may belong to a population of lysosome-like organelles.

Nucleus The nucleus is not confined to a fixed position; it may lie apically (Fig. 88) basally (Fig. 90) or centrally (Fig. 91) depending upon the cell. The nuclei vary between ovoidal in shape or more spheroidal with quite a large diameter. The nuclear membrane is, as usual, composed of two dense components enclosing a region of low density (Figs. 90, 91, 92). Electron-dense granules are in contact with both its inner and outer surfaces and irregular, less dense structures are scattered through the nucleoplasm. These appear to correspond to heterochromatin and euchromatin respectively. The nucleolus is irregular in outline and contains numerous granules of high electron density (Fig. 92) or it may appear as a compact mass as in Fig. 90; this structure may be broken up into smaller bodies (exactly the same is evident with light microscopy)(Fig. 91). The nuclear envelope is quite clearly a tripartite structure being composed of two membranes separated by a lucid space; these are respectively the inner nuclear membrane (next to the nucleoplasm space), the perinuclear space and the outer nuclear membrane in contact with the cytoplasm. The envelope is pierced by pores where the two membranes unite and the openings allow communication between the nucleoplasm and cytoplasm. Anderson and Beams (1956) showed that a number of investigators found that the nuclear membrane is not a continuous structure but is interrupted at intervals by pores, nodes, granules or ring-like formations. Most authors emphasize the structure of the membrane and its porous nature as significant in facilitating the interchange of material between the nucleus and the cytoplasm. The outer membrane has a rough outline due to the presence of attached ribonucleoprotein (RNP). The inner membrane is smoother and more regular than the outer and nuclear pores occur whenever the two membranes join. Some of the cells are binucleate (Fig. 93).

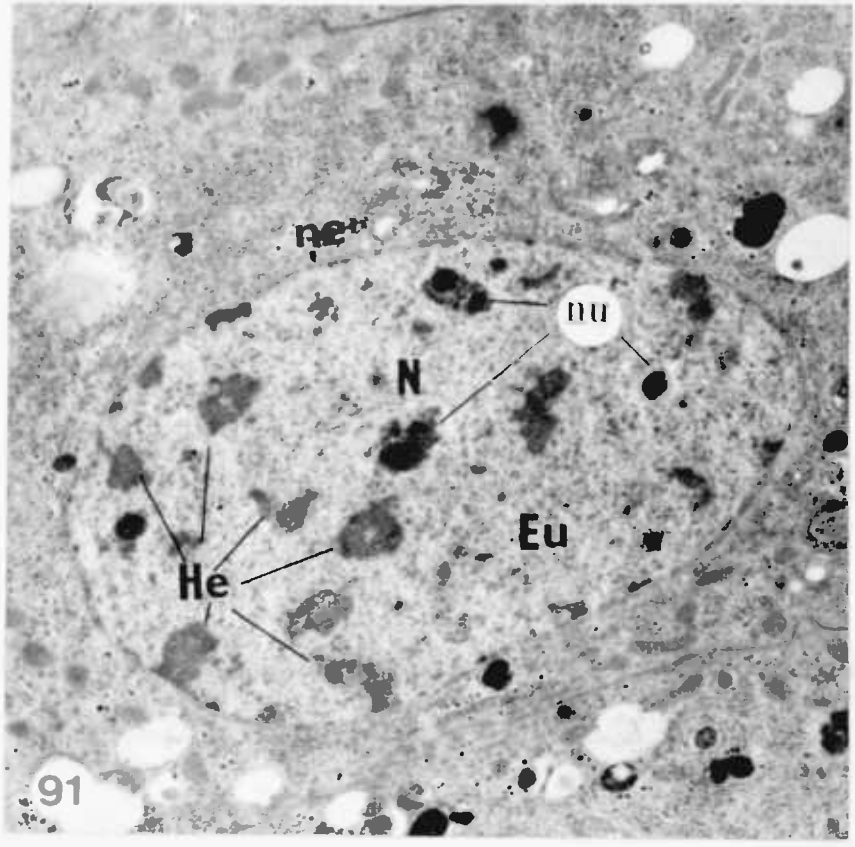


Figure 91 L.S. 1st. ventriculus, illustrating nucleus (N) located centrally with more than one nucleolus (nu), euchromatin (Eu) and Heterochromatine (He), all within nuclear envelope (ne) x 9000

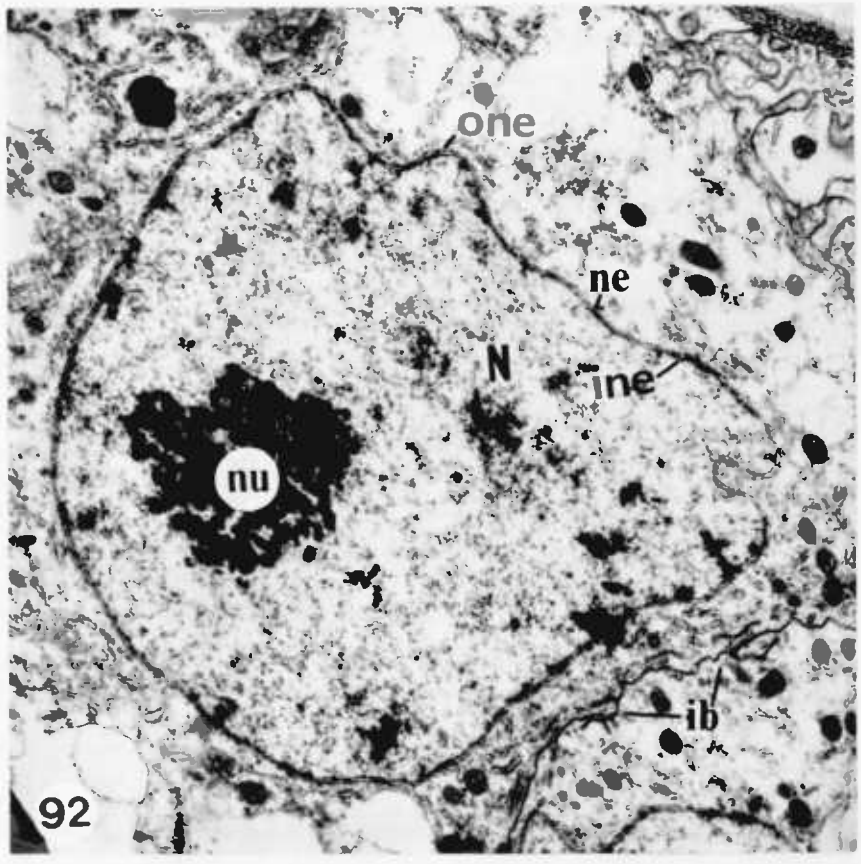


Figure 92 L.S. 1st. ventriculus, illustrating nucleus (N) of irregular shape; nuclealus (nu); inner (ine) and outer nuclear envelope (one) x 9500

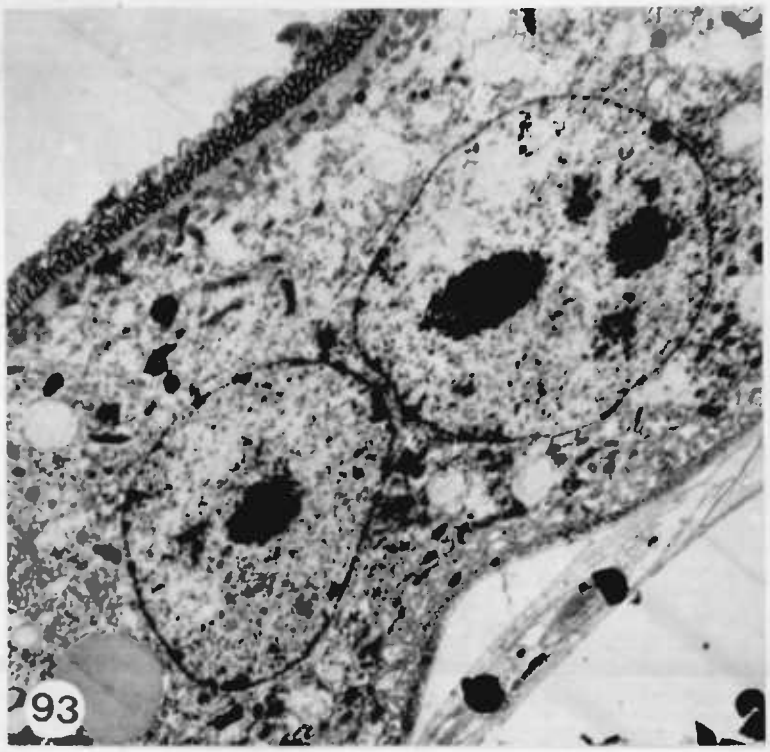


Figure 93 T.S. 1st. ventriculus, binucleate epithelial cell x 2500

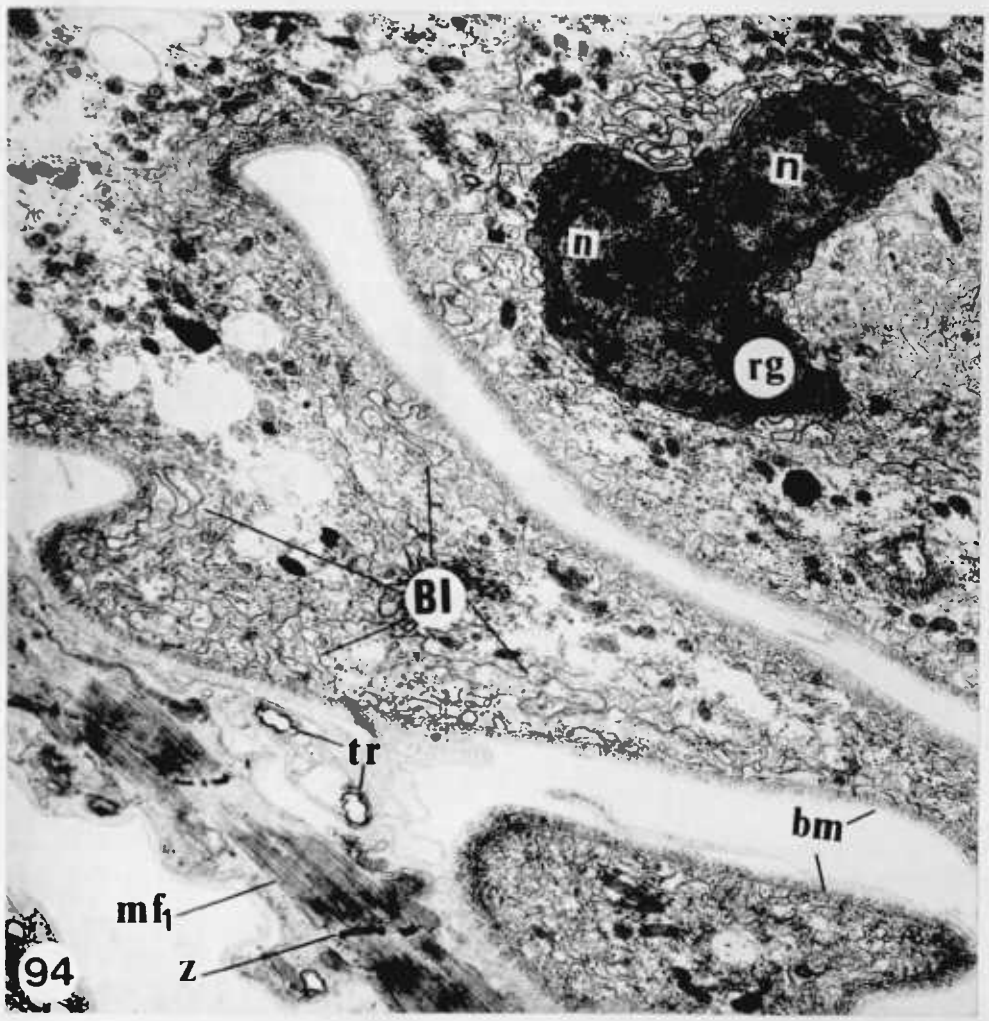


Figure 94 T.S. 1st. ventriculus, basal region representing two adjacent regenerative cell (nidi)(rg); basal invagination (BI); basement membrane (bm); circular muscle fibre (mf_1); Z-band (Z); tracheoles (tr) x 5000

Regenerative cell (Nidi) The regenerative cells in Oncopeltus are usually present at the base of the epithelial cells. They do not make contact with the basal plasma membrane and the greater part of their cytoplasm is located in the basal portion of the epithelium. The nidi cells may contain one or two nuclei. Figures 94 and 95 represent two nidi cells with large nuclei. Mitochondria are sparse and smaller than in columnar cells and the nucleus is quite large or even more voluminous than the cytoplasm, occupying almost the whole cell body. The nucleolus with chromatin granules is always very clear, the nuclear envelope is tripartite as in the main epithelial cell nucleus. The cytoplasm contains little endoplasmic reticulum but may include free ribosomes.

Basal plasma membrane and basement membrane The plasma membrane and to a less extent, the basement membrane are deeply folded, the former penetrating deeply into the basal cytoplasm. The basal cell membrane generally shows a highly convoluted appearance in section (Fig. 96), being deeply infolded into the cell at frequent intervals. The basal cell membranes differ markedly between the first and second ventriculi. In the first ventriculus (Fig. 97) the plasma membrane is not so deeply or extensively folded but in places the folds come together to form compartments. Mitochondria and vesicles are often closely associated with these compartments. In the 2nd. ventriculus (Fig. 98) the basal plasma membrane is very complex, being deeply infolded into the cell at frequent intervals. These infoldings extend apically for about three-quarters of the height of the cells; sometimes branching and anastomosing in their course to form an interconnected set of compartments. Inside the cell adjacent to the channels are numerous mitochondria. The basal plasma membrane is surrounded by a basal lamina or basement membrane, with a sinus between them. The basal plasma membrane of the 3rd. ventriculus is also highly folded to a degree intermediate between the 1st. and 2nd. ventriculus with large granules scattered in this region (Fig. 99); according to Barrini (1970) in

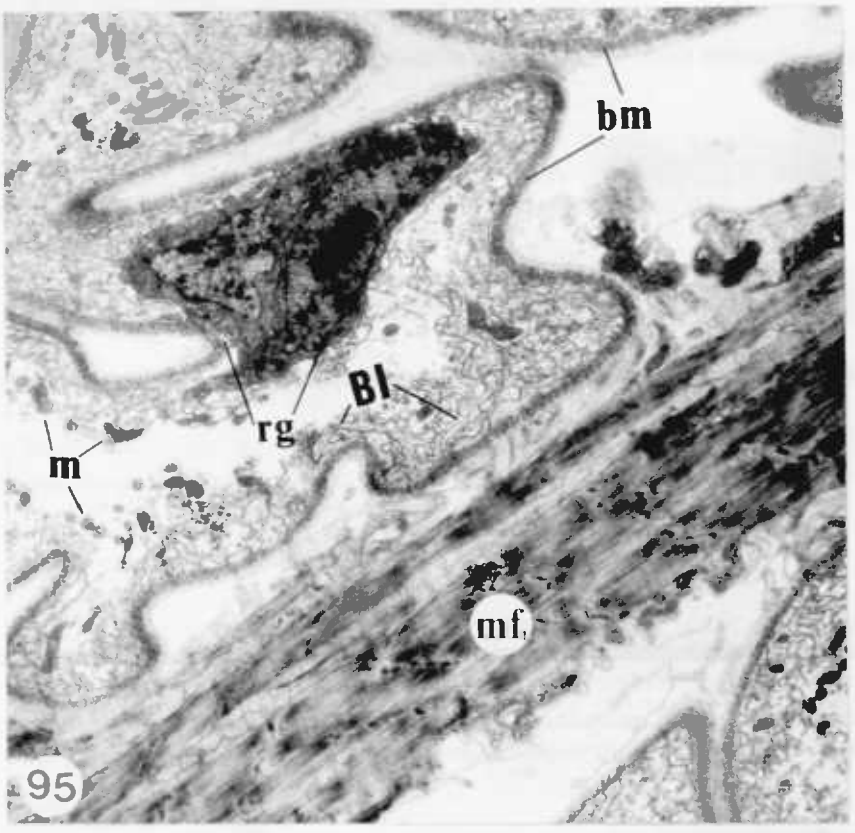


Figure 95 T.S. 1st. ventriculus, illustrating basal plasma membrane (bm) highly folded with two regenerative cells (rg), mitochondria (m), circular muscle fibre (mf,) x 5500

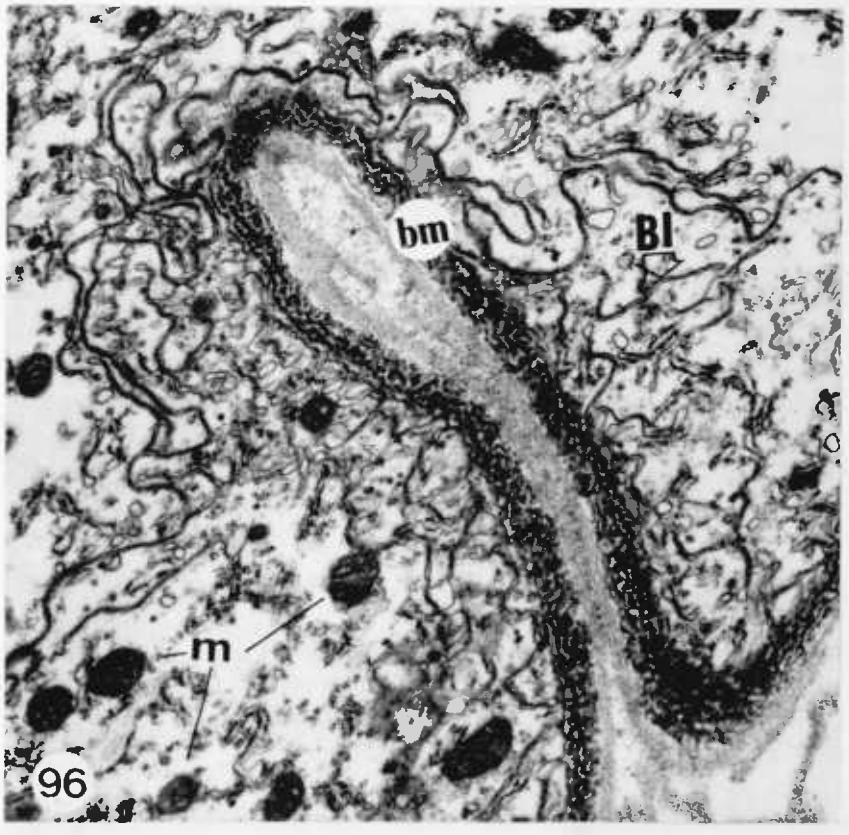


Figure 96 T.S. 1st. ventriculus illustrating basal plasma membrane with deeply infolded basal invagination (BI) and basement membrane (bm), also folded x 13 000

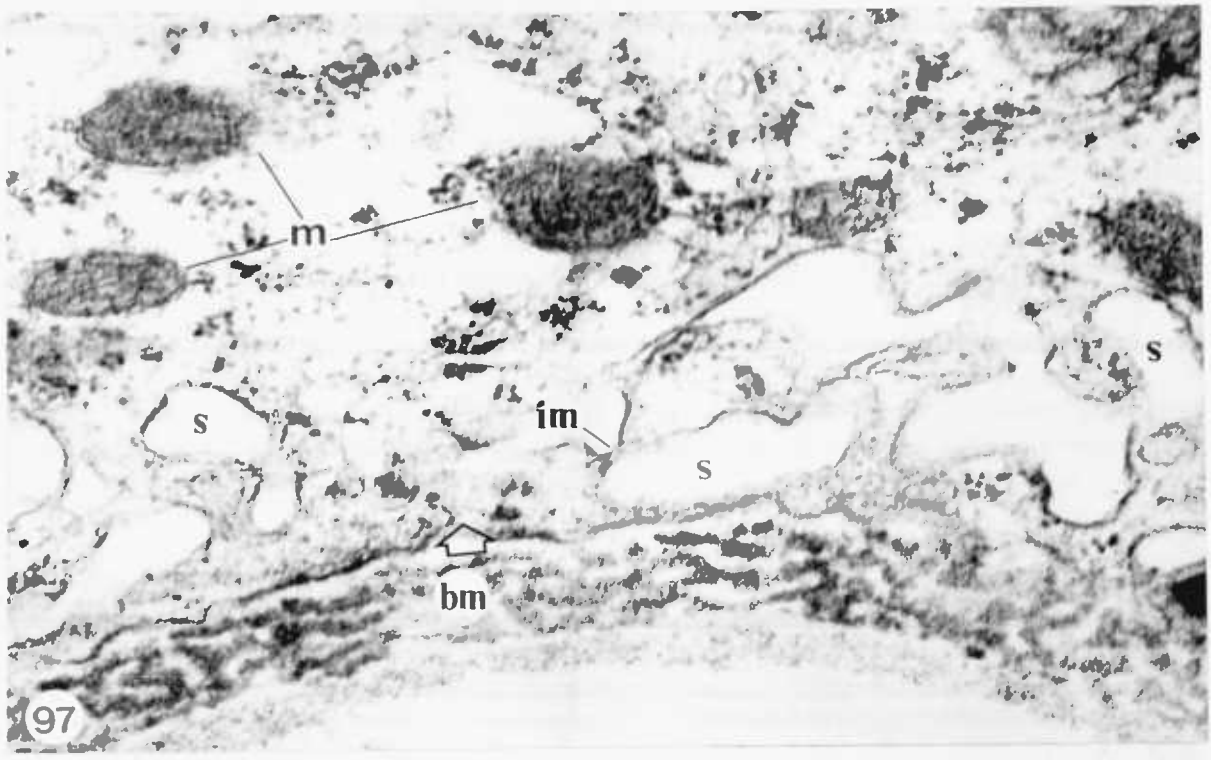


Figure 97 T.S. 1st. ventriculus; higher magnification of basal region illustrating the basal plasma membrane infolding as noted by arrow to form a very complex labyrinth which encloses spaces (s) between the infolded membrane (im) x 20 000

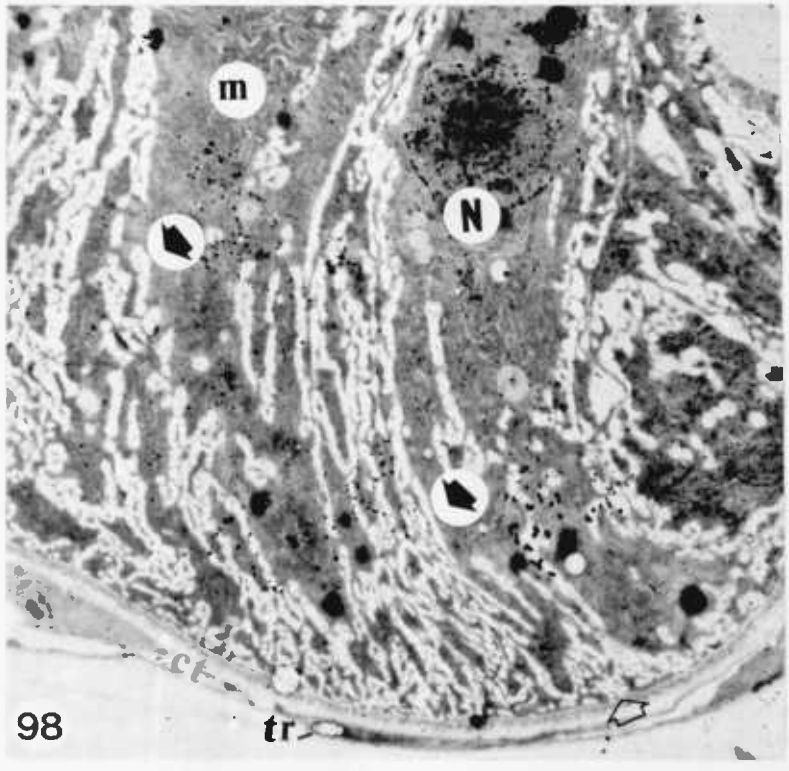


Figure 98 L.S. 2nd. ventriculus, basal region illustrating numerous deep infoldings noted by arrows and bordered by a mass of connective tissue (ct); Tracheoles (tr) x 3500

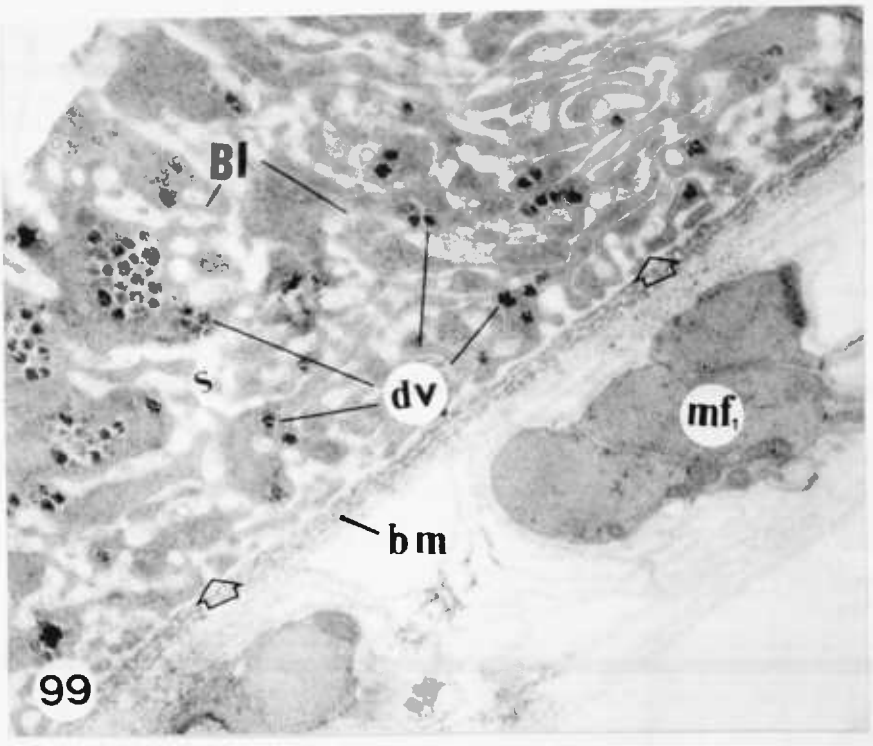


Figure 99 L.S. 3rd. ventriculus; basal region illustrating the continuity between the channels and the extracellular spaces (s). The arrows point to the openings of the channel into the extracellular space x 4500

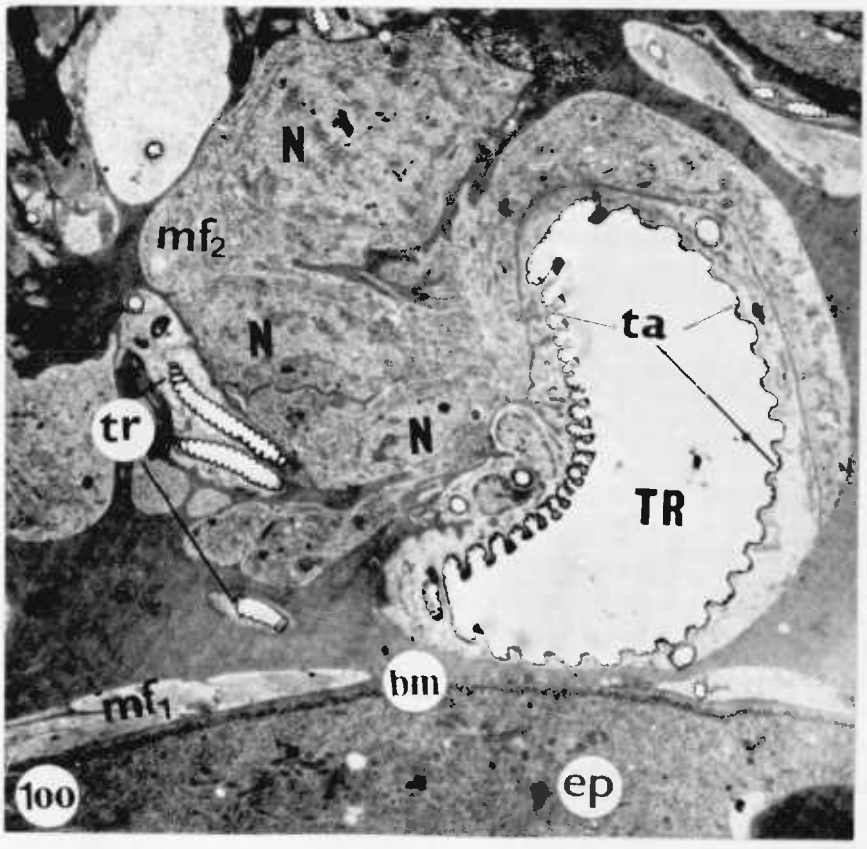


Figure 100 T.S. 1st. ventriculus to illustrate the disposition of the Tracheal and mscular supply of the basal region of an epithelial cell (ep) bordered by basement membrane (bm); circular muscle fibre (mf1) longitudinal muscle fibre (mf2); Trachea (TR); oblique profile of taenidium (ta); tracheoles (tr) x 2200

Bacillus rossius (Phasmida) large masses of glycogen are found in the midgut epithelium and to judge from his photomicrographs they are the same as those found in Oncopeltus (his photograph XXIV pp. 188).

On the external surface of the lamina is an extensive network of connective tissue, within which are tracheae and muscle bundles (Fig. 100). The muscle and tracheoles are knit together by sheets of extracellular material probably representing their respective basement membranes while the outermost of these sheets separates the entire tracheal and muscle sheath from the haemolymph. Many small tracheoles and occasional profiles of tracheae are found. In all of these the cuticular intima is helically folded to form the taenidium and it is quite clear in the lining of the trachea. The muscle fibres are arranged in circular and longitudinal arrays. The fibres closest to the epithelium run in a circular fashion around the gut. The fibrils are divided by well defined Z-bands (Figs. 94, 95). What are perhaps a great number of small synaptic vesicles clustered within an axon terminal are shown indistinctly in Fig. 101. Outside the circular muscles lie the longitudinal muscle, which contain centrally placed nuclei. These muscles perform peristaltic contractions that assist in the backward passage of food material within the gut lumen.

General Conclusion Electron microscopy has revealed the following points.

1. There is a remarkable array of finger like microvillar projections at the apical region of the midgut epithelial cells, particularly in the 6th. ventriculus, thus confirming that the latter is a midgut structure.

2. The basal plasma membrane of the 2nd. ventriculus is extensively and deeply folded; more so than the 1st. ventriculus. This may facilitate diffusion of material from the cell into the blood and thus be more highly specialized for absorption in this respect than 1st. ventriculus.

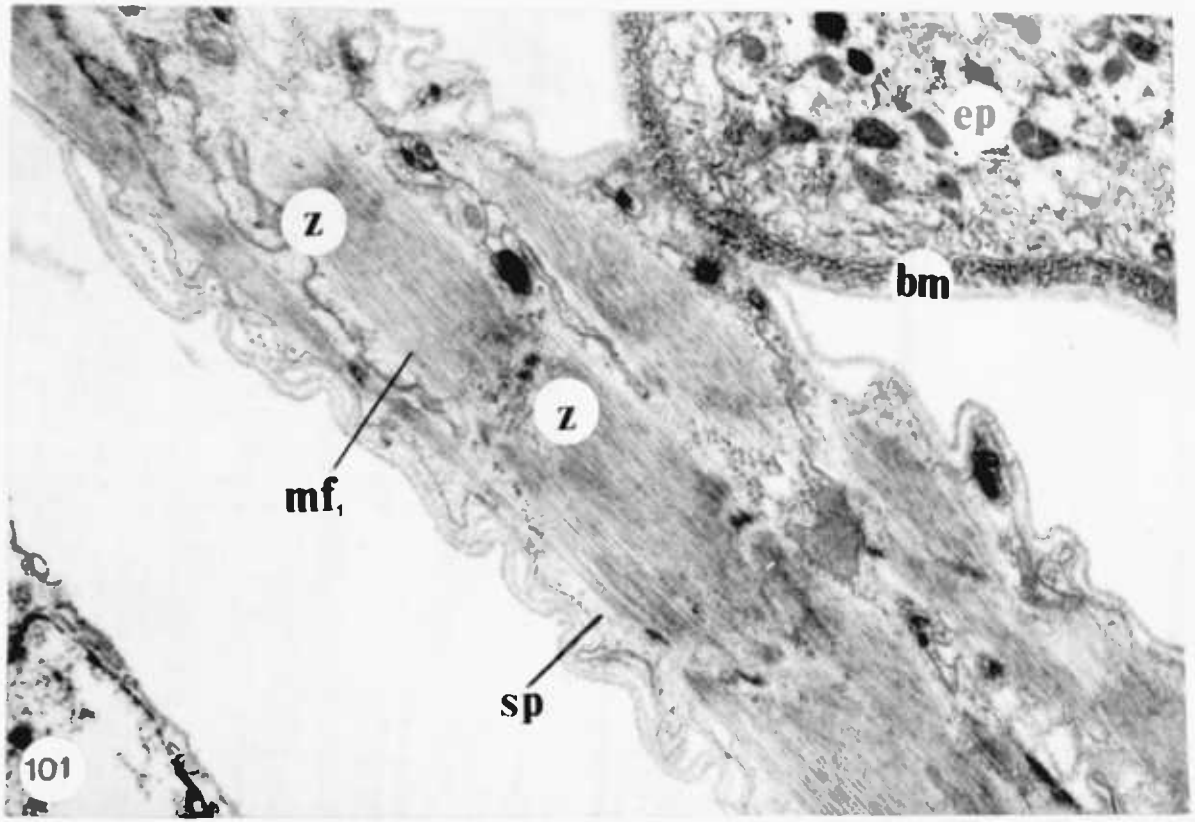


Figure 101 T.S. 1st. ventriculus to illustrate the circular muscle fibre (mf1) closest to the epithelium (ep) and peripheral to the basement membrane (bm); the narrow band of contractile material is divided into Sarcomeres by Z-bands (Z) surrounded by a thin sheet of Sarcoplasm (SP) x 5000

3. Mitochondria are accumulated apically rather than elsewhere in the 1st. ventriculus, and this agrees with the findings of Anderson and et al. (1966). He considered that ion accumulation by mitochondria in close association with plasma membranes may play a role in the active movement of potassium across the midgut. It is generally accepted that ion accumulation by mitochondria may play a central role in the transcellular movements of ions (Lehninger, 1965). The structure and position of mitochondria has been dealt with in many light microscope studies such as Sud and Sarin (1966). They studied the cytology of the epithelium midgut in Dysdercus cingulatus Fabr. and found mitochondria in the form of filaments with tip granules, dispersed throughout the cytoplasm, taking different shapes and not confined to fixed regions of the cell. Gresson (1934) found mitochondria in Periplaneta americana during degeneration. They occur only in the basal region of the cell and when the cell later regenerates the mitochondria multiply and spread from the basal part. He found no differences in recently fed and in unfed specimens.

4. Rough endoplasmic reticulum is very abundant in the 1st. ventriculus in 5 day old specimens, while in 15 day old adults the cytoplasm shows scattered ribosomal reticulum and not the dense endoplasmic reticulum of the previous age.

5. Nidi near the basal part of the epithelial cells are without any connection with the plasma membrane.

6. The apical border microvilli of the columnar midgut cells (Microvilli) is covered with an outer coat which is of interest not only in terms of the possible origin of the peritrophic membrane,

but also in respect of a peculiar form of cell surface morphology and possible activity (pp. 181, 206, 209).

7. A well developed extracellular basement membrane is present, connective tissue providing a sheath for the visceral muscles and associated tracheae.

GENERAL DISCUSSION

Some of the conclusions drawn in the present study have already been discussed in previous sections. In order to provide more detailed and critical evaluation the following topics have been selected for further discussion in the light of work by other investigators.

Subdivisions of the midgut In the literature, widely diverse terminologies have been applied to the various subdivisions of the midgut of Hemiptera such as first and second stomachs, etc., first and second segments etc., midgut region 1, midgut region 2 .... etc. and so on. In the present study the different subdivisions are designated as 1st., 2nd., 3rd., 4th., 5th. and 6th. ventriculi.

The midgut of Heteroptera has been discussed in a number of comparative studies relating the anatomical and histological structure to the major classification of Heteroptera. Yanai (1952) recognized five types of Heteropteran midgut on the basis of their anatomy. The five types were mainly based on the number of the midgut segments and the presence or absence of gastric caeca.

The midgut of Oncopeltus fasciatus, with six segments according to the present study, and with no caeca, cannot be included in any of Yanai's types. However, it is nearest to the Hydrometra type (with four segments but no caeca) which occurs in the Cimicoidea. Goodchild (1963) has criticised Yanai for grouping together forms with anatomically similar midgut appearance (e.g. Reduviidae and Asopinae or Pyrrhocoridae and Hydrometridae), since these combinations are not supported by histological evidence or by the conventional taxonomic characters.

Oesophageal valve The junction of the foregut with the midgut has been the subject of numerous studies. Many workers observed a peculiar group of cells near the junction but their opinions differed on the origin of

these cells and also whether a special groove is present anterior or posterior to them. Breakey (1936) observed a well-developed stomodaeal valve in Anasa tristis and denoted the peculiar cells by the symbol (X) as they were devoid of a striated border or a chitinous intima. He did not refer to the groove and regarded the cells as belonging to the midgut epithelium, considering them to be a ring of peritrophic membrane secreting cells. Hood (1937) said nothing of the junction and the groove in Oncopeltus fasciatus. Harris (1938), Woolley (1949), and Goodchild (1952) also made no mention of such a structure in the species they studied, but Sutton (1951) reported a special ring of cells at the anterior end of the oesophageal valve in the Corixidae. These cells were shown to be taller and more basiphil than the cells of the valve itself and separated from the rest of the valve by a groove. Sutton regarded these cells as part of the midgut because they secrete a peritrophic membrane. She regarded this region as a functionless remnant of the proventriculus of other orders. Marks (1958) also observed a special ring of cells in many Cryptocerata and claimed that they secrete a very fine chitinous intima. They were, therefore, considered by Marks to be modified foregut cells and he called them the terminal pad, separated from the midgut epithelium by a groove or "Sillon". Sutton's claim and argument were not supported by Marks (1958) or by Parsons (1957b). The present study has shown that a well-developed oesophageal valve is present in Oncopeltus fasciatus at the anterior end of the midgut.

My own observations on Oncopeltus fasciatus tend to support Sutton's interpretation. There is certainly a ring of tall cells at the junction of the outer cell layer of the invagination with the anterior end of the ventricular area. With PTAH these cells stain more strongly basiphil than the cells of the oesophageal valve invagination from which they are separated by a groove. They have a quite clear brush border.

How far this condition can be related to the interpretation of the foregut/midgut junction advanced by Henson (1931, 1946, etc.) is less clear.

According to this there is present at or near the junction a zone of persistently embryonic cells whose differentiation provides for the growth of the gut during development. These cells form the so called anterior imaginal ring or anterior interstitial ring and are characterized by their small nuclei, absence of a brush border, presence of a very delicate intima and occurrence of mitotic divisions. No such cells can be recognized in the adult of Oncopeltus. If they are present at earlier postembryonic stages they must by the adult have completed their differentiation into the definitive imaginal tissue of the gut.

The junction between mid and hindgut The boundary between the mid and hindgut has been of great interest to many workers, since its exact limits are still uncertain at the histological and embryological levels. This subject has been discussed in detail when dealing with the 6th. ventriculus (pp. 69), but it seems worth considering the present findings in Oncopeltus fasciatus in relation to those of Hood (1937) on the same insect.

a) Hood thought that the Malpighian tubules were probably part of the proctodaeum as they entered the pylorus. According to the present work, which is also based on histological observations in Oncopeltus, the so called pylorus of Hood and others is actually the most posterior part of the midgut and, therefore, referred to here as the 6th. ventriculus.

b) The Malpighian tubules have a striated border and they and their junction with the 6th. ventriculus (pylorus of Hood) have very similar histological characters. For this reason it seems probable that the Malpighian tubules in Oncopeltus are of endodermal origin, thus supporting Henson's interpretation (1932, 1937, 1946).

c) There is a well developed valve at the junction between the 6th. ventriculus and the rectum in Oncopeltus. This valve, recognised here for the first time, is given the term "ventriculo-rectal valve" on the basis of its histology and location.

Intestinal muscular layers According to many published accounts of the histology of the midgut epithelium of Heteroptera, there is a relatively uniform arrangement of muscle layers. Slight variations in the abundance of muscle fibres and the thickness of the muscle layers have been reported in individual species and in the different segments of the midgut. Hood (1937) found that in Oncopeltus fasciatus his third and fourth (i.e. my 4th. and 5th.) segments have a thick circular muscle coat. He claimed also that no longitudinal muscles are present in the "third stomach" and that only a few scattered fibres occur in the fourth. Breakey (1936) also claimed that no circular muscles occur in the first ventriculus and that they are very fragmentary in the second in Anasa tristis. Goodchild (1965), while reviewing our knowledge of alimentary canal structure in the Hemiptera did not deal with the subject. Hood (1937) also claimed that there are differences in the number of strands of circular muscle fibres, a single strand was found in the first segment of the midgut of Oncopeltus fasciatus while one strand and a partial second strand occur in the second and two strands were found in the third and fourth.

It seemed, therefore, worth comparing my observations on Oncopeltus with those of Hood (1937).

a) No constant differences in the number of muscle fibres have been observed in the different ventriculi in Oncopeltus fasciatus, but there were other differences in thickness and distribution in these fibres throughout the sections in any given ventriculus.

b) Circular and longitudinal fibres were observed in all segments of

the midgut with some differences in the abundance of the fibres in sections taken from the same or different segments. These differences may result from the interlacing of muscle fibres.

c) There is a reversal in the arrangement of muscle layers in the oesophageal valve, so that the circular muscle layer lies within the longitudinal layer in the midgut instead of being outside it as they are in the oesophagus.

Connective tissue Connective tissue is generally inconspicuous in insects and has been little studied (Ashurst, 1968). Indeed, insects are sometimes thought not to possess a cellular connective tissue comparable with that of vertebrates. Nevertheless small amounts of connective tissue are present, binding together and suspending other tissues in the body cavity. The tracheal system plays an important part in binding tissues together and most organs in the haemocoele are limited externally by a usually non-cellular membrane. In many cases the mode of connective tissue formation remains contraversial. Ashurt (1968) noted that some workers have thought that blood cells are responsible for the formation of some of these tissues while others have thought that it might be the underlying cells (epithelial cells). Yet another conception was that both the haemocytes and the underlying cells might play some part in connective tissue formation. Ashurt found that all the evidence supported the view that though in general haemocytes are not responsible for the formation of connective tissue in insects, there are some sites where the blood cells may contribute to their formation. Ashurst found fibrous elements of connective tissue similar to the elastic fibres of vertebrates, but very little is known about the physical properties of insect connective tissue. Histochemical studies of connective tissue revealed that collagen is present, embedded in a matrix of neutral muco-polysaccharides, but others have rejected the possibility that collagen is

present. There is a layer of connective tissue in the form of a basement membrane overlying all organs of the body and histochemical studies indicate that the basement membrane of the gut contains neutral mucopolysaccharides. There is a debate about the presence of fibrils; some authors have found unbanded fibrils and also those possessing distinct collagen fibrils while a third type of basement membrane has distinct banded fibrils, recognisable as collagen and embedded in an amorphous matrix.

Peritrophic membrane It has been reported that the peritrophic membrane of insects may be of two main types, either a single, uniform continuous structure or a series of concentric tubular lamellae. In the first type the peritrophic membrane is produced as a viscous secretion by a ring of cells at the junction of the fore and midgut; as it passes back it emerges through the narrow space between the invaginated oesophagus and the midgut (Wigglesworth, 1972). The cells that secrete the peritrophic membrane are columnar, devoid of a cuticular lining, and similar in appearance to midgut cells. However, they appear to develop from the stomodeum in Simulium (Gambrell, 1933), in Sciara (Butt, 1934) and in the Honeybee (Butt, 1934) (quoted in Roeder, 1953). In the second type, the cells of the midgut are thought to secrete a series of thin lamellae. The production of the lamellae has been described as the delamination of a membrane which forms at the surface of the midgut cells (Aubertot, 1932; Von Dehn, 1933; Rengel, 1903; Tchang, 1929a; quoted in Roeder). A peritrophic membrane is lacking in many insects which ingest only fluid. Thus it is said to be absent from Hemiptera (Roeder, 1953; Goodchild, 1965; Wigglesworth, 1972). Breakey (1936) found that the cells of the anterior part of the first ventriculus present a striated border in well prepared sections and are not associated with an intima, but he found and identified a ring of "peritrophic cells". Neither Rawat (1939), studying Naucoris cimicoides L. nor Hamner (1936), working on Solubea pugnax Fab. (Heteroptera; Pentatomidae), mentioned the peritrophic membrane nor did Hood (1937) find a peritrophic membrane in

Oncopeltus fasciatus. Similarly Harris (1938) studying the anatomy and histology of the Cabbage bug Murgantia (Hemiptera; Pentatomidae), found no evidence of a peritrophic membrane. But although a peritrophic membrane is apparently absent from most investigated Hemiptera, a few authors (Kershaw, 1913; Sutton, 1951; Woolley, 1949) agree that there are detectable rudiments of one. Sutton (1951) reported that a peritrophic membrane was present in the Corixidae (an atypical microphagous Heteropteran family) though Parson (1957a) was unable to confirm Sutton's claim. She has, however, detected a fragmentary membrane in Ranatra fusca P.B., but concluded that the positive tests for chitin which were reported by Sutton were due to chitin fragments in the food mass and she considered that the appearance of a membrane surrounding the food mass is not uncommon but probably results from the precipitation during fixation of a layer of freshly secreted digestive enzymes close to the epithelial surface. Goodchild (1966) found that peritrophic membranes appear frequently in the Corixidae but never in Ranatra fusca P.B. This view is also held by Kurup (1961) as a result of his studies on various Hydrocorisae and by Mukherji (1961) from work on Homoptera. Bocharova-Mesner (1959) claimed that a peritrophic membrane is present in certain immature stages of Eurygaster integriceps (Pentatomidae). She reported that such a peritrophic membrane was absent from nymphs of various ages and from actively feeding adults but it was seen in older adults hibernating or preparing to hibernate. This claim was not accompanied by histological preparations, while the description and diagramatic illustrations cannot exclude the possibility that Bocharova-Mesner was dealing with a rather prominent luminal boundary to the striated border. The peritrophic membrane is characteristic of the midgut of the majority of insects. Waterhouse (1953) found the peritrophic membrane is endodermal in origin in many insects, the endodermal midgut epithelium being capable of producing a chitinous membrane.

The absence of a peritrophic membrane in insects with sucking mouth parts is one of the bases for considering the main function of this structure

to be the protection of the epithelial cells of the gut from mechanical injury by food particles (Wigglesworth, 1972), but Waterhouse (1953) has shown that some insects which were supposed to lack a peritrophic membrane do actually deliminate extremely delicate chitin-containing membranes from their midgut epithelium.

The peritrophic membrane of many insects is too dense to be examined with the electron microscope and relatively little is known of its fine structure (Roeder, 1953). Further work is therefore likely to be important and according to some recent literature on ultrastructure, a few Hemiptera are in fact provided with a peritrophic membrane or similar structure. Peters (1968, 1969), while working on the occurrence, composition and fine structure of peritrophic membranes, found that they are not confined to insects and other arthropods, but are widely distributed in the animal kingdom. He found microfibrils in the peritrophic membrane of several insects, probably containing chitin (Peters, 1969). There are three types of microfibrils differing in texture and described as irregular dispersed, hexagonal or honeycomb, and orthogonal. No correlation occurs between the mode of formation of peritrophic membrane and the type of texture or between the type of nutrition and the texture of the microfibrils. Peters concluded that to understand the texture of microfibrils in peritrophic membranes we should increase our knowledge of the fine structure of midgut epithelium, especially of the microvilli, as well as the functions of the peritrophic membrane.

Marshall and Cheung (1970) found that the midgut of Fulgora candelaria (Homoptera : Auchenorrhyncha : Fulgoridae) has a unique plexiform surface coat consisting of two layers. The outer layer consists of membranous saccules, while the inner layer is formed from flattened sac like membranes. Cytochemically the plexiform coat has the same character as the glycocalyx in being mucopolysaccharide in nature. Enzyme activity was found in the plexiform coat but it is lower than in microvilli; this is consistent with

the fact that some enzymes are located in the plasma membrane of microvilli and few enzymes may be associated with the glycocalyx. Reger (1971) describes a similar trilaminar membranous structure covering the microvilli in the midgut of Phylloscelis atra (Fulgoridae). He interpreted it as a surface delamination product of the epithelial cells and therefore comparable to a peritrophic membrane. Recently Burgos and Gutierrez (1976) studied the intestine of Triatoma infestans (Reduviidae) and found a peculiar peritrophic membrane. The membrane forms tube-like compartments around and upon the microvilli. Richards and Richards (1977) in their review of insect peritrophic membranes found much vagueness and uncertainty in defining the structures and doubtless there are a variety of reasons for this, including the fact that the peritrophic membrane is important for many studies of parasite transmission. They suggest that workers on peritrophic membranes should become familiar with current methods in cell biology and apply modern analytical methods to avoid the complications of contaminating food residues and the presence of digestive enzymes. According to my own observation on Oncopeltus the electron microscope demonstrates a membrane delimited system of tubular spaces or cavities that are open at the luminal end which thus extends into the lumen from the spaces between the microvilli. Such a structure, which might be called a glycocalyx or a peritrphic membrane corresponds to that revealed by Burgos and et al. (1976), Marshal (1970) and Reger (1971). Its function as a protective structure is still under discussion and needs further investigations.

Striated border Newell and Baxter (1936) described in a light microscope study the free cell border in certain midgut epithelia, and they illustrated two main types : ciliated and nonciliated or rod-bordered. Each was further divided into two categories : the first into motile ciliated and nonmotile, while the second may be present as a rod border, found in vertebrates, and a brush border, found typically in insects. Beams and Anderson (1957) studied

the striated border of the insect midgut for the first time by means of the electron microscope and showed it to consist of large numbers of densely arranged microvilli. This structure is characteristic of the midgut epithelium of insects though Wigglesworth (1972) noted that such a cell border is sometimes absent. Hood (1937) claimed that a brush border occurs only in the third segment of the midgut of Oncopeltus fasciatus, as did Breakey (1936) in Anasa tristis and Woolley (1949) in Leptocoris trivittatus. Harris (1938), however, found a striated border throughout the epithelium of the midgut of Murgantia histrionica. Sutton (1951) working on some Corixidae found that one type of epithelial cell almost always had a brush border while in another type this brush border was sometimes present and sometimes absent. Parsons (1959) reported that there were two main types of midgut epithelium in the aquatic Heteroptera. In some of these (Type I, Belostomatidae, Nepidae and Corixidae) a low brush border is often seen while in the other (Type II, Naucoridae and Notonectidae) a low brush border is often seen on the younger cells and less frequently on the older ones though in places where the epithelium lacks villiforms ridges a brush border is prominent on all cells. Goodchild (1963) also noticed a difference in the thickness of the striated border in the Cinucomorphan families of Heteroptera. It is suggested that differences in the stain and fixation used may be responsible for some of the apparently contradictory results reported in previous investigations. Brlak and Kralik (1967) studied the striated border in the midgut cells of Javesella pellucida (F.) (Homoptera : Delphacidae); they found a well developed border covered with a membrane but were not able to accept this as a peritrophic membrane. The purpose of the striated border of insect midgut cells is probably to increase the area of the free cell surface on an ultrastructural scale (Smith, 1968).

In Oncopeltus fasciatus I have found a striated border in all segments and not only in the 3rd. ventriculus (4th. segment) as reported by Hood

(1937). The striated border was sometimes noted as broken or incomplete in the old or degenerating cells but it was regularly present in the young cells of normally fed specimens. The striated border shows some variation in its thickness between different regions of the same segments and there were also some differences in its thickness and appearance according to the age of the adult as well as in starved or desiccated specimens. Well defined microvilli were found at the apical ends of the 6th. ventriculus epithelium where work with electron microscope supports the light microscopical findings in considering this segment as a part of the midgut. The external surface of each microvillus is covered with fuzzy material, generally referred to as glycocalyx (Ito, 1965). The glycocalyx is thought to act as a filter to large molecules or as a protective layer against viral and bacterial infection and to prevent cellular disruption (Threadgold, 1976). The same glycocalyx was found in Oncopeltus and it may have the same function.

Mono and binucleate cells There are many contradictory views concerning this subject in Hemiptera. Yanai (1952) and Yanai and Iga (1956) studied the matter on a quantitative basis and found that the frequency of uninucleate cells was correlated with the abundance of regenerative cells in the epithelium. In the highly carnivorous Hydrocorisae nearly all the cells are uninucleate and nidi are very abundant, while in the phytophagous Pentatomidae there are few uninucleate cells and regenerative cells. The investigations of Breakey (1936), Harris (1938), Wooley (1949) and others indicated that the epithelial cells are all mononucleates. This condition was also reported by Hood (1937) in Oncopeltus fasciatus though Sporano (1959) found binucleate cells were more abundant in the same species. Goodchild (1952) claimed that all epithelial and regenerative cells are binucleate in the Miridae. On the other hand he had claimed earlier (Goodchild, 1963, 1965) that a proportion of the midgut cells in many species of Heteroptera are uninucleate. Rastogi (1960) reported mono-, bi-, tri- and quadrinucleate cells in the midgut epithelium of Lygaeus pandrus

(Lygaeidae) while Kanungo (1964) says that binucleate cells appear to be extremely rare in Dysdercus fasciatus.

This matter has been given special attention in the present work on Oncopeltus fasciatus. The number of mono and binucleate cells in the epithelium was repeatedly counted in different segments of the midgut at progressive ages of adult specimens. The results show that the epithelium is generally dominated by mononucleate cells though the percentage of binucleate cells varies in the different segments. There is also some noticeable variation in this percentage in the same segment at different ages.

The presence of mono and binucleate cells in the epithelium of the same species may have physiological implications in the secretory and absorptive activity of these cells as already indicated by some workers, such as Pradhan (1939) and Goodchild (1965). Goodchild suggested that the uninucleate cells of Hydrocorsae secrete by total cell breakdown (holocrine secretion) and thus require constant replacement where as the binucleate cells of Amphibicorisae and Geocorisae secrete without total destruction (merocrine secretion) and are longer lived. But no such correlation was observed in the present study. It is desirable that more careful large-scale studies on this subject be made to reveal whether there is some correlation between the number of the nuclei in the epithelial cells and their physiological activity. A few studies have been made on methods of counting or estimating the numbers of cells and nuclei in cross sections of the midgut, e.g. Kanungo (1966), Marrable (1962).

Types of epithelial cells This is another subject that has led to somewhat contradictory reports among different workers on the midgut epithelium of Hemiptera, quite apart from the obvious differentiation into columnar and regenerative cells. The earlier histological accounts of individual species of Heteroptera made by Breakey (1936), Hood (1937), Harris (1938), Woolley

((1949) and others indicated that their midgut epithelia were almost uniformly made up of columnar and regenerative cells with some slight variations among the columnar cells in different species or in different segments of the midgut in a given species.

Sutton (1951) distinguished two types of epithelial cells in the Corixid midgut. This distinction was based on variation in size and shape, cytoplasmic basiphilia, and the nature of the brush border. The differences were shown to depend on their location in the two segmented midgut and the behaviour of the epithelium in the presence of food. It was therefore suggested by Sutton that one type is primarily secretory while the other is absorptive. Parsons (1959), using other cytological criteria on a number of aquatic Heteroptera, concluded that "the shape of the regenerative units in a given region of the Corixid midgut is determined by the amount of the pressure exerted upon the epithelium by the contents of the lumen and consequently the cylindrical and horizontal cells of Sutton are not distinct types but different conditions of the same sort of cell". Parsons did, however, distinguish between normal, blistered and degenerate cells in the midgut epithelia of the species of the Hydrocorisae she studied. She recognized two histological patterns : Type I epithelium with villiform ridges always present and the apices of the columnar cells all interdigitating (Belostomatidae, Corixidae and Nepidae). Type II epithelium having villiform ridges present or absent; when present the apices of the columnar cells interdigitate basally or not at all. This type of epithelium was observed to occur in Naucoridae and Notonectidae. Parsons also recognized the variation which occurs in both the above types of epithelium; for example, in her type II epithelium she recognized high and medium columnar types, both with villiform ridges, and also low columnar and cuboidal types, both without villiform ridges.

Goodchild (1952, 1963a, 1965) indicates that though the epithelium of the Heteropteran midgut is formed of columnar cells, the recreting cell

types typically have bulbous tips while other cell types, probably of an absorptive nature, occur in the midgut of many phytophagous species. These may have a border extended into the lumen as a long lobe. Kanungo (1964) also reported that there is considerable variation in the shape of cells and groups of cells depending on the degree of distension of the gut, but she admitted that there were variations in the cell types of the midgut epithelium of the different segments in Dysdercus fasciatus.

One of the most interesting investigations in insect midgut histology is the finding that the apparently uniform midgut epithelium of some Dipteron larvae is actually a mosaic of histologically, functionally and embryologically distinct cell types (Waterhouse, 1957; Waterhouse and Stay, 1955; and others). Threadgold and Gresson (1962) have also revealed by electron microscopical study that there are ultrastructural differences between cells that they claim to be secretory and absorptive in the midgut of Blatta orientalis, as mentioned previously.

My own work on Oncopeltus does not seem to reveal anything corresponding to Parsons' regenerative cells, though her 'blistered cells' ('Extrusion cells' in my account) have some equivalent in my preparations. In Oncopeltus the regenerative cells are fewer and irregularly scattered, either singly or in groups of two to four, without forming distinct regenerative units or "cell-nests". This is already clear from the description above; both sections II and III and the tables giving the mean number of nidi per section (pp. 78), show that the gut exhibits a typical Geocorisan condition in their arrangement. The variation occurring in Oncopeltus depends on the segments under consideration; these have already been clarified in section II and the situation may be summarized as follows :

First ventriculus The columnar epithelial cells show a gradual change in shape of cells as discussed above (pp. 44). An anterior region, behind the oesophageal valve, varies from narrow columnar to low columnar; a middle region, the greater part of 1st. ventriculus, shows larger columnar epithelial

cells, resembling the type II epithelium of Parsons (1959) who has made a useful point covering the variation which occurred in both her types of epithelium. Finally a posterior region tends to have tall columnar epithelial cells. Due to the activity occurring in 1st. ventriculus and possibly related to enzyme secretion, this segment shows extrusion mainly in the middle region and the bulbous cells at the posterior region. These types are not confined to the region mentioned but are most common there.

Second ventriculus The columnar epithelial cells vary from low to high columnar; there are proportionally fewer extrusions showing vacuolation than in 1st. ventriculus.

Third and Fifth ventriculi Both segments show clavate columnar epithelial cells.

Fourth ventriculus Columnar epithelial cells vary from tall columnar to low columnar to cuboidal, depending on the distension of this segment.

Sixth ventriculus Various forms from tall through medium to low columnar.

Generally speaking, therefore, it appears that the epithelium of Oncopeltus, like that of many other geocorisae, has a histological pattern distinct from the Hydrocorisae, but there are similarities since Parsons' Hydrocorisan type II epithelium resembles that of some segments of Oncopeltus in the range of variation in height.

Regenerative cells The regenerative cells have been recognized by earlier workers on the histology of the midgut epithelium as small cells located or embedded at the bases of the columnar epithelial cells. Their continuous multiplication produces new cells which replace the degenerating epithelial cells. There are somewhat discordant views in the literature on the nature,

position, number and activity of these cells in different Hemiptera.

Yanai (1952) claimed that the presence of cell nests was one of the distinctive features of the midgut epithelium of Hydrocorisan Heteroptera, while their absence was characteristic of the Geocorisae and Amphibicorisae. Parsons (1959) has similar results, though with some exceptions. Goodchild (1952), while studying some Mirid species reported that multiplication of regenerative cells is probably amitotic, but he hardly ever figures regenerative nuclei in his numerous illustrations (Goodchild, 1963b, 1965). He mentioned only the results of Yanai (1952), Yanai and Iga (1956) and Parsons (1959), but apparently did not make direct observations on this topic.

In the present work, the number of regenerative cells was carefully studied in the midgut epithelium of Oncopeltus fasciatus in all segments at progressively older adult stages. In general, these small cells are embedded near the bases of the epithelial cells and are fewer than those shown by Parsons (1959) in her two types of Hydrocorisan epithelium. Furthermore, the regenerative cells of Oncopeltus are also found irregularly scattered, either singly or in groups of two to four, without forming distinct and regularly repeated units. My observations on Oncopeltus show that some of these cells undergo mitotic division in any given region of the midgut at any age of the adult and do not carry out amitotic division as reported by Goodchild in Mirid species. The percentage of regenerative cells in the epithelium also shows variation according to the age of the adults and generally declines as they grow older. The electron microscope study illustrated these regenerative cells very clearly in Oncopeltus, as explained on pp. 193.

Cytological changes and secretion This is a topic of major importance and will be discussed at somewhat greater length than the subjects dealt with above. The process of enzyme-secretion and the possible associated

cytological changes in the midgut epithelium of insects have led to an active and unsettled controversy among different workers on this subject. This matter has been reviewed by Newcomer (1914), Day and Powning (1949), Wigglesworth (1972) and others. The main point of the argument is whether the observed cytological features such as vacuolation, extrusion of material through the striated border, presence of cytoplasmic globules and so on are all expressions of secretory activity. According to the "vesicular theory" of secretion as proposed by Van-Gehuchten as early as 1890, the vesicles observed by light microscopy contain digestive enzymes which are built up within the cytoplasm and are then released into the lumen. There are several similar reports by later workers on different groups of insects, though most were based on relatively simple histological techniques. According to Duspiva (1939) feeding starved adults of Dytiscus marginalis leads within one minute to the midgut epithelial cells discharging a trypsin-like enzyme that develops in the form of intra-cytoplasmic globules. In the Heteroptera, Bogiawlensky (1925) described the rupture of the walls of the vesicles and subsequent release of secretory material into the lumen in Notonecta glauca, while Poisson (1924) figures the same process in Notonecta maculata. Hamilton (1931) described a similar disintegration of secretory vesicles in the midgut of Nepa cinerea and Pesson (1944) also considered comparable cytological phenomena to represent secretory activity in several species of Homoptera. Sutton (1951) described in blistered cells her "anucleate vesicles" in the midgut of Corixid and considered them to be secretory, enzyme-containing vesicles which separate from the cell body and pass into the lumen. Parsons (1959) while working on a number of aquatic Heteroptera critically reviewed this matter as 'blistered cells' sometimes show signs of senescence at their bases. However, the majority do not, and it therefore seemed probable that the blistered cells are those which, exhausted by their secretory activity, die during or soon after the release of their enzymes. However, Goodchild (1952) claimed to have observed cyclic merocrine secretion in the midgut of the Cacao Capsid bugs Sahlbergella, Distantiella

and Bryocoropsis, and saw no blistered cells in his material. Gresson (1934), working on Periplaneta claimed that both secretory and absorptive cells occur in the caeca and anterior region of the midgut; he found the secretory cells more numerous than the absorptive cells and believed that cells recover after discharging their granules and probably give rise to further secretions this process being repeated several times before the cells finally degenerate.

On the other hand, Franzel (1886), Peterson (1912), Woodruff (1933) and others believe the blistered cells to be artifacts of fixation or trauma as suggested by Vignon (1899).

A third view that the vesiculated cells and the cytoplasmic extrusions are senescent or degenerative rather than secretory phenomena was suggested at an early date by Henson (1929) and Tchang Yung-Tai (1929). This view has been strongly supported by the experiments of Day and Powning (1949) on Blattella germanica and reported by Waterhouse and Stay in Lucilia larvae (1955). Shinoda (1926) believed that pressure of food on the epithelium may be a normal stimulus for secretion. He also claimed (Shinoda, 1927) that in Blattella secretion is usually merocrine but becomes holocrine after a period of starvation. Pradhan (1939) found holocrine secretion in carnivorous coccinellidae and merocrine secretion in the phytophagous ones. Goodchild (1966) suggested a similar condition in Heteroptera where he said that the uninucleate cells of Hydrocorisae probably secrete by total cell breakdown (holocrine), thus requiring constant replacement, while the binucleate cells of terrestrial Hemiptera secrete without self destruction (merocrine) and are longer lived. Clearly there has been much disagreement on this subject. Khan and Ford (1962) investigated the relationships between cytology and α-glucosidase activity in Dysdercus fasciatus. They concluded that the production of cytoplasmic extrusion in the epithelium is directly related to the presence or absence of food in a particular gut region and the cytological changes were therefore held to be a response to starvation. In other words, they believed that vacuolation of cytoplasm,

shedding of globules of cytoplasm and loss of nuclei were all degenerative changes that affect the midgut cells in Dysdercus. In this view they supported Day and Powning (1949). However, Kanungo (1964) who also worked on Dysdercus fasciatus criticised Khan and Ford's results on histological grounds. She reported that when fully fed specimens of Dysdercus fasciatus were compared with those starved for 72 hours, none of the cytological changes claimed by Khan and Ford to be the result of starvation were observed. There is also a significant criticism of Day and Powning's results made by Schönfield (1958) while working on the larvae of Chaoborus. Schönfield points out that "what is secreted by the epithelium may well be an enzyme precursor which was only activated and therefore only became biochemically recognizable after the epithelium had reverted to a non-secretory and histologically uniform condition". He showed that in Chaoborus larvae at any rate there is a correlation between cytological change and secretion.

Of course it is not necessary from these interpretations and hypotheses to suggest that all insects must necessarily secrete digestive enzymes by histologically obvious means. Day and Powning's work on Blattella is quite convincing as far as that particular insect is concerned, but may not be a typical or even a common condition.

From what has been said above it appears that although many authors have observed "blistered cells" and other cytoplasmic changes in the midgut epithelium of insect, opinions vary as to their significance. Whether they are entirely artifacts or are related to secretory activity or to degenerative changes in the cytoplasm remain highly controversial. In fact some of the workers on this subject seem merely to have quoted or accepted the interpretations of Day and Powning (1949) and of Khan and Ford (1962) and thought the problem was solved (De Priester, 1971). The secretion of material by the midgut epithelium could have another significance since the midgut epithelium plays a part in the formation of the peritrophic membrane (Waterhouse, 1957, 1953; Smith, 1969; Wigglesworth, 1972 and the very

recent work on the formation of the plexiform layer considered as a peritrophic membrane : Marshall et al., 1970; Burgos et al., 1976; Reger, 1971).

The formation of constrictions and the vesiculation of the tip of the microvilli was also reported by Baccetti (1962) in Dacus oleae; and Heinrich et al. (1973) in third instar larvae of Locusta migratoria at different stages of digestion after starvation and feeding. They found that during starvation the epithelial cytoplasm is very dense and that intracellular membranes are difficult to resolve, while in feeding insects the density of the cytoplasm decreases and the microvilli give a rise to vesicles of two distinct types : large and small. The first are found budding at the apex of the microvilli, while the second occur anywhere in the brush border, forming rows like strings of beads. The cell membrane may be ruptured with its contents in the lumen, the apex of the epithelial cells swell, and portions of homogenous cytoplasm are extruded into the lumen. This work is illustrated in their Fig. a, b, c, pp. 319. Clark and Anstee (1971) provided ultrastructural evidence that in Locusta migratoria the removal of the frontal ganglion causes changes in the endocrine system which appear to influence protein synthesis and that starvation also reduced protein synthesis, causing shrunken mitochondria and greatly disorganized rough endoplasmic reticulum. An analysis of the two states (i.e. those resulting from frontal ganglionectomy and from starvation) suggests that both indicate reduced levels of protein synthesis (though the effect may be produced through different pathways). This could imply that starvation would reduce the production of digestive enzymes, as my work suggests.

One of the most satisfactory types of evidence is obtained by correlating a variety of cytological approaches with observations on the feeding activity of the insects and one of the objects of the present work has been to seek further histological evidence that might be correlated with enzymatic secretion in the midgut epithelium.

I do not wish to criticise the grounds on which Khan and Ford's work

was based since criticisms have already been made by Kanungo (1964) who worked on the same species, but I would like to summarize the basis of my own observations on Oncopeltus fasciatus.

1. Controlled periods of feeding, starvation, desiccation and combined starvation and desiccation were used for the first time to correlate a variety of cytological approaches with observations on the feeding activity.

2. Each experiment was repeated on specimens of different ages to assess the extent to which changes that might be correlated with the secretory activity of the midgut epithelium could also be related to postmetamorphic growth or senescence.

3. My results were based on many specimens prepared and fixed independently in several fixatives and stained with PTAH and MCTS.

4. Photographs and tables of various measurements were provided.

5. The light microscope finding have been put within a framework of observations made by electron microscope techniques even though time did not allow the latter to be exploited fully.

6. The following observations were evident.

   a) vacuolation, extrusion, appearance of cytoplasmic globules and bulbous enlargement of cells were observed to be more abundant in the epithelium of normally fed specimens than in starved specimens and were greatest in the first ventriculus. This agrees with Parsons' results (1959) where a higher

proportion of "blistered" cells were found in the first ventriculus of the gut after feeding in Belostoma and Ranatra. M.A. Khan (1961) also found that invertase was mainly produced in the first ventriculus of Dysdercus and this was in line with the histological evidence of Kanungo (1964). Geering-Sacker (1972) while working on the digestive proteinase activity in the midgut of adult Dysdercus fasciatus (Hemiptera) indicated the presence of proteolytic activity and he also attributed to Herzog (1967) the claim that proteinase secretion occurs in the second and third ventriculus of the midgut. This work used gut homogenates in a 1% gelatin solution and subsequent chromatography of the released amino acids and contradicts Ford (1962) who states in his studies on digestive enzymes of Dysdercus fasciatus, that the midgut of this insect has no proteinase. Together, therefore, these cytological changes seem to bear some relation to enzyme secretion.

b) The second ventriculus shows no "blistered cells" or cast off cell debris in both normally fed and starved specimens, but cytoplasmic extrusions through the striated border were reduced in starved-and-desiccated specimens. The extent of this extrusion and cytoplasmic globule formation differs in the different segments of the midgut in normally fed specimens, but the phenomena of cast off of cell debris declines at the apical ends of the cells, and the breakdown of cells has never been met in midgut epithelium during starvation and desiccation, in contrast to what was shown by Khan and Ford (1962). Differences in the extent of these cytological changes throughout the epithelia of the different ventriculi in Oncopeltus may reflect the regional differentiation of the midgut in

secretory activity as was observed by Saxena (1958) in Dysdercus koenigii, Bongers (1970) in Oncopeltus fasciatus, M.A. Khan (1961) in Dysdercus fasciatus, Bocharova-Mesner (1959) in Eurygaster integriceps and others. No alternative explanation has been given for the anatomical differentiation observed in the midgut of Heteroptera and for the cytological differences described above.

c) The process of degeneration of some epithelial cells which takes place throughout the midgut during normal feeding is most frequently seen in the older cells of the villi, i.e. those farthest from the regenerative cells. Their cytoplasm appears more basiphil and the nuclei show signs of distortion, being irregular in outline and with clumped chromatin masses. These senescent cells seem to be virtually forced out of the epithelium into the lumen by lateral pressure from younger, actively growing cells. Such an appearance seems normal in fed specimens where, especially in the middle region of the first ventriculus, the epithelium is never histologically uniform as claimed by Khan and Ford (1962). The appearance of the cytoplasmic extrusions in fed insects differs completely from the condition of the epithelium in starved specimens. There all the cells appear affected and generally collapsed, especially in starved-and-desiccated specimens. This was followed by deformation of the epithelial cells in some ventriculi, such as the complete loss of the clavate shape of the cells in the 3rd. and 5th. ventriculi. Despite prolonged starvation the epithelium never shows shedding of globules of cytoplasm or breakdown of cells such as is usually met in the midgut epithelium of normally fed specimens, especially in the first ventriculus.

d) Since mitosis was frequently observed in the regenerative cells, it appears that these cells replace the degenerated cells. It is probable that the latter are secretory cells which are, in some senses, exhausted by their secretory activity.

One can imagine the movement of the cells in a given villus during adult life to occur as follows :

The regenerative cells lie at the bases of the villus, the oldest cells are located at its tip while the young and mature cells are found nearer to the regenerative cells from which they are produced. A gradual series of mature cells lies laterally and runs up towards the tip of the villus and a given epithelial cell moves from the base of the villus to a position facing the lumen of the gut during its life.

There are thus several reasons for regarding enzyme secretion by the midgut epithelium of Oncopeltus as correlated with the appearance of cytological changes and for considering that some of the enzymes are liberated in apocrine fashion while others are released in merocrine fashion throughout the striated border.

Khan and Ford's conclusions have often been quoted in the literature and are even accepted by De Priester (1971, p. 802), despite their disagreement with his own findings on the same subject in Calliphora larvae. It will be seen from above that Khan and Ford's observations have not been critically examined and in view of my results on a related species of Heteroptera they require further investigation. Until this has been done, their interpretations can hardly carry as much weight as once seemed likely.

SUMMARY

1. The present work describes the anatomy, histology and ultrastructure of the adult midgut of Oncopeltus fasciatus. Light miscroscopy of paraffin-sectioned material has provided most of the results using phospho-tungstic acid haematoxylin and Mallory's connective tissue stain.

2. The midgut comprises six anatomically distinct segments, the 1st., 2nd., 3rd., 4th., 5th. and 6th. ventriculi.

3. The 3rd. segment is a newly recognised region, which is similar in histological and cytological features to the 5th. ventriculus and for that reason they are treated together in sections 3 and 4.

4. The 6th. ventriculus, for long treated erroneously as a part of the hindgut, is really a region of the midgut, since it shows histological differences from the region posterior to it in respect of the shape and size of the epithelium cells, definition of their brush or striated border, and development of intrinsic musculature connective tissue and basement membrane and the distribution of regenerative cells (pp. 69, 127).

5. The histology of the connections of the midgut with fore and hindgut are described and discussed. The junction near the Malpighian tubules includes a specially differentiated endodermal region, the structure of which confirms Henson's interpretation of the Malpighian tubules as endodermal in origin. For that reason we treat the extreme anterior part of the hind gut as a valve between mid and hindgut, called here the ventriculo-rectal valve.

6. Comparison of the cytology of the midgut epithelium at different stages of postmetamorphic development, made here for the first time, showed that there are histological differences through the life span in respect of cell extrusion, vacuolation and the proportion of resting and degenerating cells.

7. The frequency of binucleate and mononucleate cells was recorded quantitatively in tables in each segment.

8. Differences in the cytology of the midgut epithelium were observed in normally fed, starved, desiccated and starved-and-desiccated specimens.

9. Histological evidence of cellular activity such as vacuolation, extrusion, formation of cytoplasmic globules and development of bulbous cells, were observed in the epithelium of normally fed specimens rather than in starved, desiccated and starved-and-desiccated specimens. From this it may be concluded that cytoplasmic extrusion is correlated with enzyme secretion. Other work on this subject has been discussed critically.

10. The electron microscope has proved very valuable in revealing some of the structural features that appear to be connected with the activity of the midgut of Oncopeltus fasciatus, these being as follows.

a) Well defined microvilli (striated border) along all segments of the midgut, particularly of the 6th. ventriculus, this confirming (4).

b) Remarkable material covering the apical part of the 1st., 2nd., 4th., 6th. ventriculi (and presumably present throughout).

This may be a glycocalyx which acts as a filter for a large molecule or as a protective layer against bacterial infection as discussed on pp. 209.

c) A well-defined rough endoplasmic reticulum in young adult specimens rather than in older ones.

d) Mitochondria accumulated apically rather than elsewhere in the cytoplasm.

e) The basement and plasma membrane were deeply folded. The folds of the latter were very complex in 2nd. ventriculus rather than in first ventriculus and may facilitate diffusion of molecules from the cell into the blood, suggesting that the 2nd. ventriculus is highly specialized for an absorptive function rather than secretion as in 1st. ventriculus.

f) Vacuoles, small vesicles, dense electron opaque vesicles and the Golgi apparatus are discussed in section 5 (pp. 180).

LIST OF ABBREVIATIONS

ap.c.	=	apical cell
bin.c.	=	binucleate epithelial cell
b.m.	=	basement membrane
b.m.rid.	=	basement membrane ridge
bul.c.	=	bulbous cell
bul.end.	=	bulbous end
clav.c.	=	clavate cell
c.mus.	=	circular muscle
col.ep.	=	columnar epithelium
c.t.	=	connective tissue
c.t.n.	=	connective tissue nucleus
cub.epit.	=	cuboidal epithelium
deg.c.	=	degenerating cell
epith.c.	=	epithelial cell
extr.c.	=	extrusion cell
F.g.	=	Fore-gut
h.g.	=	Hind-gut
inf.	=	inner fold
int.	=	intima
l.mus.	=	longitudinal muscle
l.s.	=	longitudinal section
lum. of 1st. vent.	=	lumnen of first ventriculus
lum. of 2nd. vent.	=	lumen of second ventriculus
M.C.T.S.	=	Mallory's connective tissue stain
M.g.	=	Midgut
mit.div.	=	mitotic division

mon.epit.c.	=	mononucleate epithelial cell
m.tub.	=	Malpighian tubules
n.	=	nucleus
Oes.inv.	=	Oesophageal invagination
Out.f.	=	outfolded portion
Oes.	=	Oesophagus
Oes.v.	=	Oesophageal valve
P.T.A.H.	=	phosphotungstic acid haematoxylin
raph.	=	raphe
rec.	=	Rectum
reg.c.	=	regenerative cell
rest.c.	=	resting cell
reg.n.	=	regenerative nucleus
Sal.g.	=	Salivary gland
St.b.	=	Striated border
St.	=	Striation
t.col.c.	=	tall columnar cell
Tr.	=	Trachea
vac.	=	vacuoles
vac.c.	=	vacuolated cell
vent.con.	=	ventricular constriction
vent.	=	ventriculus
vent.Rect.v.	=	ventriculo-rectal valve
vil.	=	villi
y.c.	=	young cell
1st.vent.	=	first ventriculus
2nd.vent.	=	second ventriculus
3rd.vent.	=	third ventriculus
4th.vent.	=	fourth ventriculus
5th.vent.	=	fifth ventriculus
6th.vent.	=	sixth ventriculus

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