CYTOLOGY OF THE ALIMENTARY TRACT OF LUMBRICUS TERRESTRIS LINNAEUS.

by

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1. INTRODUCTION.

During the last quarter of a century a consi-:derable amount of research has been carried out on various aspects of cytology. In invertebrate animals, however, observations have been confined mainly to the nucleus and to the structure and behaviour of the cyto-:plasmic components during gametogenesis. The few accounts of the epithelial cells of the alimentary canal are restricted to one or two specific regions or to the associated glands. Gresson (1934) and Shay (1946) worked on the cytology of the mid-gut of the cockroach. Siang-Hsu (1947) studied the epithelium of the mid-gut of the larvae of Drosophila melanogaster and Subramaniam (1938) studied the Golgi apparatus in the intestinal cells of Lumbriconereis. The salivary glands of Chironomus larvae have been investigated by Parat and Painlevé (1924), Beams and Goldsmith (1930) and Gatenby (1932), and the salivary glands of the grass-hopper by Beams and King (1932). Gresson (1936) studied secretion and the cytoplasmic inclusions in the cells of the salivary glands of Tipula paludosa.

The present work was undertaken in order to study the cytology of the different regions of the ali-:mentary canal of an invertebrate animal and to deter-:mine the relationship, if any, between the morphology and disposition of the Golgi material and mitochondria and the phases of the secretory cycle. As the alimen-:tary tract of the earthworm, Lumbricus terrestris is divided/

divided into several distinct regions, it was consi-:dered to be a suitable animal for investigation. The different regions of the gut were fixed in various fixatives, sections were cut, and the epithelial cells were subsequently examined carefully. In order to study the cellular changes during different phases of the secretory cycle, certain worms were starved prior to fixation of the parts of the alimentary tract, while others were fed subsequent to a period of starvation, and thereafter examined at different stages.

There are numerous works on the histology of earthworms (including Lumbricus), but so far as the writer is aware, the cytology of the alimentary tract of Lumbricus, or of any other earthworm, has not been studied previously.

II. MATERIAL AND METHODS.

The material for the present investigation was obtained from specimens of Lumbricus terrestris collected at intervals from the autumn of 1947 until the autumn of 1948. Samples were taken from the differ-:ent parts of the gut, namely the buccal region, pharynx, oesophagus, crop, gizzard and intestine, and from the pharyngeal glands, oesophageal glands and oesophageal pouches. Both young and mature adult spe-:cimens were used in the investigation and tissues from apparently healthy individuals were selected for cytological study. Certain individuals were dissected immediately after they were collected and small parts of the different regions of the alimentary tract and associated glands were placed in the fixatives as speedily as possible; others were kept in large glass jars to which soil could be added so that it was possible to fix tissues at different periods of fast-:ing, and of feeding after previous fasting. The food given to the earthworms in the laboratory consisted of manured soil, decaying leaves and grass, and vege-:table moulds maintained in a fairly moist condition. Specimens were dissected alive as quickly as possible in normal saline; the whole operation never took more than ten to fifteen minutes. In order to avoid possible error or abnormality, more than two earthworms were used to investigate each physiological phase.

To study the origin, formation and passage of the secretory granules from the gut-epithelium to the lumen, it was necessary to obtain samples, at various times after feeding, from several specimens which had previously been starved. The resting stage of the cells of the gut-epithelium and assosciated glands was studied in animals which had been starved for three to six days. The active stage was investigated in worms which were fed after a fast of three to six days; tissues were fixed at intervals from one to twenty-four hours after food was made available.

For general histological studies, material was fixed in the fluids of Bouin and Zenker (with and without formalin), and the sections were stained with haematoxylin and eosin or with Mallory's stain. For the study of the mitochondria, Flemming's (without acetic acid), Regaud, Champy, Altamann's acid fuchsin and picrid acid, Bensley's acid fuchsin and light green and Cain's modification of Helly's fluid with potassium dichromate and methyl blue, were used. Flemm-:ing (without acetic acid), diluted to half as recom-mended by Gatenby, and Meves fluid were found to be the most suitable fixatives. The fixatives were changed after three to four hours and further fixation carried on for thirty-two to thirty-four hours. The tissues were then washed in the running tap water for two and a half to three hours. Heidenhain's iron-haematoxylin and Bensley's acid fuchsin and light green were found

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to be most suitable for staining the mitochondria. Southgate's and Mayer's methods were used to demonstrate the presence of mucus.

For observation of the Golgi material both silver and osmic methods were tried. Among the silver methods, Aoyama and Da Fano's method gave the most satisfactory and constant results. Sections were usually mounted unstained, but in a few cases they were stained with Ehrlich's haematoxylin or toned with gold chloride. Of the osmic methods, Mann-Kopsch, Kopsch, Kolatchev's and Ludford's modification of Kolatchev were tried. Kolatchev's methods was found to be most suitable for all the various tissues of the gut-epithelium, except the intestine and the oesophageal pouches in which the Golgi elements were impregnated by the silver method only. In addition to the methods mentioned above, the Golgi elements were fairly well impregnated in material fixed for twenty-four hours in a mixture of equal volumes of 2% osmic acid, 6% potassium dichromate and 1% chromic acid, washed in running tap water for ten to fifteen hours and in distilled water for half an hour, then placed in 2% osmic acid, kept at 30-38° C. for five to seven days, and finally transferred into distilled water for four to six hours. This method is a modification of Kolatchev by which the tissue is kept for a longer period in osmium tetroxide. In cases of over-impregnation, sections/

sections were satisfactorily bleached by hydrogen peroxide (20 volumes of 20% hydrogen peroxide in 100 volumes of 70% alcohol) or by turpentine. Sections prepared for the demonstration of the Golgi material were either mounted unstained or stained with neutral red with a trace of acetic acid. Observations were based chiefly on material embedded in paraffin. Sections were cut at 4-8 μ in thickness. Attempts were made to stain the mitochondria supra-vitally with Janus Green B. It was found, however, that the amount of teasing necessary to separate the epithelial elements damaged the cells to such an extent, that this method was abondoned. In neutral red preparations, the secre-:tory granules only were stained. Certain individuals were fed with iron saccharate for twelve to thirty-six hours; the gut epithelia were then fixed in equal parts of Bouin's fluid and 5% ammonium sulphide in 95% alcohol, and subsequently treated, as described by Yonge (1927), to demonstrate the presence of iron by the Prussian blue reaction and thus determine the absorptive regions of the gut.

III. ACKNOWLEDGEMENTS.

It is with great pleasure that I wish to record my personal thanks to Prof. James Ritchie for granting me research facilities to carry out this work, to Dr. R.A.R.Gresson for his constant encouragement and also for his help and valuable suggestions, and to Mr. N.Macdonald, senior technician of the Department of Zoology, for taking the photo-micrographs of my preparations.

IV. DESCRIPTION OF PLATES.

Lettering

A. F., absorbed food material.
C., cilia.
Cu., cuticle.
C. B., central body.
C. S., clear space.
D. P., discharge-pocket.
G. A., Golgi area.
G. M., Golgi material.
M., mitochondria.
N., nucleus.
Nu., nucleolus.
Oes., oesophagus.
O. G., osmiophilic granule.
O. Gl., oesophageal gland.
O. P., oesophageal pouch.
P. G., pharyngeal gland.
P. I., peritoneal investment.
P. S., pharyngeal shelf.
S. B., striated border.
S. C., salivary chamber.
S. G., secretory granule.
S. M., secretory mass.
T., tunnel.
V. C., ventral chamber.
V. N. C., ventral nerve cord.
Cl., cleft

V. THE BUCCAL CAVITY.

A. HISTORICAL.

The histology of the buccal region of Lumbricus has been described by Szüts (1913) and others. Szüts found that the buccal cavity has dorsal as well as ventral diverticula. Gurwitsch (1901) described the structure of the buccal epithelium. According to him the cells are non-ciliated and are covered with a thick cuticle.

As far as the writer is aware, there is no other contribution on the cytology of the buccal epithelium of Lumbricus.

B. METHODS.

Samples of the buccal region were taken from earthworms having constant access to food. Material was also obtained from specimens which were kept without food for four to six days. Certain specimens were starved for four to six days and then fed; tissues were fixed one hour, three hours, five hours, six hours and twenty-four hours after the worms were placed in contact with food. The tissues were fixed for thirty-six to forty-eight hours in Flemming's fluid (without acetic acid), diluted to half as recommended by Gatenby. Sections were stained successfully by Bensley's acid fuchsin and light green method. It was found that Heidenhain's iron-haematoxylin never stained/

stained the secretory granules and the mitochondria satisfactorily. Kolatchev's original formula (impregnation in osmium tetroxide for about six to seven days at 37°C.) was fairly satisfactory for the demonstration of the Golgi material. It was noticed that material from fasting specimens took a longer period to impregnate than the tissues of other individuals.

C. OBSERVATIONS.

The epithelium of the buccal region consists of short as well as elongated columnar cells with a thin cuticle. They are non-ciliated. The cell-membrane is usually distinct. The nucleus has distinct nuclear membrane and chromatin granules, and usually one to two nucleoli. The nuclei lie at different positions in the cell. Binucleate cells were present in some of the sections. Replacement cells are present throughout.

The cells of the buccal epithelium do not show very marked changes correlated with feeding and fasting. Cells in all stages of the secretory cycle are usually present, but after the intake of food, following a period of starvation, they show a slight response.

The secretory granules may be concentrated chiefly in the basal or in the lumen half of the cell. In some cases they are aggregated near the border of the cell adjacent to the lumen, or in the neighbourhood of/

of the nucleus (Pl. I, figs. 1 and 2). In a few cases, secretory granules are seen in the area occupied by the Golgi material above the nucleus. Clear spaces are usually seen in the basal cytoplasm chiefly near the basement membrane (Pl. I, fig. 1).

The Mitochondria

The mitochondria of the buccal epithelial cells of Lumbricus consist of rods of various sizes, a few granules and rarely filamentous forms. The rods are scattered throughout the cell. In the majority of cells, the lumen half contains more rods than the basal half. Granules are usually situated near the border of the cell adjacent to the lumen or near the basement membrane (Pl. I, figs. 1 and 2). The majority of the mitochondria exhibit marked polar orientation and are arranged parallel to the longitudinal axis of the cell.

The Golgi Material

The Golgi material consists of rods, filaments and granules and is situated above the nucleus. In the cells which have completed their secretory cycle and are at rest, the Golgi material occupies a smaller area than in active cells (Pl. II, fig. 5).

In cells which are actively engaged in the production of secretion, the Golgi material is hypertrophied and occupies a large area of the cytoplasm between the nucleus and the border of the cell adjacent/

adjacent to the lumen. In the majority of such cases the rods and filaments run almost parallel to the longitudinal axis of the cell and secretory granules are seen in close contact with the Golgi material (Pl. II, figs. 1, 2, and 4). Appearances indicate that during the final stages of the formation of the gra-:nules of secretion, the Golgi rods and the filaments, break up to form small rods and granules.

The Golgi material of the cells of the buccal epithelium is never in the form of a compact reticulum. Feeding or fasting does not induce a marked change in the morphology of the Golgi material.

PLATE I.

Drawings of the epithelial cells of the buccal cavity of *Lumbricus terrestris* showing secretory granules and Mitochondria.

All figures from Flemming preparations stained according to Bensley's method.

Fig. 1. Showing secretory granules in the Golgi field and accumulation of granules in the basal cytoplasm.

Fig. 2. Showing accumulation of secretory granules in vicinity of lumen.

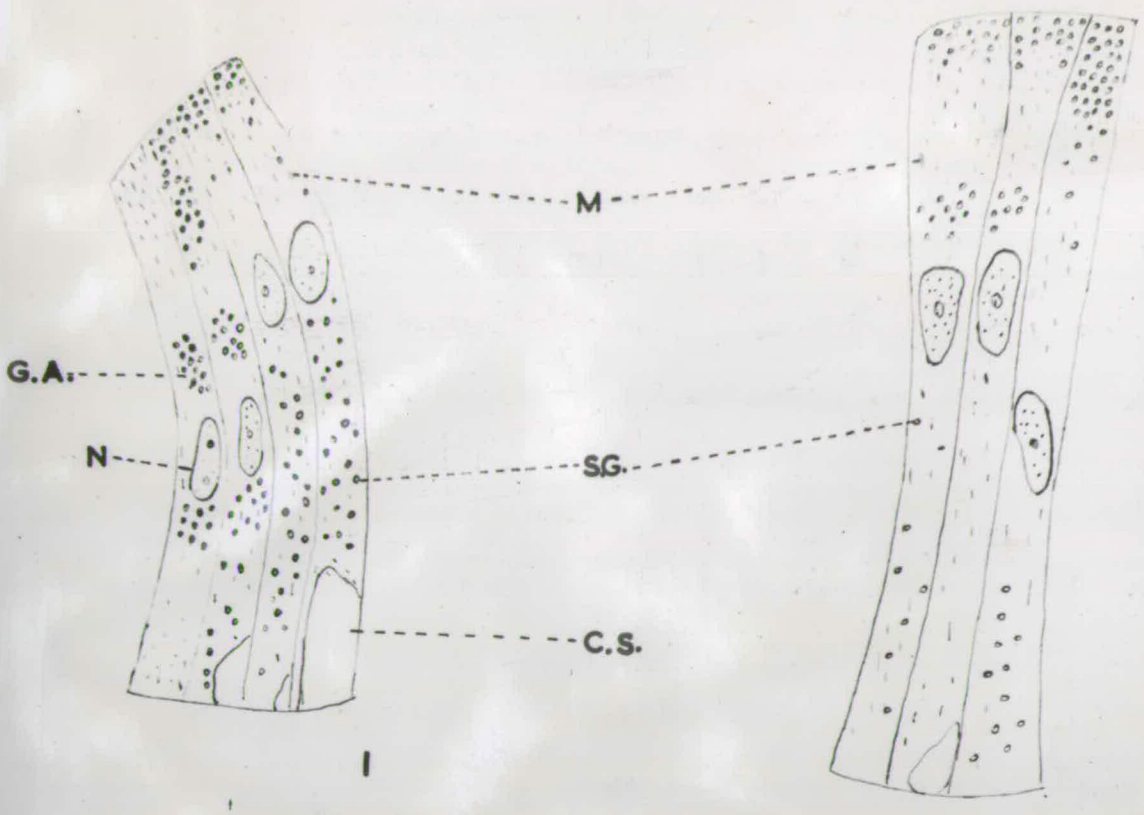

M
G.A.
N
SG.
C.S.
1
2
·01mm

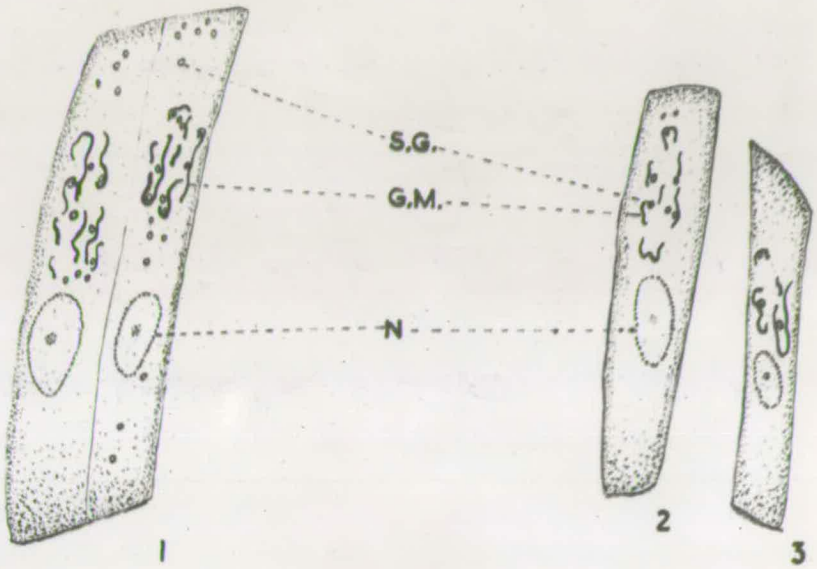
PLATE II.

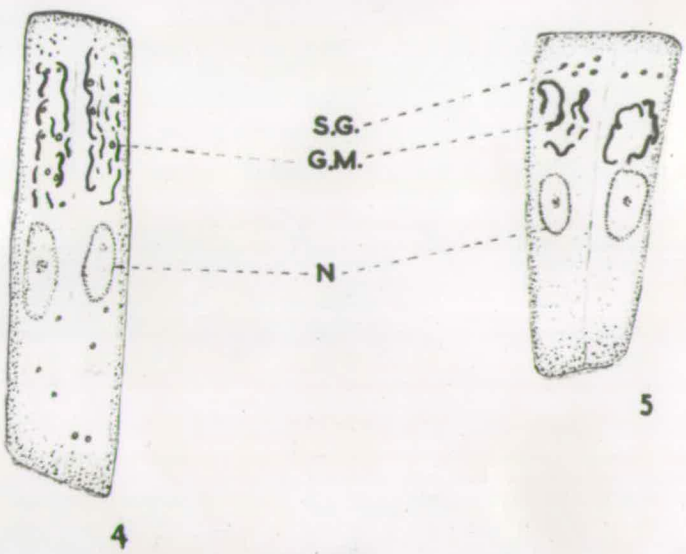
Drawings of the epithelial cells of the buccal cavity to show the Golgi material.

All figure from Kolatchev preparations.

Figs. 1, 2 and 4. Cells during secretory activity.

Figs. 3 and 5. Cells in the resting stage.





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VI. PHARYNX.

A. HISTORICAL

Much work has been carried out on the histol-:ogy of the pharynx of Lumbricus and of other earth-:worms. The pharyngeal epithelium has been described by Polowzow (1903), Mayer (1913), Menzi (1919), Keilin (1920), Stephenson (1930) and other authors. The writer has, therefore, nothing to add to the detailed work of these authors.

As far as the writer is aware, there is no previous contribution which deals with the cytology of the epithelium of the pharynx of Lumbricus.

B. METHODS.

As for the buccal epithelium.

C. OBSERVATIONS.

The pharynx is a relatively thick portion of the alimentary canal not very definitely separated from the buccal cavity in front, but more clearly defined from the narrow oesophagus posteriorly. The great bulk of the pharynx is due to its muscles and to the thick investment of glandular cells which form the pharyngeal bulb.

The pharynx of Lumbricus, possesses dorsal diverticula, one of which is of consider-:able length antero-posteriorly; its opening into the pharyngeal cavity is narrow and slit-like from/

from side to side. The cavity of the diverticulum extends laterally on each side and possesses secondary evaginations. The structure of the pharynx and the pharyngeal epithelium can be clearly seen from the photomicrographs of the transverse sections of the pharynx (Pl. III, figs. 1 and 2).

The cells of the pharyngeal epithelium are columnar and those of the dorsal epithelium, including the diverticulum, bear close-set cilia seated up on a layer of basal granules which stain deeply. Cilia are also found in patches on the ventral wall of the pharynx, the epithelium between the patches being covered by cuticle.

The cells of the lining of the pharyngeal shelf (Pl. VI, fig. 2), which separates the pharyn-:geal cavity into a dorsal or salivary chamber and a ventral chamber, are smaller and cuboidal in shape.

The dorsal portion of the pharyngeal epithe-:lium, including the diverticulum, is composed of tall, elongated ciliated cells compressed from side to side, and containing long oval nuclei each of which is pro-:vided with one or two nucleoli as well as chromatic granules. The cell-~~walls~~ membranes in most cases are not clearly visible, but the nuclear membranes are distinct. These cells are usually so crowded that in section their nuclei appear to lie at different levels. The basal

ends/

ends of the cells are very narrow and are covered with a basement membrane. The salivary ducts from the pha-:ryngeal glands, situated dorsally, extend to the vicinity of the basement membranes of the epithelial cells, and there give off numerous small ductules without walls, which appear to penetrate between the cells and terminate separately in a multitude of small pockets containing mucine, immediately beneath the free surface next to the lumen. These fine ductules with their terminal salivary discharge-pockets are clearly seen in sections and were first observed by Keilin (1920), although Stephenson (1930) failed to see them. The salivary pockets ultimately discharge their contents anteriorly into the dorsal salivary chamber of the pharynx or posteriorly into the single cavity of the pharynx. Such discharge-pockets are, however, absent among the non-ciliated epithelial cells of the ventral wall of the pharynx. Some of the ciliated cells contain bacteria.

The cells of the lining of the ventral wall of the pharynx are mostly non-ciliated, columnar in shape, and smaller than those of the dorsal ciliated epithelium. Patches of ciliated cells are present between the non-ciliated cells. The cell-membranes of the ciliated cells are more distinct than those of the dorsal epithelium and the nuclei are situated chiefly at the same level in all the cells. Replacement cells are present close to the basal membrane in both the dorsal and ventral epithelia. Binucleate epithelial cells/

cells were sometimes observed (Pl. III, fig. 3).

1. Worms with Constant Access to Food.

When an animal has constant access to food, the position, form, and arrangement of the components of both the ciliated and the non-ciliated cells of the pharyngeal epithelium vary considerably. The cell-membranes are distinct in some regions, but are indis-:tinct in others. The nuclei of some of the cells are very close to the basal membrane or lie in the basal half, others are present in the central region or in the cytoplasm adjacent to the lumen. The nuclei vary considerably in shape. These variations are correlated with the activity of the cell.

The disposition of the secretory granules varies and is related to the phases of the secretory cycle. The granules may be most numerous in the basal and scanty or almost absent in the lumen half of the cell, or they may be situated chiefly in the cytoplasm adjacent to the lumen. In certain cells the position of the granules clearly indicates that they are migra-:ting from the basal area towards the lumen (Pl. IV, figs. 1-4).

In the dorsal ciliated epithelial cells the nuclei lie mostly in the basal region and very few are in the centre or in the lumen half of the cell. These cells contain very few granules of secretion, but the discharge-pockets are full of mucine secreted by the pharyngeal glands.

2. Conditions Induced by Fasting.

The most noticeable feature seen in this material is the great accumulation of secretory gra-:nules clumped together in the cytoplasm adjacent to lumen and also scattered throughout the cell. In some cells, however, there is a greater accumulation of granules in the basal half of the cell. The nuclei usually lie in the basal part of the cell except in the epithelial cells of the pharyngeal shelf where, due to the constant activity of the cells, the nuclei lie at different levels.

Early secretory granules can be seen in the area of the Golgi zone of some cells, where they are present in close association with the mitochondria as well as the Golgi material (Pl. V, figs. 1 and 2).

In the ciliated cells of the ventral epithe-:lium accumulation of secretory granules, especially at the extreme end of the cell towards the lumen, was clearly seen.

3. The Secretory Cycle.

Following the intake of food after a fast of five to six days, the majority of both the ciliated and the non-ciliated cells become very active. The nuclei move towards the lumen side of the cell and become very irregular in shape. In most cases, the cell-membrane is indistinct or very faintly marked. Secretory granules begin to pass into the pharyngeal chamber/

chamber.

Two to three hours from the intake of food, the non-ciliated cells show a general evacuation of the secretory granules which were accumulated during the fasting stage in the lumen half of the cell. The nuclei lie in all positions from the centre to the extreme outer end of the cell. In majority of cells, fresh secretory granules begin to accumulate in the basal half of the cell (Pl. V, figs. 4 and 5). In the ciliated cells the discharge-pockets do not bulge so much as in the fasting stage as most of the mucin has passed into the lumen. Due to the closely packed condition and lateral compression of the ciliated cells of the dorsal epithelium, the origin of the sec-:retory granules could not be traced.

Five hours from the intake of food, the nuclei move downwards, and occupy an almost central position. The cells appear to be more uniform in appearance and are elongated. A light area without any distinct border becomes visible in the supra-nuclear region of cells fixed and stained for the demonstration of the mitochondria. This area increases in sharpness and size during the increase in cell activity and corres-:ponds with the area occupied by the Golgi material. The areas are negative images of the Golgi material. The early secretory granules are restricted to this zone, but later some of the secretory granules move towards/

towards the lumen side of the cell (Pl. VI, figs. 1 and 2).

Six hours from the intake of food, the lumen half of the cell seems to become almost full so far as the accumulation of the secretory granules is con-:cerned. In some cells both the basal and the lumen halves of the cell are full of secretory granules, but in most cases the accumulation in the lumen half of the cell is much greater than in the basal half, and the granules are more numerous than during any of the previous stages of feeding (Pl. VI, figs. 3 and 4). At this stage masses of secretory material from the pharyngeal gland cells enter the epithelial cells by breaking the basal membrane, and finally are evacuated into the lumen. After the material has been discharged into the lumen, the cells undergo a process of disinte-:gration (Pl. VI, fig.5.).

Twenty-four hours from the intake of food, the structure of the cells appears to be similar to that of the cells of animals with constant access to food.

In both active and resting cells clear spaces, or vacuoles-like structures, were very commonly seen near the basement membrane of ciliated and non-ciliated cells. In some of these spaces secretory granules are present (Pl. V, figs. 1 and 2).

The cells of the pharyngeal shelf resemble the non-ciliated cells of the ventral epithelium, except/

except that they are shorter and broader. They produce a secretion which appears to be similar to that of other epithelial cells. They react in a similar manner to fasting and feeding (Pl. VI, fig. 2).

4. The Mitochondria.

i. Worms with Constant Access to Food:- In animals with constant access to food the mitochondria occur in the form of short rods and granules of equal thick-:ness scattered throughout the cell. In some cells, the mitochondria are more numerous in the basal region than in the lumen half of the cell. In others, they are numerous in the lumen half and very scanty in the basal half. The granular mitochondria occur chiefly clumped together in the vicinity of the lumen in close association with the secretory granules. Some of the granules may be the cross sections of the rod-shaped forms. Granular and rod-shaped mitochondria are also found around the area occupied by the Golgi bodies and in its neighbourhood. Polar orientation was not very well marked on account of the small size of the mito-:chondria (Pl. IV, figs. 1-4).

ii. Conditions Induced by Fasting:- During the fasting stage of the various types of cells the mito-:chondria consist of small rods and granules; only very rarely are filaments present (Pl. V, figs. 1 and 2). In the basal part of all types of cells, the mito-:chondria are mostly rod-shaped; in very rare case a few/

few granules may, however, be present amongst the rods. Clumps of granular mitochondria, in association with secretory granules, are seen at the extreme end of the cell towards the lumen. The mitochondria around the nucleus are small and rod-shaped, but a few granules occur around the Golgi zone during the early stages in the formation of the secretory granules. In cells which are densely packed with secretory granules, the majority of the mitochondria are scattered at the extreme poles of the cell, but a few are also scatter-:ed in the cytoplasm, especially in the neighbourhood of the Golgi field (Pl. V, figs. 1-3).

There is no polar orientation of the mito-;chondria in the cells of the fasting animals.

iii. The Secretory Cycle:- In worms which have recently been feeding, the mitochondria in the ciliated and non-ciliated cells consist of small rods and granules; very rarely are filaments present. Those of the basal half are in the form of small rods and a few granules. The lumen half of the cell contains granular as well as rod-like forms. The number of mitochondria seems to be greatly increased as compared with those in the cells of starved worms; this is due to their fragmentation into smaller bodies during increased cellular activity. In certain cells granular mitochon-:dria are very numerous and form a dense clump in the neighbourhood of the lumen (Pl. V, figs. 4 and 5).

Two/

Two to three hours from the intake of food, the mitochondria of the epithelial cells are mostly granular with a few rod-like forms present chiefly at the extreme basal pole of the cell and scattered in the cytoplasm. Some of these granular and rod-like mitochondria are in association with the secretory granules situated around the Golgi zone and its neighbourhood. The mitochondria are much more numerous in the basal half of the cell than in the lumen half. Clumping of granular mitochondria adjacent to the cavity is frequently seen during this stage (Pl. V, figs. 4 and 5).

Four to five hours from the intake of food, the arrangement of the mitochondria seems to be greatly changed as compared to the previous stage. There are more granular and rod-shaped mitochondria in the distal half of the cell than in the basal half where thay are very few in number and mostly of the small rod-shaped variety. During this stage the nuclei seemed to have moved towards the centre, so that, in most cases, the Golgi bodies now lie immediately above the nucleus. Granular mitochondria are still seen around the Golgi zone in association with the secretory granules (Pl. VI, figs. 1 and 2).

Six hours from the intake of food, the mito-:chondria seem to be less numerous than in the previous stage. It is quite likely that, due to the great accumulation/

accumulation of the secretory granules, some of the mitochondria have become obscured. The mitochondria in the basal half of the cell appear to be mostly rod-like with a few scattered granules. Clumping of the granular and rod-shaped mitochondria in the neighbour-:hood of the nucleus is seen in some cells. At the side of the lumen very small rods and granules are present among the secretory granules (Pl. VI, figs. 3 and 4).

Twenty-four hours from the intake of food, the form and position of the mitochondria are similar to those found in worms with constant access to food.

Due to the extreme shortness of the mito-:chondria, it is very difficult to determine with certainty if the mitochondria exhibit polar orienta-:tion during the stages of secretory activity; it seems likely , however, that they are arranged in relation to the poles of the cell.

It must be noted that in all the various physiological phases, only a certain number of cells is involved in the secretory processes at any one time, and that each cell acts as an independent unit.

During all stages of cellular activity the secretory granules and mitochondria showed very mark-:ed affinity for acid fuchsin, but stained less deeply with haematoxylin.

5. The Golgi Material.

1. Worms with Constant Access to Food:- When an animal has constant access to food, some of the non-ciliated epithelial cells are in stages of activity and others are at rest. The Golgi material shows marked morphological changes which are typical of the respective phases, and are related to the participation of this cytoplasmic component in the functional activities of the cell. The morphological changes of the Golgi material are shown on Pl. VII, figs. 1-5. The Golgi elements are most numerous in the vicinity of the nucleus and are absent from the basal region.

The Golgi material in some of the epithelial cells consists of thin filaments connected together by a few very thin cross links so that in s cross sections the material appears to be in the form of a simple reticular or ring like structure (Pl. VII, figs. 1 and 2). With the onset of secretory activity, the compact Golgi bodies spread out into the cytoplasm towards opposite poles of the cell so that the reticulum becomes more complicated and forms a cylindrical structure which occupies a much greater field than before. The Golgi filaments increase considerably in thickness and are impregnated more deeply than during the resting stage. Swellings with sharp outlines are present on the osmiophilic filaments(Pl. VII, figs. 3 and 4). In the Golgi and osmic preparations secretory granules are visible in contact with the filaments.

As/

As a result of further activity of the cell the long rods of the basket-shaped Golgi apparatus break up into shorter segments, and curved Golgi rods with deeply impregnated rims now surround the secretory granules (Pl. VII,fig. 4).

In cells in which secretory activity is at its highest the Golgi substance breaks up into rods and granules which become arranged parallel to the longitudinal axis of the cell. Secretory granules of various sizes lie between the Golgi elements. At this stage the Golgi field is so large that it occupies almost the whole of the lumen half of the cell (Pl. VII, fig.5). After the discharge of the secretory bodies, the cell returns to the resting condition and the Golgi material again takes up its original position in relation to the nucleus and now appears as a simple reticular or ring-shaped structure composed of thin Golgi filaments or rods.

The Golgi material of the ciliated cells of the pharyngeal epithelium is in the form of short and long rods running parallel to the longitudinal axis of the cell. Secretory globules are found commonly in close association with the Golgi rods; the latter finally break up into granules (Pl. IX, fig.2.).

ii. Conditions Induced by fasting:- When a worm is starved for four to six days, it was noticed that the Golgi material was very much reduced in size and quantity and that it is most difficult to impregnate it successfully. Consequently, longer impregnation of about seven days in osmium tetroxide at 35-38° C., is necessary for a good result.

The Golgi material of the non-ciliated cells consists of thin long rods and threads arranged in a circular or oval ring. The rods and threads are joined together by a very few thin cross links usually of minute diameter, so that, the material appears as a simple reticular structure. It occupies a median area between the nucleus and the lumen or in a few cases lies closer to the nucleus (Pl. VIII, figs. 1 and 2, and Pl. X, fig. 1). In few cases a slight swelling on the Golgi elements is visible.

In the ciliated epithelial cell, however, there is little change in the morphology of the Golgi material as compared with that of worms with constant access to food, except that there is a slight decrease in the area of the Golgi field. The structure of the Golgi material is similar to that of the Golgi material of animals which have been feeding normally. The absence of any specific change in the morphology of the Golgi material of the ciliated cells is due to the fact that the production and the discharge of

mucine/

mucine is continuous whether the animal be in the rest-
:ing stage or in the active stage, although with the onset of secretory activity after the intake of food the production of mucine is somewhat accelerated.

iii. The Secretory Cycle:- With the onset of activity, as a result of feeding the animals after a fast, there is a marked change in the morphology of the Golgi elements of the non-ciliated cells; at the same time, the elements become much easier to be impregnated successfully, with osmium tetroxide. It appears, therefore, that there is definitely an increase in the power of the Golgi material to reduce osmium tetroxide; consequently, quicker and deeper blackening of the Golgi material is a visible mani-
:festation of physiological changes. The maximum mani-
:festation of this increased power to reduce osmium tetroxide seems to be about five to six hours after the intake of food.

The first visible change indicative of secre-
:tory activity is marked by the hypertrophy of the Golgi material. The whole mass becomes less compact and spreads out into the surrounding cytoplasm. There is a loosening of the osmiophilic threads and the field covered by the Golgi material is considerably larger than during the resting phase. Small sharply outlined swellings appear in the osmiophilic links. The reticular structure of the Golgi apparatus becomes/

becomes much more complicated, forming a cylindrical basket which stretches towards both poles of the cell, but chiefly towards the lumen. In some cells the whole of the distal half of the cell is practically filled with Golgi elements and with associated secretory granules. Some secretory granules are surrounded by deeply impregnated rims (Pl. VIII, figs. 3-6 and Pl. X, figs. 2-4).

Curved Golgi rods occur in the neighbourhood of the nucleus and early secretory granules are present in intimate connection with them. After the Golgi elements break up into rods and granules it was noticed that most of the rods lie parallel to the longitudinal axis of the cell. At this stage the elements not only fill the lumen half of the cell completely, but are also present a little below the centre of the cell (Pl. VIII, fig. 5 and Pl. X, fig. 3). Sometimes owing to movement of the nucleus towards the lumen, the Golgi bodies lie below the nucleus and thus present a picture of "reversed polarity". The topography of the Golgi material never changes, except when the elements of which it is composed come to occupy a larger area of the cytoplasm; the polarity or reversed polarity is a purely rela-:tive question, depending on the free movement of the nucleus, whose location in the cytoplasm appears to be independent of the Golgi bodies. This is confirmed/

confirmed by Pollister's observations (1938) on the orientation of the Golgi bodies.

Further fragmentation of the Golgi elements results in the breaking up of some of the rods into granules. This happens when secretory granules come into close contact with the Golgi rods. The latter become free of the main mass and move away from the nucleus. The migration of part of the Golgi material is accompanied by the movements of the granules of secretion and their concentration in the cytoplasm close to the lumen. The granules are not only first visible in close association with the Golgi elements, but the latter undergo a change of form and distribu-:tion during the phase of secretory activity - thus clearly indicating that the Golgi substance plays an active part in the process of cell secretion.

The Golgi material in the ciliated cells are in the form of long rods and threads with swellings which enclose secretory granules. It covers a slightly larger field than in the resting stage. There is no other appreciable change in the morphology of the Golgi material.

Although the nuclei of the cells of the pharyngeal shelf move towards the lumen during secre-:tory activity, the structure and disposition of the Golgi material does not change.

PLATE III.

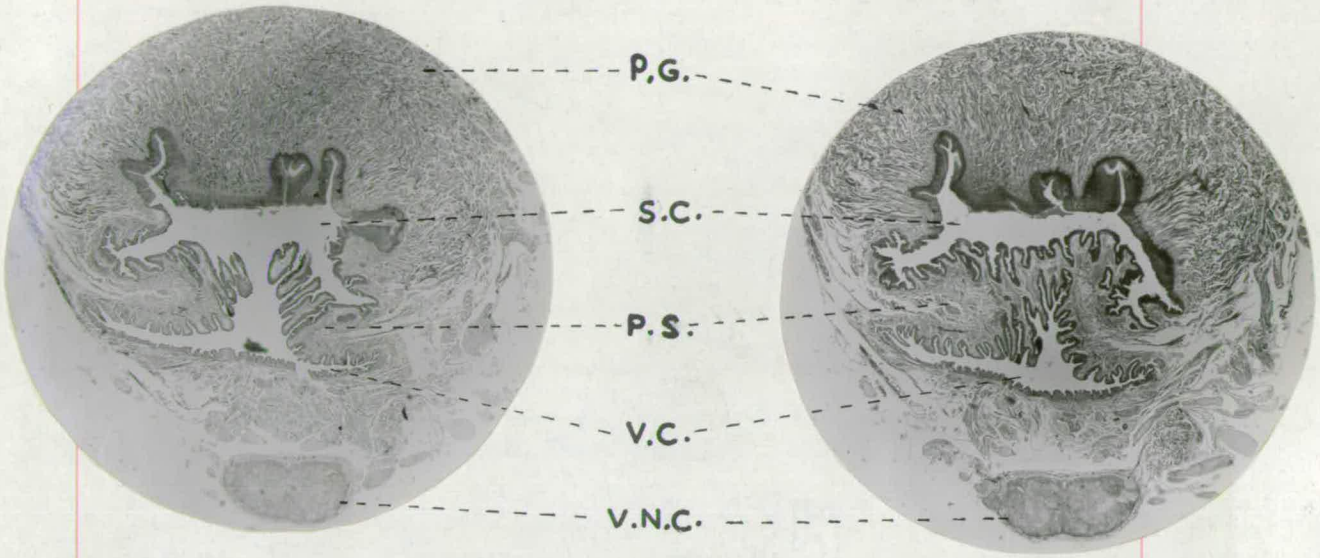
Photomicrographs of the pharynx of Lumbricus.

All from Flemming preparations.

Fig. 1. Transverse section of the pharynx showing the pharyngeal bulb, dorsal diverticula and pharyngeal shelves. The pharyngeal shelves do not meet in this section. The dorsal (or salivary) chamber communicates with the ventral chamber. x23

Fig. 2. Transverse section of the pharynx showing the same features as Fig. 1, except that the pharyngeal shelves have met so that the dorsal and ventral chambers are now completely separated from each other. Some of the secretory mass from the pharyngeal glands may be seen in the shelf on its way to the lumen. x 23

Fig. 3. Non-ciliated epithelial cells of the pharynx during starvation showing mitochondria and secretory granules. One binucleate cell can also be seen. x 1140



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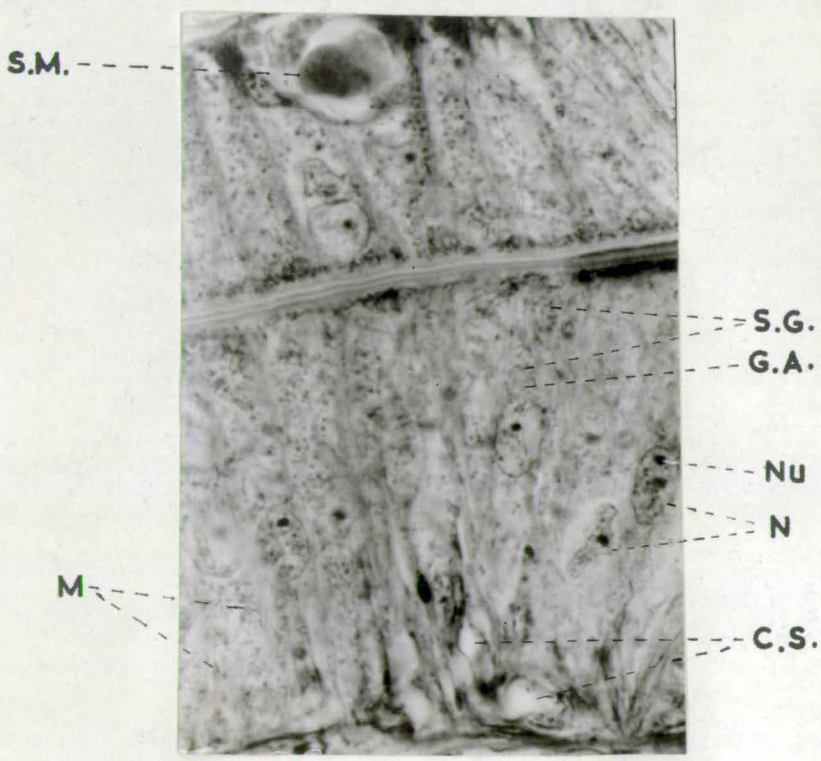
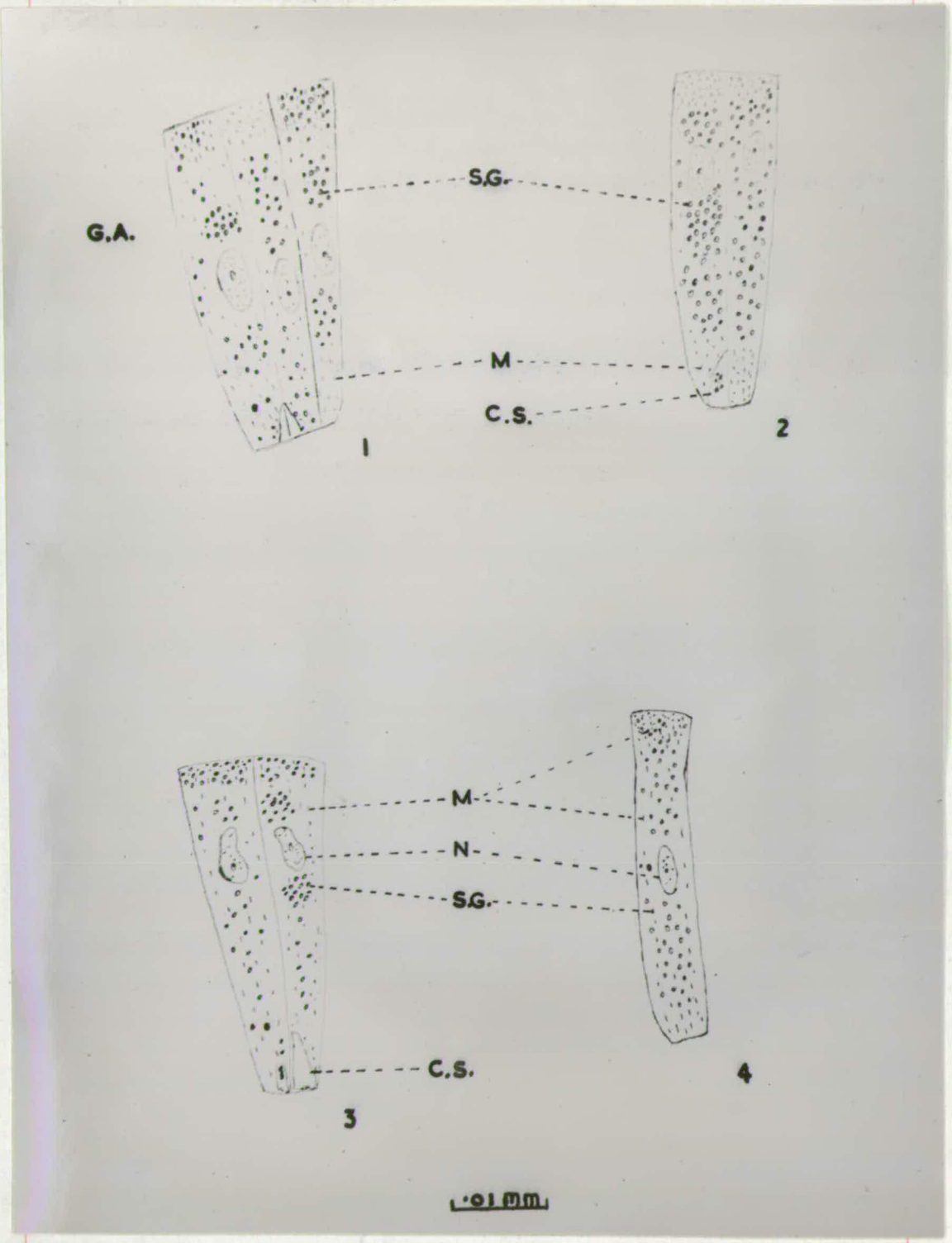


3

Drawings of the epithelial cells of the pharynx of earthworm having constant access to food.

All figures from Flemming preparations and stained according to Bensley's method.

Figs. 1-4. Showing mitochondria and secretory granules in cells in all stages of secretory activity.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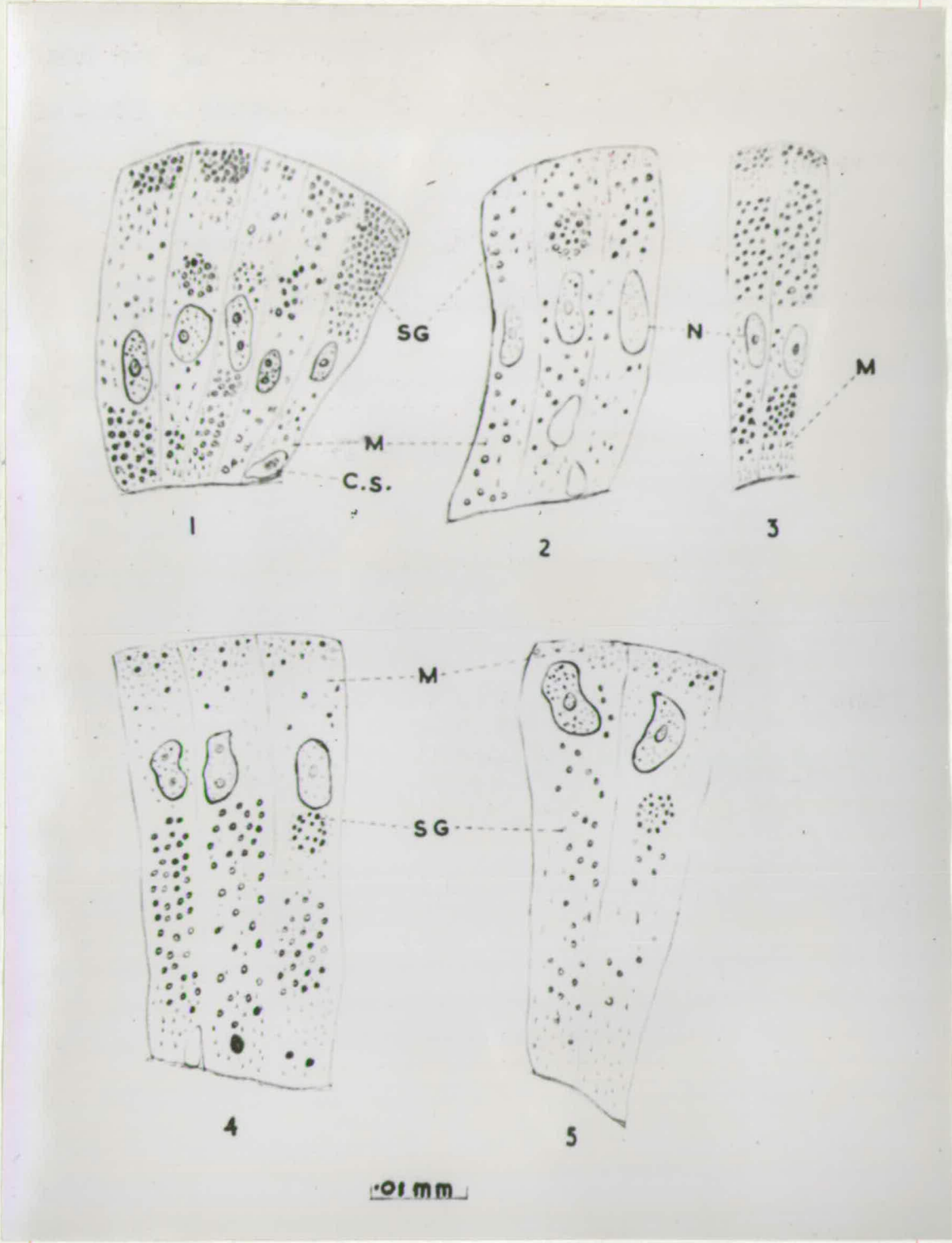
Drawings of the epithelial cells of the pharynx of earthworms. Figs. 1-3 from worms killed after five days' starvation. Figs. 4 and 5 from worms killed two hours after being in contact with food, following a fast of five days.

All figures from Flemming preparations and stained according to Bernsley's method.

Figs. 1-3. Non-ciliated cells showing gradual accumulation of secretory granules, secretory granules in the Golgi field, mitochondria and clear spaces in the basal cytoplasm of some cells.

Figs. 4 and 5 Non-ciliated cells showing evacuation of the granules from the lumen half of the cell.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Drawings of the epithelial cells of the pharynx of earthworms, killed at various periods after feeding, showing mitochondria and secretory granules.

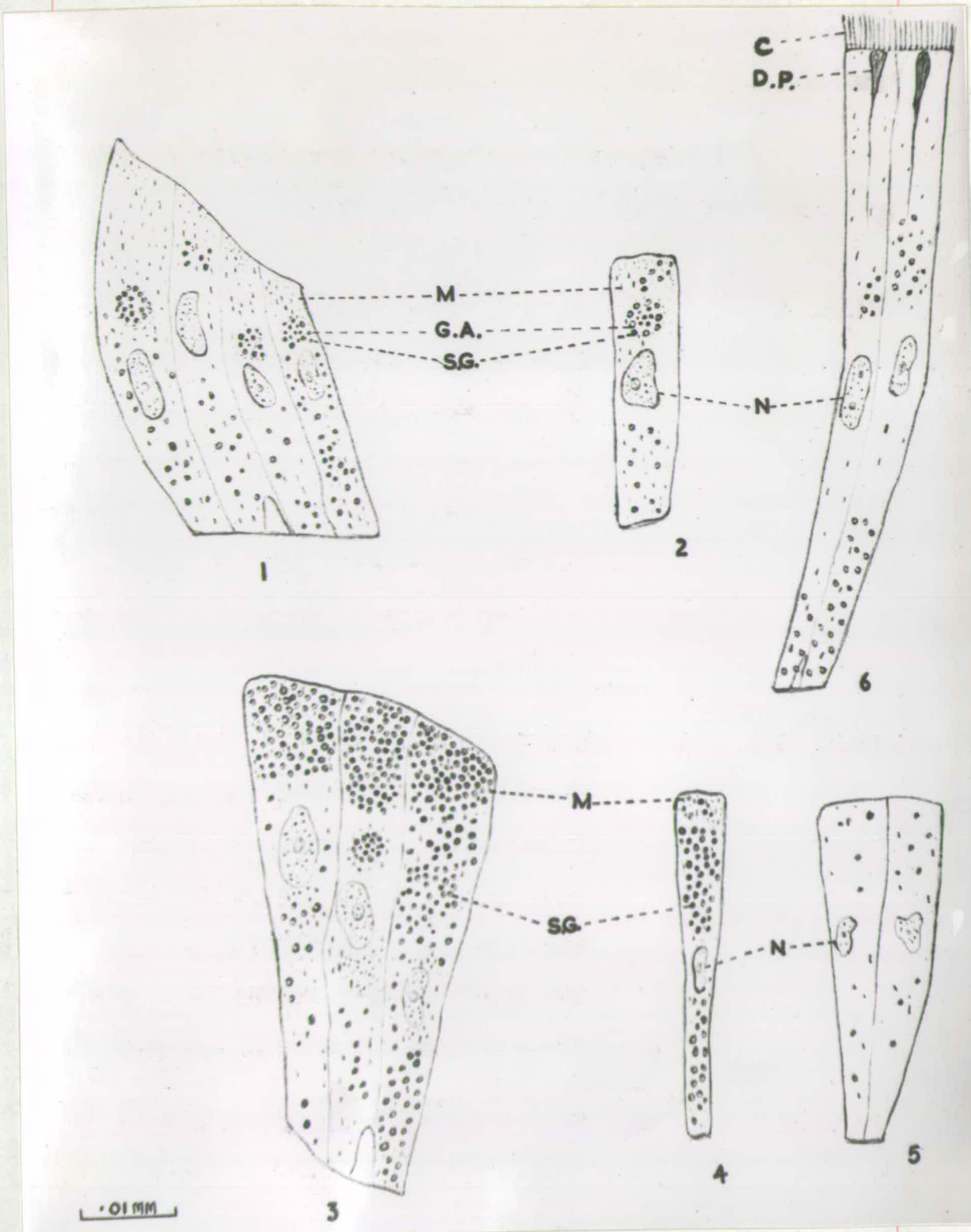
All figures from Flemming preparations and stained according to Bensley's method.

Figs. 1 and 2. Non-ciliated cells, 5 hours after feeding, showing secretory granules in the Golgi field and the origin of the earliest secretory granules in the basal cytoplasm. Mitochondria are more numerous in the lumen half of the cell than in the basal region.

Figs. 3 and 4. Non-ciliated cells, 6 hours after feeding, showing gradual accumulation of the secretory granules chiefly at the basal pole. Granular mitochondria are chiefly seen near the nucleus whereas the rods are distributed throughout the cell.

Fig. 5. Non-ciliated cell after the discharge of secretory granules showing disintegration.

Fig. 6. Ciliated cell showing gradual evacuation of secretory material 2 hours after feeding.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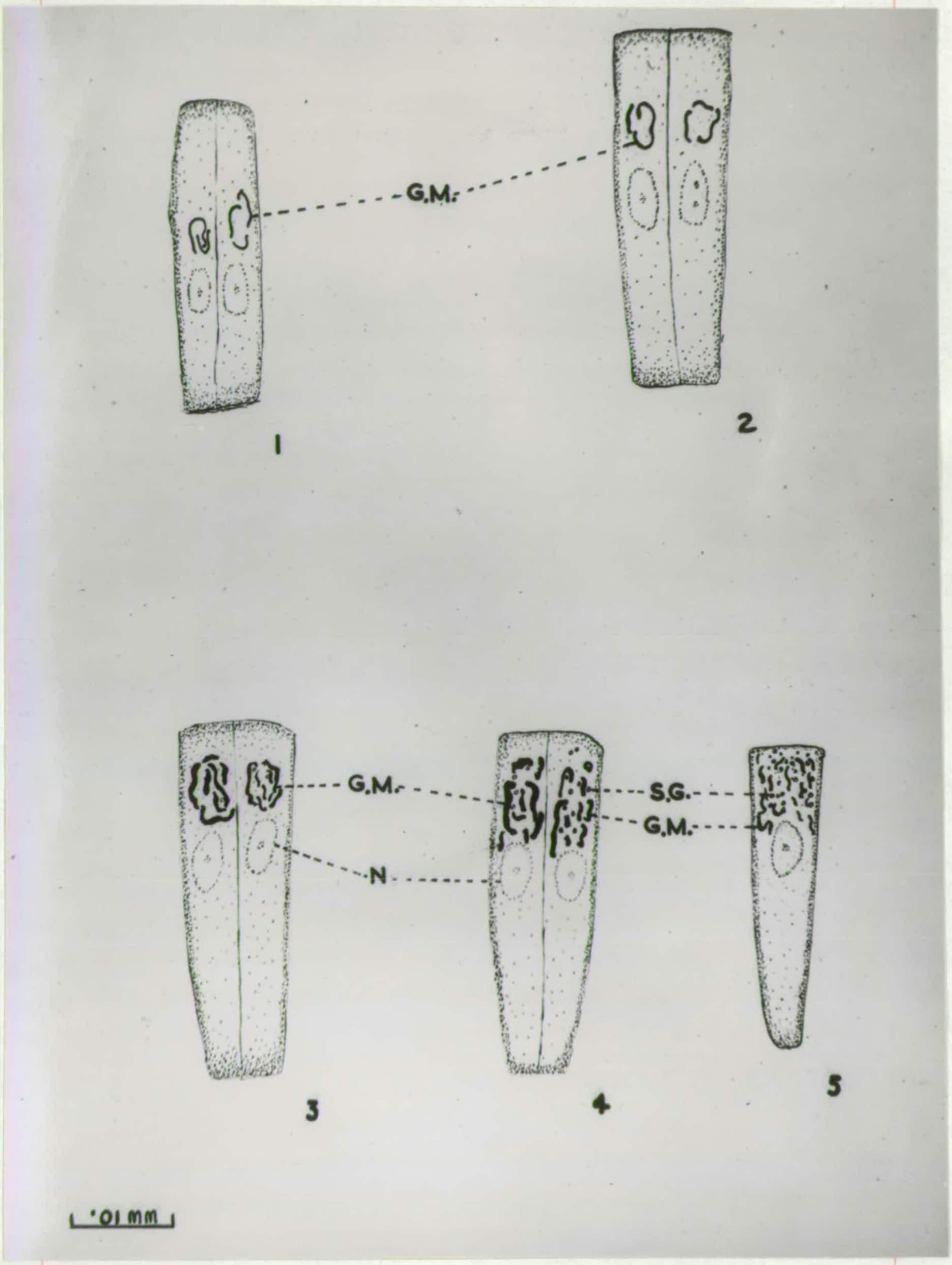
Drawings of the epithelial cells of the pharynx of earthworms having constant access to food.

All figures from Kolatchev preparations.

Figs. 1-5. Showing Golgi material of non-ciliated cells in all stages of secretory activity.

PLATE VII.

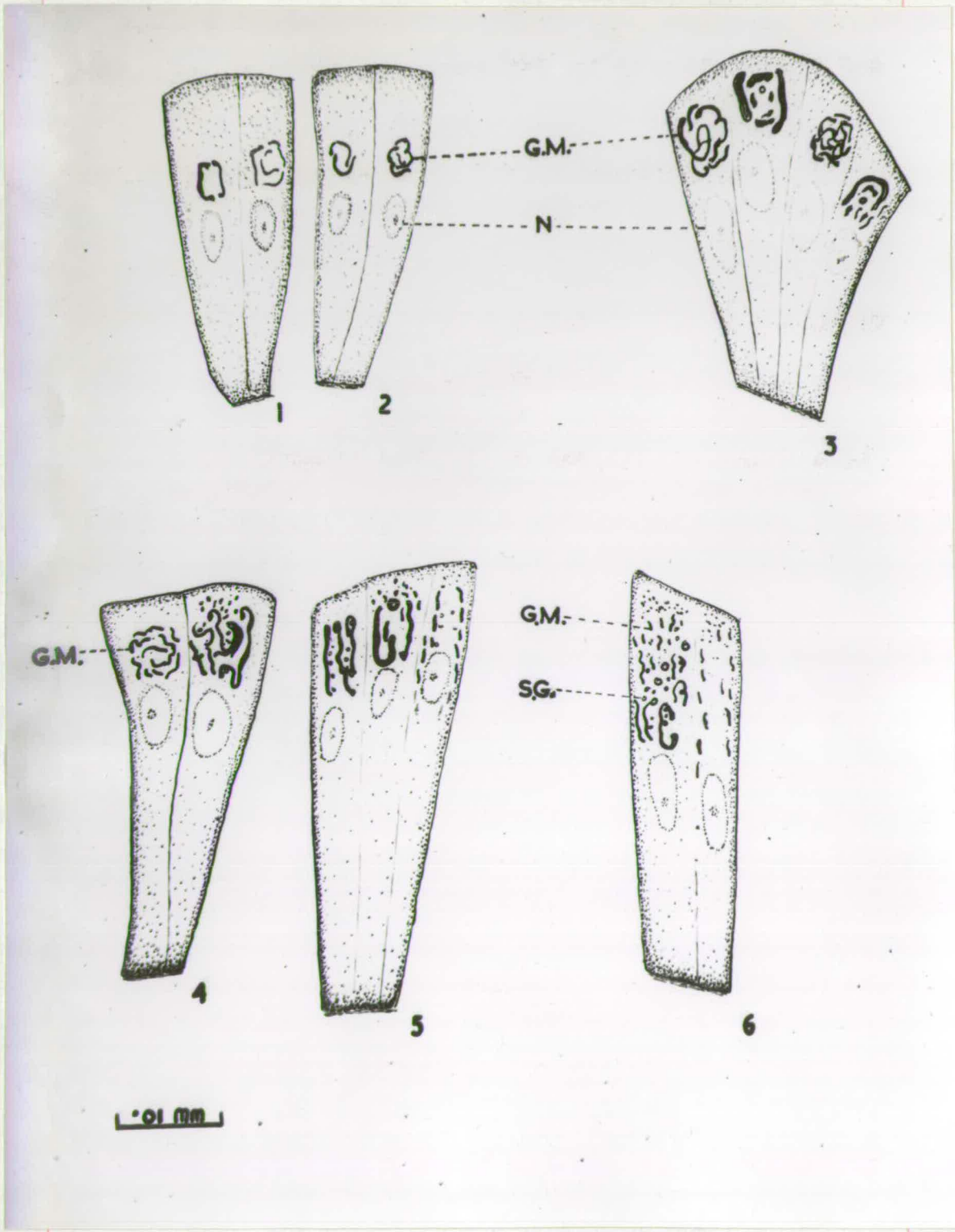


Drawings of the non-ciliated epithelial cells of the pharynx of earthworms killed after a fast of 4-5 days followed by 3-6 hours in contact with food.

All figures from Kolatchev preparations.

Figs. 1 and 2. Cells showing reduced Golgi material.

Figs. 3-6. Cells showing hypertrophy and gradual elongation and thickening of the Golgi elements leading to their fragmentation.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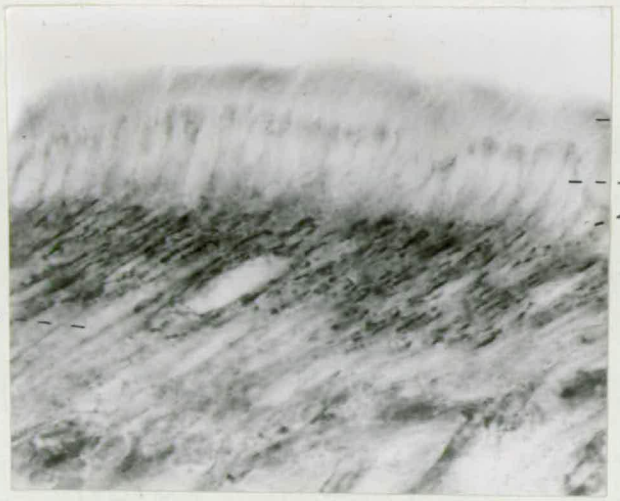
Photomicrographs of the ciliated epithelium of the pharynx showing the Golgi material and discharge-pockets. x 1140.

All from Kolatchev preparations.

Fig. 1. Showing discharge-pockets near the border of the cell adjacent to the lumen.

Fig. 2. Showing the Golgi material very clearly in the form of rods, filaments and granules. At some places the Golgi elements are seen surrounding secretory material which appears white in the section. The Golgi material is elongate and runs almost parallel to the longitudinal axis of the cell.



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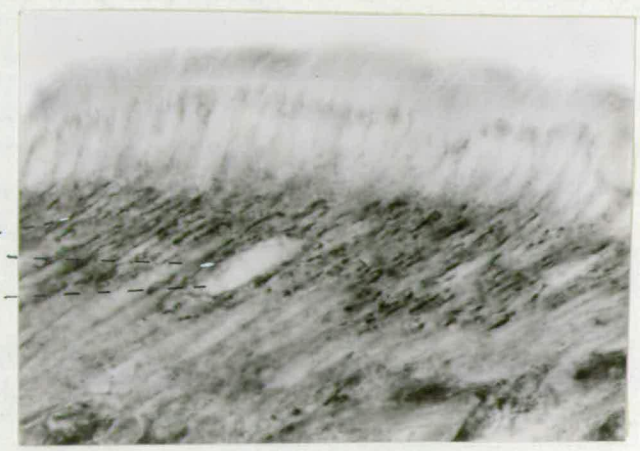


2

Photomicrographs of the non-ciliated epithelial cells of the pharynx showing the Golgi material.

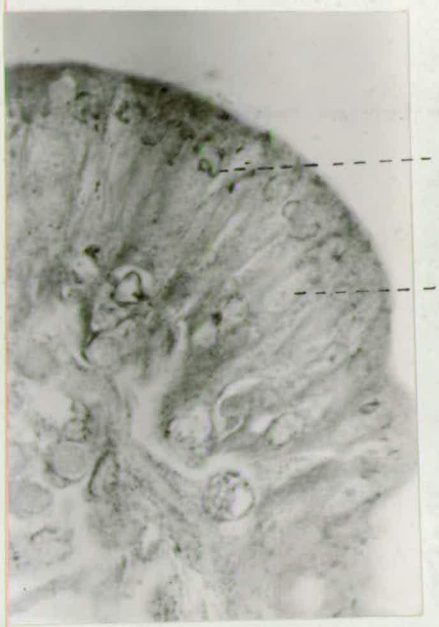
All figures from Kolatchev preparations.

Fig. 1. Showing the Golgi material in the cells of a fasting animal. The Golgi material is very much reduced. x 980

Fig. 2. Showing the Golgi material after the intake of food, following starvation. The gradual loosening up and hypertrophy of the Golgi material is seen. x 980

Fig. 3. Showing considerable elongation of the Golgi material during secretory activity. Fragmentation of the Golgi elements is seen in some cells. x 820

Fig. 4. Showing great hypertrophy and elongation of the Golgi elements during increased cellular activity. x 980



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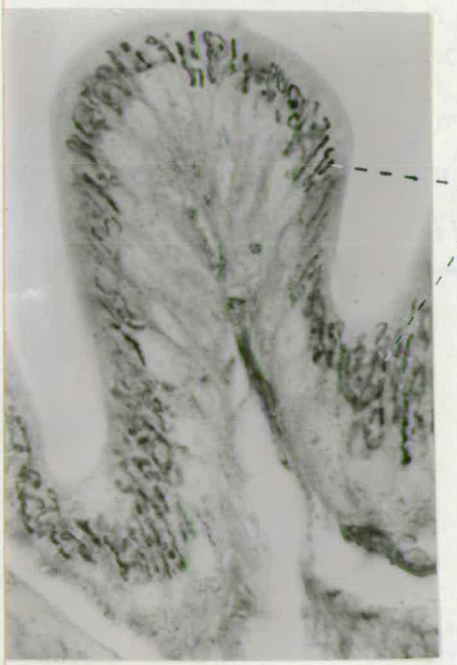

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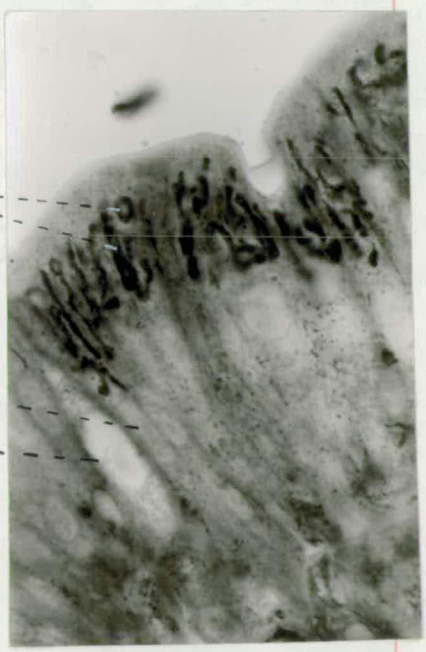

4

VII. OESOPHAGUS

A. HISTORICAL

The histology of the oesophageal epithelium of earthworms has been described by various authors in the past, notably by Ribaucourt (1901), who worked on Allolobophora turgida (A. caliginosa), and by Stephenson and Prashad (1919), who worked on A. caliginosa and Lumbricus. As far as the writer is aware, there is however no previous contribution which deals with the cytology of the epithelium of the oesophagus of Lumbricus.

B. METHODS

The methods employed for the cytological study of the oesophageal epithelium were the same as those used for the examination of the pharynx, except that certain specimens were fed after a fast of four to six days, and material was then fixed at two, three, four, five, five and a half, six, six and a half, seven, and twenty-four hours after the worms were placed in the soil. This method was adopted in order to obtain information on the secretory cycle during the stages of increased cellular activity.

For the examination of the Golgi material osmic (Kolatchev) as well as silver (Aoyama) methods were tried with successful and satisfactory results/

results.

C. OBSERVATIONS

The oesophagus is the portion of the alimen-:tary tract which succeeds the pharynx. It is a long narrow tube with segmental dilatation and intersegmental constrictions. The foldings of the oesophageal epithe-:lium attain a great degree of complexity in this animal and form glandular structures like the oesopha-:geal diverticula (or oesophageal pouches) in segment ten and the oesophageal glands in segments eleven and twelve. On the whole this region of the alimentary canal is highly vascular. The transversely folded epi-:thelium consists of tall columnar cells with a thick cuticular lining. Cilia or rodlets are not found except in the region of the oesophageal pouch.

The epithelial cells situated in the part of the oesophagus where the oesophageal pouches and oeso-:phageal glands open are more elongated than those situated more posteriorly. Binucleate cells were some-:times observed.

1. Worms with Constant Access to Food

When an animal has constant access to food, the structure and arrangement of the components of the cells of the oesophageal epithelium vary greatly depending upon the phases of the secretory cycle.

The cell membranes are distinct in some regions/

regions but indistinct in others. The outline of the nucleus may be regular or irregular, but in all cases the nuclear memrane is distinct. The nucleus may lie in the extreme basal region or occupy a position close to the lumen half of the cell. One or two nucleoli and chromatin granules are present.

The arrangement of the secretory granules varies in the different cells and is related to the phases of the secretory cycle. The granules may be most numerous in the basal half and scanty in the lumen half of the cell; in other cells they may be numerous in the lumen half and scanty in the basal half. In some cells the migration of the secretory material from the basal area towards the lumen is clearly shown, and in certain cells their accumulation in close asso-:ciation with the mitochondria ~~can be observed in close association with mitochondria~~ around the Golgi zone.

2. Conditions Induced by Fasting

In worms which have not been fed for some days the accumulation of the secretory granules, chiefly in the lumen half of the cell, is the most noticeable cytological feature. In some cells, however, the granules are more numerous in the basal region than towards the lumen, and in this case the formation of the secretory granules in the Golgi area can be easily seen (Pl. XI, figs. 1-3). The nuclei are mostly regular in shape and oval in outline, and occupy a more or less central position in the cell. There are, however,/.

however, a few cells in which the nuclei are irregular in outline, and in which the nuclei lie in the basal or in the lumen part of the cell. The nuclear membrane, usually one ~~nucleus~~ nucleolus, or rarely two, and chromatin material are clearly visible. Cell membranes are distinct in most cases. As the cells are packed with secretory material they are not uniform in appearance throughout the sections.

3. The Secretory Cycle.

Following the intake of food, after a fast of four to six days, the majority of the cells become very active. During the early part of the active phase, the nuclei become irregular in shape and move towards the lumen. In most cases the cell membranes are less distinct than during the resting condition.

Two hours from the intake of food, some of the cells show general evacuation of the secretory granules which were accumulated during the fasting period in the lumen half of the cell, while in others secretory granules from the basal part have migrated towards the lumen. Fresh secretory material appears in the basal part of some of the cells (Pl. XI, figs. 4 and 6).

Three hours after a worm has commenced to feed there is little appreciable change from the preceding phase except that the secretory granules are either totally discharged into the lumen or they fill the entire distal half of the cell; in most cases the basal half contains very few secretory granules, but in a few cases it is still full of granules. In some cells/

cells secretory granules are accumulated in close vic-:inity to the nuclear poles (Pl. XII, figs. 1 and 2).

Four hours from the intake of food, a few secretory granules are still visible in the vicinity of the nucleus and fresh secretory material begins to accumulate in the basal region of the cell. Early sec-:retory granules appear in the Golgi field and migration of secretory granules from the basal region is visible in some of the cells. Owing to the discharge of the accumulated secretory granules the cells are now more uniform in appearance (Pl. XII, fig. 3)

Five to five and a half hours from the intake of food, secretory granules have again accumulated in the cells. The nuclei may be pushed towards either end of the cell and thus lie at different levels. The arrangement of the secretory granules and their accumulation resembles that of the resting phase. Cells from which most of the accumulated secretory granules have been discharged into the lumen appear to be much elongated and uniform in shape with regular oval nuclei which occupy a central position (Pl. XII, figs. 4 and 5).

Six to seven hours from the intake of food, some of the cells have discharged the secondary accum-:ulation of secretory granules and consequently are of uniform appearance with the nucleus lying chiefly in a central position. After the secretion is discharged into the lumen the cells undergo a process of disintegration. In certain cells, however, secretory granules are still present in the lumen half and early secretory granules

are/

are situated in the Golgi field, and, in some cases, in close proximity to the opposite pole of the nucleus. The appearance of the cells is somewhat similar to that of cells of animals with constant access to food (Pl. XII, fig. 7).

Twenty-four hours from the intake of food the appearance of the cells is similar to that of the cells of animals with constant access to food.

In both active and resting phases, clear spaces, or vacuole-like structures, are very commonly seen near the basement membrane. Masses of mucus secretion are also present either in the basal or in the lumen half of the cell. Secretory material from the chromophil cells, lying in the outer cavities of the villi, are commonly seen entering the epithelial cells of the oesophagus; its entry appears to be brought about through the rupture of the basement membrane. This secretion ultimately escapes into the lumen.

It must be noted that the production of secretory granules never ceases completely during any stage of fasting or feeding nor does complete evacuation of the granules take place from a cell at any one time.

4. The Mitochondria

i. Worms with Constant Access to Food :- In animals with constant access to food, the mitochondria are chiefly in the form of rods and granules; a few filaments of equal thickness are scattered through the cytoplasm. Some of the granules may be the cross section of the rods and the filaments. The mitochondria are arranged more or less at random without any polar orientation/

orientation.

Cells in all stages of the secretory cycle are present. The behaviour of the secretory granules, mitochondria and Golgi material during the different phases of the secretory cycle is described in the following sections, and consequently need not be repeated here. In worms which have received a meal after a prolonged fast the secretory response is much more marked than in worms with free access to food.

ii. Conditions Induced by Fasting:- During the resting stage the mitochondria consist of long rods and filaments and a few granules (Pl. XI, figs. 1-3). In the basal part of the cell the mitochondria are in the form of rods and filaments. The majority of the filaments are present in close proximity to the base-:ment membrane, but a few occur scattered throughout the cytoplasm. In the lumen half of the cell the mito-:chondria are mostly in the form of rods and granules. In some cells the lumen part contains more mitochon-:dria than the basal region. The rods and filaments are always of equal thickness but some of the rods are longer than the others. Granular mitochondria are chiefly found in the vicinity of the nucleus, or in its immediate neighbourhood, in association with the secretory granules. A few granular mitochondria are also seen adjacent to the lumen. In cells which are densely packed with secretory granules, the majority

of/

of the mitochondria are situated at the extreme poles of the cell.

There is no definite polar orientation of the mitochondria during this stage.

iii. The Secretory Cycle:- During the increased cellular activity which results from feeding after fasting, the most noticeable feature observed is the fragmentation of the filamentous mitochondria into long rods and finally the fragmentation of all the rods present into short rods and granules. This results in an increase in the number of mitochondria. Polar orientation of the mitochondria becomes strongly marked through the arrangements of the rods parallel to the longitudinal axis of the cell (Pl. XII, figs. 1-7).

Two hours from the intake of food, the mitochondria consist of small rods and a few granules scattered all through the cytoplasm (Pl. XI, fig.5). The lumen half contains more mitochondria than the basal half of the cell. Some of the rods and granules are seen in association with the secretory granules either around the Golgi field or in the vicinity of the nucleus. In some cells granules also occur in the distal part of the cell close to the lumen. There are, however, in some cells, a few long rod-shaped mitochondria as well as the smaller rods. These rod-shaped mitochondria/

mitochondria are most numerous in the basal half of the cell. No filamentous forms are seen during this stage.

Three hours from the intake of food, it was noticed that further fragmentation of the mitochondria has taken place as a result of which there are more granular mitochondria present than during the previous stage (Pl. XII, figs. 1 and 2). These granules lie chiefly in the vicinity of the nucleus and near the border of the lumen. A few rod-shaped mitochondria are found in the lumen half of the cell. In the basal half of the cell there are small rod-shaped and a few grannular mitochondria; filamentous forms are totally absent from the cell during this stage. Around the area occupied by the Golgi bodies, very small rod-shaped and grannular mitochondria are seen lying in close association with the secretory granules.

Four hours from the intake of food, the majority of the mitochondria are concentrated towards both poles of the cell (Pl. XII, fig. 3). There are now more rod-shaped mitochondria and fewer granules present than during the preceding phases. A few small rod-shaped mitochondria are situated in the cytoplasm in the neighbourhood of the nucleus. The rods are, however, arranged chiefly towards the extreme poles of the cell, although a few rods are scattered through the rest of the/

the cytoplasm. A few long rods occur amongst the other rods. The granular mitochondria occur chiefly around the Golgi field and close to the basement membrane.

Five to five and a half hours from the intake of food, the mitochondria are in the form of small and long rods and a few granules. The granules are situated chiefly in the neighbourhood of the lumen, and the rods occur in both halves of the cell. Due to the great accumulation of the secretory granules during this stage, it is difficult to determine the exact arrangement of the mitochondria but they appear to be concentrated chiefly at the extreme poles of the cell (Pl. XII, fig. 4). This is probably due to the pressure exerted by the secretory granules. A few granular and rod-shaped mitochondria are seen in close vicinity to the nucleus. In cells from which secretory material has been discharged into the lumen, or which contain very few secretory granules, the mitochondria can be clearly seen. In such cells the mitochondria in the basal region, chiefly near the basement membrane, consist of long and short rods, while the majority of those in the lumen half are in the form of short rods with very few long rods scattered amongst them. Granu-:lar mitochondria are also seen in the lumen half of the cell chiefly in the vicinity of the nucleus (Pl. XII, figs. 5 and 6).

Six to seven hours from the intake of food, the/

the mitochondria are mostly rod-shaped and are uni-:formly distributed through the cytoplasm (Pl. XII, fig. 7). A few granular forms are also seen around the Golgi field in close association with the early secretory granules. Both short and long rod-shaped mitochondria and occasionally a few filamentous forms are found during this stage which therefore resembles to some extent the cells of animals with constant access to food.

Twenty-four hours from the intake of food, the form and position of mitochondria are similar to those found in worms with constant access to food.

It must be noted that in all the various physiological phases, only a certain number of cells is involved in the secretory process at any one time and that each cell acts as an independent unit.

During all stages of cellular activity, the secretory granules and mitochondria showed very marked affinity for acid fuchsin, but stained less deeply with iron-haematoxylin.

5. The Golgi Material

During the different physiological phases, the Golgi material of the oesophageal epithelial cells resembles in its morphology and behaviour that of the non-ciliated cells of the pharyngeal epithe-:lium.

i. Worms with Constant Access to Food:- When an animal has constant access to food, the Golgi material in some cells consists of thin filaments connected to-:gether by a very few thin cross-links, so that in sections it appears to be in the form of incomplete rings or a compact reticular structure. In other cells, which are more active physiologically, a loosening of the material takes place; the filaments become thicker and occupy a greater area than in the resting phase. This process continues and results in the Golgi material being broken up into smaller elements and granules. The larger elements now lie almost parallel to the longitudinal axis of the cell and in some cases occupy the entire lumen half of the cell. The granules usually lie at the extreme distal end of the cell in the neighbourhood of the lumen. This loosening and frag-:mentation of the Golgi material seems to run parallel to the rate of increase in cellular activity.

ii. Conditions Induced by Fasting:- During the phase of fasting the Golgi material is very much reduced in size and quantity and there is no appreciable difference in its morphology from that of the resting non-ciliated cells of the pharyngeal epithelium (Pl. XIII, figs. 1-6 and Pl. XIV, fig. 1).

iii. The Secretory Cycle:- With the onset of secre-tory activity, resulting from feeding after a fast, the Golgi material shows changes similar to those observed/

observed in the non-ciliated cells of the epithelium of the pharynx. During this period it becomes easier to impregnate it successfully with osmium tetroxide than during the fasting stage. The maximum manifesta-:tion of this increased power to reduce osmium tetrox-:ide seems to be about six or seven hours from the intake of food. By that time the Golgi material is broken up into rods and granules occupying a much greater area than during any of the preceding stages. Curved Golgi rods in close association with secretory granules are commonly seen during the phase of cellular activity. The morphological changes in the Golgi material are shown in Pl. XIII, figs. 7-11 and Pl. XIV, figs. 2-4.)

PLATE XI.

Drawings of the epithelial cells of the oesophagus of earthworms killed after a fast of four days followed by two hours in soil. Showing the mitochondria and the secretory granules.

All figures from Flemming preparations stained according to Bensley's method.

Figs. 1-3. Cells during the fasting period showing accumulation of secretory granules and mitochondria.

Figs. 4 and 6. Cells of animals in contact with food for two hours showing gradual evacuation of the secretory granules and the fragmentation of the mitochondria.

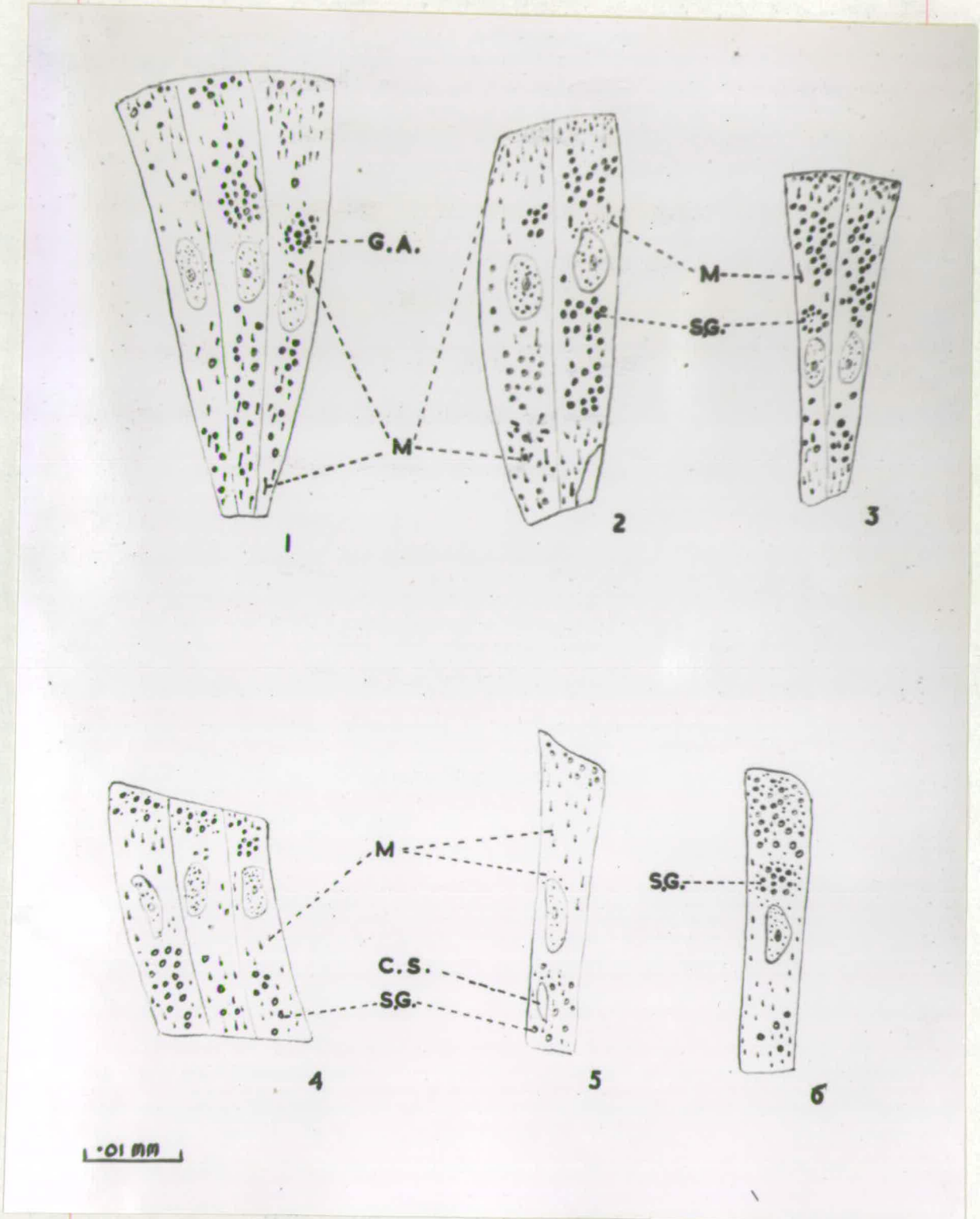
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PLATE XII.

Drawings of the epithelial cells of the oesophagus of worms killed after feeding showing mitochondria and secretory granules.

All figures from Flemming preparations stained according to Bensley's method.

Figs. 1 and 2.	Cells, 3 hours after feeding, showing the positions of the nuclei, secretory granules and granular mitochondria.
Fig. 3.	Cells 4 hours from the intake of food.
Figs. 4 and 5.	Cells 5 hours after feeding.
Figs. 6 and 7.	Cells 6 hours after feeding.

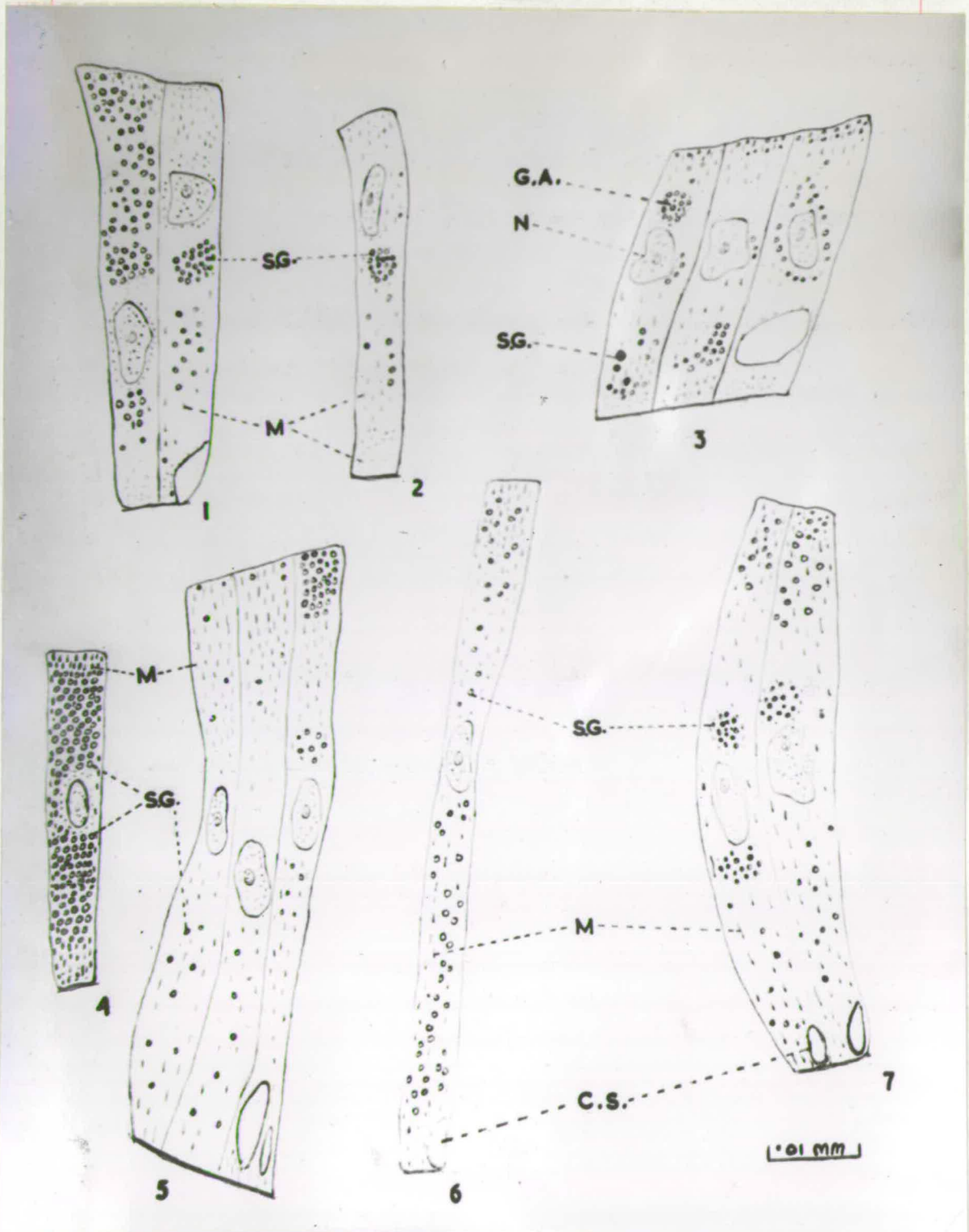
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PLATE XIII.

Drawings of the epithelial cells of the oesophagus showing the Golgi material.

All figures from Kolatchev preparations.

Figs. 1-6. Cells of fasting animals showing greatly reduced Golgi material.

Figs. 7-11. Cells 3-7 hours after feeding, showing the gradual hypertrophy and loosening of the Golgi material leading to fragmentation of some of its elements.

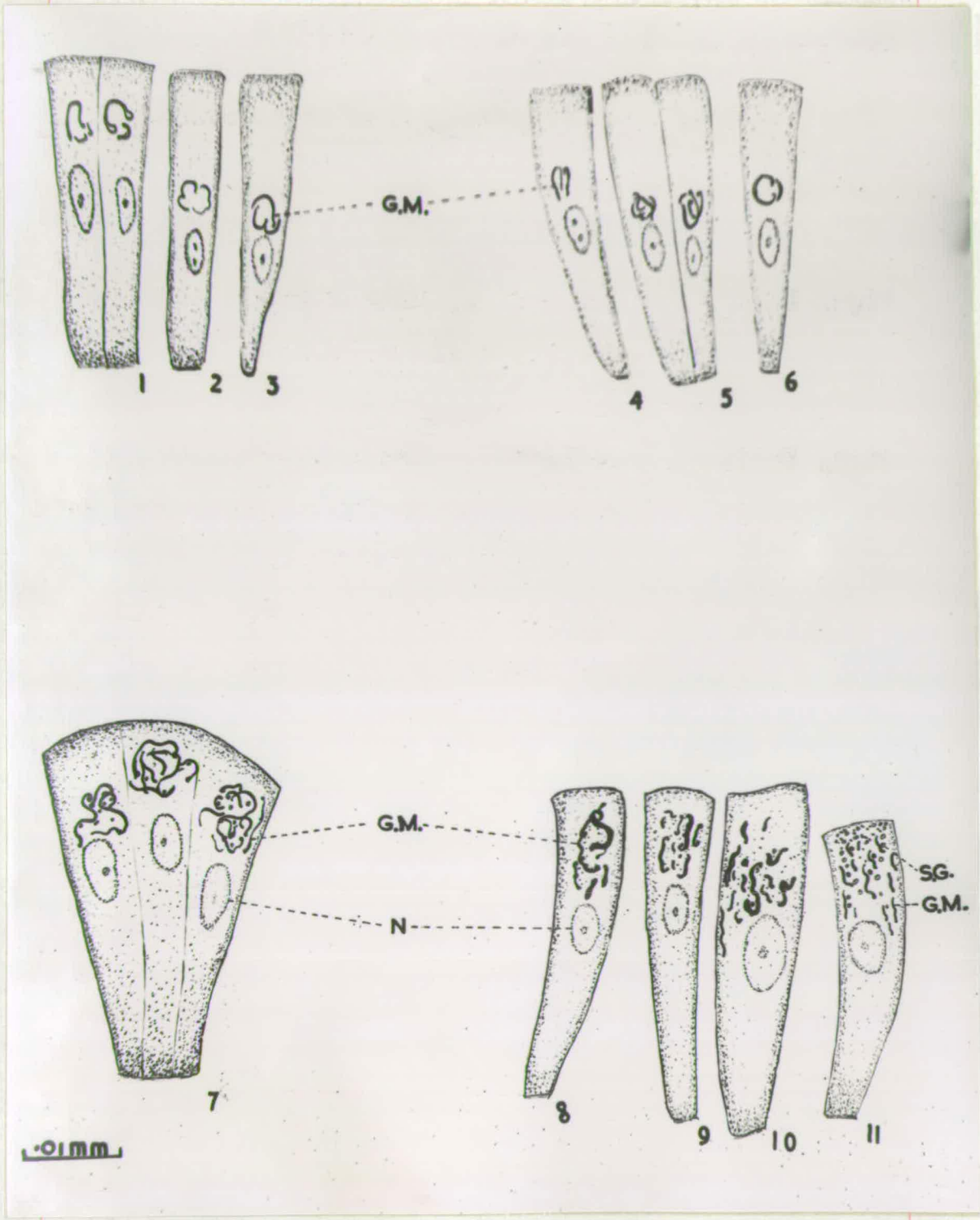
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PLATE XIV.

Photomicrographs of the oesophageal epithelium showing Golgi material, mitochondria and secretory granules.

Figs. 1-4 From Kolatchev preparations. x 980

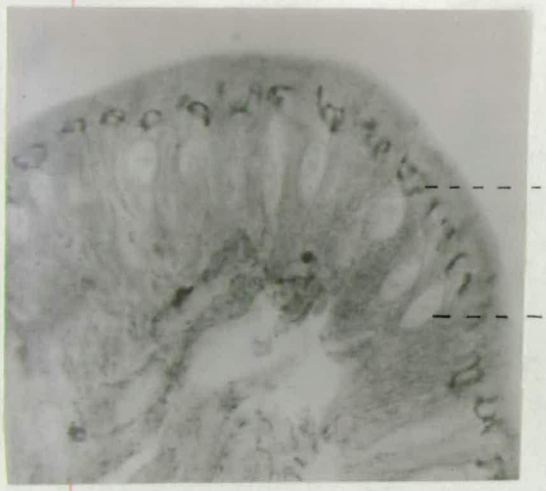
Fig. 5 From Flemming preparation. x 1380

Fig. 1. Showing the Golgi material during a period of starvation.

Figs. 2 and 3. Showing the gradual elongation and and hypertrophy of the Golgi material which follows the intake of food.

Fig. 4. Showing fragmentation of the Golgi material, 6 hours after the intake of food, following starvation. The Golgi rods run parallel to the longitudinal axis of the cell.

Fig. 5. Showing mitochondria and secretory granules. Secretory granules are also seen in the Golgi field in some cells. Clear spaces are seen in basal region of some of the cells.



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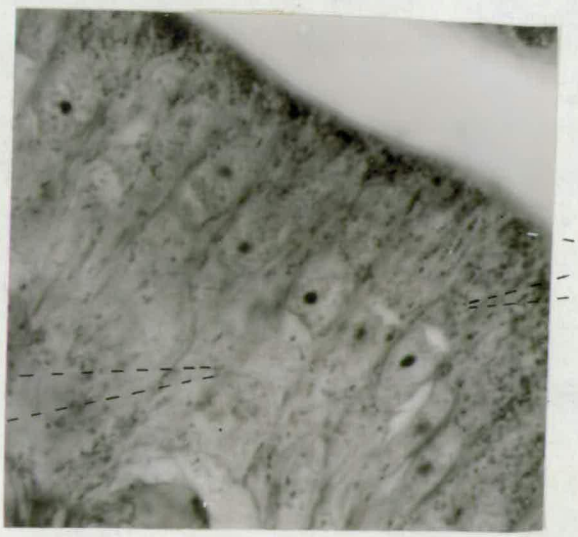

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VIII. CROP.

A. HISTORICAL

The epithelium of the crop of Lumbricidae has been described by various authors notably by Dequal (1910) and Ribaucourt (1901). Dequal, working on Octolasium complanatum, found that the epithelial cells have a striated border and are covered with a thin cuticle which is insoluble in KOH and is not fibrillar in structure. Stephenson states, in his monograph of the Oligochaeta, that the food seems to undergo no important change in this region and does not stay there long.

The cytology of the epithelium of the crop of Lumbricus has not been studied previously.

B. METHODS

The methods employed for the cytological study of the epithelium of the crop were almost the same as those used for the examination of the pharynx and the oesophagus.

While staining the sections with acid-fuchsin and light green, according to Benseley's method, it was noticed that those regions of the cells in which the accumulation of the secretory granules is at its maximum become overstained. This renders cytological investigation extremely difficult, and in order to overcome the difficulty, acid fuchsin-stained sections were treated for fifteen seconds to two minutes with a very/

very dilute solution of sodium carbonate in distilled water (1-2 drops of saturated aqueous solution of sodium carbonate in 20 c.c. of distilled water). Sections were constantly watched under the microscope and when excess acid fuchsin was removed, they were washed with distilled water and counterstained with light green. This method gives a very precise and satisfactory staining of the mitochondria and the secretory granules.

For the examination of the Golgi material, osmic (Kolatchev) as well as silver (Aoyama) methods were tried with successful results.

C. OBSERVATIONS

In Lumbricus the oesophagus is followed by a rounded thin walled chamber, the crop, which occupies segments xvi-xvii. The epithelial cells lining the crop are tall, cylindrical and columnar; they have striated border covered by cuticle. The cells of the whole epithelium are uniform in size and the cell-membranes are usually distinct during all phases of activity. The nuclei are mostly oval in shape and regular in outline with distinct nuclear membranes; one to two nucleoli and chromatin granules are present. Clear spaces, or vacuoles, are usually seen in the basal region of the cells during all phases of cellular activity (Pl. XV, figs. 1-3 and 5, and Pl. XVIII, fig. 1).

1. Worms with Constant Access to Food

When an animal has constant access to food, some of the epithelial cells are in stages of activity and others are at rest. Consequently, the arrangement of the secretory granules and the position of the nucleus varies in different cells. The changes, which are correlated with the secretory cycle, are described in the following sections.

2. Conditions Induced by Fasting

During the resting phase, produced by starving the worms for some days, the cells, due to a marked accumulation of secretory granules close to the lumen, are club-shaped (Pl. XV, figs. 1-3 and Pl. XVIII, fig. 1). Clumps of secretory granules are also situated close to both poles of the nucleus. The majority of the cells contain very few secretory granules in the basal region except the clump in close proximity to the nucleus. There are, however, a few cells in which secretory granules are situated uniformly throughout the cytoplasm.

In cells with an accumulation of secretory granules in the lumen half, the nuclei are situated either in the central cytoplasm or in the basal region. In these cells the position of the nucleus appears to be governed by the pressure exerted by the secretory granules. In a few cells, secretory granules are numerous in the basal part but are scanty in the cytoplasm near/

near the lumen; in these cases, the nuclei are situated in the lumen half of the cell.

3. The Secretory Cycle

As a result of feeding after a fast, cellular activity is considerably increased. Most of the secretory granules observed, during the fasting stage, in the lumen side of the cell have been discharged so that the majority of cells are no longer club-shaped. Migration of secretory granules from the basal part to the lumen region of the cell is clearly seen especially in material fixed for the examination of the Golgi material (Pl. XVII, figs. 3-5 and Pl. XVIII, fig. 2). In the mitochondrial preparations early secretory granules are visible in the Golgi zone.

Two to three hours from the intake of food, the cells show a gradual evacuation of the secretory material. Some secretory granules are present close to one pole, or both poles, and in the cytoplasm adjacent to the lumen. In some cases a few secretory granules are visible in the region, close to the basement membrane, while in some others, the basal region is full of granules (Pl. XV, fig. 5 and Pl. XVI, figs. 1 and 2).

Six to seven hours from the intake of food, the cells contain more secretory granules in both the basal and lumen regions. In some cells secretory granules from the basal region have now accumulated in the lumen half of the cell and a secondary accumulation is taking place in the basal cytoplasm. Concentration of secretory material/

material near the poles of the nucleus is still seen in some cases (Pl. XVI, figs. 3 and 4). Some of the cells at this stage resemble those observed in animals with constant access to food.

Twenty-four hours from the intake of food, cells in all stages of the secretory cycle are present.

4. The Mitochondria

i. Worms with Constant Access to Food:- In animals with constant access to food, the mitochondria of the active cells are chiefly in the form of granules with a few very small rods distributed throughout cytoplasm; no filaments are present. Due to the very small size of the mitochondria polar orientation is not very well demonstrated.

Cells in all stages of the secretory cycle are present. The behaviour of the mitochondria and the Golgi material during the different phases of cellular activity is described in the following sections and need not be repeated here.

ii. Conditions Induced by Fasting:- During the fasting period, the mitochondria consist chiefly of thick rods and very few granules. The majority of the mitochondria are situated in the lumen half of the cell; the basal half contains very few rod-like mitochondria. Some rods are in close association with the secretory granules and are situated either around the Golgi field or in the neighbourhood of the poles of the nucleus (Pl. XV, figs. 1,2 and 4). In some of the cells, which contain very few secretory granules, rod-shaped/

rod-shaped mitochondria are concentrated chiefly in the neighbourhood of the nucleus and the Golgi field and a few rods are scattered through the rest of the cytoplasm, particulary in the lumen half of the cell. In others, the rods are scattered uniformly in the lumen half but are very scanty in the basal region of the cell (Pl. XV, figs. 1-4).

Granular mitochondria are few during the inactive phase; they occur chiefly in the neighbour-:hood of the Golgi field and at the opposite pole of the nucleus.

The rod-shaped mitochondria seem to be slightly thicker than those present during the inactive stage of the epithelial cells of the pharynx and the oeso-:phagus. The majority of the mitochondria are arranged parallel to the longitudinal axis of the cell but a few sometimes occur at random without any definite orientation (Pl. XV, figs. 1-4).

iii. The Secretory Cycle:- With the onset of cellular activity, fragmentation of most of the rod-shaped mitochondria takes place. The mitochondria are now chiefly in the form of granules, but a few very small rods are also present.

Two to three hours from the intake of food, the mitochondria consist mostly of granules and, occasionally, a few very small rods which are present either in the neighbourhood of the nucleus or close to/

to the lumen. The granules are situated throughout the cytoplasm, but are most numerous in the Golgi field and in the neighbourhood of the nucleus where they are in close association with secretory granules. In a few cases granules are present adjacent to the lumen (Pl. XV, fig. 5 and Pl. XVI, figs. 1 and 2).

Six to seven hours from the intake of food, the majority of the mitochondria are still granular, but the rods have increased slightly in size and in number (Pl. XVI, figs. 3 and 4).

Twenty-four hours from the commencement of feeding, cells in all stages of activity are present.

5. The Golgi Material

The Golgi material of the epithelial cells of the crop consists of thick rods and filaments connected together by thin cross links, which in sec-:tions appear to be in the form of straight or curved thick rods. These rods may be long or short.

i. Worms with Constant Access to Food:- During this period, all stages of the secretory cycle are seen, and the Golgi material shows marked morphologi-:cal changes indicative of its participation in the functional activity of the cell. The epithelium of the crop is very good material for demonstrating the close association of the secretory granules with the Golgi material. Migration of the earliest secretory granules from/

from the basal region to the Golgi field, and from the Golgi zone to the lumen side of the cell is very clearly shown.

ii. Conditions Induced by Fasting:- The Golgi material of the cells of animals which have been starved for some days consists of small curved rods and filaments situated close to the nucleus. In the majority of the cells it occupies a relatively small area, but in certain cells the Golgi elements are more loosely arranged so that they spread over a greater area than is usual (Pl. XVII, figs. 1 and 2; Pl. XVIII, fig. 1). This may be due to the fact that even in the fasting stage the production of secretory granules never wholly ceases.

On the whole, in the fasting animal, the Golgi material never seems to be in the form of a compact ring-like structure as observed in the case of the the pharynx and the oesophagus.

iii. The Secretory Cycle:- As a result of feeding cellular activity is accelerated and the secretory response is much more marked than in worms with free access to food. Consequently, the Golgi material increases in amount and occupies a greater area than in the cells of the fasting animal. Loosening of the rods and the filaments takes place and they now lie roughly parallel to the longitudinal axis of the cell. The migration of secretory material from the basal region to the area of the Golgi field can be traced; granules/

granules are visible in close association with the elements of the Golgi material (Pl. XVII, figs. 3-5 and Pl. XVIII, fig. 2). With a further increase of cellular activity the Golgi elements break up into smaller rods and granules (Pl. XVII, figs. 4 and 5).

As a result of the fragmentation of the Golgi material some of the rods lie parallel to the longi-:tudinal axis of the cell. Some of these occur on either side of the nucleus. Granules are also present close to the nucleus. There are, however, some Golgi elements in the form of long rods and filaments which have greatly increased in size during the secretory cycle (Pl. XVII, figs. 4 and 5).

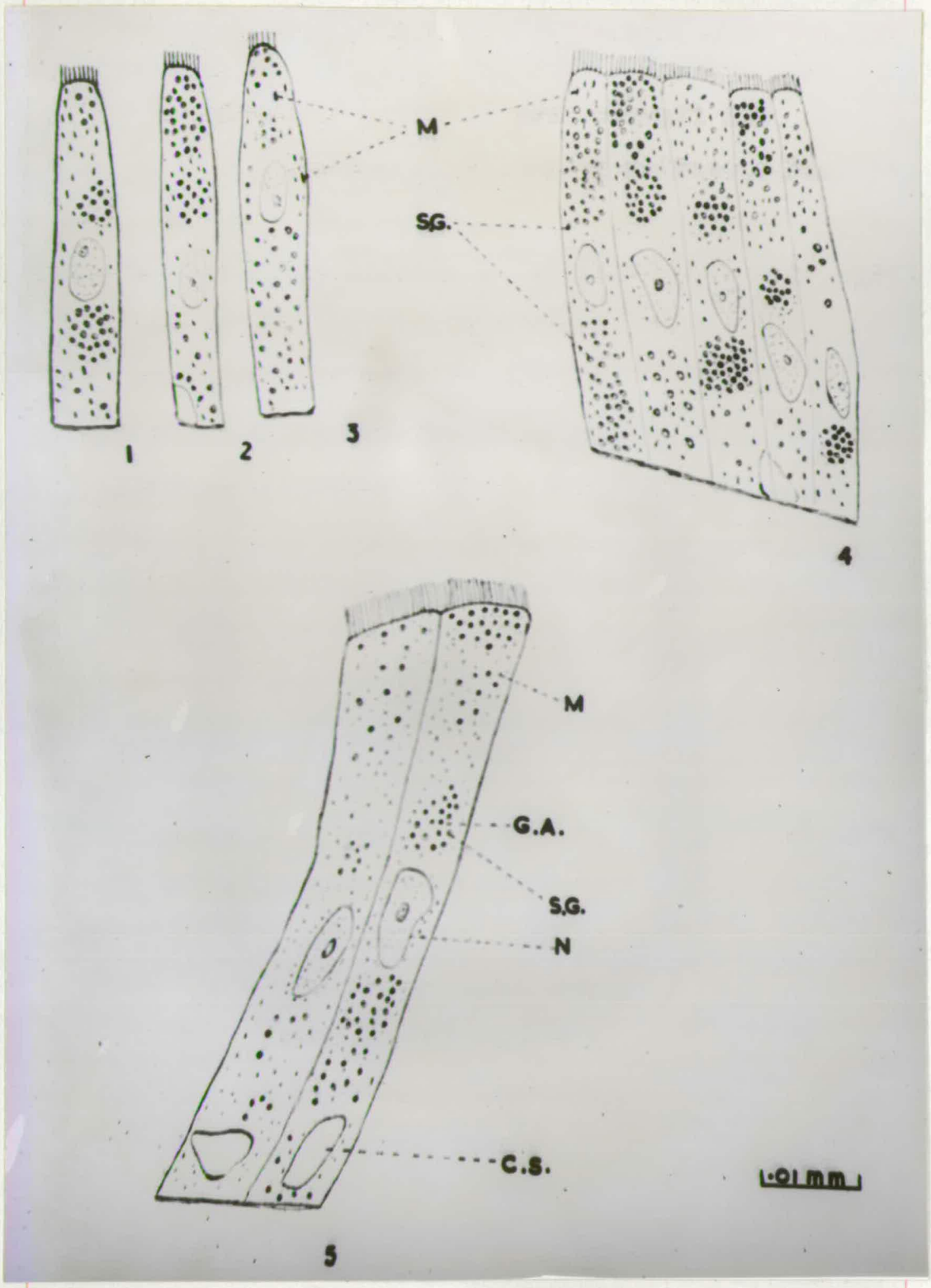
The increase in the area occupied by the Golgi material during the period of increased cellular activity, and the elongation and final fragmentation of the Golgi material after coming into contact with secretory granules are evidently signs of its parti-:cipation in the process of secretion.

Drawings of the epithelial cells of the crop showing mitochondria and secretory granules.

All figures from Flemming preparations stained according to Bensley's method.

Figs. 1-4. Cells of animals which were starved for 6 days showing the great accumulation of the secretory granules chiefly at the lumen pole, and the occurrence of secretory granules in clusters in the immediate neighbourhood of the nucleus. The mitochondria are more numerous at the lumen pole than in the basal region.

Fig. 5. Cells from animals killed after two hours contact with soil, showing gradual evacuation of the secretory granules, occurrence of fresh granules in the basal cytoplasm, and accumulation in the Golgi field.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Drawings of the epithelial cells of the crop, worms killed after feeding, showing the mitochondria and secretory granules.

All figures from Flemming preparations stained according to Bensley's method.

Figs. 1 and 2. Cells of worms which have been in soil for 3 hours, showing the evacuation of the accumulated secretory granules from the lumen pole of the cell, and the gradual accumulation of fresh secretory granules in the basal cytoplasm. Concentration of early secretory granules in the Colgi field is well marked.

Figs. 3 and 4. Cells of worms which have been in soil for 6 hours, showing gradual accumulation of secretory granules at either or both poles. Some secretory granules are still present in the Golgi field.

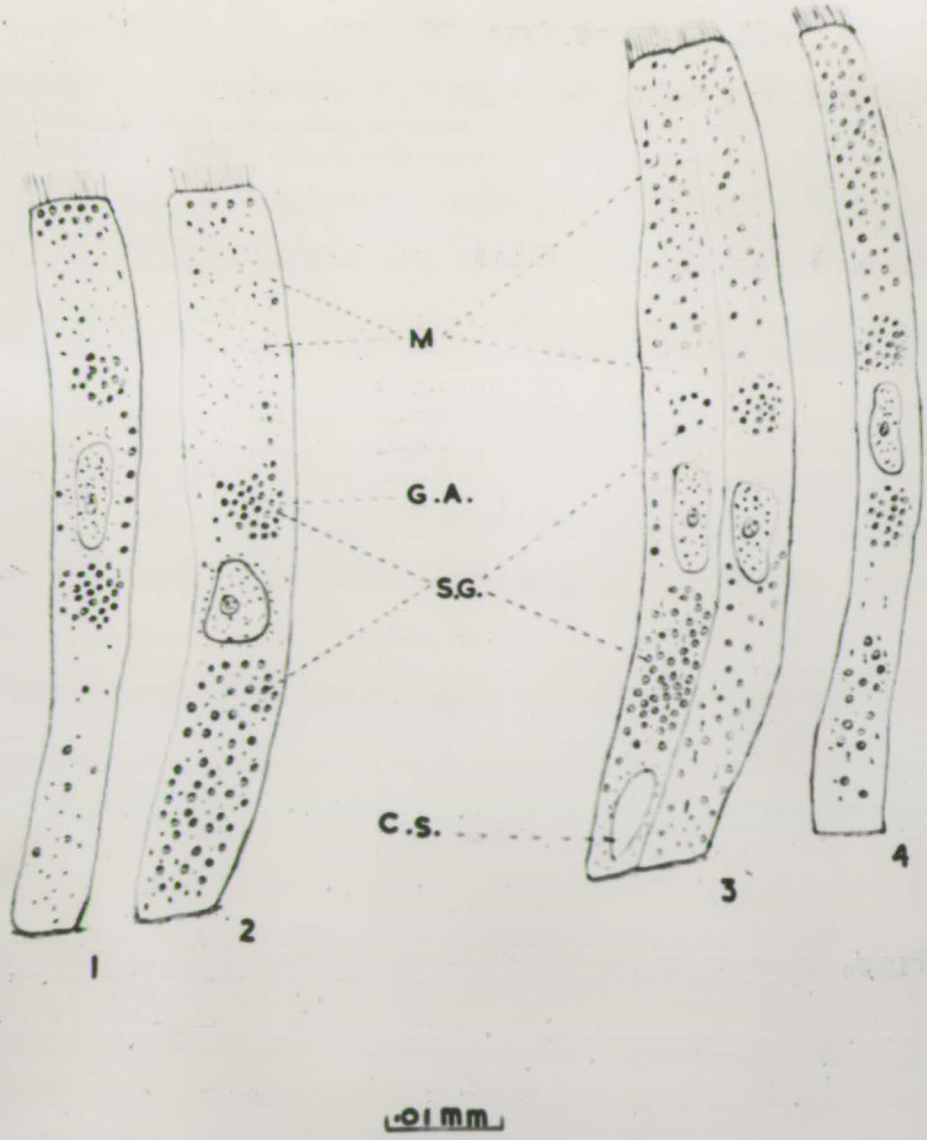
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PLATE XVII.

Drawings of the epithelial cells of the crop showing the Golgi material.

All figures from Kolatchev preparations.

Figs. 1 and 2. Cells of animals which were starved for 6 days showing reduced Golgi material, secretory granules chiefly at the lumen pole of the cell and in the Golgi field.

Figs. 3-5. Cells of worms which were in soil for 5 hours showing the hypertrophy of the Golgi material and the migration of the early secretory granules from the basal region to the Golgi field. A few mature secretory granules are seen in some of the cells in process of migration towards the lumen.

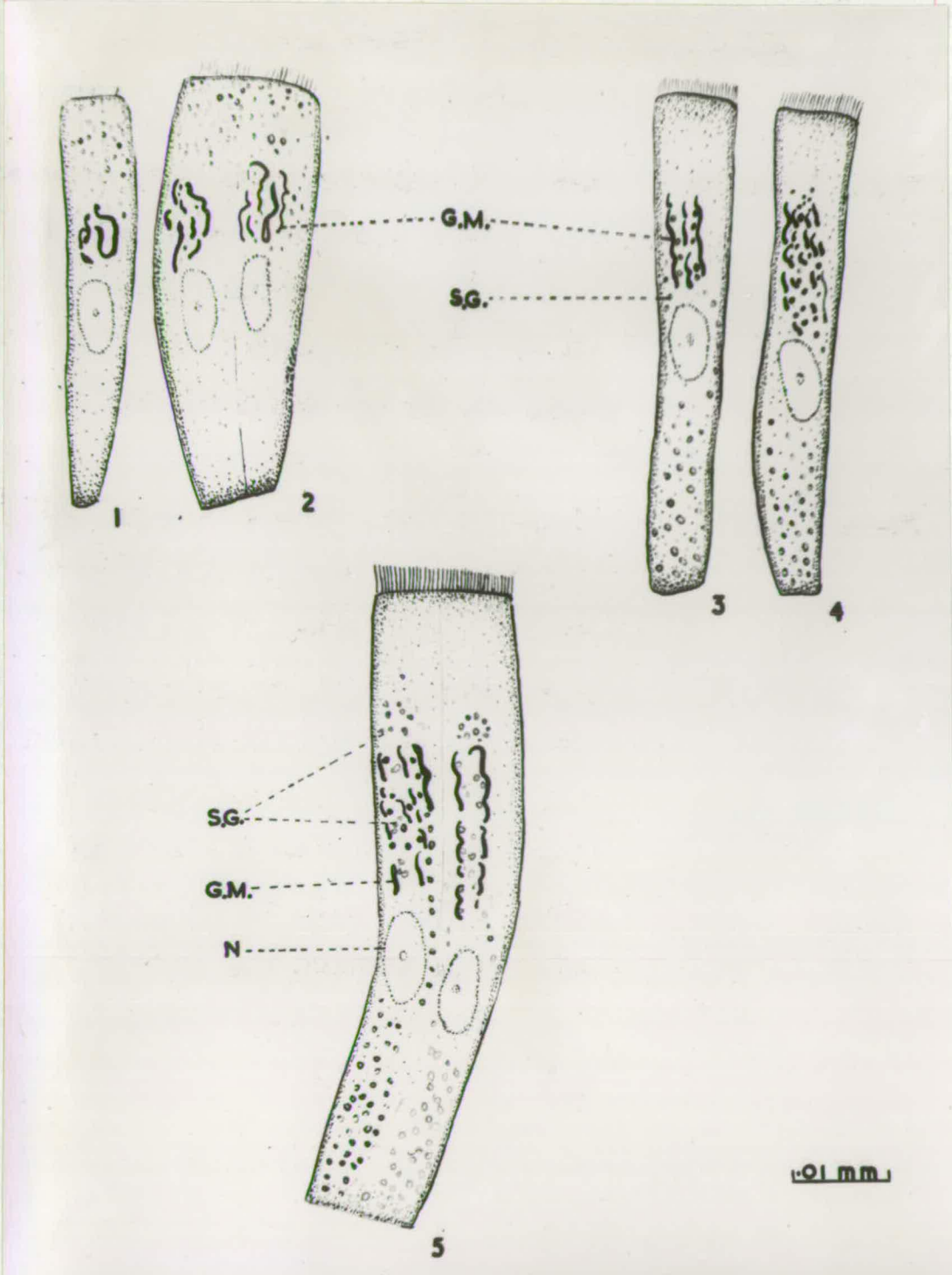
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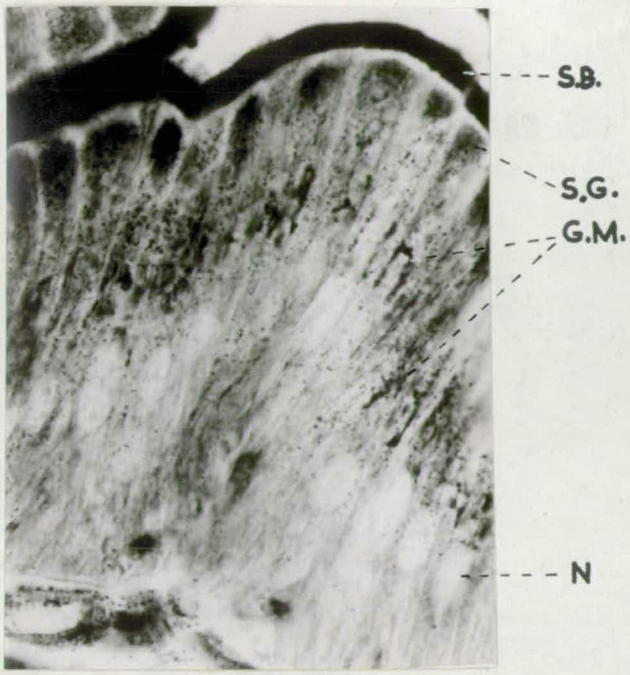
PLATE XVIII.

Photomicrographs of the epithelium of the crop. x 980

All figures from Kolatchev preparations.

Fig. 1. Showing Golgi material in cells of fasting worm. The lumen half of the cell contains more secretory granules than the basal region.

Fig. 2. Showing the Golgi material in the cells of worms which were in soil for 4 hours. The secretory granules from lumen region of cells have been discharged, and accumulation of fresh secretory granules in basal cytoplasm is seen. Migration of early secretory granules from the basal cytoplasm to the Golgi field is demonstrated in some cells where they are situated in close contact with the Golgi material.



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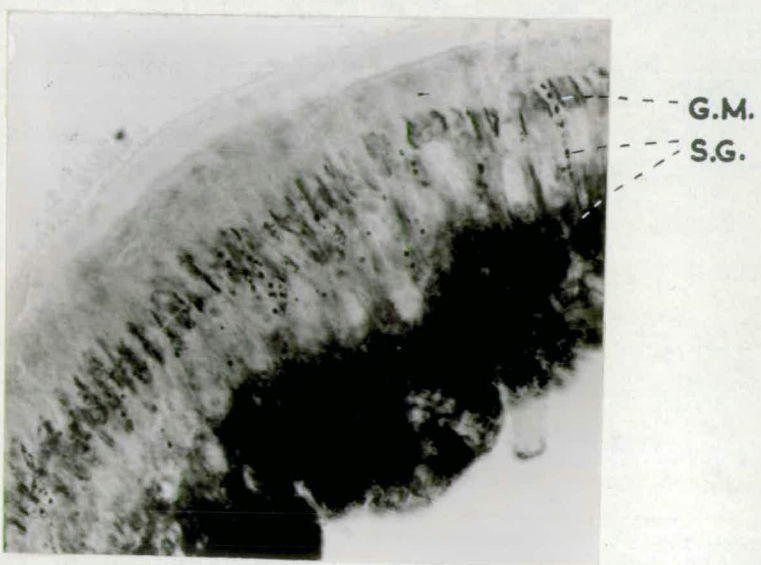

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IX. GIZZARD

A. HISTORICAL

The gizzard is a thick-walled muscular organ of the alimentary canal whose main function is the tituration of the food matter into a finer state of division.

The histology of the gizzard of Lumbricidae has been worked on by Dequal (1910), Ribaucourt (1991), Mayer (1913) and others. Dequal, working on Octolasium complanatum, describes the epithelial and cuticular coats of the gizzard as being similar to those of the crop, the cuticle, however, is thicker. Mayer describes the epithelium as being ciliated and consisting of intra-:cellular fibrillae which are mostly attached to the basement membrane. Ribaucourt states that the epithel-:ium forms villosities in the posterior part of the gizzard of Allolobophora chlorotica, and in the anterior part of that of Lumbricus terrestris. Both muscular layers of the alimentary wall are thickened, especially the circular layer which, according Ribaucourt, is composed of striated fibres. Beddard, in his mono-:graph (1895) denies that these fibres are striated.

There is no previous contribution on the cytology of the gizzard of earthworms.

B. METHODS.

The methods employed were the same as in the case of the crop. Great difficulties were, however, encountered/

encountered with the osmic methods; this was because the epithelium is covered by a thick layer of cuticle. The highly reducing power of the keratinoid material exhausts the osmium tetroxide very quickly and the Golgi material is left unblackened and the mitochondria are not well fixed. Small pieces of the material must be used and the fixative must be changed frequently in order to obtain uniform and complete fixation of the cytological components.

For the examination of the mitochondria and the secretory granules, Flemming's fluid (without acetic acid) diluted to half, as recommended by Gatenby, gave satisfactory results. For the Golgi material both osmic (Kolatchev) and silver (Aoyama and Da Fano) methods were found to give fairly good results.

The mitochondria and the secretory granules showed marked affinity for acid fuchsin, but stained less deeply with iron-haematoxylin.

C. OBSERVATIONS

In Lumbricus the gizzard immediately followed the crop and occupied segments xvii-xix. The epithelial lining of the gizzard consists of a single layer of columnar cells, arranged in protruding lamellae, which form simple elongated crypts which are filled with keratinoid secretory material. The epithelial cells near the apices of the lamellae are elongate and possess oval nuclei, whereas the cells between the apices/

apices are shorter and cuboidal with large spherical nuclei. The nuclear membrane is distinct, and chroma-:tin granules are always present in the nucleus. The nucleus is usually situated in the centre of the cell, but in a few cases lies in the basal or the lumen half of the cell. The outlines of the nuclei are mostly regular. Cell-membranes are distinct throughout. The epithelial cells contain intracellular fibrillae.

Cells situated near the apices of the lamellae show gradual degenerative changes resulting finally in their disintegration and death. The cells are ciliated and are covered by cuticle which is very thick in places (Pl. XXI, figs. 1 and 2). The cuticle is formed by the secretion of the epithelial cells lining the tubular crypts.

Examination of the cells showed that those situated at the bottom of the crypts or the lower part of the lamellae, and also where the cuticular covering is not very thick, are the only normal cells with respect to the behaviour of their cytological compo-:nents during the stages of the secretory cycle.

Fasting and feeding do not induce any marked morphological changes in the cell components of the epithelium of the gizzard. The formation of the secre-:tory granules is continuous under all physiological phases, although if worms are fed for about four to five hours following a fast of a few days duration, the secretory response appears to be more marked for a short/

short period than in those which have constant access to food.

Binucleate epithelial cells were sometimes observed.

In some cells secretory granules are found chiefly concentrated adjacent to the lumen and at one or both poles of the nucleus, or in its immediate neighbourhood. In others they are more numerous in the basal region and scanty in the lumen half of the cell (Pl. XIX, figs. 1 and 2). Secretory granules are also situated in the Golgi field in some of the cells. In some cells globule-like secretory material, much larger than the usual secretory granule, is present in the basal part of the cell chiefly in the neighbourhood of the basement membrane (Pl. XIX, fig. 1). These large globules appear to be present chiefly in cells which are active physiologically, and probably represent an early stage in the formation of the secretory material. The globules are later reduced in size.

The Mitochondria

The mitochondria in the epithelial cells of the gizzard consist of thin rods and a few granules. The majority are situated chiefly in the lumen half of the cell, where they are concentrated either adja-:cent to the lumen or near the Golgi field in close association with secretory granules. A few rods and granules/

granules are, however, are scattered throughout the cytoplasm. In some cases a few rods and granules occur in the immediate neighbourhood of the nucleus chiefly towards its longitudinal poles (Pl. XIX, fig. 1 and 2). As already stated, fasting and feeding do not induce any change in the general arrangement of the mitochondria.

Due to the very small size of the mitochon-:dria polar orientation is not clearly demonstrated

The Golgi Material

The Golgi material is well developed in the normal cells of the gizzard. It consists of small rods or filaments with a few granules arranged in a loose net-like structure which is situated at one pole of the nucleus. Secretory granules are present in the Golgi field (Pl. XX, figs. 1-3 and Pl. xxi, fig. 3). In some cells elongation and fragmentation of the Golgi elements had taken place, and consequently the Golgi material occupied a much greater area than in the other epithelial cells. Granules and small curved rods are now present. The rods and filaments lie almost parallel to the longitudinal axis of the cell (Pl. XX, figs. 1 and 2). It is concluded that these cells are physiologically active.

In cells which are physiologically senile, the Golgi material is very much reduced in amount and/

and in some cases had almost completely disappeared (Pl. XX, fig. 4 and Pl. XXI, fig. 3).

Like the mitochondria, the Golgi material does not show any morphological change as a result of feeding worms which have previously been starved. All the various stages of the secretory cycle are shown in the cells of animals which have been fasting or feeding.

PLATE XIX.

Drawings of the epithelial cells of the gizzard.

All figures from flemming preparations stained according to Bensley's method.

Fig. 1. Cells of the apices showing accumulation of secretory granules at the basal as well as the lumen poles. Mitochondria are distributed throughout the cell.

Fig. 2. Cells between the apices, showing very few secretory granules in the basal cytoplasm and the concentration of secretory granules in the neighbourhood of the nucleus, Golgi field and the lumen.

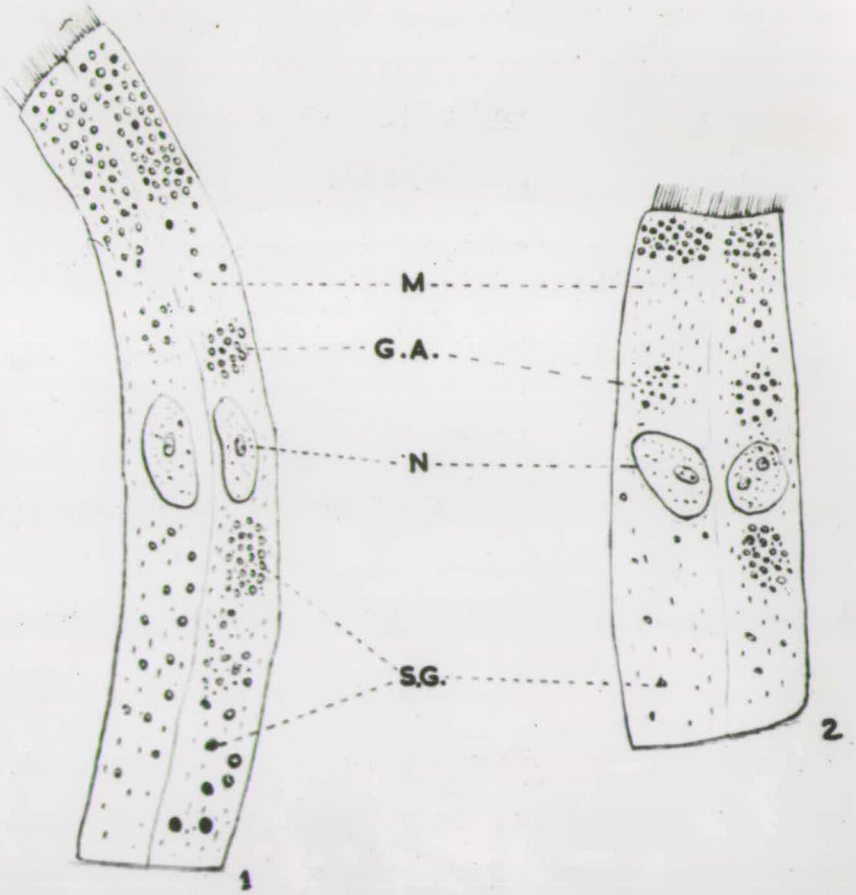
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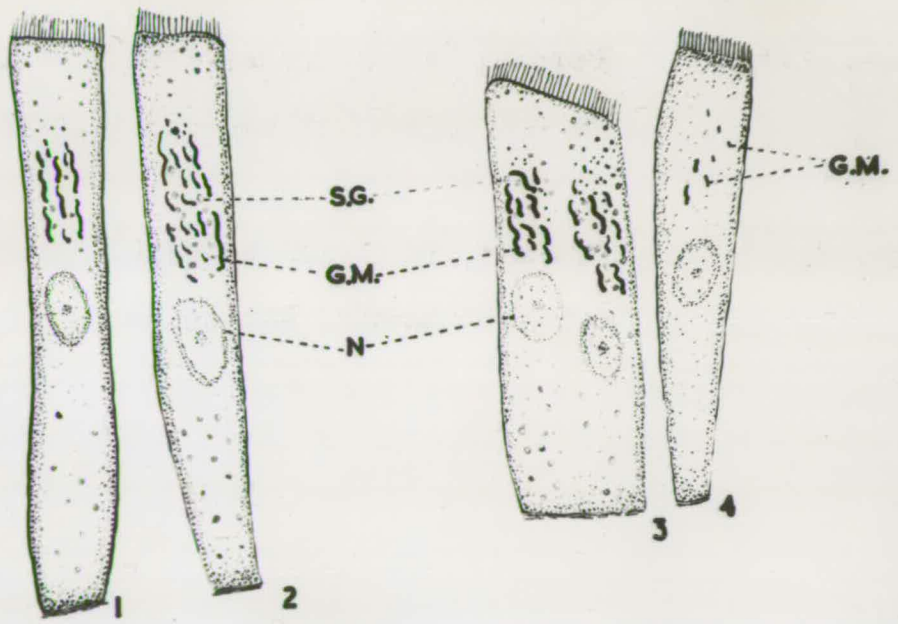
PLATE XX.

Drawings of the epithelial cells of the gizzard.

All figures from Kolatchev preparations.

Figs. 1-3. Showing the Golgi material in close association with secretory granules.

Fig. 4. Showing the Golgi material in a physiologically senile cell.



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Photomicrographs of the gizzard of Lumbricus.

Figs. 1 and 2 from Zenker's (picro-formal) preparation stained with haematoxylin and eosin.

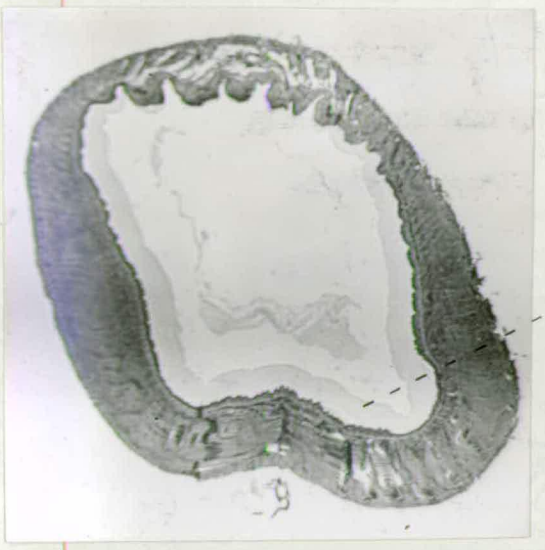
Fig. 3 from Kolatchev preparation.

Fig. 1. Transverse section of the gizzard, showing its general histological appearance. The varying thickness of the cuticular layer is well marked, and the ciliated free border of the epithelium is also visible. x 18

Fig. 2. A part of the epithelium from fig. 1, showing the presence of cilia at the border of the cells below the cuticle. x 1060

Fig. 3. Showing the Golgi material in the epithelial cells of fasting animal. x 980

PLATE XXI.



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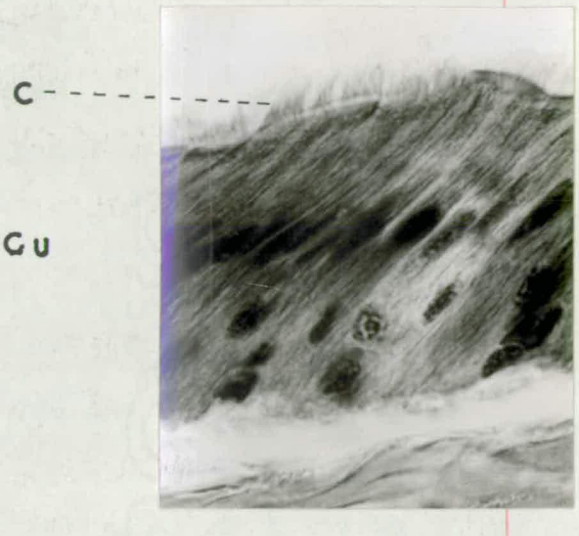

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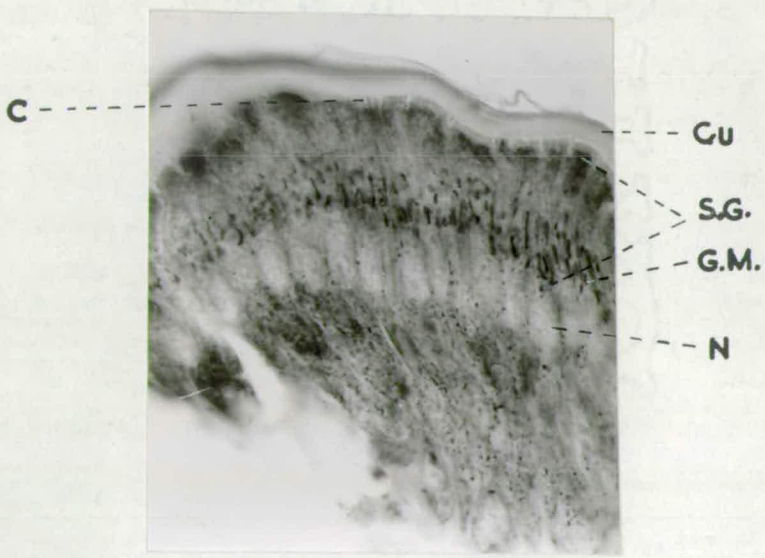

3

X. INTESTINAL EPITHELIUM.

A. HISTORICAL

The gizzard of Lumbricus is followed by the intestine which is the longest and widest portion of the alimentary canal. Like the oesophagus, it bulges segmentally and is constricted intersegmentally at the insertion of the septa.

Histological and cytological studies have been carried out on the intestinal epithelium of various animals, but as far as the writer is aware, there is no published work on the cytology of the intestinal epithelium of Lumbricus.

The histology of the epithelial lining of the intestine of the Lumbricidae has been described by various authors, notably by Willem and Minne (1899), Greenwood (1892), Schneider (1908) and more recently by Millott (1948). According to them, it consists of ciliated and glandular (or non-ciliated) cells.

According to Willem and Minne, the glandular cells are club-shaped and contain round particles of secretion. These cells are most numerous on the typh-:losole, and fewest in the ventral region. Greenwood and Millott found that they are very variable in size, form, relative prominence and in the number of inclu-:sions. Millott describes the investment of the gland cells by a sheath of four to five ciliated cells.

The ciliated cells are yellow, slender, at

times/

times compressed between the glandular cells, and hence of somewhat variable shape. According to Willem and Minne, the yellow colour is due to a number of extremely small granules which disappear in alcohol. The basal corpuscles of the cilia are visible. Gurwitsch (1901) states that very distinct rodlets are present in these ciliated cells, and that their cha-:racter changes periodically. As the secretion of the glandular cells passes into the lumen, the cilia dis-:appear by degrees and the rodlets become smaller; finally both cilia and rodlets vanish. Joseph (1902) believes that the rodlets are in reality the basal portion of the cilia. According to him some cells have rodlets only but lack cilia and basal corpuscles; these cells are transformed into ciliated cells, and during this process a basal corpuscle associates itself with one of the rodlets and a cilium grows out through the rodlet, which thus forms a useful support for the base of the cilium. Joseph, who did not identify the worms he investigated, found rodlet epithelium on the dorsal wall of the gut, including the typhlosole, and ciliated epithelium elsewhere. According to Greenwood (1892), the extent of the ciliation varies at differ-:ent times and in different phases of activity. The ciliated cells contain intracellular fibrillae.

Millott studied in living material the method by which the secretion of the intestinal gland cells (non-ciliated cells) of L. terrestris is extruded to the/

the lumen of the intestine. He observed a precise se-
:quence of events, and believes that extrusion
involves two series of events. He considers that first
of all the ciliated cells play an important part in
the process by pressing against the gland cells, thus
constricting them and forcing out some of their
contents. In this way they tend to limit the rate of
discharge. When a particular stage in the life of the
non-ciliated cells is reached, a portion of their
secretory contents is discharged through channels
which are formed between the ciliated cells. The
ciliated cells assist in the formation of these cha-
:nnels by invaginating and thus exerting a force which
tends to pull apart their free ends. According to
Millott the extrusion from the intestinal gland cells
may involve co-ordinated activity in both gland cells
and ciliated cells. He describes the structure of the
ciliated cells and states that they contain intracellu-
:lar fibres and pore-rings, the latter were previously
described by Gurwitsch (1901).

Opinion is divided regarding the presence of
'replacing cells' in the gut epithelium.

B. METHODS

The procedure adopted for the study of the
mitochondria and of the general histology of the intes-
:tinal epithelium was almost identical with that used
for the other parts of the alimentary canal. In order
to/

to determine the absorptive regions of the intestine, certain worms were fed with iron saccharate. Later, sections of fixed material were treated with 10% aqueous solution of potassium ferrocyanide for about ten minutes and then for a few minutes with distilled water, containing a trace of HCl. In this way the presence of iron was demonstrated by the prussian blue reaction.

For the examination of the Golgi material, it was found that only silver methods (Aoyama and Da Fano) gave good results. Osmic methods (Kolatchev in its original formula and its modification by Ludford and by Mann-Kopsch) were tried, but failed to impregnate the Golgi material at all even after prolonged treatment. It appears, therefore, that the fat globules of the intestinal epithelial cells reduce the osmium tetroxide very quickly. Some of the silver preparations were stained with Ehrlich's haematoxylin.

It should be noted that sodium sulphite, as recommended by Aoyama, stains the cytoplasm too deeply and thus render the study of the Golgi material and its association with the secretory granules very difficult; consequently the amount of sodium sulphite was reduced to about one half, or less, the original quantity recommended, and this gives a light golden colouration to the cytoplasm.

Samples were taken simultaneously from different regions of the intestine of worms under different physiological conditions. Three regions- the anterior, middle/

middle and posterior part of the intestine were selected in order to study the cell components and their behaviour during secretion and absorption.

C. OBSERVATIONS

In view of the considerable amount of work done on the histology of the intestine of Lumbricus and of other worms, the writer has little to add to previous accounts.

The cells of the lining epithelium of the intestine of Lumbricus are ciliated and non-ciliated and are arranged in a single row. In some cases the non-ciliated cells may be surrounded by three to four, or more, ciliated cells. The non-ciliated cells are full of secretory granules of various sizes, and as already stated are club-shaped (Pl. XXII, figs. 2-4). The nuclei occupy different positions at different times and are oval to spherical in shape and regular or irregular in outline. Usually one to two nucleoli and chromatin granules are present (Pl. XXII, figs. 2-4 and 9). The cell and nuclear membranes are usually distinct.

The ciliated cells are tall and slender, and very much compressed laterally by the non-ciliated cells.

The typhlosole is well developed in Lumbricus and extends throughout the length of the intestine except in the last fifteen to twenty segments where it is absent. In the mid-region of the intestine it is very/

very much enlarged and broad, so that the lumen is much reduced.

1. Worms with Constant Access to Food.

All stages of the secretory cycle were observed in both ciliated and non-ciliated cells.

2. Conditions Induced by Fasting.

(a) Anterior Intestine

The non-ciliated cells, during the period of fasting show considerable accumulation of secretory granules and are club-shaped in appearance when the secretory granules are situated chiefly in the lumen half of the cell, or they are much broader from side to side when the whole cell is full of granules; consequently the nuclei occupy different positions in the different parts of the epithelium (Pl. XXII, figs. 1 and 2).

The ciliated cells are very much compressed by the non-ciliated cells especially in the region of the typhlosole. The nuclei usually lie in the centre of the cell, although in some cases they are situated either in the basal or in the lumen part of the cell (Pl. XXII, fig. 1). The secretory granules are sca-:ttered through the cytoplasm and the cells appear club-shaped.

(b) Mid-Intestine

The non-ciliated cells of the typhlosole

as/

as well as of the ventral epithelium show great accu-:mulation of secretory granules throughout the entire cell (Pl.XXIII, fig. 3). Due, in all probablity, to the large amount of accumulated material, some of the cells have discharged their secretion into the lumen (Pl. XXIX, fig. 3).

In certain cells secretory granules, preparatory to their discharge, are present just inside the free border. The cells from which secretory material has been evacuated contain granules and large globules in the basal region although a few are also present in the lumen half chiefly in the neighbourhood of the nucleus. The nucleus does not occupy the same position in all the cells of the mid-intestine; it usually lies in the central region or between the centre of the cell and the lumen.

There is little change in the ciliated cells as compared with those of the anterior region of the intestine. Some of the cells are, however, more uni-:form in appearance. Secretory granules are seen in the Golgi field and in other regions of the cell.

(c) Posterior Intestine

The non-ciliated and the ciliated cells of the posterior region of the intestine are more uniform in appearance than are those of the anterior and the middle regions of the intestine (Pl. XXIV, figs. 1 and 2). Secretory granules are present, but they are not/

not so numerous as in other regions. The number of non-ciliated cells is not great in this part of the intestine. Each cell usually lies between four to ten ciliated cells. Secretory granules in the non-ciliated cells are mostly accumulated in the lumen half of the cell, whereas in the ciliated cells they are mostly in the basal region.

3. The Secretory Cycle

(a) Anterior Intestine

Two to three hours from the intake of food, the secretory granules which accumulated during the period of fasting have already been discharged into the lumen from most of the ciliated cells. The gra-:nules from the basal region have moved to the lumen side of the cell, chiefly towards the Golgi field or in the immediate neighbourhood of the nucleus. There are, however, some cells which are still completely full of secretory granules, but in the majority of cases one half of the cell is practically free of secretory material (Pl. XXII, figs 5-8). New secre-:tory granules have appeared in a few cells. With the further increase in secretory activity, secretory granules begin to accumulate in the lumen half of the cell (Pl. XXII, fig. 7).

The non-ciliated cells, however, show very little change and still contain secretory granules, chiefly in the lumen part of the cell, although there is a decrease in the amount of secretory granules (Pl. XXII, fig.4).

Six hours from the intake of food, the majo-:rity of the ciliated cells show a general evacuation of the secretory material from their lumen half. There are a few cells, however, in which the secretory gra-:nules are accumulated chiefly in this region. In cells from which secretory material has been evacuated, clumps of secretory granules appear in the basal region (Pl. XXII, fig. 12). Some of these clumps are close to the nucleus or near the basement membrane (Pl. XXII, figs. 11 and 12). These granules in the basal part are, therefore, fresh (or secondary) sec-:retory material. The cells are uniform in appearance and the nucleus usually lies in the centre.

In the cells of the ventral epithelium, the secretory response seems to be rather slow. The lumen region of the cell still contains an accumulation of secretory granules, although in some cells, the gra-:nules have been evacuated from the lumen region (Pl. XXII, fig. 10).

The non-ciliated cells show a greater accu-:mulation of secretory granules than in the preceding phase; the granules are situated mostly in the lumen half of the cell (Pl. XXII, fig. 9).

Eighteen to twenty-four hours from the commencement of feeding, the cells in all stages of secretory cycles are present.

(b) Mid-Intestine

Two to three hours from the intake of food, the non-ciliated and ciliated cells of the middle region of the intestine show a general evacuation of the accumulated secretory granules similar to that of the anterior part of the intestine. In some cells almost all the secretory material has been evacuated, while in a few cells practically no discharge of secretory material has taken place (Pl. XXIII, figs. 4-6).

Six hours from the intake of food, the ciliated and non-ciliated cells show an accumulation of secretory granules chiefly in the lumen side. Accumulation of secretory granules in the immediate vicinity of the nucleus has taken place in some cells. The nuclei occupy various positions, but the majority are in the lumen half of the cell (Pl. XXIII, figs. 7-9).

Eighteen to twenty-four hours from the intake of food, cells in all stages of secretory cycles are present. Some of the ciliated cells contain absorbed food material. Those granules of absorbed food material are small in the lumen half of the cell, but become bigger as they move towards the basement membrane (Pl. XXV, fig. 1).

(c) Posterior Intestine

The secretory response in the posterior part of/

of the intestinal epithelium is very slow. Two hours from the commencement of feeding many of the ciliated cells are full of secretory granules; some cells, however, show a partial evacuation of the accumulated secretory material. The basal half of most of the cells is full of secretory material. In some cases secretory granules are situated in the Golgi field. The nuclei lie at different levels, but chiefly in the centre of the cell or a little closer to the lumen (Pl. XXIV, figs. 3&5).

The non-ciliated cells contain very few secretory granules in the lumen half but they are numerous in the basal half of the cell (Pl. XXIV, fig.4).

Six hours from the intake of food, secretory granules are present either in the basal or in the lumen region of the ciliated cells. In the lumen regions of the cells of the typhlosole, some granules are much smaller than others. These small granules are situated chiefly in the neighbourhood of the lumen. The nuclei are oval and elongated (Pl. XXV, fig. 3).

There is no appreciable change in the non-ciliated cells from those seen in the preceding case.

Eighteen to twenty-four hours from the intake of food, cells in all stages of the secretory cycle are present.

Absorptive granules similar to those observed in the mid-intestine are present. They are more numerous than in the cells of the mid-intestine (Pl. XXV, fig. 4 and Pl. XXXI, fig. 2).

4. The Mitochondria

i. Worms with Constant Access to Food:- The cells of animals with constant access to food are in all stages of secretory activity. Consequently, the mitochondria differ in form and arrangement in the different cells. The stages of the secretory cycle are described in the following sections.

ii. Conditions Induced by Fasting

(a) Anterior Intestine

In the ciliated cells, the mitochondria consist of rods and a few granules. The rods are usually most numerous in the region immediately adja-:cent to the lumen, although in some cells they are concentrated at both poles. In both cases, small rods are scattered without any definite arrangement in the in the remainder of the cytoplasm. The granules, which are very few, are found chiefly in the neighbourhood of the lumen (Pl. XXII, fig. 1).

In the non-ciliated cells, which are full of secretory granules, the mitochondria occur chiefly in the form of small rods and granules. The granules are scattered throughout the entire cell. The rods are more numerous in the basal region but a few rods occur in the lumen region of the cell. A few small rods are, however, present in the near neighbourhood of the nucleus and the accumulated secretory material. In cells which contain very few secretory granules, rods

are/

are found throughout the cytoplasm (Pl. XXII, figs. 1 and 2).

The majority of the mitochondria are arranged parallel to the longitudinal axis of the cell.

(b) Mid-Intestine

In the ciliated cells of the middle region of the intestine, the mitochondria are in the form of rods and granules situated chiefly near the basement membrane and adjacent to lumen. In the cells of the typhlosole, granular mitochondria with a few rods are situated in the lumen region, but rods only are present in the basal region of the cell (Pl. XXIII, figs. 1 and 2).

In the non-ciliated cells, the mitochondria are usually in the form of very small rods and granules which are situated mainly at the extreme poles of the cell. A few small rods are, however, present in the rest of the cytoplasm (Pl. XXIII, fig. 3).

The mitochondria are not arranged parallel to the longitudinal axis of the cell.

(c) Posterior Intestine

The mitochondria of the ciliated cells consist of rods only. The rods are of different sizes and are more numerous in the lumen region than in the basal part of the cell. The mitochondria of the basal region are arranged parallel to the longitudinal axis of the cell, but in the lumen region they do not show polar orientation (Pl. XXIV, figs. 1 and 2).

In the non-ciliated cells, the mitochondria are in the form of small rods and granules which occur chiefly at the extreme poles of the cell (Pl XXIV, fig. 6). In a few cells they are scattered throughout the entire lumen region of the cell.

iii. The Secretory Cycle

(a) Anterior Intestine

Two to three hours from the intake of food, the mitochondria of the ciliated cells are in the form of very small rods and granules. The rods, which are very small, are in the majority of cells arranged uni-:formly throughout the cytoplasm. In some cells, however, the rods are most numerous at the extreme poles. The granules are located in the lumen half of the cell chiefly towards the outer pole (Pl. XXII, figs. 5-8).

In the non-ciliated cells the mitochondria are mostly glandular and are scattered throughout the entire cell. A few very small rods are situated close to the basement membrane and also scattered throughout the cytoplasm (Pl. XXII, figs. 3 and 4).

Six hours from the commencement of feeding, the mitochondria of the ciliated cells are still in the form of rods and granules. The rods appear to be slightly longer than in the previous case. Granules occur all through the cytoplasm and are in association with/

with the secretory granules (Pl. XXII, figs. 11 and 12).

In the non-ciliated cells, the mitochondria are, as in the preceding stage, chiefly in the form of granules; a few very small rods are also present. Due to the presence of secretory granules, which comple-:tely fill the major part of the cell, the mitochondria are located at the extreme poles of the cell (Pl. XXII, fig. 9).

Twenty-four hours from the intake of food, cells in all stages of cellular activity are present.

(b) Mid-Intestine

Two to three hours from the intake of food, the mitochondria of the ciliated cells are in the form of rods scattered uniformly through the cytoplasm. Some of the rods are longer than the majority. In a few cells the mitochondria are situated in the imme-:diate neighbourhood of the nucleus. In cells in which the lumen region is full of secretory granules, the mitochondria in this part of the cell are in the form of small rods. All the mitochondria are arranged parallel to the longitudinal axis of the cell (Pl. XXIII, figs. 4 and 5).

In the non-ciliated cells the form and arrangement of the mitochondria closely resemble the conditions observed during the resting phase. They consist of small rods and granules located chiefly towards/

towards the opposite poles of the cell. In addition, a few rods and granules are scattered through the remainder of the cytoplasm (Pl. XXIII, fig. 6).

Six hours from the commencement of feeding, the mitochondria of the ciliated cells are chiefly in the form of small rods and granules; the longer rods previously present in the vicinity of the lumen appear to have undergone fragmentation. A few larger rods are sometimes seen mainly in the basal region. In the majority of the cells the basal region contains rod-shaped forms, whereas, in the lumen region, both rods and granules are present. In cells which possess very few secretory granules in the lumen half, rods and granules are aggregated in the neighbourhood of the lumen, but a few rods and granules are distributed through the adjacent cytoplasm. A few rods and granules occur around the Golgi field of some cells. In cells in which the lumen half is full of secretory granules the mitochondria, usually short rods and granules, are more or less uniformly distributed in that region of the cell (Pl. XXIII, figs. 7 and 8).

In the non-ciliated cells there is no appre-:ciable change in the form and arrangement of the mitochondria as compared with the preceding stage (Pl. XXIII, fig. 9).

Eighteen to twenty-four hours from the intake of food, the cells are in all stages of cellular activities./

activities.

(c) Posterior Intestine

Two to three hours from the intake of food, the mitochondria of the ciliated cells are in the form of rods and a few granules. As in the fasting stage, the rods are of different sizes. Their distri-:bution shows little change from the fasting stage, but in some cells they are arranged uniformly through the whole cell. Granular mitochondria, which were not present in the fasting stage, are present chiefly in the lumen region (Pl. XXIV, fig. 3).

In the non-ciliated cells, the mitochondria are in the form of thin short rods and granules situa-:ted mainly as in the fasting cell, except that they are less numerous towards the basal pole, and a few rods and granules are scattered throughout the cell (Pl. XXIV, fig. 4).

Six hours from the commencement of feeding, the mitochondria of the ciliated cells are in the form of small rods, arranged uniformly through the cell, whereas in the non-ciliated cells they show little change from the preceding stage (Pl. XXV, figs. 2 and 3).

Twenty-four hours from the intake of food, cells in all stages of secretory cycles are present.

5. The Golgi Material

The Golgi material of the intestinal epithelial cells consists of filaments, rods and granules. It usually occupies an area between the nucleus and the lumen, but leaves the space immediately above the nucleus free.

i. Worms with Constant Access to Food:- When an animal has constant access to food, some of the cells are active and others are at rest; consequently the Golgi material presents variations in the form and size which are described in the following sections. These variations are more marked in the cells of the typhlosole than in those of the ventral epithelium. Consequently, the following account is based mainly on observations carried out on the cells of the typhlosole. When one set of epithelial cells is active the cells of the neighbouring areas are in the resting stage.

ii. Conditions Induced by Fasting

(a) Anterior Intestine

The Golgi material of the non-ciliated cells consists of thick rods, filaments and granules. The rods may be short or long. In cells in which secretion is accumulating, some of the Golgi elements are elongate and lie close to the periphery of the secretory material,/

material, while the remainder are situated directly above the nucleus. Some of the granules and short rods are in close contact with the secretory granules (Pl. XXVI, figs. 1 and 2, and Pl. XXVII, fig. 1). When cellular activity is at its maximum, the Golgi elements become very much elongated, but later break up into short rods and granules which are distributed through the secretory material; a few rods and granules are also situated at the periphery of the secretory mass (Pl. XXVI, fig. 2). When the cells become full of secretion, the Golgi material assumes its normal shape and becomes very much reduced in amount so that it occupies a much smaller area than before (Pl. XXVI, fig. 1). In this material the majority of the non-:ciliated cells contain secretory granules which accum-:ulate throughout the period of fasting.

In the ciliated cells, the Golgi material consists of rods and filaments which run almost parallel to the longitudinal axis of the cell. Some of the Golgi rods are curved while others are straight and elongate (Pl. XXVI, fig. 3, and Pl. XXVII, fig. 1). These cells are very much compressed laterally by the non-ciliated cells, especially in the region of the typhlosole; consequently, it is difficult to study the assosciation of the secretory granules with the Golgi elements.

It should be noted that in both ciliated and non-ciliated/

non-ciliated cells of worms which have not been fed, the Golgi material in this part of the intestine is usually not reticular or basket-like in structure.

(b) Mid-Intestine

The non-ciliated cells in this region of the intestine are not so numerous as in the anterior part. The Golgi material does not show any appreciable morphological differences from that of cells in the anterior region (Pl. XXVIII, figs. 3 and 4).

In the ciliated epithelial cells, the Golgi material is in the form of rods and filaments which are either arranged as a loose or a compact reticulum, or are in the form of elongated elements which run parallel to the longitudinal axis of the cell. Some of the rods are, however, curved. The Golgi material does not extend over a large area and the rods and filaments lie close together (Pl. XXVIII, figs. 1 and 2, and Pl. XXIX, fig. 1).

(c) Posterior Intestine

The Golgi material of the non-ciliated cells resembles in its morphology that of the cells of the anterior region. Some of the small rods and granules are in close association with the secretory granules, while others are situated at the periphery of the sec-
:retory material. As in the other regions of the intestine/

intestine when the cell is full of secretory granules, the Golgi material of some cells is a compact structure.

The Golgi material of the ciliated cells consists of short and long rods which are either straight or curved. In some cells, the Golgi material is reticular in form, while in others the elements are elongate and run parallel to the longitudinal axis of the cell (Pl. XXXI, fig. 1). The ciliated and non-ciliated cells of the ventral epithelium impregnate more deeply than those of the typhlosole.

iii. The Secretory Cycle:- During increased cellular activity, brought about by feeding worms after a period of fasting, the Golgi material shows marked morphological changes. At the same time it becomes easier to impregnate than that of animals which have not been fed.

(a) Anterior Intestine

The Golgi material of the non-ciliated cells, after a period of two hours from the intake of food, consists of rods and granules with a few filaments. As the accumulated secretion is discharged into the lumen, the Golgi material comes to occupy a much smaller area than formerly (Pl. XXVI, figs. 7 and 10). In some cells, however, the appearance of the Golgi material, and the area occupied by it, is almost the same/

same as during the fasting stage. In some cells, the rods are broken up into granules which lie in contact with the secretory material. In a few cells, the Golgi material did not impregnate well, and in these cells it appears to be in the form of small vesicles. It may be that during this stage of cellular activity, it is much broken up into spherules.

There is no considerable change in the Golgi material of the ciliated epithelial cells during this early stages of activity, except that there is a slight elongation of the Golgi rods. In cells in which the Golgi material previously appeared as a reticulum, the whole structure becomes slightly loosened. In some cells the Golgi rods have become broken up to form smaller rods. New secretory granules have made their appearance into the cytoplasm (Pl. XXVI, figs. 4-6).

Six hours from the commencement of feeding, there is a marked increase in the amount of Golgi material present both in ciliated and non-ciliated cells, but particularly in the latter, and as fragmentation of the rods and threads has taken place they now occupy a much greater area than previously (Pl. XXVI, figs. 8, 9 & 11-14 and Pl. XXVII, fig. 2). More secretory granules have appeared in the cytoplasm chiefly in the basal region and in the Golgi field (Pl. XXVI, fig. 12 and Pl. XXVII, fig. 2). In the non-ciliated cells, the longer filaments and Golgi rods have broken up into shorter rods and granules which/

which are scattered through a greater area of the cell, and in some cases almost extends over the entire area between the nucleus and the lumen. The gradual fragmentation of the Golgi material can be easily observed during this stage (Pl. XXVI, figs. 8, 9 and 11). In some cells, however, the Golgi material is in a compact form, which indicates that secretory activity has ceased and that the cell is now in the resting phase (Pl. XXVI, fig. 10).

In the ciliated cells the Golgi material consists of thick and elongated rods which are situated parallel to the longitudinal axis of the cell. More secretory granules have appeared in the cytoplasm, chiefly in close association with the Golgi rods and granules (Pl. XXVI, figs. 12-14). In some cells, however, the Golgi material is still in a compact form.

Eighteen to twenty-four hours from the intake of food, ciliated and non-ciliated cells in all stages of activity are present.

(b) Mid-Intestine

Two hours from the intake of food, the Golgi material in some of the ciliated and non-ciliated cells is very lightly impregnated, and secretory granules, in considerable number , have appeared in the basal cytoplasm as well as in the Golgi field. In some of the/

the ciliated cells a close association of the Golgi rods and the secretory granules is evident (Pl. XXVIII, figs. 5 and 6). In some of these cells the Golgi material is in the form of elongate rods running parallel to the longitudinal axis of the cell (Pl. XXVIII, fig. 5). There is, however, no appreciable change in the Golgi material of the non-ciliated cells during this early stage of activity.

Six hours from the commencement of feeding, there is considerable increase in the thickness and in the size of the Golgi rods and threads in the ciliated cells (Pl. XXVIII, fig. 7).

In the non-ciliated cells, the Golgi rods are elongate and surround the secretory material. In some of the cells further fragmentation of the Golgi rods has taken place, so that the number of rods is reduced and granules are numerous (Pl. XXVIII, fig. 8 and Pl. XIX, fig. 2).

The Golgi material of the ciliated cells consists of long and short rods which are thicker than some of those present during the previous stages. Secretory granules occur in the Golgi field in associ-:ation with the Golgi rods (Pl. XXVIII, fig. 7 and Pl. XXIX, fig. 2).

Eighteen to twenty-four hours from the intake of food, cells in all stages of secretory activity are seen. Granules of food material which have been absorbed from the lumen are present in the ciliated cells./

cells. These granules are small in the lumen region but are larger in the basal region of the cell (Pl. XXVIII, fig. 9).

(c) Posterior Intestine

Two hours from the intake of food, the Golgi material of the epithelial cells show marked secretory response and impregnation of the Golgi material takes place more quickly than during the fasting stage. In the majority of the ciliated cells, as in some of the cells in material taken from starved worms, the localized Golgi material has spread out from its original posi-:tion and appears to have increased in amount, as well as in the thickness of the individual rods. New secre-:tory granules are present in the basal part of the cell, while in some cases, a few granules occur in the Golgi field (Pl. XXX, figs. 1-3). The Golgi material of the ventral epithelial cells has much thicker and shorter rods than the cells of the typhlosole (Pl. XXX, fig. 4).

There is no appreciable difference in the Golgi material of the non-ciliated cells as compared with that of the mid-intestinal epithelial cells.

In some of the epithelial cells of the typh-:losole, the impregnation of the Golgi material is very poor.

Six hours from the intake of food, the Golgi material of the ciliated as well as the non-ciliated cells/

cells is in the form of thick rods. More secretory granules have appeared in the ciliated cells, and in some cases they are clearly seen inside the Golgi field (Pl. XXX, figs. 5 and 6). In a few ciliated cells, the Golgi elements are elongate and occupy a larger field than in other cells (Pl. XXX, figs. 6 and 7) In some cells, however, the Golgi material is very much reduced in amount and compact in appear-: ance; this is probably due to the onset of the rest-:ing phase (Pl. XXX, fig. 8).

Eighteen to twenty-four hours from the intake of food, the epithelial cells show all stages of cellu-:lar activity. Some of the ciliated cells contain granules of absorbed material which are smaller in the lumen region than in the basal part of the cell (Pl. XXXI, fig. 2).

Drawings of the epithelial cells of the anterior region of the intestine.

All preparations from Flemming preparations stained according to Bensley's method.

Figs. 1 and 2. Ciliated and non-ciliated cells during the fasting period, showing accumulation of the secretory granules and the mitochondria.

Figs. 3 and 4. Non-ciliated cells of worms 2 hours after the intake of food, showing mitochondria and secretory granules.

Figs. 5-8. Ciliated cells of worms 2 hours after the intake of food, showing gradual evacuation of the secretory material and the mitochondria.

Figs. 9-12. Non-ciliated and ciliated cells of worms 6 hours after the intake of food, showing the gradual formation and accumulation of secretory granules. The mitochondria are uniformly distributed.

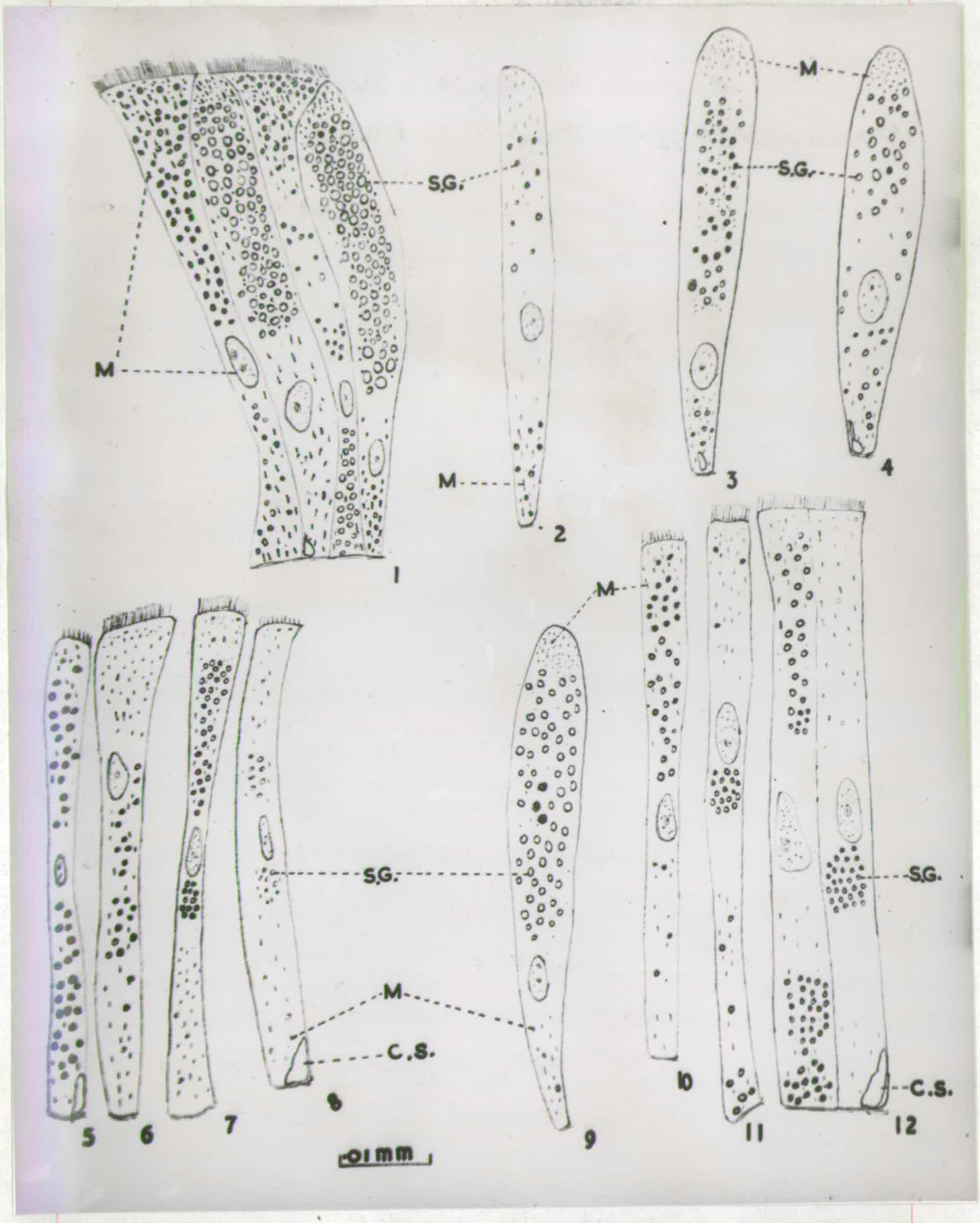
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PLATE XXIII.

Drawings of the epithelial cells of the middle region of the intestine.

All figures from Flemming preparations stained according to Bensley's method.

Figs. 1-3. Ciliated and non-ciliated cells during the period of fasting, showing accumulation of secretory granules and the arrangement of the mitochondria chiefly at opposite pole of the cell.

Figs. 4-6. Ciliated and non-ciliated cells of worms 2 hours after the intake of food, showing gradual evacuation of secretory granules and the uniform arrangement of the mitochondria.

Figs. 7-9 Ciliated and non-ciliated cells of worms 6 hours after the intake of food, showing gradual accumulation of secretory granules and the mitochondria. In the non-ciliated cell, the mitochondria are in the form of short rods and granules.

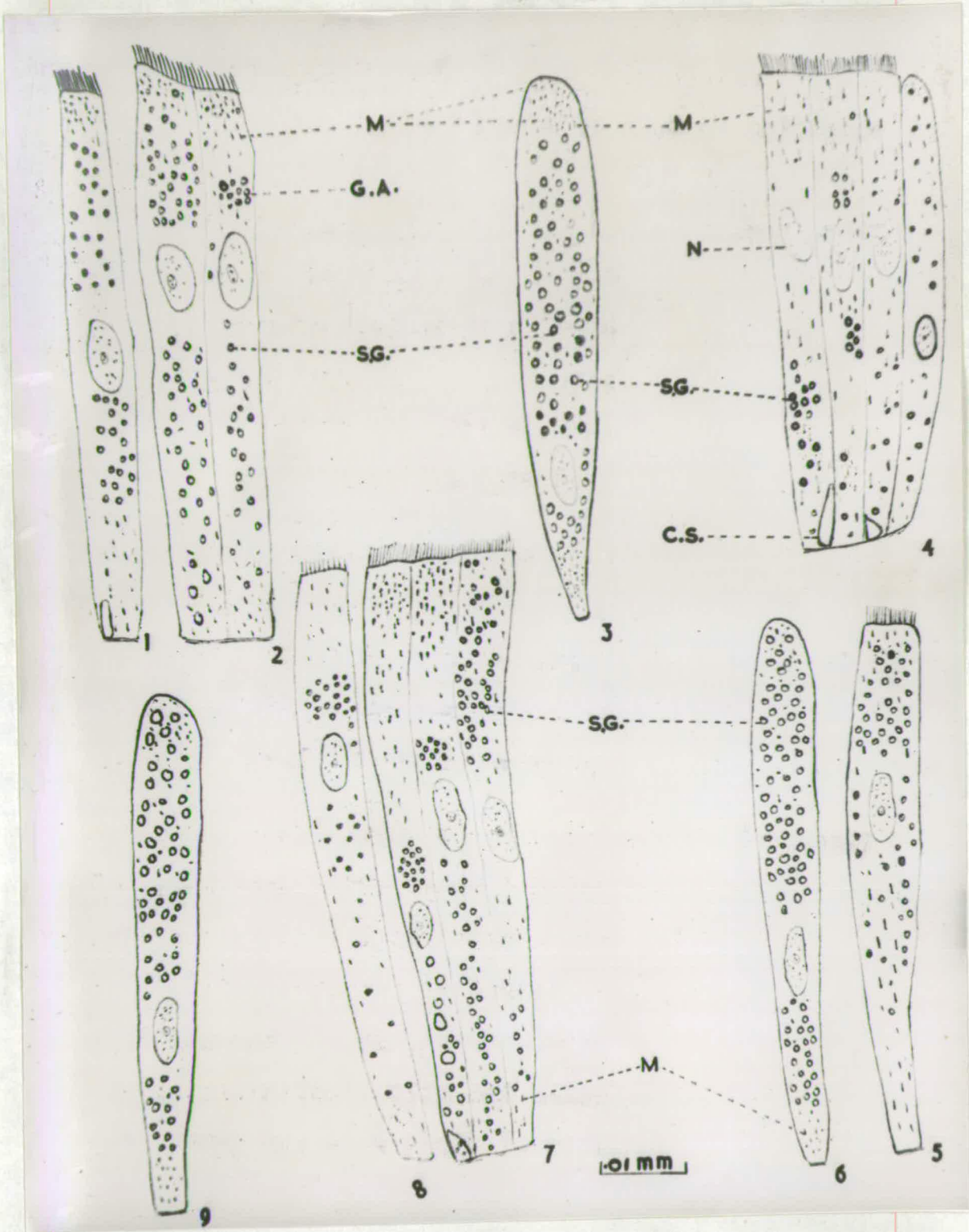
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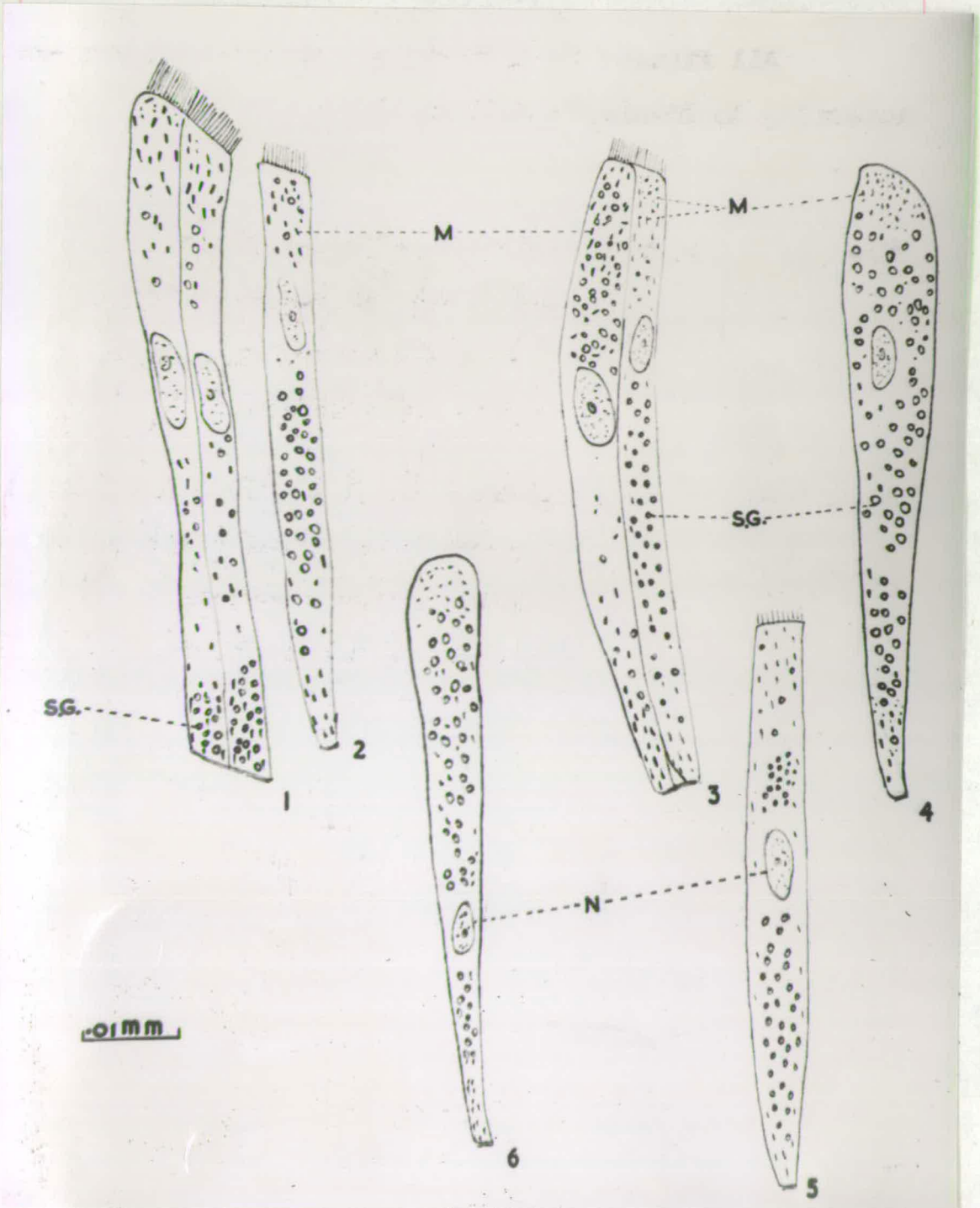
PLATE XXIV.

Drawings of the epithelial cells of the poste-
:rior region of the intestine.

All figures from Flemming preparations stained according to Bensley's method.

Figs. 1, 2 and 6. Ciliated and non-ciliated cells during the period of fasting, showing the secretory granules and the mitochondria.

Figs. 3-5. Ciliated and non-ciliated cells of worms 2 hours after the intake of food, showing secretory granules and mitochondria.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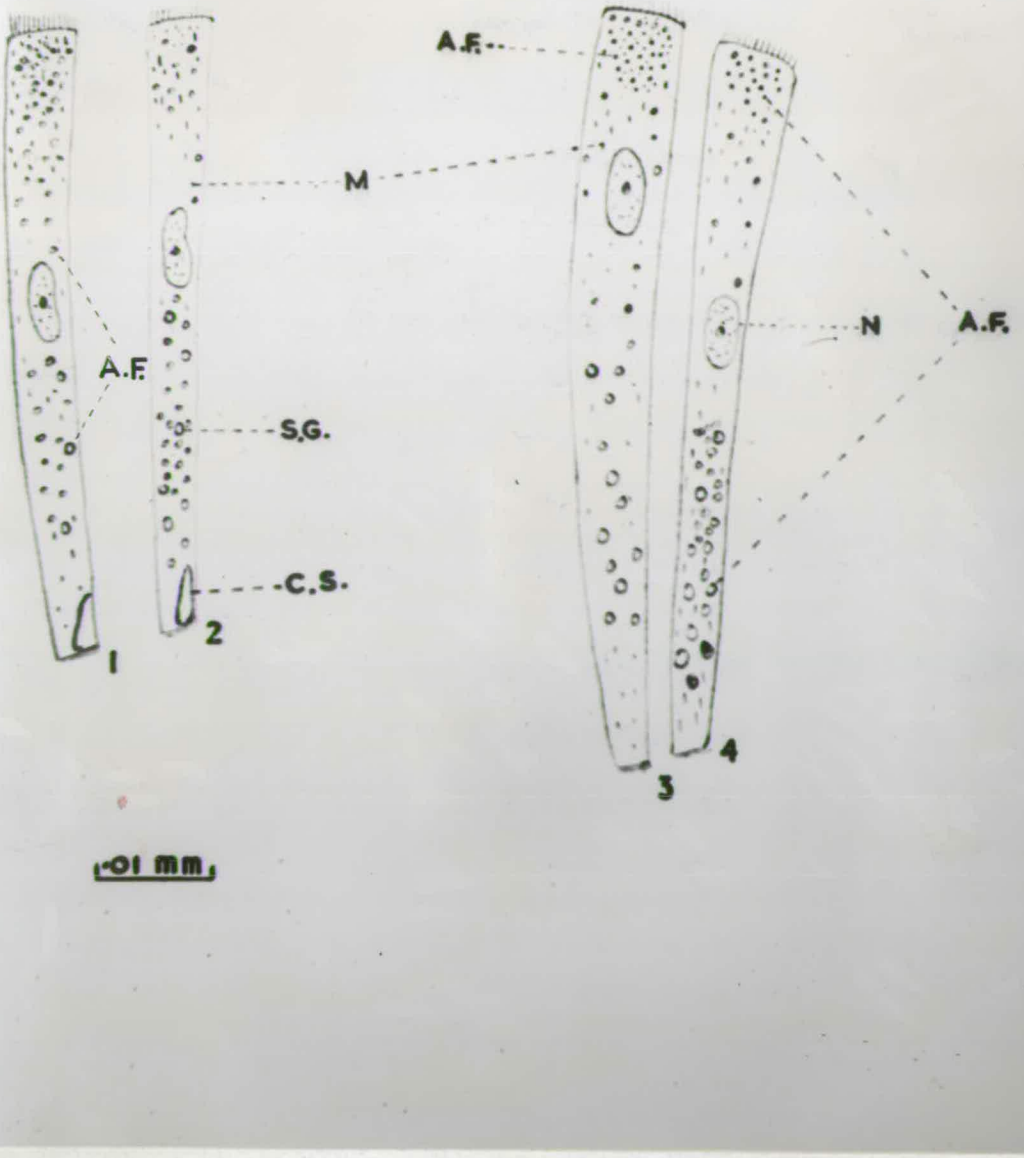
Drawings of the epithelial cells of the middle and the posterior regions of the intestine of worms killed after the intake of food.

All figures from Flemming preparations stained according to Bensley's method.

Fig. 1. Ciliated cells of the mid-intestine of worms 24 hours after the intake of food, showing granules of absorbed food material.

Figs. 2 and 3. Ciliated cells of the posterior intestine of worms 6 hours after the intake of food, showing mitochondria, the absorbed food material and secretory granules.

Fig. 4. Ciliated cell of the posterior intestine 24 hours after the intake of food, showing granules of absorbed food material.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Drawings of the epithelial cells of the anterior region of the intestine, showing the Golgi material.

All figures from Aoyama preparations stained with Ehrlich's haematoxylin.

Figs. 1-3. Ciliated and non-ciliated cells during the fasting period.

Figs. 4-7. Ciliated and non-ciliated cells of worms 2 hours after the intake of food.

Figs. 8, 9 and 11. Non-ciliated cells of worms 6 hours after the intake of food, showing increased hypertrophy and fragmentation of the Golgi material and association of secretory granules with the Golgi material.

Fig. 10. Non-ciliated cells of worms 6 hours after the intake of food, showing reduced amount of Golgi material after the completion of cellular activity.

Figs. 12-14. Ciliated cells of worms 6 hours after the intake of food, showing gradual hypertrophy of the Golgi material, and the secretory granules.

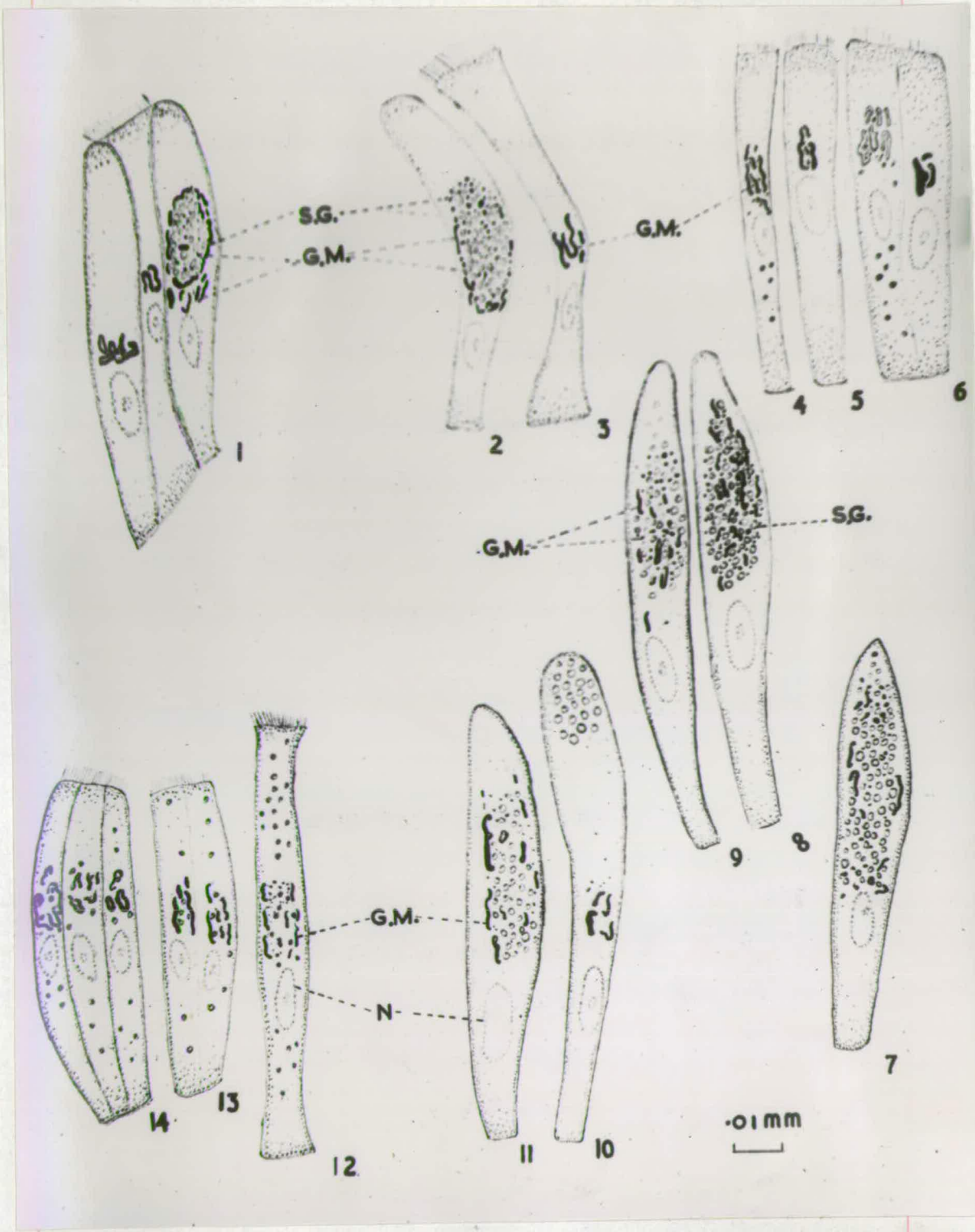
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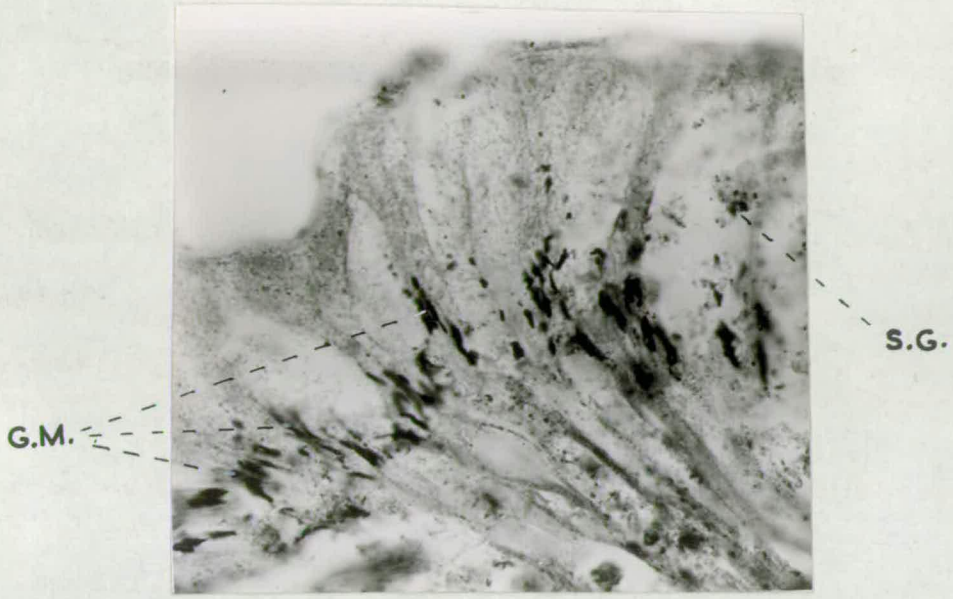
PLATE XXVII.

Photo micrographs of the anterior intestinal epithelium of earthworms.

All figures from Aoyama preparations.

Fig. 1. Showing the Golgi material in ciliated and non-ciliated cells of fasting worms. In some of the non-ciliated cells the Golgi elements surround the secretory granules. x 980

Fig. 2. Showing the Golgi material in ciliated and non-ciliated cells, 6 hours after the intake of food. The Golgi material is very much broken up. x 660



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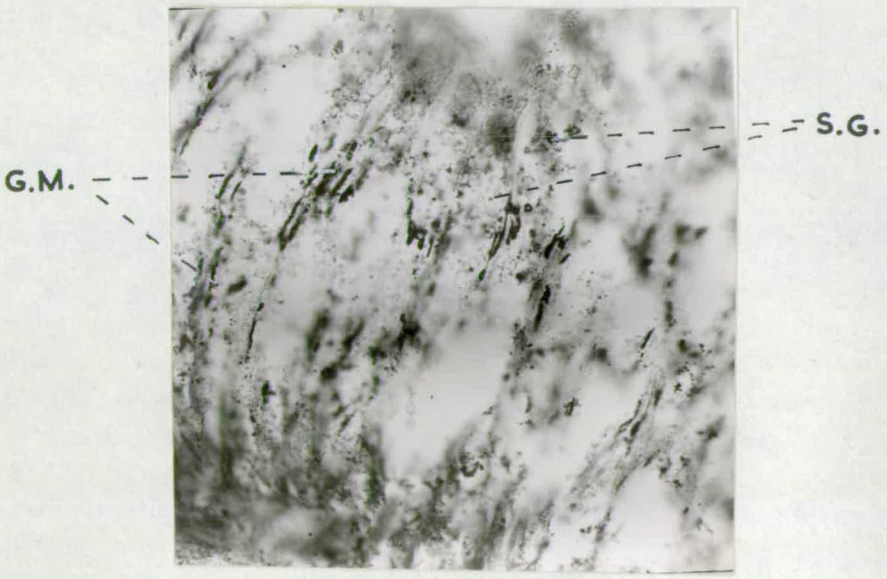

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Drawings of the epithelial cells of the middle region of the intestine showing the Golgi material.

All figures from Aoyama preparations stained with Ehrlich's haematoxylin.

Figs. 1-4. Ciliated and non-ciliated cells during the fasting period, showing reduced Golgi material in the ciliated cells. In one ciliated cell the Golgi material is in the form of vesicles.

Figs. 5 and 6. Ciliated cells of worms 2 hours after the intake of food, showing the Golgi material in association with secretory granules.

Figs. 7 and 8. Ciliated and non-ciliated cells of worms 6 hours after the intake of food, showing hypertrophy and fragmentation of the Golgi material.

Fig. 9. Ciliated and non-ciliated cells of worms 24 hours after the intake of food, showing the Golgi material and the granules of absorbed material.

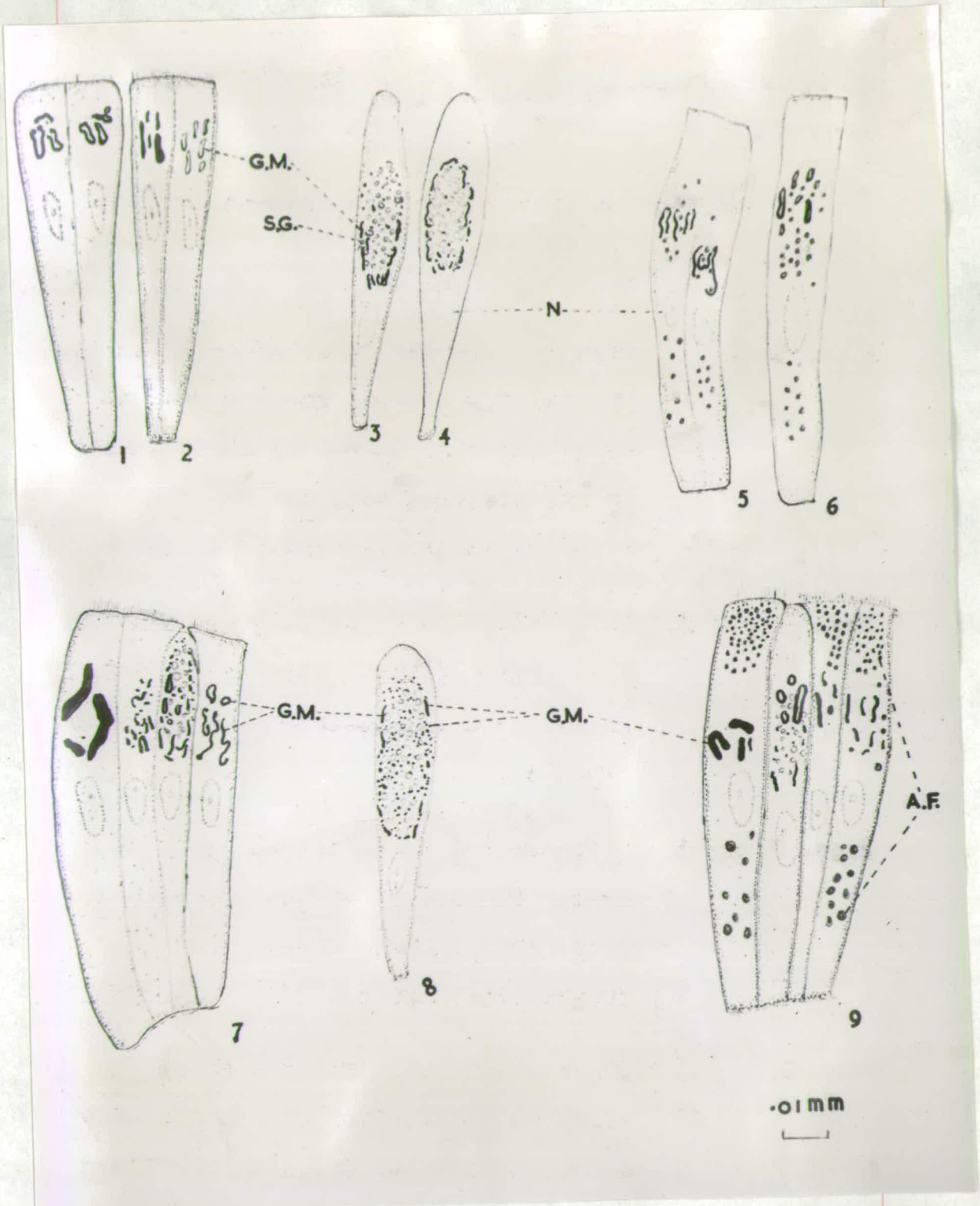
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PLATE XXIX.

Photomicrographs of the mid-intestinal epithelial cells.

Figs. 1 and 2 from Aoyama preparations.
Fig. 3 from Flemming preparation.

Fig. 1. Showing Golgi material in ciliated and non-ciliated cells during a period of fasting. The Golgi material is very compact. x 980

Fig. 2. Showing great hypertrophy of the Golgi material, 6 hours after the intake of food following a fast. x 980

Fig. 3. Showing secretory granules in different phases of their discharge from the non-ciliated cells, after a fast of 6 days. x 180

1

2

3

Drawings of the epithelial cells of the posterior region of intestine of worms killed after the intake of food, showing the Golgi material.

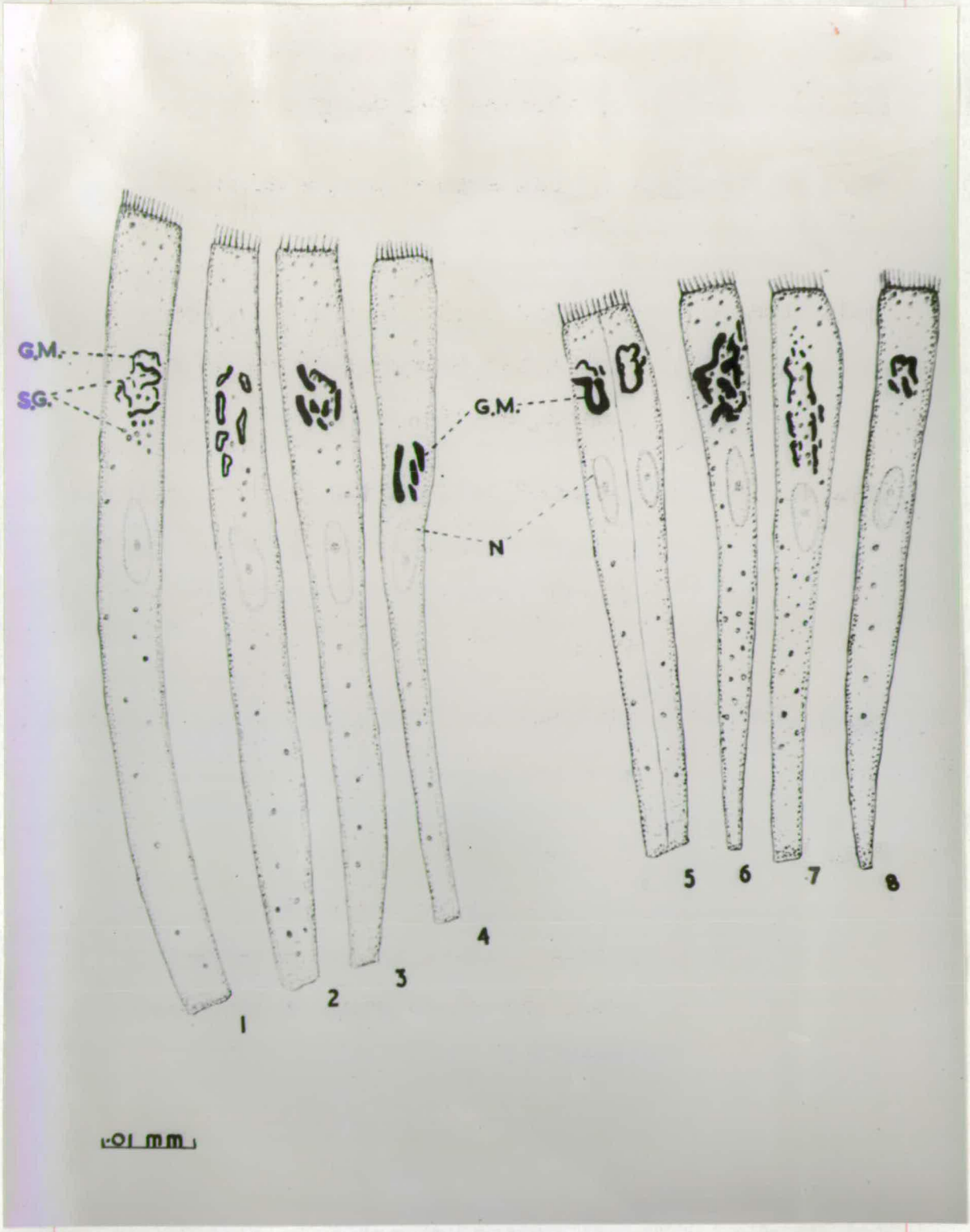
All figures from Aoyama preparations.

Figs. 1-3. Ciliated cells of the typhlosole region two hours after worms were placed in soil.

Fig. 4. Ciliated cell of the ventral epithe-:lium 2 hours after the worms were placed in soil, showing thicker Golgi rods.

Figs. 5-7. Ciliated cells 6 hours after worms were placed in soil.

Fig. 8. Ciliated cell 6 hours after worms were placed in soil, showing the reduced amount of Golgi material after the completion of secretory activity.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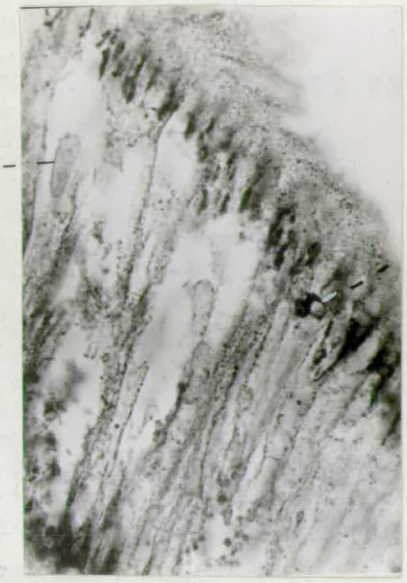
Photomicrographs of the posterior region of the intestinal epithelium. x 980

All figures from Aoyama preparations.

Fig. 1. Showing the Golgi material in ciliated and non-ciliated cells during a period of fasting.

Fig. 2. Showing hypertrophy of the Golgi material, 24 hours after the intake of food. Granules of absorbed food material are also seen; these are larger in the basal region than in the lumen region of the cell.



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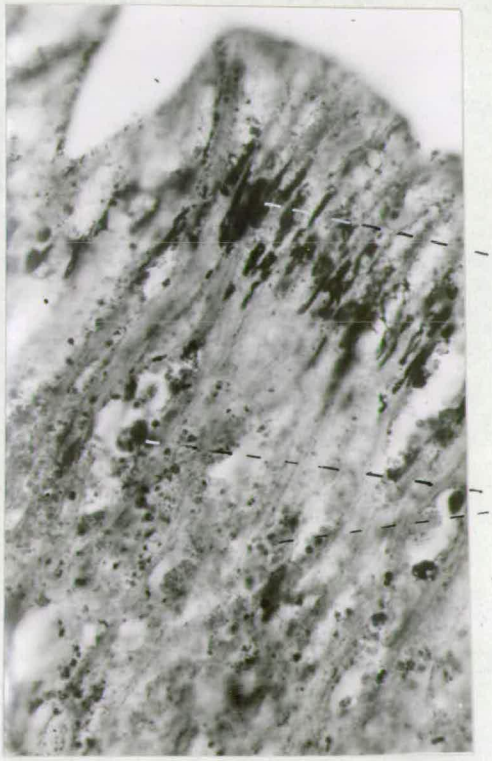

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XI. PHARYNGEAL GLANDS.

A. HISTORICAL

The salivary glands of vertebrates and inver-:tebrates are favourable material for histological and cytological studies. The cytoplasmic components of the salivary glands has been studied in the larvae of Chironomus by Beams and Goldsmith (1930), Gatenby (1932) and by Parat and Painlevė (1924), in the grasshopper, Rhomlea microptera, by Beams and King (1932), and in Tipula paludosa, by Gresson (1936).

There is no published papers on the cytology of the pharyngeal glands of earthworms.

The histology and funtions of the pharyngeal gland-cells of earthworms has been studied previously, notably by Stephenson (1917), who worked on Pheretima (Megascolecidae), Allolobophora caliginosa, and Bimastus parvus (Lumbricidae), by Keilin (1920), in Allolobophora chlorotica, Eisenia foetida and on a species of Lumbricus, and by Lankester (1864) in Lumbricus terrestris.

Willem and Minne (1899) worked on the chemi-:cal composition of the secretion of the pharyngeal gland-cells of Lumbricid worms and found that besides the general secretion of mucin, some of the gland-cells secrete a proteolytic ferment which dissolves fibrin in an alkaline medium with the formation of peptone.

Stephenson calls the pharyngeal gland-cells 'chromophil cells', since the cell body stains in part more deeply than the surrounding tissues. Conse-:quently the groups of gland-cells are always immediately visible in stained sections and at once attract atten-:tion. There is general agreement as to the true salivary nature of the pharyngeal gland-cells which are believed to secrete mucin for the lubrication of the food, as well as enzymes for its digestion. There is, however, conflict of opinion as to the manner in which the secretion passes into the pharyngeal cavity. Keilin believes that the products of secretion are collected in a system of salivary ducts lying in the conductive musculo-vascular portion of the pharynx, and that these ducts divide into innumerable fine ductules which penetrate between the ciliated epithe-:lial cells and terminate in discharge-pockets near the free surface of the cells. The salivary secretion accumulates in these pockets before it is discharged into the dorsal or salivary chamber of the pharynx. Stephenson, however, does not agree with these accounts and states that the secretion passes along 'mucin ducts', although he could not find the walls of these ducts as definite structures.

As previously stated in the account of the pharyngeal epithelium, the writer has been able to see clearly small salivary ductules in the ciliated cells/

cells terminating in discharge-pockets as described by Keilin.

As so much work has been done on the histo-:logy of the pharyngeal glands, the writer has little to add to the previous detailed descriptions.

B. METHODS

The methods employed were almost similar to those carried out for the study of the pharyngeal epithelium.

For the demonstration of mucin, sections were treated according to the formula of Southgate and Mayer.

As these gland-cells show great affinity for stains, overstaining occurs when acid fuchsin is employed, thus presenting considerable difficulty in the study of the cell components. In order to over-:come this difficulty, the sections, previously stained with acid fuchsin, were treated for one to two minutes with a very dilute solution of sodium carbonate, as employed in the case of the crop and the gizzard. This method gives a very delicate and precise stain for the secretory granules and the mitochondria. Like the cells of the alimentary canal the cells of the pharyngeal gland show a greater affinity for acid fuchsin than for iron-haematoxylin.

Unlike the pharyngeal epithelium, the pharyngeal gland-cells do not give satisfactory results/

results with silver methods. As only osmic methods were satisfactory for the demonstration of the Golgi material, Kolatchev's formula, with an impregnation period of four to five days at about 37° C., gave very good results.

C. OBSERVATIONS

The pharyngeal gland-cells are situated on the dorsal and the lateral surfaces of the pharyngeal bulb. A few cells are, however, also present on the ventral side of the pharyngeal chamber in the near neighbourhood of the ventral epithelium. In the deeper posterior portion of the pharyngeal mass, the cells are fewer in number and occur in groups between the connective tissue of the bulb. These cells are poly-:morphic, being either triangular, spherical or crescent-shaped. The individual cells are not situated very close together but are separated from one another by clefts (Pl. XXXII, fig. 1). As stated by Stephen-:son, their outlines are not very definite and they are frequently continuous at their periphery with an amorphous or fibrillar coagulum-like substance, which partly fills up the space between the cells. The peripheral regions of the cells stain more lightly than the deeper cytoplasm.

The cells which are very conspicuous, because of their great affinity for dyes, have big nuclei/

nuclei situated either at the centre or at one end of the cell. The nuclear membrane is distinct, and one very large nucleolus and chromatin granules is usually present. The nucleus is generally regular in outline and spherical to oval in shape (Pl. XXXII, figs. 1 and 2). The cells are usually found in aggregates of six to sixteen, or more, surrounded by a very thin peritoneal investment which is more prominent in the anterior portion of the pharyngeal bulb than towards the middle or the posterior region. When the cell is full of mature secretory granules, the cell-~~wall~~ membrane dis-:appears or is broken by the pressure of the accumulated secretory material and loose their membranes, when all the secretory material inside the peritoneal invest-:ment seems to move towards the pharyngeal chamber and is finally discharged. The secretion is either discharged into the lumen through the dorsal ciliated epithelium or through the cells of the pharyngeal shelf (Pl. III, figs. 1 and 2). Some of the secretion is also poured into the lumen through the ventral epithelium.

Examination of the secretory material reveals that not only is all the secretion produced by a group of cells discharged at the same time, but that the nuclei of these cells are also passed into the lumen (Pl. XXXII, fig. 8 and Pl. XXXV, fig. 2). It is concluded, therefore, that the cells disinte-:grate after they become full of secretion. The nuclei present/

present in the secretory mass are very irregular in outline and at times the nuclear membrane is indistinct and the chromatin granules are thicker than in the nuclei within the cells (Pl. XXXII, fig. 8). The secretory mass contains small as well as large secre-:tory granules and in some cases a few mitochondria.

Fasting and feeding induce very little change in the morphology of the cell components since the production of mucin is constant and continuous.

1. Worms with Constant Access to Food.

When an animal has constant access to food, some of the cells are more active than the others, consequently the cell components behave differently in different cells. Their behaviour is described in the following sections.

2. Conditions Induced by Fasting.

During this stage some of the cells show accumulation of secretory granules, while in others formation of secretory granules is taking place. Secretory granules are chiefly seen in the neighbour-:hood of the nucleus, particularly at one of its poles, presumably in the Golgi field. When the secretory granules become mature, they are found aggregated towards the periphery of the cell. In some cells a few big spherical secretory granules are scattered in the cytoplasm and are surrounded by a number of mitochondria./

mitochondria. When the cells become full of secretory granules, the cell membrane disappears (Pl. XXXII, figs. 1-8).

3. The Secretory Cycle

Two to three hours from the intake of food, the cells usually show a few secretory granules sca-:ttered without any special arrangement throughout the cytoplasm. There are, however, a few cells in which the secretory granules accumulated during the stage of fasting are still present. In some cells secretory granules are situated at the periphery of the cell (Pl. XXXIII, figs. 1-3).

Five hours from the commencement of feeding, the secretory granules have increased in number (Pl. XXXIII, figs. 4-6). Some of the early secretory gra-:nules are in close vicinity to the nucleus near the Golgi field. One or two of these secretory granules are larger than the others. The nuclear membrane of a few cells has disappeared, and in some cases the nucleolus is no longer visible. In some cases the nuclear material seems to be greatly changed and the chromatin granules are much thicker and more prominent than previously. A few of the nuclei are slightly irregular in outline.

Six hours from the intake of food, the secre-:tory response seems to be still greater. More mature secretory granules are scattered in the cytoplasm, and/

and some of the granules are in small clumps (Pl. XXXIII, figs. 7-11).

Twenty-four hours from the intake of food, cells in all stages of the secretory cycle are present.

4. The Mitochondria

i. Worms with Constant Access to Food:- The mitochondria are in the form of thick rods of various lengths and of a few granules. Their behaviour during the different stages of cellular activity is described below.

ii. Conditions Induced by Fasting:- The mitochondria consist of short thick rods and a few granules. In some cells a few long rods are scattered in the cytoplasm. In most of the cells some mitochondria are situated close to the nucleus where accumulation of secretory granules has not taken place (Pl. XXXII, fig. 1). In some of the cells, clumps of granular and rod-shaped mitochondria surround a large spherical secretory mass, probably composed of mucin, situated some distance from the nucleus (Pl. XXXII, figs. 2-4). A few rods are scattered close to the border of the cell.

iii. The Secretory Cycle:- With the intake of food, the secretory response seems to be much more marked than in cells from animals with constant access/

access to food. The mitochondria become thicker, but do not show polar orientation during any stage of ce cellular activity.

Two to three hours from the intake of food, the mitochondria in the majority of cells become shorter and fewer in number as compared with the pre-:ceding phase. In some cells the secretory granules have increased in number and the mitochondria are scanty. Very few mitochondria are seen in the neigh-:bourhood of the nucleus. In the cells of the extreme dorsal region of the pharyngeal mass, there seems to be little appreciable change in the structure or arrangement of the mitochondria as compared to cells examined during the fasting stage. In some cells there are very few secretory granules but the mitochondria, chiefly in the form of rods, are more numerous than in the cells of fasting animals (Pl. XXXIII, figs. 1-3).

Five hours from the commencement of feeding, the number of mitochondria is greatly increased. They are still in the form of small rods; some of which are situated near the nucleus in close association with the secretory granules. In cells which contain more secretory granules than the majority, the mito-:chondria are few in number (Pl. XXXIII, figs. 4-6).

Six hours from the intake of food, the mito-:chondria are in the form of short and long rods, situated/

situated chiefly in association with the secretory granules which are present in small clumps near the nucleus or in the more distant regions of the cytoplasm (Pl. XXXIII, figs. 7 and 9). In some cells the mitochondria are arranged in the peripheral cytoplasm (Pl. XXXIII, fig. 8).

Twenty-four hours from the commencement of feeding, cells in all stages of the secretory cycle are present, and the arrangement of the mitochondria is correlated with these stages.

5. The Golgi Material

The secretory granules and the cytoplasm of the pharyngeal gland-cells do not impregnate so deeply with osmium tetroxide as the granules and cytoplasm of the epithelial cells. As the cells of the pharyngeal glands are, more or less, continuously active in the production of mucin, the Golgi material shows very little morphological change as a result of fasting and of feeding after a fast.

The Golgi material consists of short and long rods, filaments and granules scattered through the cell (Pl. XXXIV, figs. 1, 2 and 6 and Pl. XXXV, fig. 1). It has a distinct tendency to spread throughout the cell and thus comes into contact with the scattered secretory granules. It must be noted, however, that the Golgi material in the gland-cells of the pharynx is never in the form of a compact network or a reticulum/

reticulum as in the epithelial cells during the rest-:ing phase.

During the fasting stage the Golgi material is chiefly in the form of rods and filaments; granules are not numerous (Pl. XXXV, figs. 1 and 2). The rods are usually curved, but a few may be straight. Some of the larger secretory granules , which are situated in association with thickenings of the Golgi threads, have a vesicular appearance. The larger secretory vesicles are surrounded by ramifications of the Golgi material which lie all around the periphery of the cell (Pl. XXXIV, fig. 3). In cell in which the accumulation of the secretory material is at its highest, the Golgi material is very scanty and consists of a few threads with small thickenings (Pl. XXXIV, fig. 7).

During the stage of increased cellular activity which results from the intake of food, after a fast of four to six days, the most noticeable feature observed in the morphology of the Golgi material is the considerable increase in the amount of the mate-:rial, which now occupies a greater area than during the other phases. Fragmentation of the Golgi threads and rods takes place, as a result there are more rods and granules than before. Some of the curved Golgi rods become straight and elongate (Pl. XXXIV, fig. 6 and Pl. XXXV, fig. 1). The rods usually appear to be scattered through the entire cell. In some cells the majority/

majority of the Golgi elements are situated around the nucleus (Pl. XXXIV, fig. 4). The longer rods and the threads are present chiefly in one half of the cell. In some cases Golgi rods and granules surround secretory granules (Pl. XXXIV, fig. 5). The Golgi granules are scattered throughout the cytoplasm, but are less numerous at the extreme peri-:phery than elsewhere in the cell.

Drawings of the pharyngeal gland-cells of earthworms killed after a fast of 4 days, showing mitochondria and secretory granules.

All figures from Flemming preparations stained according to Bensley's method.

Fig. 1. Showing gland-cells separated from each other by clefts. The cells show large nucleus with a very big nucleolus in each. Mitochondria are seen arranged chiefly at the periphery of the cell.

Fig. 2. Cell to show mitochondria arranged around the secretory material.

Figs. 3-5. Cells to show gradual accumulation of the secretory material and reduction in the size and number of the mitochondria.

Fig. 6. Showing cell, which is full of secretory granules, and has very thick chromatin granules. The nucleolus has disappeared.

Fig. 7. The nuclear membrane has disappeared and the cell is full of secretory granules.

Fig. 8. Secretory mass enclosed in a thin peritoneal investment containing irregular nuclei, thick chromatin granules and faint nuclear membranes of the gland-cells.

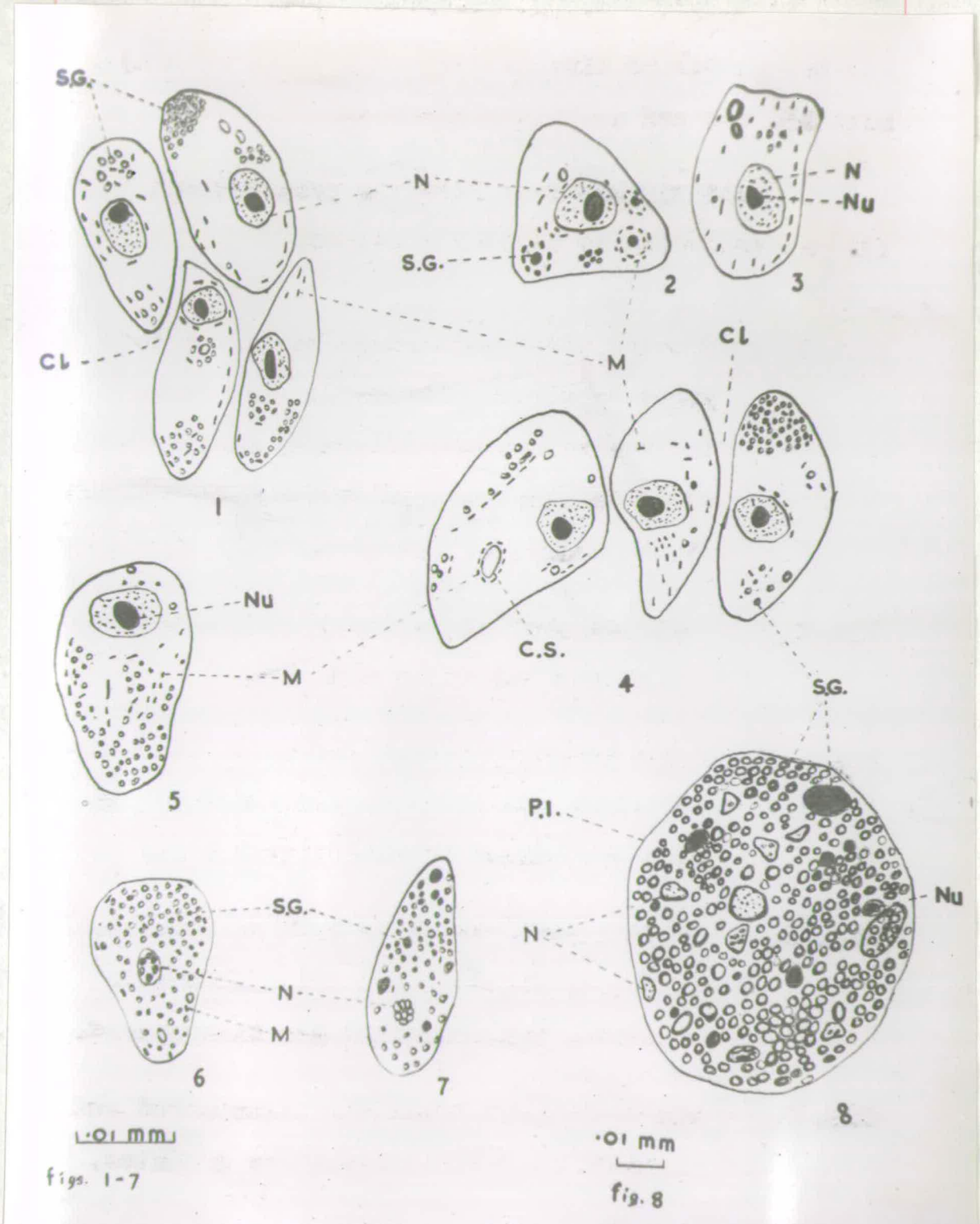
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fig. 8

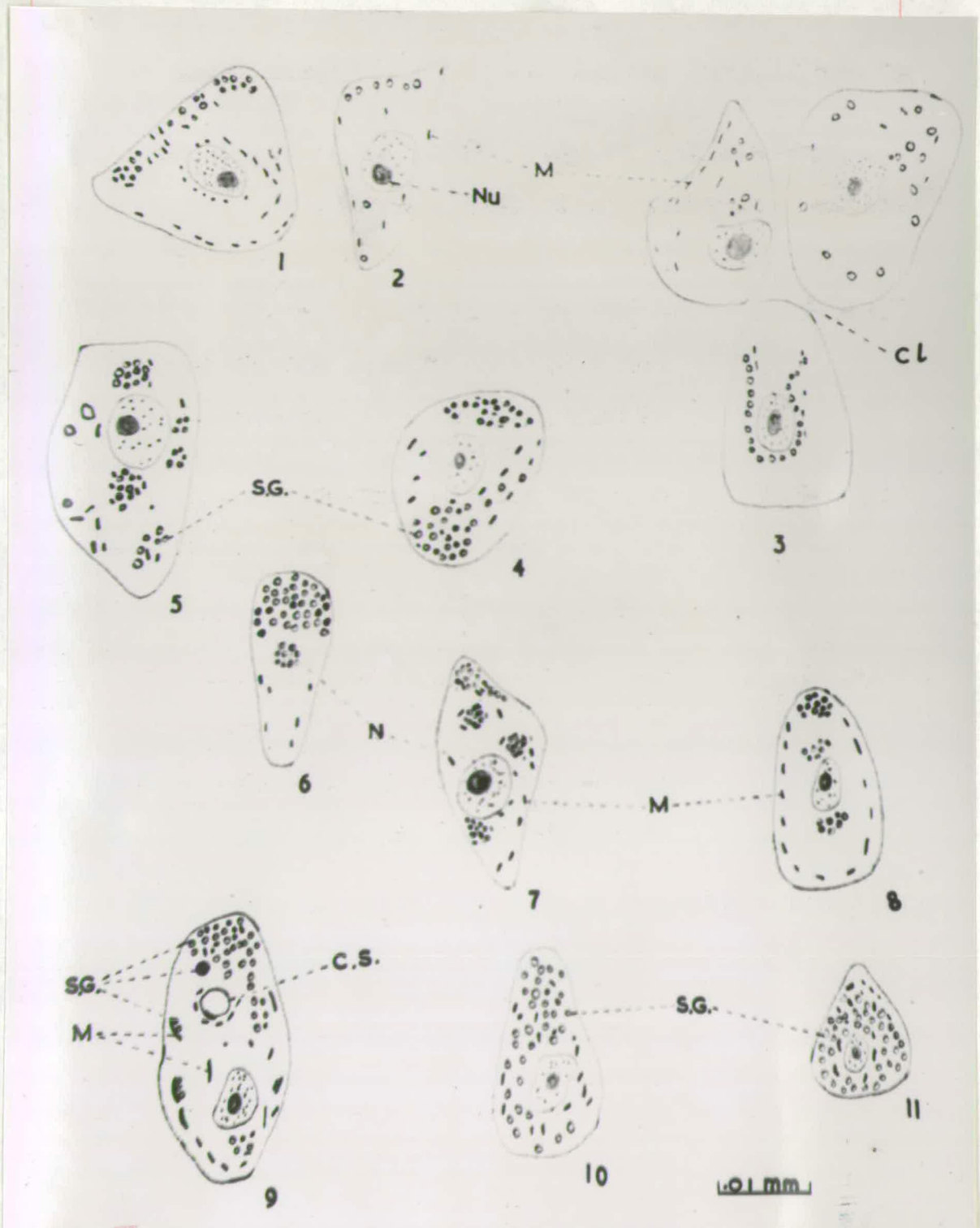
Drawings of the cells of the pharyngeal gland of earthworms, killed at various periods after feeding.

All figures from Flemming preparations stained according to Bensley's method.

Figs. 1-3. Cells of worms, which have been in soil for 3 hours, showing the arrangement of mitochondria and secretory granules.

Figs. 4-6. Cell of worms, which have been in soil for 5 hours, showing the arrangement of mitochondria and secretory granules.

Figs. 7-11. Cells of worms, which have been in soil for 6 hours, showing the arrangement of the mitochondria and the gradual accumulation of secretory granules.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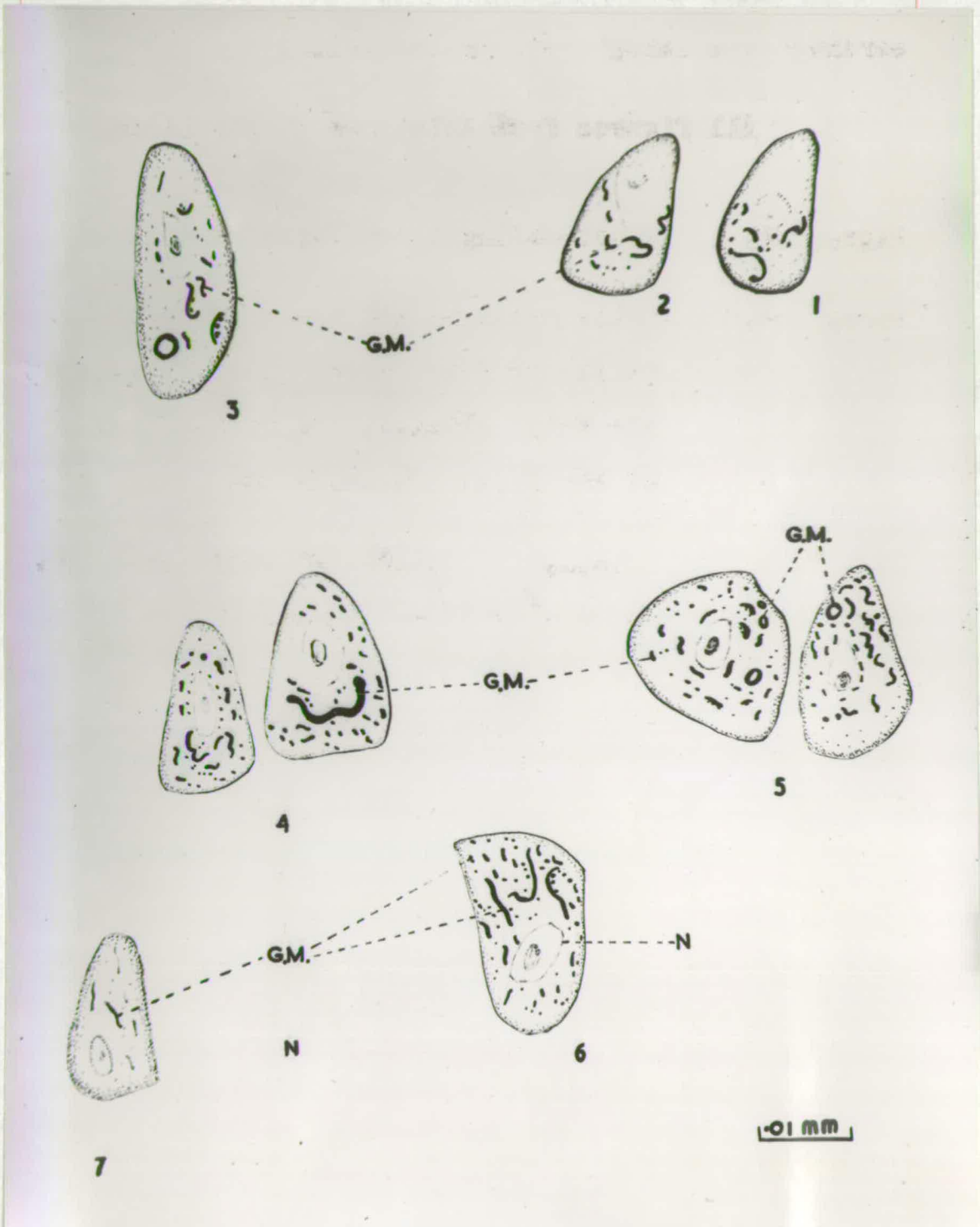
Drawings of the pharyngeal gland-cells of earthworms showing the Golgi material.

All figures from Kolatchev preparations.

Figs. 1-3. Cells during the fasting period.

Figs. 4-6. Cells of worms 3-6 hours after the intake of food, showing hypertrophy of the Golgi material and fragmentation of the Golgi filaments and rods.

Fig. 7. Showing Golgi material very much reduced in thickness and in amount in cell in which there is a great accumulation of secretory material.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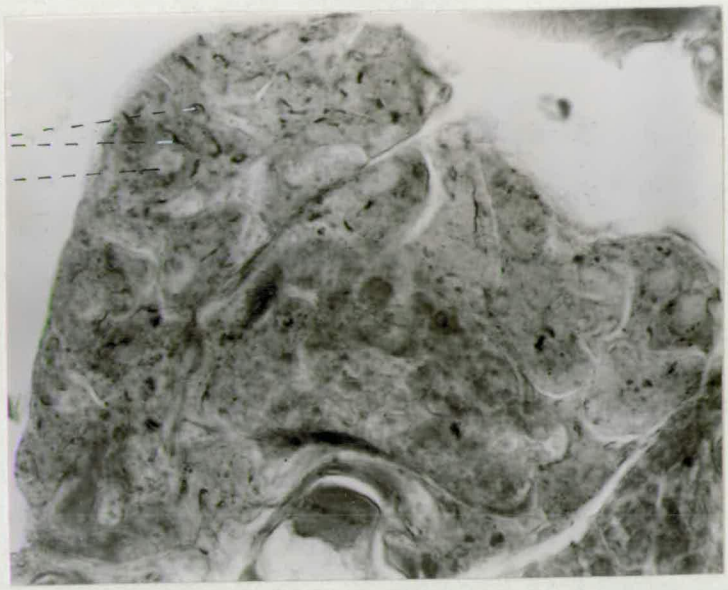
Photomicrographs of the pharyngeal gland-cells, showing Golgi material, mitochondria and secretory granules.

Fig. 1 from Kolatchev preparation.
Fig. 2 from Flemming preparation.

Fig. 1. Showing the Golgi material of the gland-cells after the intake of food. It is in the form of rods, filaments and granules scattered throughout the cell. x 980

Fig. 2. Showing mitochondria and secretory granules in gland-cells. In the centre an accumulated mass of secretory material is seen together with the nuclei of the disintegrated cells. The large nucleolus of the gland-cell is clearly seen in some cases. x 1140



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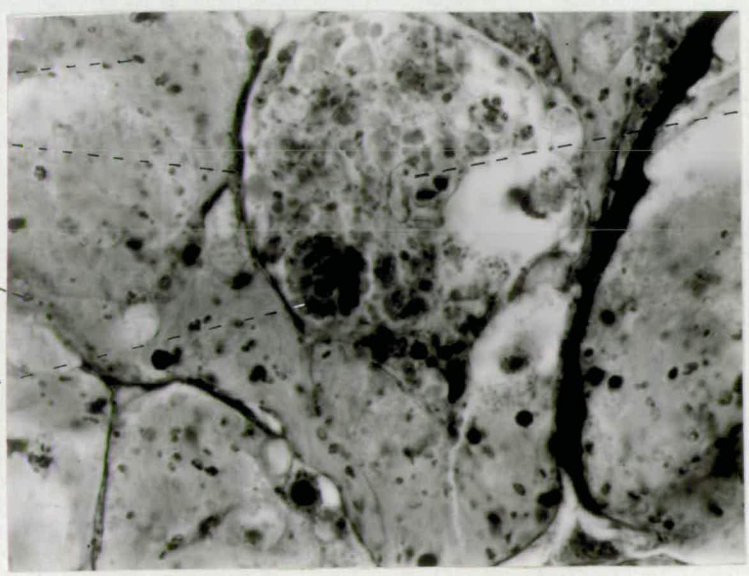

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XII. THE OESOPHAGEAL POUCHES AND GLANDS.

A. HISTORICAL

The oesophageal glands of earthworms have attracted the attention of several workers in the past and various theories have been put forward to explain their functions.

The earliest paper on the oesophageal glands of the Lumbricidae is that of Lankester (1864), who worked on the anatomy of the glands of Lumbricus terrestris and L. agricola, chiefly the former. He notes the presence of a pair of glands in segment xii and two pairs in segment xiii, and presumes the function of the first pair to be connected with the formation of the egg capsule, or alternatively for disposing of any superabundance of mineral matter in the blood. He believes the second and third pair to be digestive and states - "The use of milky secretion contained in the second and third pair may be in the process of digestion; indeed, this appears most probable, but the properties of secretion cannot be determined." Stephenson and Prashad (1919), who worked on Allolobophora caliginosa and Lumbricus sp. describe the presence of lateral diverticula of the gut (oeso-:phageal pouches) in segment ten and a pair of oeso-:phageal glands in each of the segments xi and xii of Lumbricus. The glands communicate with the oesophagus through the oesophageal pouches. Smith (1924) made similar observations on Lumbricus terrestris.

Opinion is widely divided on the function of the oesophageal glands. Darwin (1881) considered the excretion of lime to be their chief function although they are partly digestive in the sense that the calcium carbonate neutralises the acidity of the gut-contents. According to Combault (1907 & 1909) the function of these glands is the absorption of oxygen and the excretion of carbon dioxide. Michaelsen (1895), however, believes that their function is the absorption of nutriment and that the excretion of lime is a secondary function. The fact that these glands are very vascular and that the surface of the epithelium is greatly increased by the epithelial folding, indicates an absorptive function. Stephenson (1930) rejects an absorptive function of the oesophageal glands on grounds of their location, which is anterior to the gizzard and intestine, and their segregation from the alimentary tract, only the oesophageal pouches being in direct communication with the oesophageal cavity, and that only by one end of a long tunnel - conditions which render the access of food to the supposed absorptive surface more difficult. He believes the chief function of the gland to be excretion of carbon dioxide and often, at any rate, of lime, which besides fixing the carbon dioxide probably has a further use in helping to neutralise the contents of the gut.

Harrington (1899) states that in a secretory

cycle/

cycle the protoplasm of the cell first increases in amount, so that the cells project as club-like processes, lime granules appear in its interior, the cytoplasm degenerates and the granules are thrown out. The cytoplasm may be nearly all used up in the process, the thickness of the epithelium is much reduced and this layer may disappear almost down to the level of the blood-sinus. The nuclei also collapse or are cast out into the gland cavity during active secretion. The replacement of the nuclei, he believes, takes place by the migration of corpuscles from the blood stream into the glandular layer.

There are no contributions on the cytology of the oesophageal pouches and glands of Lumbricus.

B. METHODS

For the study of the mitochondria, oesopha-:geal pouches are very good material when fixed in Flemming (without acetic acid). They do not impregnate satisfactorily with any of the osmic methods employed for the study of the Golgi material. Consequently, only silver methods (Aoyama and Da Fano) were employed. These gave fairly satisfactory results. Some of the silver preparations were stained with Ehrlich's haematoxylin.

The oesophageal glands presented considerable difficulties in the study of the mitochondria, Golgi bodies and other cell components, chiefly because of the/

the very small size of the cells. With silver methods the cells become black, and thus renders observation very difficult. Kolatchev and Mann-Kopsch methods, however, gave more satisfactory results.

C. OBSERVATIONS

(a) Oesophageal Pouches

The present investigations show that the oesophageal pouches have a totally different histolo-:gical appearance to that of the oesophageal glands (Pl. XXXVI, figs. 1-5 and Pl. XXXXI, fig. 1).

The inner epithelium of the oesophageal pouches is thrown into a number of longitudinal folds or lamellae (Pl. XXXVI, figs. 1 and 2, and Pl. XXXXI, fig. 1). The free ends of these folds unite to form a series of tunnels. The epithelium of these tunnels consists of more or less cubical cells which become more prominent posterior to segment ten, while those of the free borders (posterior to segment ten) are usually in the form of columnar cells with a distinct layer of rodlets (Pl. XXXXI, figs. 1 and 2). The cells lining the tunnels contain more secretory granules in all phases of the secretory cycle than the elongated cells lining the lumen of the oesophageal pouch. The tunnels are at all times full of milky white secretion.

1. Worms with Constant Access to Food

In this material the cells of the oesophageal pouches are in all stages of activity and rest.

2. Conditions Induced by Fasting

The majority of the cells of the oesophageal pouch bordering the lumen are club-shaped whereas those lining the tunnel are more or less cuboidal (Pl. XXXVII, figs. 1,2,4 and 5). Secretory granules are, in the majority of the cells, accumulated either in the basal or in the lumen region of the cell. Vacuoles, with or without secretory material, are commonly seen in the basal half of the cell (Pl. XXXVII, fig. 1). The cells lining the tunnel are usually full of secretory granules. A light colourless area, free of mitochondria and secretory granules, is usually present at the outer pole of the cell close to the lumen of the oesophageal pouch. Its nature was not determined. The cells lining the tunnel show a great accumulation of secretory granules without any definite arrangement.

3. The Secretory Cycle

When worms are allowed to feed, after a period of fasting, the cells of the oesophageal pouch show increased cellular activity. The accumulated secretory granules are discharged into the lumen; consequently the cells are uniform in appearance (Pl. XXXVIII, figs. 1-3). The nuclei move slightly towards the lumen and usually come to occupy a central position. With further increase of cellular activity, fresh/

fresh secretory granules appear and masses of secretory material are visible between the epithelial cells on their way to the lumen (Pl. XXXVIII, fig. 2). The light area, already noticed was observed in this material, but is not well marked.

4. The Mitochondria

i. Worms with Constant Access to Food:- The cells of animals with constant access to food are in all stages of secretory activity. Consequently, the mitochondria differ in form and arrangement in the different cells. Their behaviour during the secretory cycle is described in the following sections.

ii. Conditions Induced by Fasting:- When an animal has fasted for a few days, the mitochondria consist of filaments and rods of various lengths but of equal thickness (Pl. XXXVII, figs. 1-3 and Pl. XXXXI, fig. 2). Although some of the mitochondria are arranged parallel to the longitudinal axis of the cell, on the whole, they do not exhibit well marked polar orientation. In the majority of the cells they are more numerous in the basal region than in the lumen region. The filaments, which are usually curved, are situated throughout the cell, but in some cases they are most numerous close to the basement membrane. The rods are scattered throughout the cell. Granular mitochondria are very seldom seen, but when present are usually found close to the lumen or around

the Golgi field. In the cells lining the tunnels, the mitochondria consist chiefly of small rods and in addition a very few filaments (Pl. XXXVII, fig. 4).

iii. The Secretory Cycle:- Examination of worms at different times after the intake of food, showed that there is a marked secretory response. The mitochondria are thicker and more numerous than in the cells described above, and, in the majority of cells, the lumen half contains more mitochondria than the basal half. Polar orientation is very well marked (Pl. XXXVIII, figs. 1-3). The filaments are now uniformly distributed throughout the cell. The rods are scattered throughout the cytoplasm but are more numerous in the region of the lumen than elsewhere. The granules, which usually are very few, are found in the lumen half of the cell. Some of these granules may be the cross-section of rods and filaments. In cells which are full of secretory granules the mitochondria are reduced in number (Pl. XXXVIII, fig. 4).

In the cuboidal cells lining the tunnels, there is no appreciable change in the form and arrangement of the mitochondria, except that filaments are absent from cells (Pl. XXXVII, fig. 5).

5. The Golgi Material

i. Worms with Constant Access to Food:- The behaviour of the Golgi material during the phases of secretory/

secretory activity and of rest is described in the following sections.

ii. Conditions Induced by Fasting:- When an animal is in a fasting condition, the Golgi material is usually in the form of a reticulum, which may be either compact or loose. The reticulum consists of thick rods and filaments connected together by thin links (Pl. XXXIX, fig. 1). The rods and filaments are usually curved. In some cells, chiefly in the cuboidal cells of the tunnel, the Golgi material is situated slightly above the nucleus.

iii. The Secretory Cycle:- Feeding after a fast does not induce a very great change in the morphology of the Golgi rods and filaments. The reticulum, however, becomes loose and the rods slightly thicker and longer (Pl. XXXIX, figs. 4, 5 and 8). With increase of cellular activity new secretory granules become visible and later migrate to the lumen half of the cell(Pl. XXXIX, figs. 2 and 3).

During all phases of cellular activity, the Golgi material in some of the epithelial cells is in the form of vesicles, some of which are sometimes connected together by a very thin thread (Pl. XXXIX, figs. 6 and 7). Complete fragmentation of the Golgi material does not appear to take place at any stage of the secretory cycle.

(b) Oesophageal Glands

In view of the considerable amount of work carried out on the histology of the oesophageal glands of Lumbricus, it is not necessary to describe their histological structure in detail.

In cross-section, the glands show numerous transverse folds or lamellae extending from side to side across the gland (Pl. XXXVI, figs. 3-5). The cells are small and are arranged on these lamellae. The nucleus is very long, the nuclear membrane and chromatin granules are distinct; and one nucleolus is usually present (Pl. XXXX, fig. 5).

Due to the very small size of the cells of the oesophageal glands, the secretory material, mitochondria and the Golgi material could not be satisfactorily studied. The cells were active in all the material examined during the present investigation. It is concluded, therefore, that secretory activity is not influenced by fasting or feeding.

Very small granules which blacken with osmium tetroxide are present in some of the cells, while in others large spherical bodies are present (Pl. XXXX, figs. 1, 2, 3 and 4, and Pl. XXXXII, figs. 1 and 2). It would appear that some of the small granules are early stages in the formation of the large spherical bodies, which do not blacken so deeply in osmic preparations/

preparations and contain a central body (Pl. XXXX, figs. 3 and 4, and Pl. XXXXII, fig. 1). The spherical bodies are without doubt secretory in nature. They are discharged into the lumen and accumulate between the lamellae (Pl. XXXVI, figs. 3 and 5, and Pl. XXXXII, fig. 2). Most of the cytoplasm and the nucleus passes into the lumen along with the secretory gra-:nules. Consequently more than half the total number of the cells examined were in a state of collapse (Pl. XXXVI, fig. 5 and Pl. XXXXII, figs. 1 and 2).

Study of the mitochondria was particularly difficult, but very small rods were seen in certain cells which were slightly larger than their neighbours. It is possible that some of the granules present are mitochondria, but all the granules are of approximately the same size and do not differ in staining properties (Pl. XXXX, fig. 5).

Both silver and osmic preparations reveal the presence of small granules scattered through cells in the early stages of secretory activity. Some of the granules are probably Golgi bodies and others are small granules of secretion. Small osmiophilic and argento-:philic granules were observed lying over the outer part of the large spheres of secretory material. These granules are identified as Golgi bodies (Pl. XXXX, figs. 3 and 4, and Pl. XXXXII, figs. 1 and 2).

It was not possible to obtain further informa-:tion on the mitochondria and Golgi material of the cells of the oesophageal glands.

PLATE XXXVI.

Photomicrographs of transverse sections passing through the oesophageal pouches and glands.

All figures from Flemming preparations.

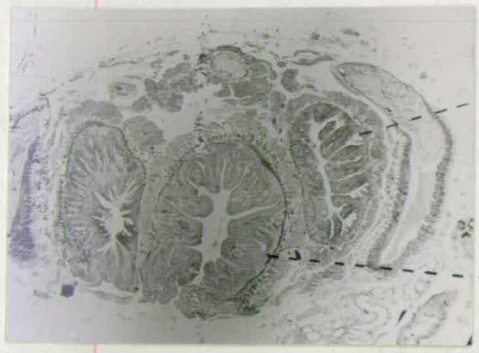
Fig. 1. Showing the position of oesophageal pouches, with respect to oesophagus, in segment ten. x 23

Fig. 2. Showing the opening of the oesophageal pouch into the oesophagus. x 50

Fig. 3. Showing the oesophageal pouch at the sides of the oesophagus, and oesophageal glands on either sides of the oesophageal pouch. The great vascularity of the oesophageal gland can be seen clearly. x 35

Fig. 4. Showing the opening of the oesophageal glands into the anterior end of the oesophageal pouches. x 23

Fig. 5. Showing the opening of the oesophageal glands into the oesophageal pouches, and the transverse folds in the oesophageal glands. x 35



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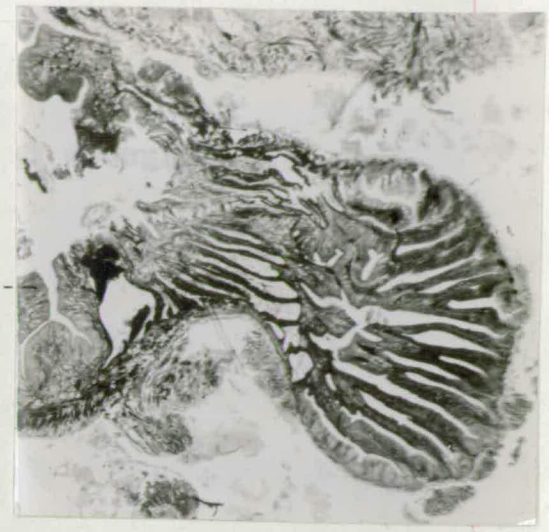

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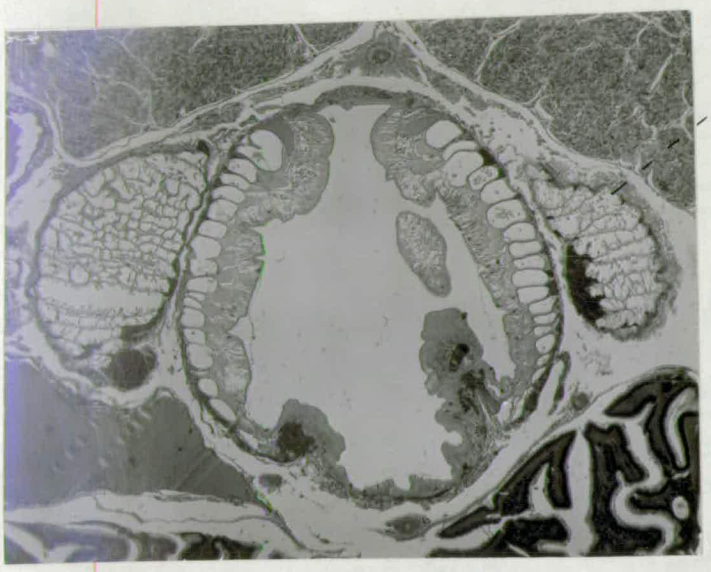

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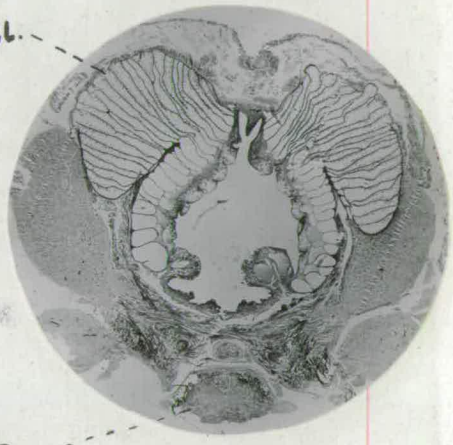

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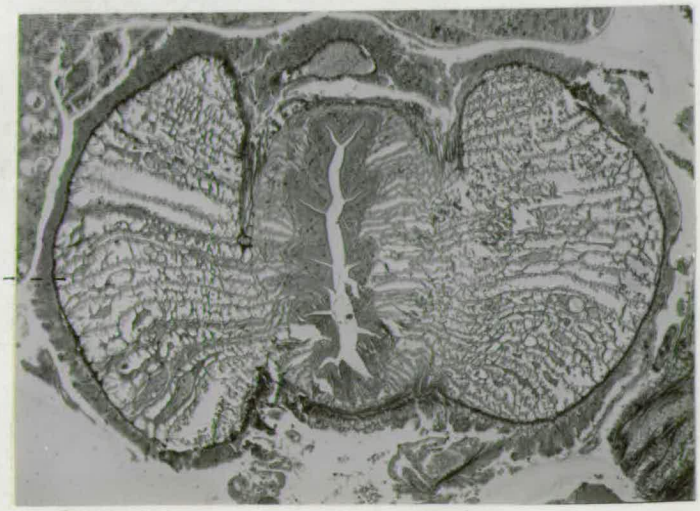


5

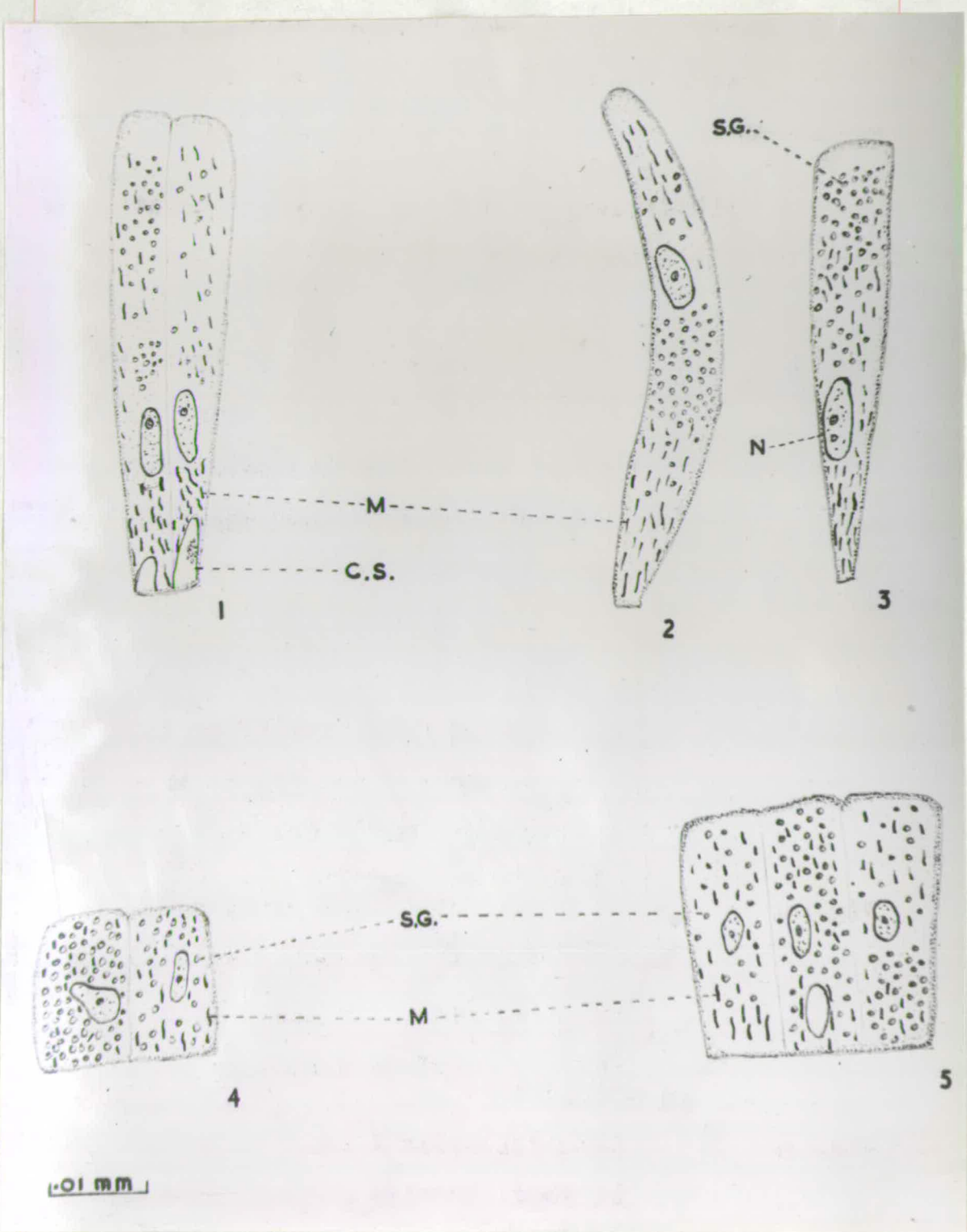
Drawings of the epithelial cells of the oesophageal pouch showing mitochondria and secretory granules.

All figures from Meves preparations stained with iron-haemotoxylin.

Figs. 1-3. Cells during a period of fast, showing secretory granules and mitochondria. The basal half of the cell contains more mitochondria than the lumen region. Clear spaces, with or without any secretory material, are seen in some cells close to the basement membrane. A light colourless area, free of mitochondria and secretory granules, is seen at the outer pole of the cell close to lumen.

Fig. 4. Cuboidal cells lining the tunnel. Showing great accumulation of secretory granules and a few small mitochondria during a period of fast.

Fig. 5. Cuboidal cells 4 hours after the intake of food, showing mitochondria and secretory granules.


S.G.
N
M
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1
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Drawings of the cells of the oesophageal pouches of earthworms killed 4 hours after being placed in soil.

All figures from Meves preparations stained with iron-haemotoxylin.

Figs. 1 and 3 Cells showing almost complete evac-:uation of the secretory granules. Nucleus has moved upwards towards the lumen. Mitochondria most numerous in the region of the lumen.

Fig. 2. Showing mass of secretory material between the cells in the basal region. Mitochondria are more numerous in the lumen half of the cell than the basal half.

Fig. 4. Showing secretory granules in the basal and in the lumen half of the cell. Mitochondria are smaller and not numerous.

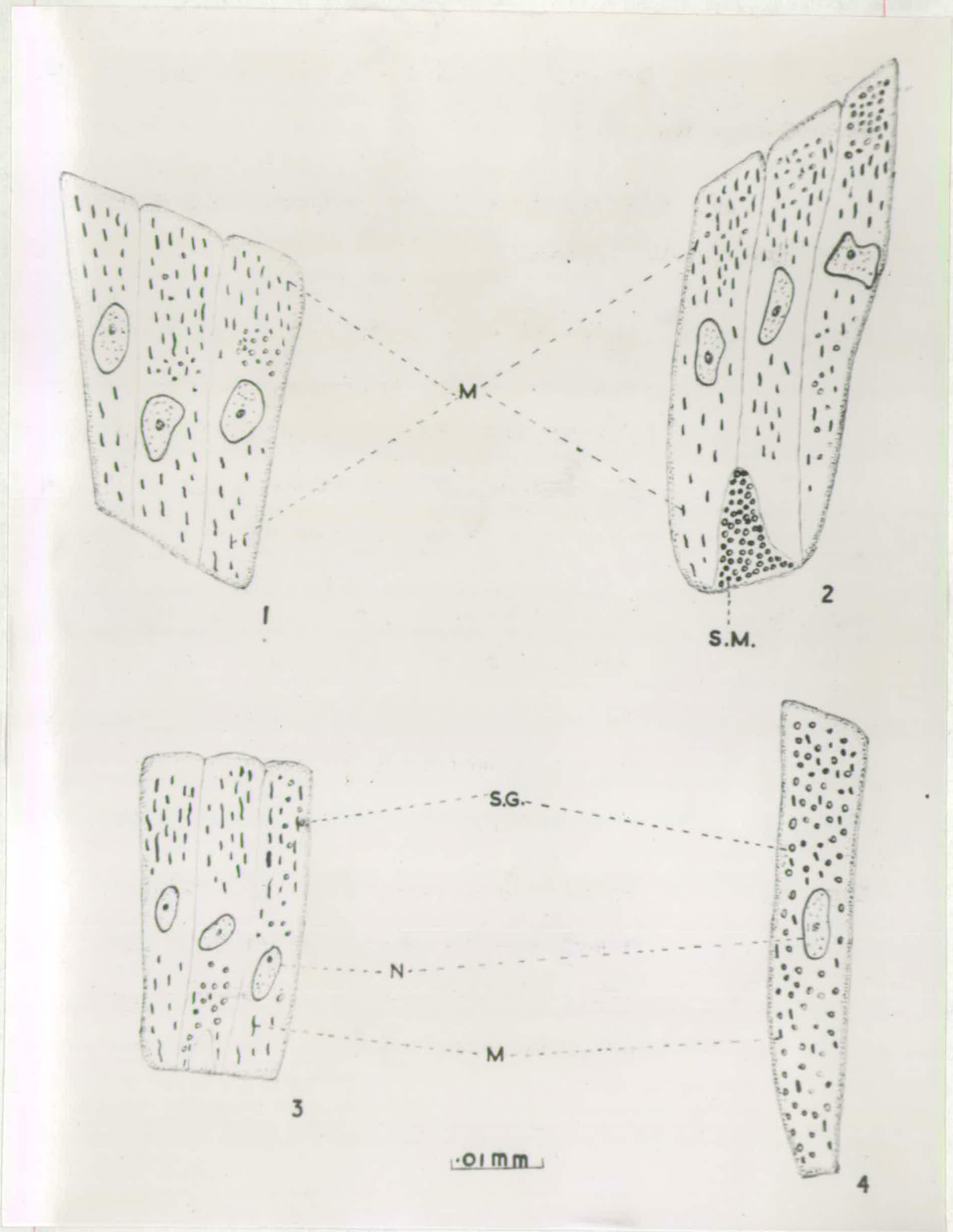
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PLATE XXXIX.

Drawings of the cells of the oesophageal pouches showing the Golgi material.

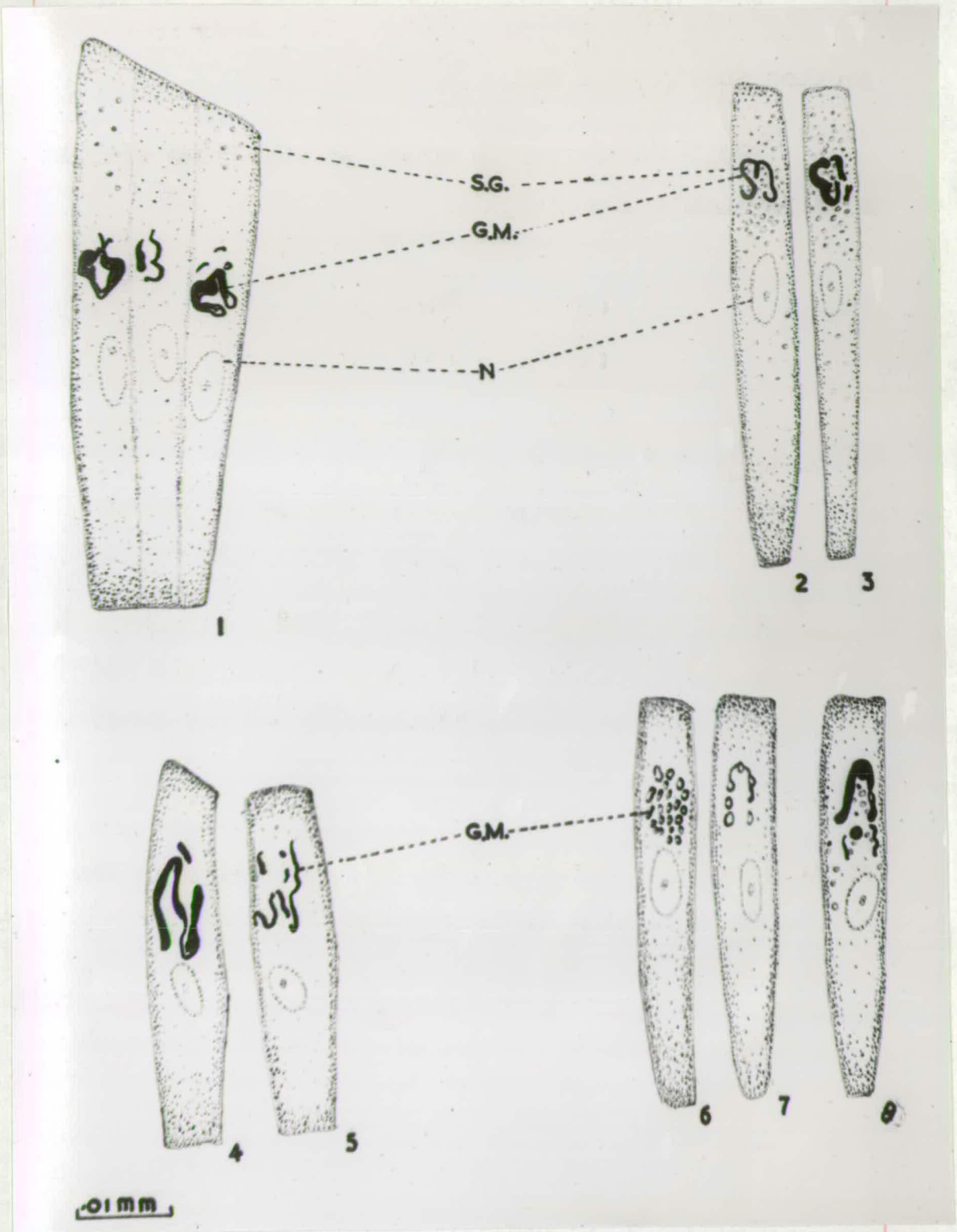
All figures from Aoyama preparations stained with Ehrlich's haematoxylin.

Fig. 1 Cells in the fasting stage, showing reduced Golgi material.

Figs. 2 and 3. Cells of worms which were in soil, showing association of the Golgi material with the secretory granules.

Figs. 4, 5 and 8. cells of worms which were in soil, showing hypertrophy of the Golgi material.

Figs. 6 and 7. cells showing Golgi material in the form of vesicles.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Drawings of the oesophageal gland-cells of earthworms showing Golgi material, mitochondria and secretory granules.

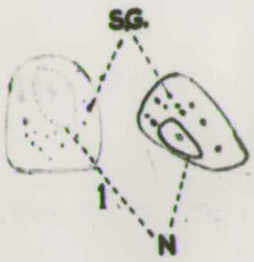
Figs. 1-4 from Aoyama and Kclatchev preparations.

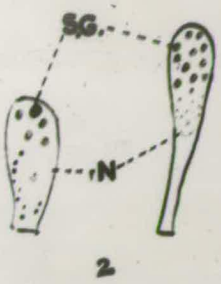
Fig. 5 from Flemming preparation.

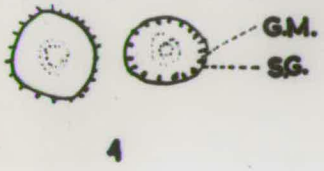
Figs. 1 and 2. Showing the gradual accumulation of the secretory granules, and the considerable elongation of the gland-cells following the accumula-:tion of the secretory granules.

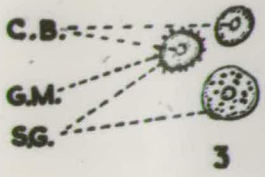
Figs. 3 and 4. Showing the Golgi material (osmiophilic granules) situated over the outer part of the secretory granules. Central body of the secretory granule is seen.

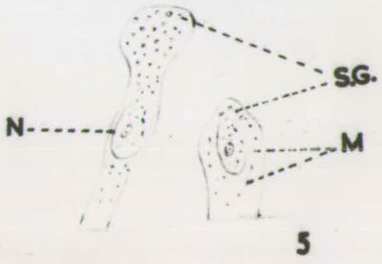
Fig. 5. Showing the mitochondria and secretory granules in the gland-cells.











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Photomicrographs of the oesophageal pouch of earthworms.

All figures from Meves preparations.

Fig. 1. Showing the oesophageal pouch in the 10th. segment. The transverse lamellae and the tunnels are seen clearly. x 80

Fig. 2. Cells of the oesophageal pouch during a period of fasting, showing the filamentous and rod-shaped mitochondria. x 1140

1

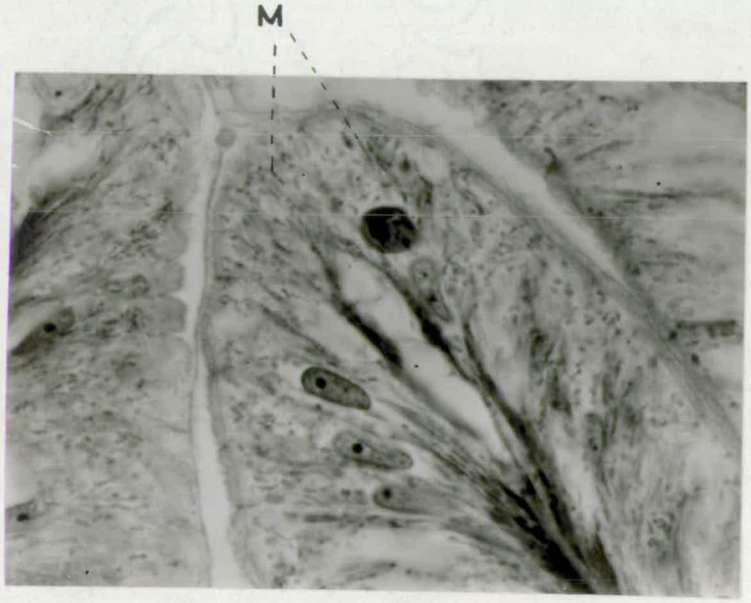

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PLATE XXXXII.

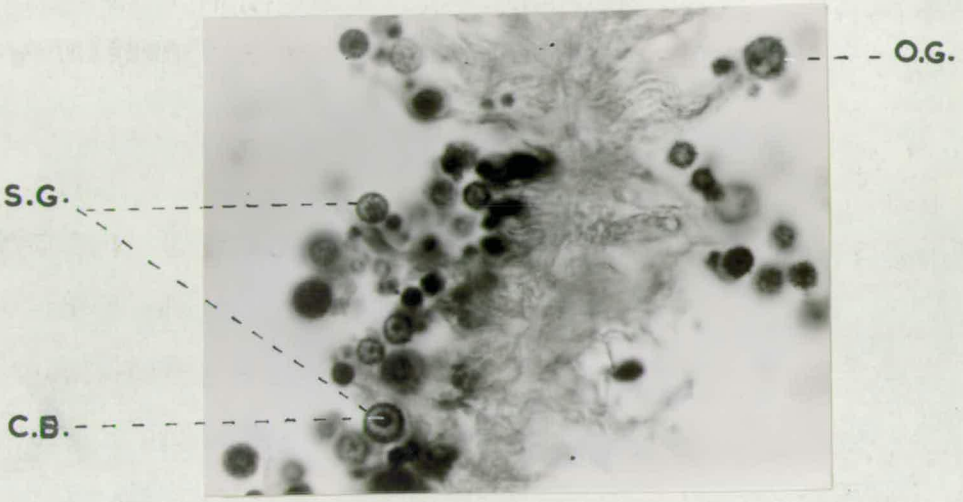
Photomicrographs of the oesophageal gland cells of Lumbricus showing the Golgi material.

All figures from Kolatchev preparations.

Fig. 1 x 1380

Fig. 2 x 1140

Figs. 1 and 2. Showing the Golgi material (osmiophilic granules) situated in contact with the secretory granules of the gland-cells. Some of the secretory granules show a central body very clearly.



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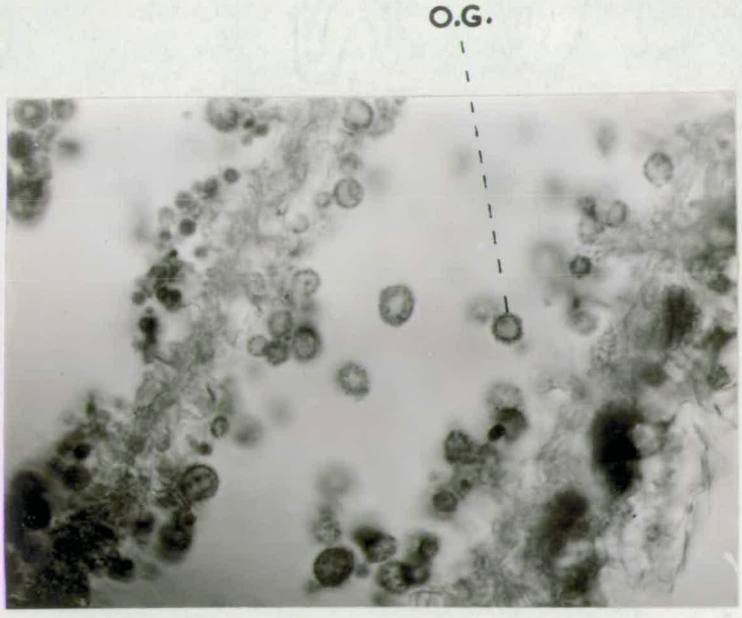

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XIII. DISCUSSION.

The mitochondria and the Golgi material have been studied more than any of the other extra-nuclear components. Many cytologists believe that they are concerned with the production of secretion and other cell products, but there is no general agreement as to the exact part which they play in these processes.

It is now universally agreed that the mitochondria are elements of definite form and are present in all animal cells. They may occur in the form of filaments, rods, or granules, capable of independent movement in the cytoplasm. The thickness of the mitochondria is said to be constant in individual cells. In tissue culture-cells (Bourne, 1942) they appear to be in a state of constant movement which may be of two types; a wriggling movement of the mitochondria themselves, or the migration of the individual rods etc. from one part of the cell to another. The migratory movements may be due to varying electric charges on the mitochondria and the cell membrane. Kingsbury (1912) and others consider that the mitochondria act as respiratory centres in the cell. This supposition rests on the fact that fat solvents, such as alcohol, acetone and acetic acid which reduce the rate of the respiration of the cell, also dissolve the mitochondria. Bourne (1942) states that/

that these fat solvents also dissolve the Golgi mate-:rial and the cell membrane; hence it is very difficult to assign to the mitochondria alone a respiratory function. Joyet-Lavergne (1938) states that the mito-:chondria possess great oxidising and reducing power, and believes that this is on account of a 'redox' system composed of vitamin A and glutathione. He used gold chloride, silver nitrate, chromic acid, picric acid, potassium permanganate and m-dinitroben-:zol to demonstrate the reducing power of mitochondria. In further support of this view, Joyet-Lavergne states that young red blood cells of vertebrates have a higher rate of respiration and a greater number of mitochondria than old erythrocytes. Bourne, however, regards the evidence put forward in favour of a respir-:atory function of the mitochondria to be insufficient, but admits that it is quite likely that they do play an important part in cell-respiration.

Horning (1926) believes that in Amoeba the mitochondria are concerned with the production of digestive enzymes. In Opalina (1925), he states, protein granules are formed under the influence of mitochondria. Horning (1925) points out that in the case of the pancreas the mitochondria are responsible for the production of zymogen granules, and that here instead of producing enzymes, as in Amoeba, for intra-cellular/

cellular digestion, they produce enzymes for extracellular digestion. He concludes that the function of the mitochondria in all animal cells is the same, and that they are, therefore, the seat of enzymatic activity. This view is shared by various other workers, but there is no general agreement that the mitochondria are concerned with the production of enzymes.

Cramer and Ludford (1925) have shown that the process of fat absorption in the mammalian intestine is assosciated with hypertrophy of the Golgi apparatus, but that the mitochondria remain unchanged throughout the cycle. In the thyroid gland both the Golgi material and the mitochondria enlarge during secretion, and Cramer and Ludford (1926) consider that the Golgi apparatus is probably actively engaged in the actual production of secretion, and that the changes in the mitochondria produce corresponding variations in surface energy within the cytoplasm, and thus affect redistribution of the lipoids. Later, Ludford (1927) states that synthesis of the enzymes occurs at the mitochondrial-cytoplasmic interface; the resulting products continually diffuse into the cytoplasm preventing an accumulation at the surface of the mitochondria which would inhibit further synthesis. At the surface of the Golgi apparatus, the elaborated products are concentrated into/

into droplets preliminary to their elimination. According to this view the Golgi material plays a purely physical role, namely condensing the products elaborated by the enzymes secreted by the mitochondria. Bourne is of the opinion that the mitochondria are probably involved in proteolytic and, in the case of germinating seeds, diastatic activity. Gresson (1948) thinks that there is reason to believe that mitochondria play an important part in the formation of secretion, and that enzyme activity takes place at the surface. Hirsch (1932) and Duthie (1934) believe that substances are separated out under the influence of the mitochondria in the basal cytoplasm of the cells of the pancreas, and that the young granules of secretion later on move to the region of the Golgi material, and are there transformed into mature secretory granules. Later, Ries (1935) con-:firmed their observations on the living pancreas.

Gresson states that it is possible that the mitochondria are concerned in some way with yolk-formation . It is well known that they form the sheath of the axial filament of the middle-piece of the sperm, and it is possible that they play some part in the physiology of the spermatozoon. In non-secretory cells, Gresson believes that the mitochondria take part in the formation of some of the cytoplasmic constituents and in the general metabolism of the cell.

Since the Golgi apparatus was first observed by Golgi in 1898, there has been much work on various aspects of this important cell-component. With a few exceptions the Golgi material cannot be seen in the living cell; consequently there has been considerable controversy in the past regarding its actual existence. The majority of modern cytologists believe that it is a definite structure or substance present in nearly all animal cells. Opinion, however, is widely divided concerning its structure and functions.

Many workers now believe that the Golgi mat-:erial seldom occurs as a true network or reticulum, but that it consists of a number of separate elements which may lie close together. Recently Baker (1944), as a result of his experiments with vital stains, has come to the conclusion that the Golgi material consists of vacuoles which contain fluid, and are surrounded by, or in contact with, lipoidal substances. Thus he be-:lieves the reticular appearance of the Golgi material in fixed cells to be an artifact caused by the shrink-:age of the vacuoles which are, in fact, the real Golgi structures in the living cells. Gresson (1948) states that the Golgi material consists of an outer argento-:philic and osmiophilic part and an inner argentophobic and osmiophobic region, and that it is made up of pro-:teins and lipoids.

As to the function of the Golgi material it is now universally agreed that in gland cells it takes/

takes part in the formation of a specific secretion, but the precise part played by the Golgi material in the process of secretion has not yet been established. Kirkman and Severinghaus (1938) believe that the Golgi material acts as a condensation membrane; others believe that it is concerned with the synthesis of various cell products. Hirsch (1939), who supports the latter view, thinks that the internum, or inner part, of the Golgi material absorbs vitamin C, and that later secretary granules originate in the internum. Bourne (1942), while agreeing that the Golgi material may absorb vitamin C, disagrees with Hirsch's views re-:garding the direct part played by the Golgi material as a synthesising agent. Another view is held by Subramaniam (1934, 1935. and 1937), who thinks that the Golgi material is chiefly concerned with the production of intra-cellular enzymes, which, later on, give rise to various products such as mucin, fat, yolk, etc.. He also believes that the Golgi substance may produce different materials at different times in the same cell. Gresson (1948) and Nath (1933) have claimed that in the eggs of some animals, chiefly invertebrates, vesicular Golgi bodies are finally transformed into fatty yolk globules. According to Ludford and Cramer (1925), the Golgi material syn-:thesizes fat from absorbed fatty acids and glycerol.

Gresson (1948) reviews some of the literature of the subject and believes that the chief function of the/

the Golgi material is the elaboration of substances which are formed by its synthetic action, or possibly by the production of intra-cellular enzymes. He also believes that the Golgi material takes part in the for-:mation of a wide variety of cell-products. He supports this belief by the fact that the during the secretory cycle of gland-cells, the Golgi material shows marked hypertrophy and thus occupies a larger area than during the resting or non-secretory stage. Further, small secretary granules come into contact with the Golgi material and portions of the Golgi material lose their connection with the main mass and move towards the lumen. When secretory activity is completed and the secretory granules are discharged into the lumen, the Golgi material assumes its original position and form.

Reference to the literature of the subject shows that there are few published papers on the cyt-:ology of gland cells of invertebrates. Gresson (1936) claims that in the salivary gland-cells of Tipula paludosa, a small clear space appears in the basal cytoplasm of fixed material which later increases in size until it extends to the border of the lumen. This clear space, he suggests, is a vesicular area into which a lipoid secretion has separated out from the basal cytoplasm. He believes that the mitochondria and the Golgi material take part in the origin of this secretory substance. He also suggests that these cell components take part in the formation of the secretion of/

of the cells of the mid-gut and hepatic caeca of Periplanata orientalis (1934). Subramanian (1938) worked on the intestinal cells of Lumbrico- nereis , and observed an increase in the number of Golgi grains during secretory activity, and an intimate relationship between the first secretory granules and the chromophobic region of the Golgi batonnets. Siang-Hsu (1947) studied the mid-gut epithelium of Drosophila melanogaster, and states that there is no visible evidence to suggest that the young secretory granules are separated out under the influence of mitochondria. According to him the Golgi material of the epithelial cells is in the form of crescent and rings, and the secretory granules are first visible inside this structure. He also claims that the Golgi material increases in amount during the growth of the secretory granules.

To summarize : There is considerable evidence that both mitochondria and Golgi material are concerned with the formation of granules of secretion and with the origin of other substances. There is, however, no general agreement as to the part which they play in the process. They may both be actively concerned with the synthesis of various cell products, or the materials which arise elsewhere in the cell may collect at the surface between these cell components and the cytoplasm. However, these points cannot be cleared up by the methods of research hitherto employed.

The present work was undertaken in order to study the structural and functional aspects of the mitochondria and the Golgi material of the various gland cells in the alimentary tract of Lumbricus terrestris during different physiological phases. As a result of this work much information was ob-:tained which has not hitherto been recorded for Lumbricus or any other earthworm.

When a worm has constant access to food, the cells show all stages of secretory activity. When one group of epithelial cells is undergoing increased cellular activity, another group of cells, adjacent to it on either side, is resting. This in-:dicates that when the secretory cycle is completed, a cell undergoes a period of rest, and that, under normal conditions, neighbouring cells, which are previously at rest, become active. The structure and arrangement of the mitochondria and the Golgi material, therefore, varies in the different cells.

In order to study cells under conditions of inactivity, it was found necessary to starve the worms, which were kept under moist conditions, for four to six days. It is of interest that worms starved for shorter periods did not show any apprec-:iable change as compared with individuals with con-:stant access to food.

Examination of material fixed after fasting showed that the majority of the cells (except those

of/

of the buccal region, pharyngeal gland, ciliated epithelial cells of the pharynx, gizzard, non-ciliated cells of the intestine, and of the oesophageal glands and pouches) were inactive, and that their components exhibited morphological features which differed from those observed in cells of animals fixed after feeding. A considerable accumulation of secretory material is the most noticeable feature during the inactive phase. The secretory material is situated either in the basal cytoplasm or very close to the lumen, and in some cases in the neighbourhood of the nucleus and in the Golgi field. This accumulation of the granules indicates that their production continues during the period of fasting but is retarded to some extent. It appears that when there is no contact with food, acc-:umulation of the secretory granules is the natural result.

The mitochondria in the epithelial cells of worms which have been fasting are aggregated towards the poles of the cell and also in the region adjacent to the nucleus, while a few occur around the Golgi field. The majority are rod-shaped but a few granules are occasionally present. In the majority of cells the mitochondria of the basal cytoplasm are longer than those of the lumen region. There is no marked polar orientation. The aggregation of the mitochondria at both poles of fasting cells may be due to the pressure exerted by the accumulated secretory material. The

Golgi/

Golgi material, which is now difficult to impregnate, especially in the pharynx and the oesophagus, is very much reduced in amount and individual elements are much thinner than in active cells.

The accumulation of the mitochondria in cer-:tain regions of the cell, the absence of polar orien-:tation, and the changes in t he Golgi material indicate that the cells are less active than when food is present in the alimentary canal.

When an animal is allowed to feed following a period of starvation, the majority of the cells enter upon a period of secretory activity; consequently, as a result of stimulation, the cytoplasmic components exhibit a different morphological pattern. Changes such as fragmentation and polar orientation of the mitochondria, hypertrophy and fragmentation of the Golgi material, are indications of the increased metabolic activity of the cell.

A gradual increase in cellular activity was obtained by feeding worms for one to twenty-four hours after a previous fast. The secretory granules which had accumulated during the period of fasting are dis-:charged into the lumen during the early stages of sec-:retory activity, and fresh secretory granules appear in the basal cytoplasm chiefly in the neighbourhood of the basal membrane. These early granules later migrate towards the nucleus and then into the Golgi field. In both mitochondrial and Golgi preparations of/

of material from all regions, the migration of the secretory granules from the basal cytoplasm to the Golgi field was clearly demonstrated in some of the cells. In both the fasting and the feeding phase clear spaces, which often contain a little secretory material, were commonly observed near the basement membrane. In some cases these spaces were surrounded by mitochondria. This indicates that the secretory material is probably separated out from the cytoplasm under the influence of the mitochondria.

The remarkable thickness of the elements of the Golgi material, its regular fragmentation at the highest peak of secretory activity, the migration of the early secretory granules from the basal cytoplasm to the Golgi zone, where they come into close contact with the Golgi elements, strongly suggests that the Golgi material plays a specific and vital part in the process of cell-secretion.

The present investigation demonstrates clearly that in the gland cells of the elementary tract of Lumbricus the earliest secretory granules first arise in the basal cytoplasm, chiefly close to the basement membrane, and that they migrate to the Golgi zone where, under the influence of the Golgi elements, the final stage in the formation of the mature secretory granules takes place.

The present observations on the origin of secretory/

secretory granules agrees with Hirsch and with Duthie's conclusions regarding the formation of the earliest secretory granules in the basal cytoplasm of the cells of the pancreas of the mouse and their further growth in the Golgi zone. It appears, therefore, that in both vertebrates and invertebrates the origin of the secretory granules in the gland cells is essentially similar. Chodnik (1947 and 1948), however, claims that the newly formed secretory granules in the cells of the alimentary tract of the domestic fowl are first visible in the Golgi field. It may be that Chodnik failed to observe the very young secretory granules in the basal cytoplasm of the gut epithelial cells of the fowl, or that, owing to their chemical nature the young granules are invisible with the technique employed. This would seem to be a more likely explanation than that there is any fundamental difference in the process of secretion in the earthworm and fowl.

In the ciliated epithelial cells of the pharynx there is no appreciable change in the morphology of the cytoplasmic components. This is probably due to the fact that the production of mucin is constant and is not affected by fasting or feeding. Similarly there is no appreciable change in the cells of the buccal cavity, the gizzard, the non-ciliated cells of the intestinal epithelium, and the oesophageal glands and pouches.

The work recorded here is in general agreement with the observations of other cytologists regarding the behaviour of the mitochondria and Golgi material of gland cells. The present investigations on the gland cells of the alimentary tract of Lumbricus is the first attempt to carry out a cytological study of the entire alimentary canal of an invertebrate animal. The writer believes, therefore, that it yields new information regarding the secretory cycle, absorption, certain histological details of the gut of the earth-worm, and on the behaviour of the cytoplasmic components correlated with the phases of physiological activity. It is hoped that this account may serve as a stimulus for further research on the cells of invertebrates, particularly on the functional aspects of the Golgi material and the mitochondria. There is still room for much research, not only on the form and behaviour of these important cell components, but on the development of new techniques designed to yield further knowledge of their finer structure and chemical nature.

XIV. SUMMARY.

Buccal Cavity

1. The epithelial cells are columnar, non-ciliated and lined by a thin cuticle.

2. The mitochondria of the epithelial cells are in the form of rods and granules. They show little change correlated with functional activity. Polar orientation is a constant feature in the majority of the cells.

3. The Golgi material of the epithelial cells lies above the nucleus, and is in the form of rods and filaments. The functional stage is marked by the hypertrophy of the Golgi material leading to its final fragmentation into short rods and granules.

4. Secretory granules are first visible in the basal cytoplasm close to the basement membrane. Later they migrate to the Golgi zone and assume their final form. The production of secretory granules is a constant feature during feeding and fasting.

Pharynx

1. The epithelial cells are of two types, namely ciliated and non-ciliated. The cells of the dorsal epithelium are ciliated, but the ventral epithelium consists of ciliated and non-ciliated cells. The non-ciliated cells of the pharyngeal shelf are broader and/

and less elogated than those of the ventral epithe-
:lium. Salivary ducts and discharge-pockets are present in the dorsal epithelium.

2. The mitochondria of both the ciliated and non-ciliated cells are in the form of short rods and granules. Granules occur chiefly near the nucleus and in the vicinity of the lumen, whereas the rods are distributed throughout the cell. Due to fragmenta-
:tion the functional stage is marked by an increase in the number of mitochondria.

3. The Golgi material in the ciliated cells consists of thin filaments, rods and a few granules. The filaments and rods are arranged parallel to the logitudinal axis of the cells. There is little change in the morphology or location of the Golgi material during secretory activity. The Golgi material of the non-ciliated cells is very much reduced in amount during the resting stage. During cellular activity it undergoes marked hypertrophy and finally breaks up into rods and granules. Reversed polarity of the Golgi material was sometimes observed; it is due to the migration of the nucleus during increased cellular activity.

4. Secretory granules arise in the basal region of ciliated and non-ciliated cells from where they migrate to the Golgi field. Feeding stimulates the evacuation of the accumulated material and immediately stimulates new production.

Oesophagus

1. The epithelial cells are ciliated, columnar and lined with a thin cuticle.

2. The mitochondria consist of rods and granules, and rarely a few filamentous forms. The functional stage is marked by fragmentation and polar orientation of the mitochondria.

3. The Golgi material resembles in morphology, arrangement and behaviour that of the non-ciliated cells of the pharynx.

4. Secretory granules arise in the basal cyto-:plasm. Feeding stimulates the production of new granules and evacuation of the stored granules.

Crop.

1. The epithelial cells are tall, cylindrical and columnar; they possess a striated border covered by a cuticle. The cells are of uniform size.

2. The mitochondria are in the form of thick rods and a few granules. The functional stage is marked by fragmentation of the rods into granules. Polar orientation is a constant feature.

3. The Golgi material consists of thick rods and filaments and a few granules. During feeding there is a slight increase in the amount of Golgi material; fragmentation takes place.

4. Considerable accumulation of the secretory granules/

granules in the lumen region of the cell takes place during the fasting period. The functional stage is well marked by the evacuation of secretory granules from the cell and the fresh origin of granules in the basal region. Migration of granules to the Golgi field is clearly demonstrated.

Gizzard

1. The epithelial cells are arranged in protruding lamellae which form elongated crypts, filled with keratinoid secretory material. Cells of the apices of the lamellae are columnar and elongate, whereas those between the apices are short and cuboidal. The cells are ciliated and covered by a thick cuticle.

2. The mitochondria consist of thin short rods and granules. The functional stage is not well marked.

3. The Golgi material is in the form of small rods, filaments and a few granules. Feeding does not induce any appreciable change.

4. Secretory granules arise in the basal region; their production is constant during feeding and fasting. Some of these granules, in the later stage of their formation, are visible in the Golgi field.

Intestinal epithelium

1. Ciliated and non-ciliated cells are present. According to the present observations, secretory granules/

granules are formed in ciliated and non-ciliated cells.

2. The mitochondria in the ciliated cells of the anterior and middle regions of the intestinal epithelium, are in the form of rods and granules. In the posterior region rods only are present. In the early stages of cellular activity fragmentation of the mitochondria occurs. Non-ciliated cells, which are numerous in the anterior region and scanty in the posterior region, contain rod-shaped and granular mitochondria. The functional stage is marked by the fragmentation of the mitochondria.

3. The Golgi material in the ciliated cells is in the form of rods and filaments. The functional stage is marked by the hypertrophy of the Golgi material and its fragmentation into granules. In the non-ciliated cells the Golgi material is in the form of rods, filaments and granules; it surrounds the accumulated secretory granules. It breaks up during secretory activity.

4. The earliest secretory granules in both types of cells are first visible in the basal cytoplasm. They migrate to the Golgi field. Feeding stimulates the evacuation of the granules and the formation of fresh granules. The non-ciliated cells, however, do not show much change, and the production of secretory granules is more or less constant.

5. The cells of the anterior region of the intestinal/

intestinal epithelium are not concerned with the absorption of food. The absorptive regions are the mid-intestine and to a greater extent the posterior intestine. Absorption is carried on by the ciliated cells only.

Pharyngeal glands

1. Due to their great affinity for dyes, the cells of the pharyngeal glands are very conspicuous. They are polymorphic. Each cell possess a large nucleus and one very large nucleolus.

2. The mitochondria are in the form of thick rods and granules. Feeding does not induce much change in their form or disposition. Polar orientation was not observed.

3. The Golgi material is in the form of thin filaments, rods and granules scattered throughout the cell. Feeding does not induce much change except that fragmentation of some of the filaments and rods takes place. The Golgi material of these cells is never in a compact form.

4. The cells of the pharyngeal glands secrete mucin as well as digestive enzymes and are thus salivary in nature. When a cell becomes full of sec-:retory granules, the cell-membrane usually disappears and the secretion is discharged along with the nucleus; this results in the disintegration of the cell. The production of secretory granules is constant.

Oesophageal pouches and glands

1. The histological appearance of the oesophageal pouches and the oesophageal glands is totally different. The cells of the pouches are glandular.

2. The mitochondria in the cells of the oesophageal pouches are in the form of rods and filaments which break up into smaller bodies during secretory activity.

3. The Golgi material in the cells of the pouches consists of rods, filaments and granules. The functional stage is not well marked.

4. Production of secretion in the pouches and in the glands is constant.

XV. CONCLUSIONS.

1. The mitochondria and the Golgi material are active components of the cell. In most of the gland cells of the alimentary canal of Lumbricus, they show a marked rythmic behaviour which is correlated with the various physiological stages of the cell.

2. The secretory granules first arise in the basal cytoplasm in the neighbourhood of and probably under the influence of the mitochondria. They migrate to the Golgi field, come into direct contact with the Golgi elements, and are transformed into the mature granules of secretion. It is concluded that both the mitochondria and the Golgi material play a vital role in the formation of the secretion of the gland cells of the alimentary tract of Lumbricus.

3. The oesophageal pouches are glandular in form and in function.

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