

A Light and Electron Microscope Study of the Epithelial Cells of the Gut of *Fasciola hepatica* L.

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ABSTRACT

The structure of the epithelial cells of the alimentary tract of *Fasciola hepatica* was investigated by means of light and electron microscopy. Tissue prepared for electron microscopy was fixed in 1 per cent osmium tetroxide, buffered with veronal to a pH of 7.4, and embedded in butyl methacrylate with 1 per cent benzoyl peroxide as a catalyst. Polymerisation was carried out at 60°C. The majority, if not all, the epithelial cells pass through both absorptive and secretory cycles. The free ends of absorptive cells possess fine protoplasmic processes that project into the lumen of the gut. These are apparently concerned with the absorption of nutriment. In electron micrographs, the protoplasmic (absorptive) processes are frequently seen to be in the form of tubular loops both ends of which arise from the same cell. The free end of a process is often expanded into a ribbon-like structure. Each process possesses an external limiting membrane and an internal membranous ultrastructure. When a cell becomes glandular in function, the protoplasmic processes seem to become less numerous. The plasma membrane is invaginated into the basal part of an absorptive cell. In the neighbourhood of the lumen of the gut where two tall cells are in contact, bands of amorphous cytoplasmic material are in contact with each cell membrane.

INTRODUCTION

Although the general histological features of *Fasciola hepatica* are known, a study of the minute structure of its cells, other than those of the gonads, has not previously been attempted. The histology of the alimentary canal and the nature of the food of this animal has been investigated by some earlier workers, and brief references to their main conclusions are given by Stephenson (4). In view of the incomplete nature of the earlier work and lack of knowledge of the cytology of trematodes generally, we considered that a study of the epithelial cells of the intestine of *F. hepatica* would yield results of interest. Consequently, an investigation of the structure of the gut epithelium and of the fine structure of its cells was carried out.

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Material and Methods

Living flukes (*F. hepatica*) were removed from the livers of sheep. Small pieces containing parts of the alimentary tract were dissected out and immediately placed in fixing fluid. Several histological fixatives were used in a preliminary investigation and Bouin's picroformal (saturated aqueous solution of picric acid 75 cc., formol 25 cc., acetic acid 5 cc.) was found to be the most satisfactory. Sections were cut at 5 μ and 7.5 μ in thickness and stained with iron haematoxylin. Eosin or light green was used as a counterstain.

For work with the electron microscope, living flukes were dissected in 1 per cent osmium tetroxide buffered with veronal to a pH of 7.4. Small pieces containing parts of the intestine were quickly transferred to tubes containing the fixing agent. The tissue was embedded in a mixture of 85 per cent *n*-butyl and 15 per cent methyl methacrylate with 1 per cent benzoyl peroxide. Polymerisation was carried out at 60°C.

OBSERVATIONS

Light Microscopy:

The wall of the intestinal crura, or caeca, consists of a single layer of epithelial cells, a basement membrane and a thin external layer of muscle fibres. When food is absent, or present in small quantities, the majority of the epithelial cells are tall, being columnar and pyramidal in form (Fig. 1). A few short cells usually occur singly or in small groups distributed amongst the other elements of the epithelium. When a large amount of food is present, the epithelium adjacent to it consists of very short or flat cells. In many of the sections of the lateral branches of the gut short cells are not present and the columnar and pyramidal cells are taller than those of the caeca. Consequently, the lumen of a lateral branch may be reduced to a narrow slit. If, however, a large quantity of food is present, the lumen is usually a spacious cavity and the epithelial layer is composed of short cells (Fig. 2).

The boundaries between the very short epithelial cells are impossible to determine with certainty. Slender processes arise from the inner margin of a cell and project into the lumen of the crura or their branches. These processes vary in length, but that of the longest is roughly equal to the height of the cell from which they arise. It was not possible, however, with the light microscope to determine with certainty the nature of these cell processes. As, when food is present, considerable areas of the gut are composed of flat cells, we conclude that they are concerned with the absorption of nutriment. The surface area of the free ends of the epithelial cells is considerably increased by the presence of the cell processes. We shall, therefore, refer to the latter as the absorptive processes.

If the crura or their lateral branches contain small quantities of food, short columnar and short pyramidal cells are present in some regions of the epithelium (Fig. 3). Absorptive processes which resemble those of the flat cells project into the lumen.

When food is absent from parts of the gut the majority or all of the epithelial cells in these regions are elongate, being roughly columnar, pyramidal, and sometimes club-shaped. The club-shaped cells contain a secretion that is passed into the lumen (Fig. 4). A careful study of the different types of epithelial cells, suggests that the gland cells arise through the transformation of flat cells. We con-

clude that the sequence of events is as follows. A flat cell increases in height and becomes roughly pyramidal or columnar in form (Fig. 3). Later, secretion accumulates in the cytoplasm between the nucleus and the lumen. As it increases in amount it extends towards both the lumen and the basal region, so that the half or two-thirds of the cell adjacent to the lumen increases in diameter and the cell becomes club-shaped (Fig. 4). When a large area of the lining of the gut is composed of gland cells, the latter are often arranged in folds each consisting of three, four, or more cells. Neighbouring folds are separated by one or two short columnar cells.

During the early stages of the accumulation of secretion, absorptive processes, similar to those of the flat and small pyramidal cells, are clearly visible. In the later phases of glandular activity they are less numerous and less clearly defined. While the determination of the exact arrangement of the processes lies beyond the limits of the light microscope, appearances suggest that some, at least, are sloughed off the cells from which they originate.

As flat, short columnar, short pyramidal, and tall absorptive cells are present, we believe that, like the gland cells, the taller types originate from flat cells. The taller absorptive cells may occur in small groups amongst the gland cells (Fig. 5) or may constitute the epithelium of a wide area of the gut. In the latter case they are arranged in folds each of which is separated from adjacent folds by one or two short columnar cells (Fig. 1). Relative to the length of the cells from which they arise, the absorptive processes of the tall cells are considerably shorter than those of the flat cells.

Absorptive cells occur throughout the intestine. Gland cells are often present in sections taken from the anterior and middle parts of the body but are few or absent in preparations of the more posterior regions of the gut. Epithelial cells in process of division were not observed in any of our sections.

Electron Microscopy:

All types of absorptive cells are shown in our electron micrographs but, although sections were cut from different regions of the gut of a large number of individuals, gland cells are not represented. An examination of histological preparations indicates that gland cells are much less numerous than absorptive cells and that they are often absent

from considerable areas of the intestine. Numerous small dense bodies are present in the vicinity of the nucleus of some of the short cells seen in electron micrographs. It is probable that these cells represent an early phase of the secretory cycle but that differentiation has not proceeded far enough to allow of a more positive identification.

The plasma membrane is visible in micrographs of both short and tall absorptive cells. As in the case of histological preparations, the boundary between neighbouring flat cells is not well defined. The part of the plasma membrane adjacent to the basement membrane is a dense structure that is shown clearly in micrographs of the taller cells. It possesses a number of invaginations extending from the vicinity of the basement membrane into the neighbouring cytoplasm (Fig. 6). Where two cells are in contact their outlines are irregular and their membranes are in close contact (Fig. 8). In the vicinity of the lumen, the membranes of two neighbouring tall cells, when in contact, are accompanied by a number of dense bands of amorphous material resident in the cytoplasm and in contact with the membranes (Figs. 7 and 9). Similar structures are not associated with the plasma membrane of the middle, basal part, and free end of a cell.

An examination of micrographs of the free ends of short and tall absorptive cells reveals the structure of the absorptive processes previously observed under the light microscope. These processes are frequently visible as loops, each end of a loop being in contact with the same cell (Figs. 8 and 14). Occasionally a small loop lies within a series of three or four progressively larger loops (Figs. 8 and 14). Owing to their delicate nature, the majority of the absorptive processes present in ultrathin sections are not shown throughout their entire length, and often a single point of attachment only is visible. It was not possible, therefore, to determine with certainty whether or not the loops are usually arranged in series in which each member, except the outer one, lies within a larger loop. It is possible that single processes are also present. Such a condition might arise through a break in a loop resulting in two single structures. Very occasionally forked processes were observed (Fig. 10).

In cross-section the processes appear as round or oval bodies according to the angle at which they are cut (Fig. 10). We conclude that the processes are often tubular in form throughout their length,

but that their free ends may be expanded to form thin ribbon-like structures. A bulb-like termination sometimes present at the end of a process is, we believe, a section through an expanded region of a process (Figs. 10, 11, and 13).

A few very small loops and minute projections are present on the free surface of some of the cells examined (Fig. 14). This suggests that new processes are formed during the absorptive phase and that these rapidly increase in size.

An absorptive process is limited externally by a membrane which consists of two dense components separated by a less dense area (Figs. 9 to 14). The two dense components lie close together and are seldom shown clearly in electron micrographs. The outer one, at least, appears to be continuous with the plasma membrane. The interior of a process is continuous with the cytoplasm of the cell and, in general, is slightly denser than the latter. In micrographs of high resolution thin internal membranes, extending across the interiors of some of the processes, are visible. These, and perhaps other membranous structures, are shown in transverse sections of the processes, but their exact arrangement could not be determined (Figs. 10 and 13).

A number of round, oval, and elongate dense bodies are present in the cytoplasm (Figs. 7, 9, 12, and 14). Some of these represent material recently absorbed from the lumen, or else are storage bodies, and others are probably mitochondria. The latter are poorly defined.

The nuclei of absorptive cells are clearly shown (Fig. 12). The nuclear membrane is a dense structure which follows a somewhat irregular course. The nucleolus contains a considerable amount of dense material, and granules and irregularly shaped bodies are distributed through the nucleoplasm.

The basement membrane is a relatively thick structure upon which the plasma membrane of an epithelial cell rests (Fig. 6). It possesses an internal structure of irregularly shaped lacunae containing little dense material.

DISCUSSION

As gland cells are never present in a part of the alimentary tract containing food, the discharge of secretion and the transformation of gland cells to flat absorptive cells must take place very rapidly. If we are correct in concluding that the absorptive processes undergo retrogressive changes when secretion accumulates within a cell, the changes from a secretory to an absorptive cycle must involve

the rapid formation of new absorptive processes. It is unlikely that the discharge of secretion and the formation of new absorptive processes occur as a result of actual contact with food. Were this the case, it is reasonable to suppose that gland cells would sometimes be seen amongst the cells of the stretched epithelium. As cells that could positively be identified as stages of the transformation of a secretory to a flat cell were never observed, we suggest that the presence of food in an anterior region of the gut acts as a stimulus resulting in the discharge of secretion from gland cells situated more posteriorly. It is possible, of course, that some cells are absorptive throughout the whole or the greater part of their lives and that they undergo changes in height governed by the presence or absence of food.

Sommer (3) and Müller (2) recorded that the intestinal epithelium of *Fasciola hepatica* is composed of a single layer of cells and that the latter vary in height. Müller concluded that the cells lack differentiation, other than that of height, and are concerned with both secretion and absorption. According to him the cells become flat after the discharge of their secretory product and tall after the completion of the absorptive phase. Stephenson (4) pointed out that Müller's conclusions were "mainly based on the assumption that a short cell is necessarily a small one." The former computed the volume of tall and short cells and claimed that "cell volumes are virtually independent of cell heights," and that Müller's conclusions were incorrect. Stephenson, however, stated that Müller was probably right in concluding that each cell is both secretory and absorptive in function. He added that the various phases "cannot be correlated with certainty with any gross changes in the form of the cells." Our conclusions regarding the changes in height of the epithelium agree in general with those of Müller. We believe, however, that tall cells lacking secretion are still capable of absorbing material from the lumen of the gut.

Protoplasmic processes arise from the free ends of the epithelial cells of the gut of some trematodes (Dawes, 1). Sommer (3) described these structures as pseudopodia and stated that they make their appearance when food is present in the intestine of *Fasciola*. According to Müller (2) they are fine and hyaline and are present in some parts of the

alimentary canal but are absent from others. Our electron micrographs demonstrate, for the first time, the structure of the protoplasmic (absorptive) processes and their relationship to the cells from which they arise. As far as we are aware, similar protoplasmic processes have not been described for the cells of any other animal. It is apparent that the possession of these structures will greatly increase the surface area of the part of an epithelial cell in contact with food in the lumen of the gut. We had some difficulty in determining the nature of the absorptive processes. Frequently, they seem to be in the form of tubular loops with both ends attached to the same cell. In many cases, however, the free end of a process is somewhat flattened and ribbon-like. This flattening is suggested by variation in the diameter of a process as seen in electron micrographs and a reversed distribution of density.

The presence of bands of dense amorphous cytoplasmic material associated with the plasma membranes of parts of cells in contact in the vicinity of the lumen of the gut, is in marked contrast to the absence of these structures in other regions of the same cells. As these bands are not visible at the free surface of epithelial cells, from which absorptive processes arise, they do not appear to be connected morphologically with the latter. They do not seem to be associated with any cytoplasmic structure other than the plasma membrane.

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EXPLANATION OF PLATES

PLATE 99

FIG. 1. Photomicrograph of transverse section of part of wall of intestinal caecum to show tall columnar absorptive epithelial cells (*T*). These cells are arranged in folds. A few short cells (*S.C.*), probably in early phase of secretory cycle, are visible between the folds. The tall cells are provided with protoplasmic (absorptive) processes (*A*). *M*, muscle layer of gut wall.

FIG. 2. Photomicrograph of three short epithelial cells. *A*, absorptive processes.

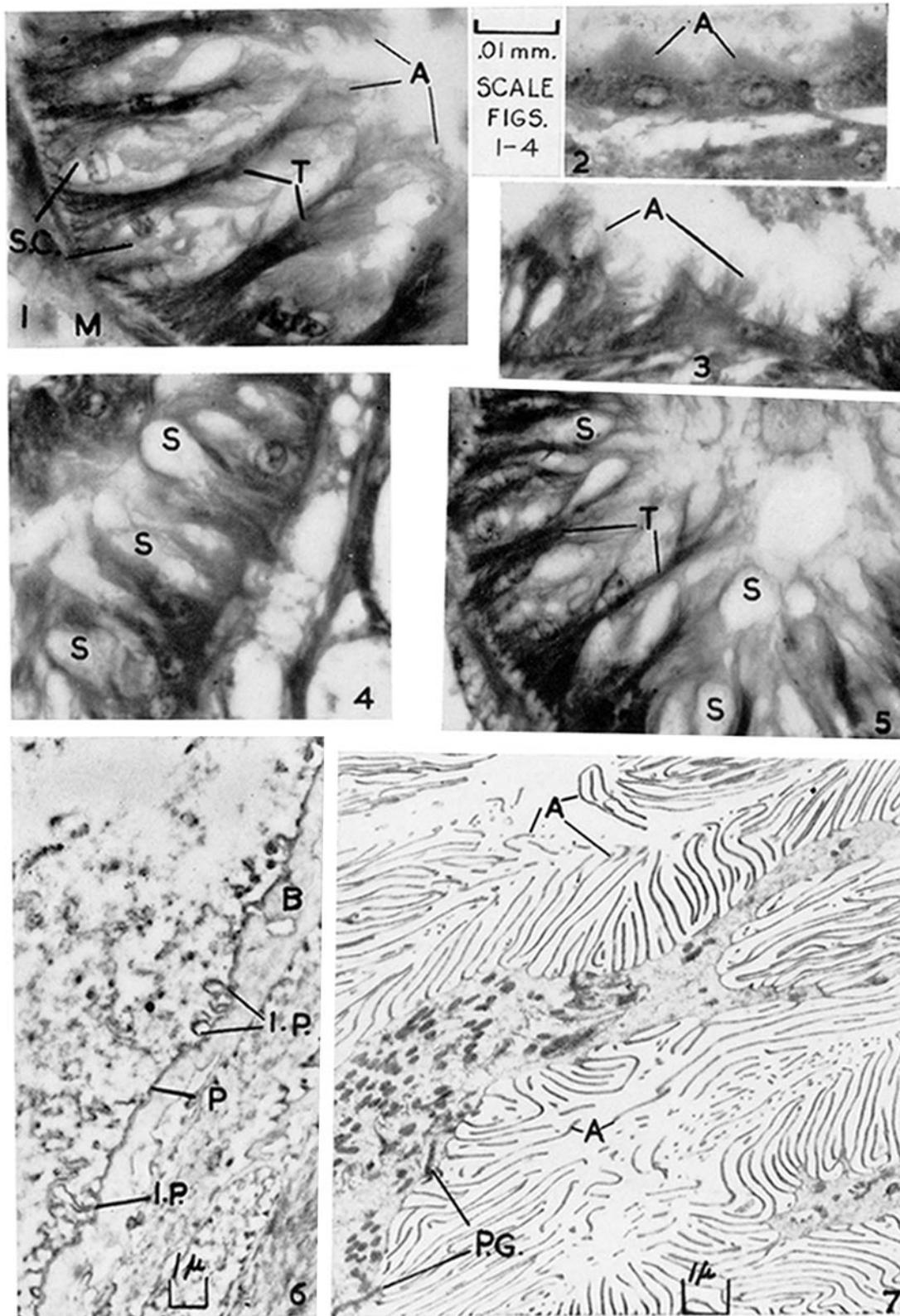
FIG. 3. Photomicrograph to show short pyramidal epithelial cells.

FIG. 4. Photomicrograph of section of lateral branch of caecum to show gland cells. The expanded free ends of the cells contain secretion (*S*).

FIG. 5. Photomicrograph to show gland cells (*S*) and tall absorptive cells (*T*).

FIG. 6. Electron micrograph of basal part of absorptive cells to show plasma membrane (*P*) and basement membrane (*B*). *I.P.*, Intracellular invaginations of the plasma membrane. $\times 5,807$.

FIG. 7. Electron micrograph of free ends of absorptive cells to show absorptive processes (*A*) and short regions of the plasma membranes of two cells in contact (*P.G.*). $\times 6,895$.



(Gresson and Threadgold: Epithelial cells of *F. hepatica*)

PLATE 100

FIG. 8. Electron micrograph of parts of free ends of absorptive cells to show absorptive processes (*A*) and plasma membrane (*P*). At (*A.I*) two complete loops are shown, one lying within the other. $\times 7,317$.

FIG. 9. Higher magnification of parts of two cells in contact to show their plasma membranes and associated bands of amorphous cytoplasmic material (*P.G.*). $\times 26,707$.

FIG. 10. Electron micrograph of absorptive processes cut at various angles. *I.N.*, internal structure of the absorptive processes. $\times 29,260$.

FIG. 11. Electron micrograph of free ends of absorptive cells to show absorptive processes. $\times 24,146$.

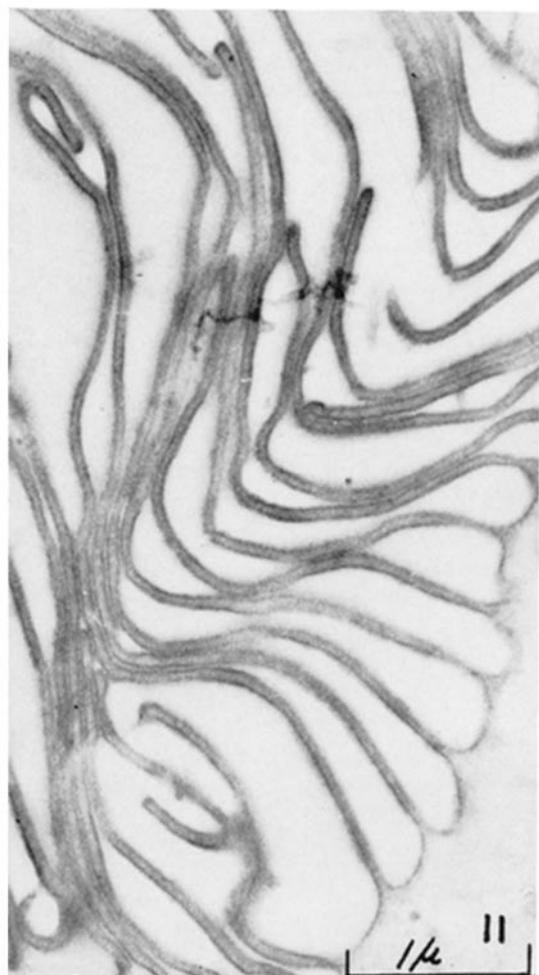
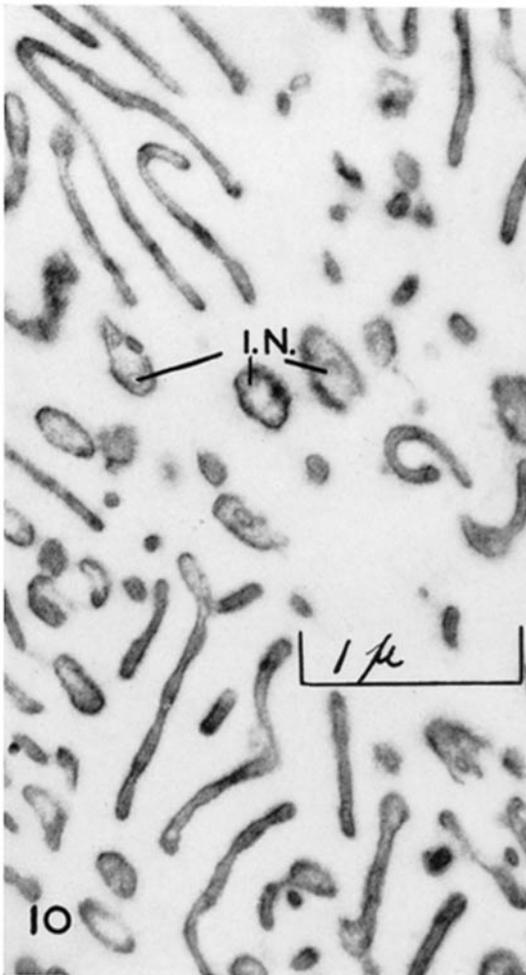
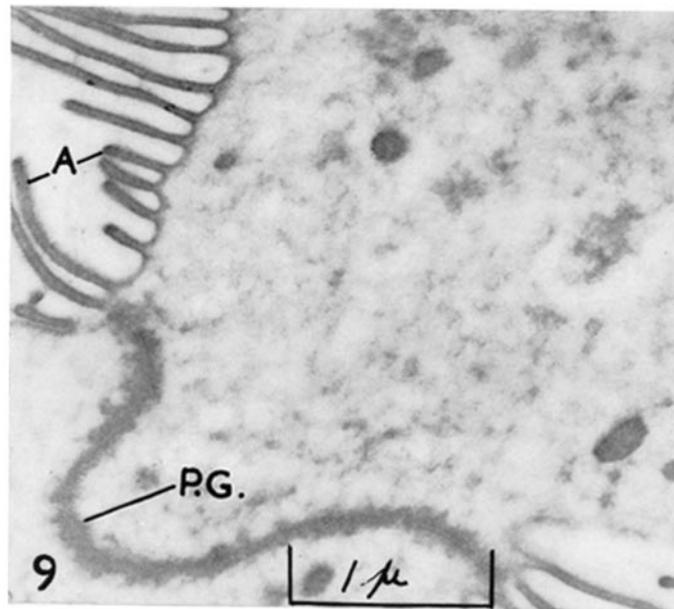


PLATE 101

FIG. 12. Electron micrograph of part of short pyramidal cell. *N*, nucleus; *Nu*, nucleolus. $\times 6,480$.

FIG. 13. Higher magnification of parts of the absorptive processes shown in Fig. 11. $\times 39,600$.

FIG. 14. Electron micrograph of free end of absorptive cell. At *A.1*, an absorptive process is shown within a larger loop. At *A.2*, a bulge on the surface of the cell probably represents an early stage in the formation of a loop. $\times 16,560$.

