STUDIES ON THE BIOLOGY OF THE SEA URCHIN

Ⅳ. Histological Observation of the Food Canal of *Strongylocentrotus intermedius*

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Anatomical and histological descriptions of the food canal of the sea urchin given by Hamann (1887), Chadwick (1900), and Stott (1955), may be incomplete and certain features require confirmation, bacause they have not presented any descriptions concerning the reserved materials and other biochemical elements, which seem necessary in a discussion of the functional aspects of the food canal. Although ***Strongylocentrotus intermedius*** is a common subtidal species in much of its range along the coast of Hokkaido, no detailed anatomical or histological studies of its food canal appear to have been made.

Consequently, the present report is an account of studies undertaken to clear up some of the existing gaps in knowledge of the histology of the food canal, and to correlate by means of histochemical techniques the structual and functional aspects of the cells comprising the lining epithelium of the food canal.

Before proceeding further, the writer is pleased to record his indebtedness to Prof. T. Tamura of Hokkaido University, for his continued encouragement and guidance. Acknowledgement is also due to Mr. Y. Ogawa and Mr. H. Kimura for the supply of living materials.

Material and Method

For most of the experiments reported here, sea urchins were collected at Sumiyoshi, Hakodate. In a few instances specimens were obtained from Ishiya, along the coast of Volcano Bay, southern Hokkaido. They were adult specimens ranging in size from 40 mm to 70 mm in test diameter.

They were dissected and portions of the food canal were fixed in a fixative for further study. These materials were subsequently processed and imbedded as appropriate for use of the following histological and histochemical techniques.

For general orientation of cell structure, tissues were fixed in Bouin's fixative, imbedded in paraffin, and sectioned at 8 μ. These sections were stained with Heidenhain's iron alum haematoxylin or Delafield haematoxylin.

For demonstration of glycogen and related compounds, fixation was in Gender's Allen-Bouin. After preparation of paraffin sections, the periodic acid-Schiff (PAS) routine was followed. Control slides expsoed to the action of a buffered solution of saliva were

used for differentiation between glycogen and other PAS-positive substances. For the recognition of mucin and other similar compounds, paraffin sections of tissues fixed in 10% formallin were stained overnight in a very dilute aqueous solution of toluidine blue and dehydrated with 50% alcohol. This routine brings about a metachromatic staining of acidpolysaccharide elements.

For recognition of lipid, tissues fixed in formal-saline, were imbedded in gelatin, and frozen sectioned at 10 μ. Sections were mounted in 1% gelatin solution then coloured with Sudan Ⅲ.

Alkalin phosphatase activity was detected in tissue fixed in 80% alcohol, and carried through the routine of Gomori.

Observation

The food canal of the sea urchin has four parts: pharynx, oesophagus, gut, and rectum. The gut has two loops; the first loop is clockwise, looking from the mouth, and the second doubles back upon the first one. The first loop differs from the second loop in having different nutritive functions and cell contents as pointed out in the following description. Accordingly these loops are indicated in terms of ***stomach*** and ***intestine*** respectively in the present paper.

The wall of the food canal is relatively simple; the presence of four main layers of tissue is observed throughout the whole length of the gut. In respect to histology, they consist of an outer epithelium of flat epithelial cells whose distal surface is bathed by coelomic fluid, a layer of definite bands of circular muscle and of longitudinal muscle cells, a layer of connective tissue of varying thickness, and an inner epithelium of a single layer of extremely tall and slender cells. The inner epithelial layer contains several distinct sorts of gland cells described below in detail. The epithelial lining is chiefly responsible for the thickness of the wall. The thickness of the epithelium varies markedly, it may ranged 50 - 200 μ.

The following description is concerned with elucidation of the cellular constituents and their characterization of the lining epithelium of the various parts, in terms of their histological and histochemical details.

Pharynx: The pharyngeal wall is deeply grooved, therefore it may be enlarged and contracted during the passage of food materials. There are also attachments of connective tissue running from the lips to the compasses of Aristotle's lantern. In the mid-pharyngeal region the well-marked grooves in the radial regions are almost exclusively lined with numerous mucous gland cells, and these together with secretory cells with strongly basophilic granules were distributed at random amongst simple epithelial cells (Fig. 1).

Mucous gland cells average about 5 μ in diameter and vary in height from 100 μ to a maximum of about 200 μ, depending upon their location and upon the size of the

animal. Their nuclei are broadly oval-shaped, measuring about 3 μ × 5 μ, and are approximately situated at the middle third of the cells. The space is filled with granules which stained slightly with haematoxylin. At a variable distance from the base, the cell narrows and finally opens into the lumen of the pharynx at a restricted stoma (Fig. 1). The basophilic granules are comparatively larger and more numerous in the basal portions of the cell: above the nuclear region they become smaller and more sparse.

The material composing these granules reacts vigorously to the Schiff reagent after treatment with buffered saliva (Fig. 2), but does not stain metachromatically with dilute toluidine blue. The cells covering the grooved region consist of granules with strongly positive reaction to the Schiff reagent after salivary digestion, while they do not stain metachromatically with dilute toluidine blue in acid solution. From the above data, it seems a justifiable conclusion that the content of these cells is to be regarded as neutral mucopolysaccharides.

The secretory cells with strongly basophilic granules were observed over the free ends of the epithelium; these cells were usually expanded to a diameter many times that of the neighboring cells. The granules of the cells exhibited PAS-negative reaction but stained strongly with haematoxylin. It may be assumed that these cells discharge fluid from the basophilic granules within them, and that the fluid is mainly responsible for the relatively greater acidity of the pharynx as compared with the rest of the food canal (Fuji, unpublished data).

In non-stained materials, a large number of reddish-purple-coloured granulocytes, measuring about 15 μ in size, are notable in the connective tissue layer and the basal region of the inner epithelium. Perhaps they are echinochrome-containing amoebocytes, and it is suggested that some respiratory action may be carried out there. Moreover, abundant agranulocytes localize in a similar region. Their size measures about 7 μ in diameter. When they are stained with haematoxylin, their cytoplasm appears as clear and as faint basophilic in character (Fig. 3).

Oesophagus: The oesophagus has well-developed circular and longitudinal muscles in its wall, and its inner epithelium is a continuous layer of mucous cells. Two types of the secretory gland cells are responsible for the production of secretion (Fig. 4). One type of the secretory cells possesses the strongly basophilic granules situated from a region near the basement membrane to the free edge of the cell, althouth these granules are larger and more numerous in the apical region of the cell. These granules were strongly stained with haematoxylin, whilst they gave a very faint PAS-reaction (Fig. 5). The other type contains numerous granules limited to the apical portion of the cell. In addition to being PAS-positive, their materials are basophilic but are not stained metachromatically with dilute toluidine blue. In all secretory cells, the nuclei lie at various depths in the epithelium, from near the distal end to the middle of the cells.

Stomach: The inner epithelium of the stomach region is composed of cylindrical cells. These cells are of somewhat variable length (100 – 150 μ) and gave rise to ridges on the inside wall of the stomach. Their nuclei are similar in size and shape to those of the secretory cells in the pharynx, and lie at various depths in the tall cells, from a point near the basal end to the middle of the cells.

The outstanding feature of the secretory cells is their content of secretory granules. These very tiny, yellowish, refringent granules lie in clumps in the slightly expanded region adjacent to the nucleus, but they have not been observed below the nucleus; they may extend in one or many rows completely to the free end of the cell (Fig. 6). These secretory spherules are encountered very frequently in the stomach epithelium alone, and there is never observed even a trace of such materials in the rest of the gut.

Several histochemical techniques were carried out to demonstrate some additional characteristics in various histological components of the food canal. Small deposits of glycogen are distributed in the basal portions of the cells (Fig. 7), especially their occurrence is more numerous in the hinder half of the stomach. After removal by salivary digestion, of all PAS-reactivity attributable to glycogen, several sites retain strongly positive reactions. These sites are limited to the connective tissue basement and the surface region of the inner epithelium of the stomach wall (Fig. 8). In addition to showing the above reaction, these sites are faintly basophilic but do not stain metachromatically with toluidine blue.

In a normal, well-fed animal these cells contain some deposits of lipids, which stain in pink with Sudan III. The lipids forming moderate-size droplets, are distributed in the basal portions and are usually lacking from the distal quarter of the cells (Fig. 9).

Alkalin phosphatase activity appears to be largely limited to the free edge of the inner epithelial region, but it is weak or lacking in the deeper portions of the epithelium (Fig. 10). This enzymatic activity is stronger in the fore half region than in the hinder one.

Larger eosinophilic granulocytes, measuring about 10 – 15 $\mu \times$ 15 – 25 μ, are sometimes found to be imbedded within the inner epithelium. Such granulocytes show yellowish-coloured in non-stained materials. The absorption of ingested material was tested by feeding the animals on starch solution containing carbon particles (ca. under 5 μ in size). Fed individuals were left for definite periods after which the food canal was fixed with Bouin's fluid. Normal paraffin imbedded sections were employed. In the stomach region, within the epithelium one or more carbon particles lay inside the granulocytes, which were distributed heavily near the free edges of the epithelium (Figs. 11 & 12). However, transverse sections from all parts of the pharynx and oesophagus of specimens fed on carbon particles did not exhibit the presence of any granulocytes. The intestine and rectum of animals fed with carbon particles showed absence of the granulocytes from the epithelial layer.

Intestine and Rectum: The intestine differed from the stomach in its relatively thinner walls, and lack of yellowish, refringent granules and of any basophilic granules.

Detailed examination shows the intestine to have an inner epithelium composed of cylindrical cells measuring approximately 70 – 150 μ in height (Fig. 13). The cytoplasm was clear, homogeneous and stained faintly with haematoxylin. The nuclei lie at various depths in the epithelium.

Histologically the rectum was similar to the intestine except that the inner epithelium was more thick, measuring about 30 – 70 μ in height of the cells (Fig. 14).

In the fore half of the intestine, glycogen and lipid deposits are shown in the basal region of the inner epithelium, while alkalin phosphatase activity is limited at the free end of the epithelium (Fig. 15). Glycogen and lipid deposits are never found in the rectum. Alkalin phosphatase, however, still appears in the marginal region of the inner epithelium, although its activity is weak.

Discussion

The histology , morphology and functioning of the food canal in the sea urchin has been the subject of several investigations varying in accuracy and in the interpretation of the results observed. In his book, "Vergleichende chemische Physiologie der niederen Tiere," Fürth (1903) gave a rough description concerning the food canal of the sea urchin. The account of Lasker & Giese (1954) of the nutrition of the purple sea urchin, *Strongylocentrotus purpuratus*, includes the food canal, but there is little communication of histological information. Concerning the cell population of the epithelium there is no analysis. Recently, Stott (1955) published in some detail the structure and function of the food canal and associated haemal canals in *Echinus esculentus*. Stott's findings on the secretory cells in the pharyngeal epithelium agree with the present writer's findings on *S. intermedius* in so far as the presence of two kinds of secretory cells is concerned. However, there will be noticed that the writer's findings differ from Stott's findings in some morphological descriptions of these secretory cells. In *Echinus esculentus*, the basophilic granules and mucous granules in the secretory cells are localized uniformly over the whole of the cells, from the figures presented by Stott, but the location of these cells was not stated, while in *S. intermedius* as shown in the previous section, the mucous gland cells contain slightly basophilic granules, which are larger and more numerous in the basal portion than above the nuclear region of the cell. Moreover, secretory cells with strong basophilic granules are observed over the free ends of the epithelium and they are located dominantly near the grooved region. From Stott's (1955) description and figures the wall of the oesophagus is composed simply of a continuous layer of tall, narrow, cylindrical mucous secreting cells. Using certain histological and histochemical techniques, the present author observed that two types of secretory cells are responsible for the

production of this secretion; one forms a neutral mucopolysaccharide only, whilst the other contains remarkably basophilic granules. From the above findings, the author considers that the latter produce an acid secretion which may be responsible for the relatively greater acidity (ca. pH 6.2) of the oesophagus, like that in the pharyngeal region.

The inner epithelium of the stomach consists of long columular cells which contain chains of tiny yellowish granules. These granules are distributed in the stomach epithelium proper. Digestive enzymes of the stomach extract predominate in their activity over those in the rest of the food canal (Fuji, unpublished data). From superficial morphology of the gut, without any observation of enzymatic activity, Delanuay (1931) has suggested that the first half of the gut itself possesses an active digestive function. From above information, it seems probable that these granules located in the stomach epithelium are discharged though the free surface and break down presumably to provide the extracellular enzymes of the lumen.

Accoding to Hilts & Giese (1949), chemical analysis demonstrated an appearence of some amounts of glycogen in various tissues of the sea urchin, but most appears in the stomach. Lasker & Giese (1954) on the basis of chemical analysis pointed out that a considerable store of glycogen exists in the food canal. Glycogen and lipid deposits are histochemically demonstrable in the hinder half of the stomach and in the fore half of the intestine of the sea urchin used in the present investigation. The conclusion that these materials are food reserves rests upon the evidence that it is possible to demonstrate a detectable decrease after five week's starvation. Therefore, the above opinions concerning the storage matter derived from chemical analysis are also justfied by the results obtained from the present investigation. From the appearence of alkalin phosphatase activity at the free end of the gut epithelium, it is strongly suggested that the gut possesses an absorptive function. Stott (1955) and other previous workers did not demonstrate any appearence of some biochemical elements in the food canal by reliable techniques. It seems necessary to discuss the functional aspects of the food canal.

In the case of experimental feeding with carbon granules, the inner epithelium of the stomach contains more numerous granulocytes in comparison with that of an animal obtained from the natural habitats. The results of the feeding experiments with carbon granules suggest that absorption of carbon takes the form of migration of the granulocytes loaded with carbon through the stomach wall into the haemal canals. Kindred (1924, 1926) observed that certain granulocytes of the sea urchin, *Arbacia*, liberate their protein or lipid inclusions throughout the organism and that they take up nutrient matter from the digestive system and transform it into the cytoplasmic granules. In consideration of the above information, it may be indubitable that amoebocytes in the stomach epithelium participate in nutrition to a greater or lesser degree by ingesting particles and absorbing dissolved nutrient.

The present observation concerned with the histological and histochemical complement of the food canal may be summarized as follows. The oesophagus has well-developed circular and longitudinal muscles in its wall; its inner epithelium is a continuous layer of mucous cells. Consequently, it is assumed that food materials ingested are propelled to the stomach along the oesophageal region by a powerful peristalsis of those circular and longitudinal muscles. Forthermore, the mucus secreted from its inner epithelium may assist the propulsion of the food by providing lubrication. Proteins and carbohydrates in the ingested material are digested and absorbed in the stomach region. Some of the nutritive substances are elaborated into reserves of polysaccharide and lipid nature, while others are transported to the other organs for storage or for use. The intestine and the rectum act as absorptive organs and a conduction tube for the undigested food materials.

Few investigations have been made of the haemal system which may serve as an avenue for nutritive substances between the gut and other organs (Stott, 1955), associated with the coelomic fluid which has been suggested as a nutritive pathway (Greenfield et al., 1958; Giese et al., 1959). The physiology of the haemal system and of the coelomic fluid is quite unknown from the viewpoint of a possible nutritive role.

Summary

Histological and histochemical observations in different parts of the food canal have been carried out in *Strongylocentrotus intermedius*. The results may be summarized as follows:

(1) The wall of the food canal is composed of an outer peritoneum; layers of circular and longitudinal muscle; a layer of connective tissue of varying thickness; and an inner epithelium generally composed of very tall, slender cells.

(2) Two kinds of secretory cells have been recognized in the pharyngeal and oesophageal wall; one produces a mucoid secretion and the other an acid secretion. Numerous echinochrom-containing amoebocytes and agranulocytes are distributed in the connective tissue layer and the basal region of the pharyngeal inner epithelium.

(3) The stomach is lined with an inner epithelium containing of yellowish granules, which are discharged from its surface to provide extracellular enzymes. Large eosinophilic granulocytes, which are assumed to participate in digestion by ingesting particles and absorbing dissolved nutrient substances, are sometimes found to be imbedded within the stomach inner epithelium. The intestine and the rectum, however, have never been found to contain even a trace of any secretory granules nor of any amoebocytes in the inner epithelium.

(4) Lipids and glycogen, which are reserve materials, appear to be deposited in the hinder half of the stomach and the fore half of the intestine. They are very strong at the deeper portion of the inner epithelium. Starvation for five weeks results in an

appreciable decrease of the reserves.

(5) Alkalin phosphatase activity is shown at the free border of the inner epithelium in the stomach, intestine, and rectum.

(6) It may be certainly concluded that the stomach functions in digestion of food, in absorption of the products of digestion, and that the intestine and the rectum act as absorptive organs and a conducting tube for the undigested food materials.

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EXPLANATION OF PLATES

Photomicrographs of transverse sections through the various parts of the food canal of *Strongylocentrotus intermedius*

PLATE I

Fig. 1. Mid-pharyngeal region showing the two kinds of secretory cells. A mucous stoma is indicated by the arrow. Bouin-Haematoxylin. ×250

Fig. 2. Part of the same region. Note basal concentration of PAS-positive materials, which have not been removed by salivary digestion. ×250

Fig. 3. Amoebocytes in the basement and connective tissue layer of pharynx. RG, Red-coloured granulocytes containing echinochrome: AG, Agranulocytes. Peritoneum at lower right. Bouin-Haematoxylin. ×500

Fig. 4. Cross section through the oesophagus. Peritoneum at lower left, lumen off upper right. Bouin-Haematoxylin. ×500

Fig. 5. Same region showing heavy concentration of PAS-positive materials, after salivary digestion. PAS-technique. ×500

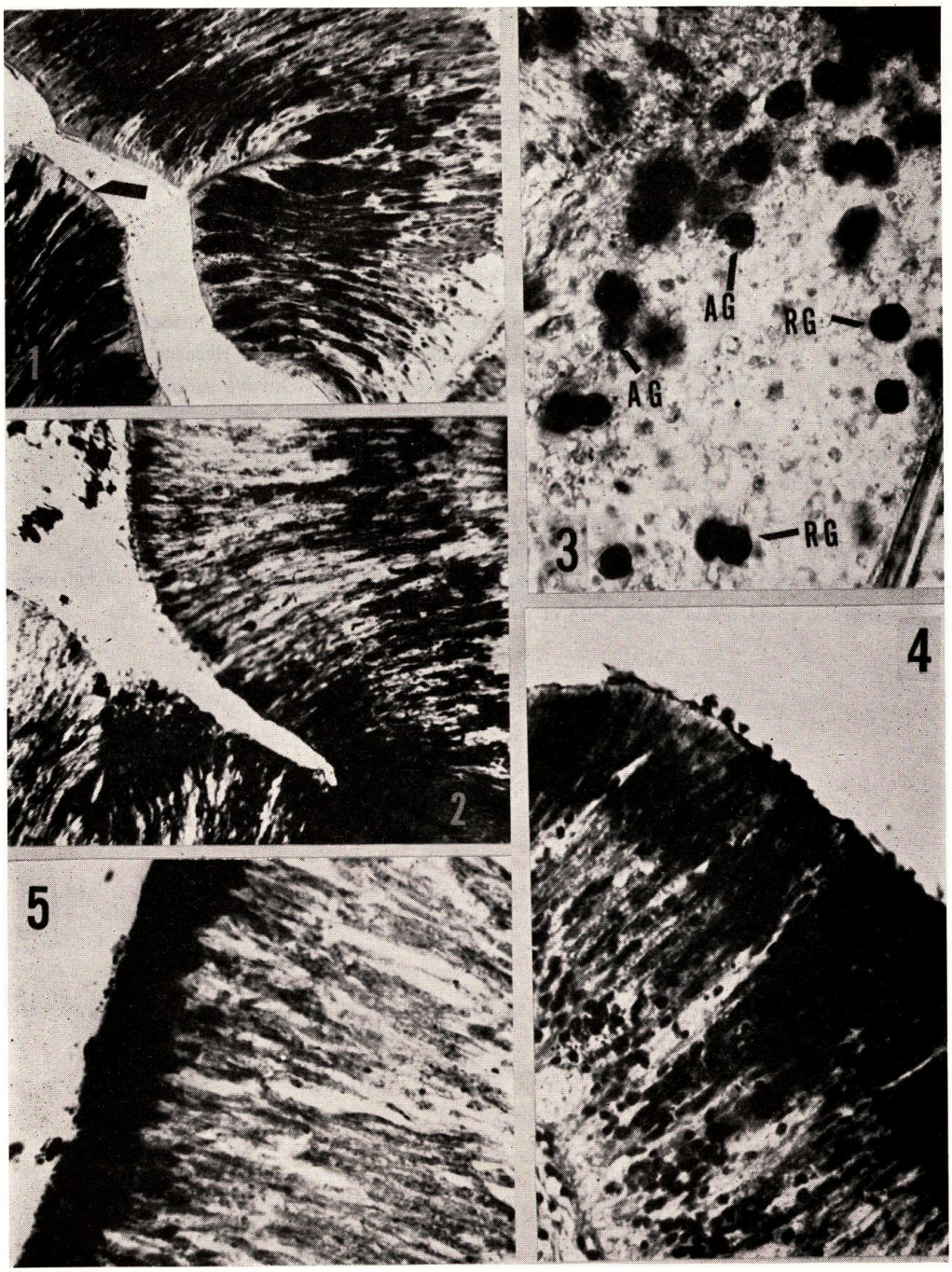

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PLATE II

Fig. 6. Cross section through the stomach. The arrow indicates secretory granules extended in one or many rows completely to the free end (left side) of the cell. Bouin-Haematoxylin. ×500

Fig. 7. Basal portion of stomach cells showing glycogen deposits. Arrows indicate small deposits of glycogen. Peritoneum at lower right. PAS-technique. ×500

Fig. 8. Cross section through the stomach region, showing PAS-positive material after salivary digestion. Peritoneum at lower left, lumen of stomach at upper right. PAS-technique. ×370

Fig. 9. Basal region of stomach. Lipid deposits are black. Peritoneum at lower right. Formal-salin: Frozen section coloured with Sudan III. ×500

Fig. 10. Cross section through stomach showing alkalin phosphatase activity. Note concentration of activity at apical region (upper left) of the inner epithelium. Gomori technique. ×370

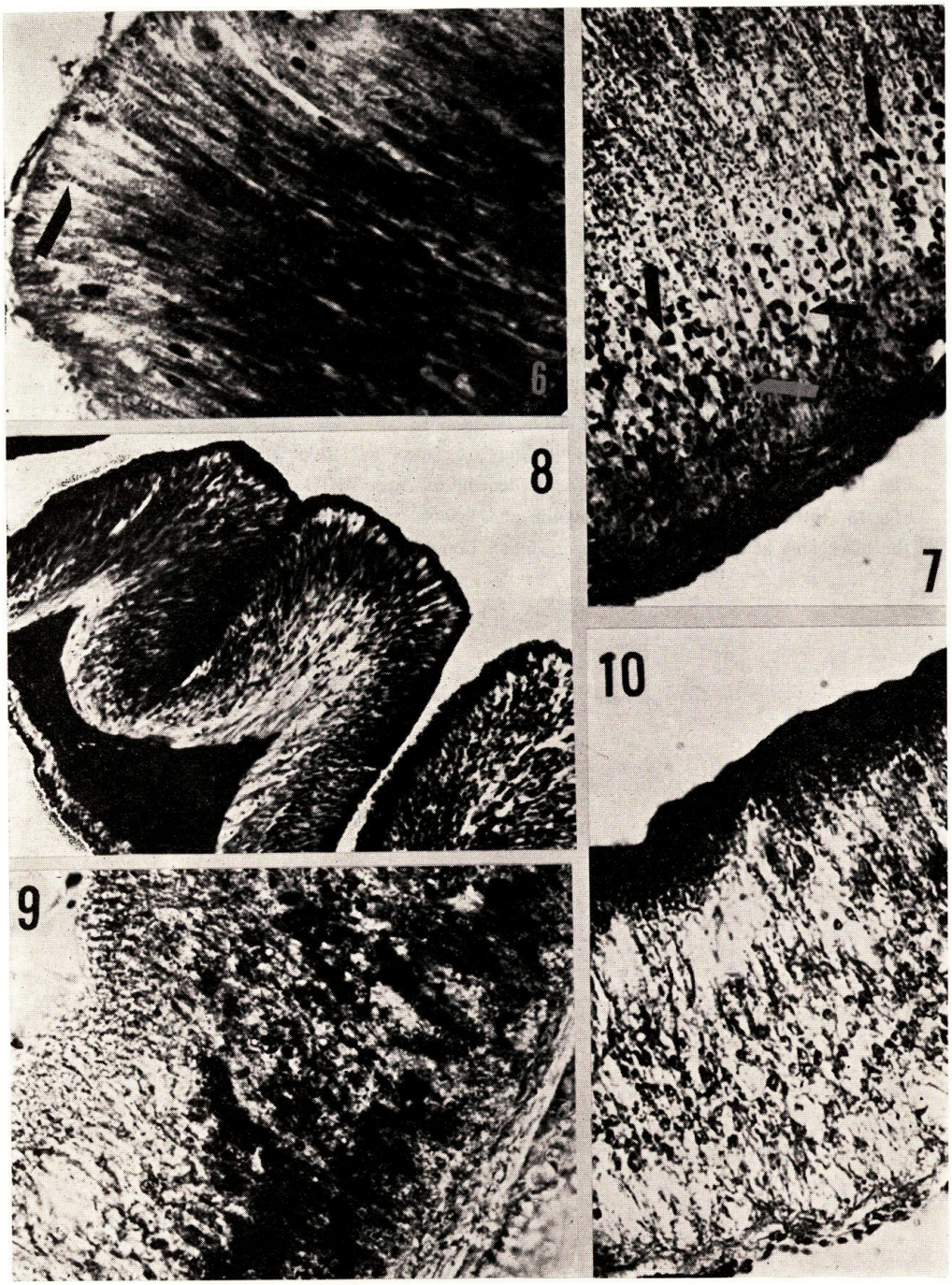

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PLATE Ⅲ

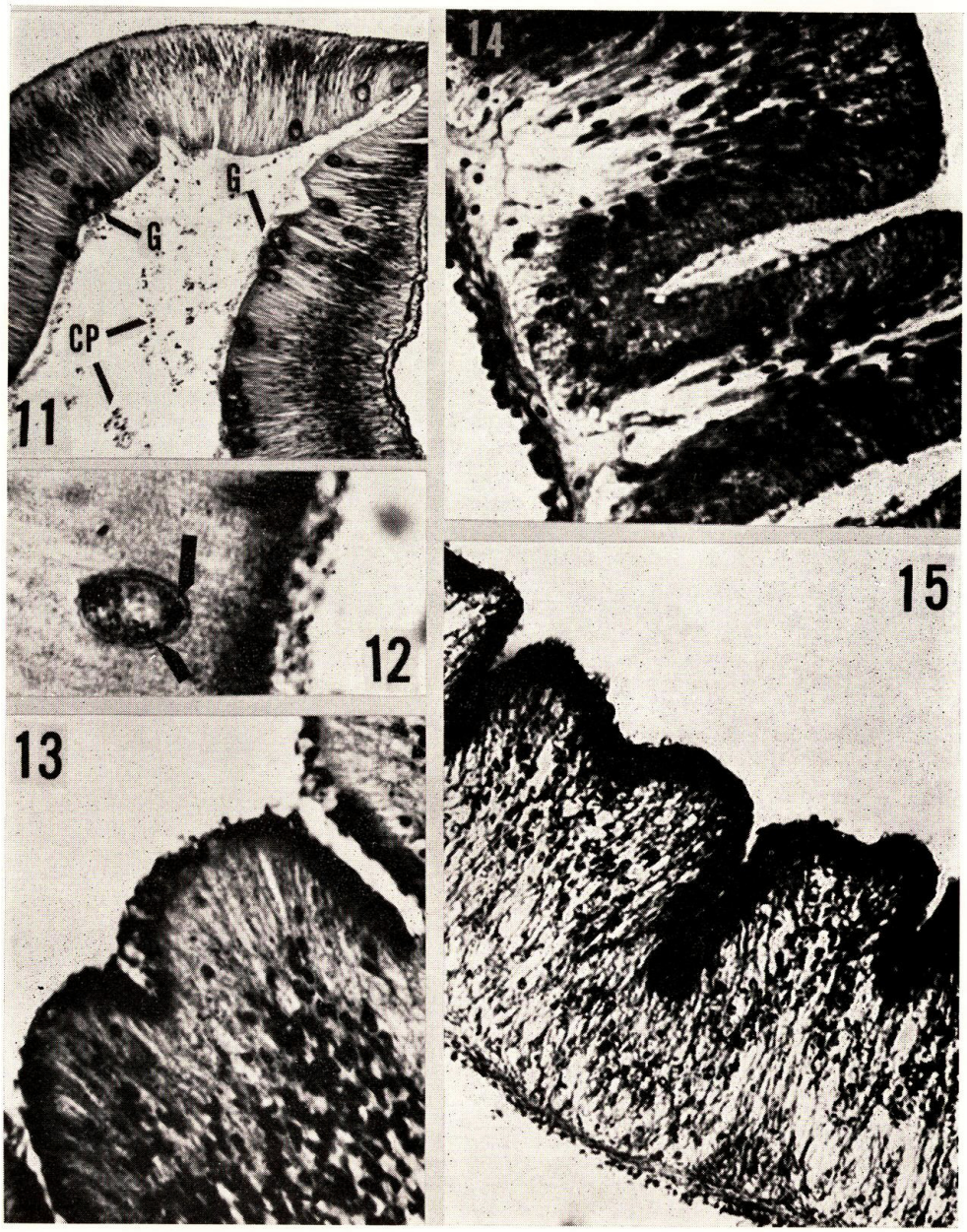
Fig. 11. Stomach region showing heavy distribution of large granulocytes. G, Granulocytes; CP, Carbon particle in the lumen of stomach. Bouin-Haematoxylin. ×120

Fig. 12. Granulocyte imbedded within the inner epithelium of stomach. Arrows indicate carbon particles in the granulocyte. Bouin-Haematoxylin. ×680

Fig. 13. Cross section through intestine. Lumen at upper left. Bouin-Haematoxylin. ×500

Fig. 14. Cross section through rectum. Peritoneum at lower left. Bouin-Haematoxylin. ×500

Fig. 15. Intestinal region, showing alkalin phosphatase activity. Localization of alkalin phosphatase is the black area at apical margin (upper right). Gomori technique. ×370



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