Light Microscopic Study on Endocrine Cells in the Gastrointestinal Tract of Sheep

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ヒツジの胃腸管における内分泌細胞の光顕的研究

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Introduction

It is a well known fact that endocrine cells have the two functions of secretion and neurotransmission. Research on the gastrointestinal endocrine cells are being actively performed. Furthermore their fine structure and functions have been studied precisely with electron microscopy. SATO *et al.* (1976), SATO *et al.* (1978), ALUMETS *et al.* (1977), YAMADA *et al.* (1979), LARSSON *et al.* (1977), BUFFA *et al.* (1978), SUNDLER *et al.* (1977), BAETENS *et al.* (1976) and FUJITA *et al.* (1971, 1973) reported histological studies of the gut endocrine cells in horses, cats, birds, human beings, dogs and rats respectively.

But only a few studies concerning endocrine cells of the gastrointestinal tract in ruminants have been accomplished (ONAITO *et al.* 1977). In this paper histological observations of endocrine cells of the ovine gastrointestinal tract are described.

Materials and Methods

For these experiments, fresh specimens were taken from the gastrointestinal tract of seven normal sheep. The sample was taken for eleven parts of gastrointestinal tract, while samples from the ansa sigmoid were taken at three different levels for more extensive study (Fig. 1). For light microscopy, BOUIN's solution and 10% neutral buffered formalin (0.1 M phosphate buffer, pH 7.3), were used as fixatives. Sections were treated with the following methods known to stain gut endocrine cells. The specimens fixed in BOUIN's solution were stained with HELLERSTRÖM-HELLMAN's silver impregnation (HELLERSTRÖM and HELLMAN, 1960), and GRIMELIUS's silver impregnation (GRIMELIUS,

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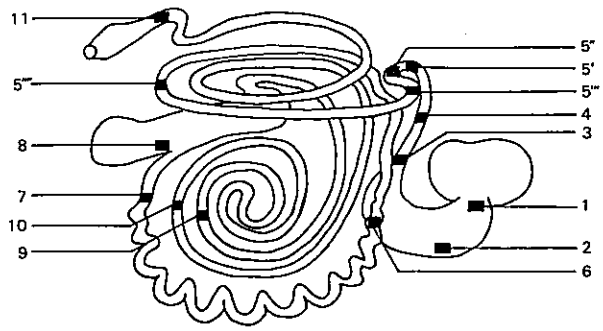


1	Cardia	5′′′′	Caudal duodenal flexure
2	Fundus	6	Jejunum
3	Pylorus	7	Ileum
4	Duodenum just behind the pylorus	8	Caecum
5′	Anterior portion of ansa sigmoid	9	Centripetal gyri
5″	Central portion of ansa sigmoid	10	Centrifugal gyri
5‴	Posterior portion of ansa sigmoid	11	Rectum

Fig. 1. Sampling portions in the gastrointestinal tract of sheep

1968), whereas the materials fixed in neutral buffered formalin solution were performed with SEVIER-MUNGER's silver impregnation (SEVIER and MUNGER, 1965), MASSON-HAMPERL's argentaffin reaction (SINGH, 1964) and lead-haematoxylin staining method (SOLCIA *et al.*, 1965).

Results

The frequency of the endocrine cells in the unit areas (cell number/1.25 mm^2) of the different regions of the gastrointestinal tract was studied and the results are represented in (Fig. 2).

GRIMELIUS's silver impregnated cells were found to be numerous in the cardiac and fundic mucosa (Fig. 3 and 4) and frequent in the duodenum, especially most frequent in the center of the ansa sigmoid (Fig. 6), but few found in the pyloric mucosa (Fig. 5). These cells decreased from the jejunum (Fig. 8) to the caecum (Fig. 9) and slightly increased in the colon and rectum (Fig. 10). In the stomach and duodenum these reacting cells occurred in the lower half portion of the epithelium, while in the duodenal regions were strongly concentrated at the base of the glands (Fig. 6), and scattered in other portions of the gastrointestinal tract (Fig. 7). Occasionally very few cells were observed in the BRUNNER's glands. The argyrophil cells were recognized as black or brownish-colored cells. These reacting cells were predominantly oval or pyramidal, often reaching the glandular lumen with a short cytoplasmic process in the pyloric, duodenal and jejunal portions (Fig. 8).

HELLERSTRÖM-HELLMAN's silver method was considered to stain specifically the D and D_1 cells. Although the pattern of the frequency of HELLERSTRÖM-HELLMAN's positive

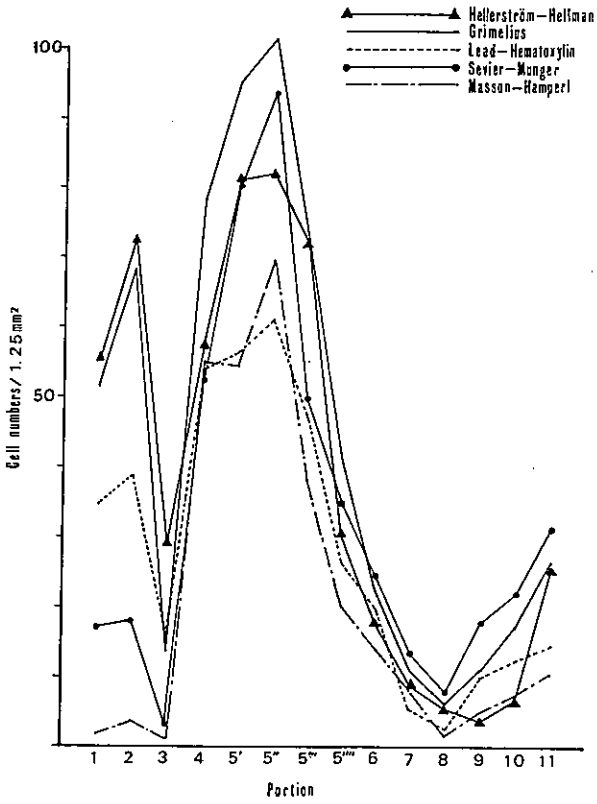


Fig. 2. The frequency of endocrine cells in the different regions of the sheep gastrointestinal tract. Each line shows cell number stained with each method of the unit areas (cell number/1.25 nm^2)

cells resembled those stained with GRIMELIUS's silver method, in the stomach these positive cells were most remarkable in comparison with those stained with other methods (Fig. 11–12). These impregnated cells were found to be numerous in the duodenum (Fig. 13 and 14), however did not show remarkable increase in the region of the ansa sigmoid in addition to being lowest in the caecum (Fig. 16) and colon and little more frequent in the rectum (Fig. 17). These cells were black-colored and were predominantly oval or short-process in shape (Fig. 14 and 15). Positive cells were also found in the lower half portion of the glands, sometimes a few could be seen in the upper portion as well.

Few SEVIER-MUNGER's reacting cells were seen in the stomach, especially in the pyloric mucosa (Fig. 18 and 19). However in the duodenum (Fig. 20) these reactive cells showed progressive increase being numerous in the center of the ansa sigmoid (Fig. 21). Comparing with the other staining methods, the frequency of these cells was highest in the duodemun, jejunum, decreasing in ileum, (Fig. 22), caecum, and again increasing in colon and rectum (Fig. 23). On the other hand, very few cells were observed in the BRUNNER's glands. Numerous oval or spindle-shaped cells were scattered in the upper

portion. These reactive cells were chiefly black-colored, but occasionally were seen as brownish-colored. With this staining method, the cells also reached the glandular lumen with a short cytoplasmic process; so-called "open" type cells were often observed in the pyloric and duodenal portions as well as with other silver methods.

The cells stained with MASSON-HAMFERL's argentaffin technique showed a characteristic frequency. Argentaffin and enterochromaffin cells were rarely seen in the stomach or having a weak reaction (Fig. 24). Nevertheless, black-stained cells showed a sharp increase in frequency in the duodenum, reaching the peak at the center of the ansa sigmoid (Figs. 2 and 25). In other regions of gastrointestinal tract, these argentaffin cells showed lowest frequency in all staining methods. It must be pointed out that oval, fusiform, and spindle-shaped cells were also found in the lower half portion of the glands (Figs. 25, 26 and 27).

Relatively numerous lead-haematoxylin positive cells were found in the stomach (Fig. 27) and it was of interest that these positive cells were second most numerous in the pyloric portion (Fig. 29). In the duodenum these cells showed the lowest frequency in all staining methods, but showed their peak in the center of the ansa sigmoid (Figs. 30 and 31). Round and oval cells and occasionally spindle-shaped cells were observed chiefly in the lower half portion of the glands (Fig. 32).

Discussion

Many reports have been published on the endocrine cells of the gastrointestinal tract in various domestic animals. Hardly any detailed investigation, however, has been carried out concerning the gastrointestinal endocrine cell of ruminants, particularly those of the sheep. In the present study on the gastrointestinal endocrine cells of seven normal sheep, they showed a considerable high frequency in the cardiac and fundic mucosa, especially the highest in the duodenum. In pyloric portion, however the frequency of the endocrine cells was low. It was the lowest in the caecum, but showed higher frequency in the colon and the rectum.

SATO *et al.* (1976, 1978) reported that they found numerous endocrine cells especially in the duodenal portion, but rarely in the caecum and colon, and somewhat numerous in the rectum of horses. ALUMETS *et al.* (1977) reported that many somatostatin cells were found in the antral mucosa of cats, pigs, and human beings, more numerous in the fundic region of rabbits and pigs. Also they reported that those of cats, pigs and human beings were densely present in the duodenum, but few in rats and dogs.

The somatostatin cells, the so-called "D" cells stained with HELLERSTRÖM-HELLMAN's silver method were found to be the most numerous in the duodenum and frequent in the fundic mucosa. It was reported that gastrin cells were observed to be numerous in the pylorus, but less in the duodenum of human beings (LARSSON *et al.*, 1975).

YAMADA *et al.* (1979) found numerous gastrin reacting cells in the pyloric region of birds. In the pyloric portion our data showed very low frequency of the total endocrine cells in all five staining methods. But gastrin cells may be numerous in pyloric portion because gastrin cells with lead-haematoxylin and GRIMELIUS's staining methods showed relatively high frequency in this region.

On the other hand, our investigation also showed the numerous profiles of the open type in the duodenum, and that various endocrine cells concentrated strongly in the ansa sigmoid. The open type cells with cytoplasmic process were demonstrated to be numerous in the duodenum in argentaffin and argyrophil staining techniques. FUJITA and KOBAYASHI (1971, 1973) classified the GEP (gastroenteropancreatic endocrine system) endocrine cells into open and closed types as to whether the cytoplasmic process reaches the lumen or not, and hypothesized that the open type cell is considered as the recepto-secretory cell which receives chemical information from the lumen by its apex to control its own hormonal secretion.

From these facts, the authors considered that in the portion of the ansa sigmoid the flow of food may be slowly moved, and that various endocrine cells as neurotransmitters may have an important relation to the secretion of the pancreas and to other different endocrine cells. In the present study, it was of interest that anatomically the opening portion of the common bile duct and pancreatic duct were located behind the ansa sigmoid. The functional meanings of the ansa sigmoid in the duodenum is still unknown but may be of importance for the digestive physiology of sheep.

We are of the opinion that is may be on account of the differences among the species. Further study is necessary to solve the precise distribution of the endocrine cells with the immunohistochemical and ultrastructural techniques.

Summary

Endocrine cells in the gastrointestinal tract of sheep were studied by light microscopy. Many kinds of endocrine cells were demonstrated with five specific staining methods.

1) In the stomach endocrine cells using four staining methods omitting MASSON-HAPERL's argentaffin reaction were shown to be frequent in the cardia, more frequent in the fundus, and very few in the pylorus. Only MASSON-HAMPERL's reacting cells were found rare in the mucosa of the stomach and showed very weak reaction.

2) In duodenum endocrine cells were seen to be the most numerous, especially concentrated strongly in the center of the ansa sigmoid; in addition they were lowest in the caecum and little more frequent in the colon and rectum.

3) The reacting cells reached the glandular lumen with the cytoplasmic process, the so-called open type cells, were observed well observed in the pylorus, duodenum and jejunum.

4) The sheep differed from other animals studied previously that numerous endocrine cells were found in the duodenum, especially in the ansa sigmoid, although few in the

pylorus.

References

1) ALUMETS, J., F. SUNDLER and R. HÅKANSON: Cell Tiss. Res., **186**: 465-479 (1977).
2) BAETENS, D., C. RUFENER, B. C. SRIKANT, R. UNGER, R. DOBES and L. ORCI: J. Cell Biol., **69**: 455-464 (1976).
3) BUFFA, R., C. CAPPELLA, P. FONTANA, L. USELLINI and E. SOLCIA: Cell Tiss Res., **192**: 227-240 (1978).
4) FUJITA, T. and S. KOBAYASHI: Z. Zellforsch., **116**: 52-60 (1971).
5) GRIMELIUS, L: Acta. Soc. Med. Upsel., **73**: 243-270 (1968).
6) HELLERSTRÖM, C, and B. HELLMAN: Acta. Endocrinol., **35**: 518-532 (1960).
7) HELMSTAEDTER, V., C. TAUGNER, G. E. FEURLE and W. G. FORSSMAN: Histochemistry, **53**: 35-41 (1977).
8) KOBAYASHI, S. and T. FUJITA: Igaku Shoin, Tokyo,: 49-58 (1973).
9) LARSSON, L.-I., R. HAKANSON, N. O. SJÖBERG and F. SUNDLER: Gastroenterol., **68**: 1152-1159 (1975).
10) LARSSON, L.-I., F. SUNDLER, J. ALUMETS, R. HÅKANSON, O. B. SCHAFALITZKY, D. MUCKADELL and J. FAHRENKRUG: Gell Tiss. Res., **181**: 361-368 (1977).
11) ONAPITO, S. J., W. J. DONAWICK and A. M. MERRIT: American J. Vet. Res., **39**: 1455-1458 (1978).
12) SATO, H., T. YAMASHITA J. YAMADA and M. MISU: Res, Bull. Obihiro Univ., **10**: 79-93 (1976).
13) SATO, H., T. YAMASHITA, J. YAMADA and M. MISU: Ses. Bull. Obihiro Univ., **11**: 25-41 (1978).
14) SEVIER, A. C. and B. L. MUNGER: J. Neuropath. and Exp. Neur., **24**: 139-135 (1968).
15) SINGH, I.: Anat. Anz., **115**: 81-82 (1964).
16) SOLCIA, E., C. CARELLA and G. VASSALLO: Histochemie, **20**: 116-126 (1969).
17) SUNDLER, F., R. HÅKANSON, R. A. HAMMER, J. ALUMTS, R. CARRAWY, S. E. LEEMAN and E. A. ZIMMERMAN: Cell Tiss. Res., **178**: 313-321 (1977).
18) YAMADA, J., M. YOSHINO, T. YAMASHITA, M. MISU and N. YANAIHARA: Arch. histol. jap., **42**: 33-39 (1979).

摘　　要

ヒツジ7頭より採取した第四胃3部位および腸11部位の粘膜を消化管内分泌細胞のための5種の組織化学的方法を用いて検索し，次のような成績を得た。

1) 胃における内分泌細胞はGRIMELIUS法，HELLERSTRÖM-HELLMAN法，SEVIER-MUNGER法および鉛ヘマトキシリン法にそれぞれ陽性を示し，それら内分泌細胞は染色法による多寡はあるが，概して噴門腺部粘膜にやや多く，胃腺部粘膜には多数出現していた。しかし幽門腺部粘膜ではいずれの染色法においても陽性細胞が極端に減少していた。MASSON-HAMFERL法で銀親和性内分泌細胞が胃の3粘膜部位とも極めて少数であった。

2) 十二指腸粘膜には5種の染色法で陽性を示す細胞が多数認められ，とくにS状ワナに極めて多数出現していた。腸の内分泌細胞は空回腸粘膜で急激な減少を示し，盲腸粘膜でもっ

とも少ないが，結直腸粘膜で再びやや増加する傾向が認められた。

3) いわゆる〝開放型〟を示す内分泌細胞が幽門腺部，十二指腸および空腸の粘膜でよく観察された。

Explanation of Plates

Plate I

Fig. 3. Many argyrophil cells of gastric pits in the cardiac mucosa. BOUIN, GRIMELIUS, X 500
Fig. 4. Many silver reacting cells in the lower half portions of the fundic glands. BOUIN, GRIMELIUS, X 500
Fig. 5. A few argyrophil cells in the pyloric mucosa. BOUIN, GRIMELIUS, X 500
Fig. 6. The most numerous positive cells at the base of the center of the ansa sigmoid. BOUIN, GRIMELIUS, X 500
Fig. 7. Many positive cells in the portion of the caudal duodenal flexure. BOUIN, GRIMELIUS, X 500
Fig. 8. Three open type cells at the base of the jejunal mucosa. BOUIN, GRIMELIUS, X 10000

Plate II

Fig. 9. Two argyrophil cells in the mucosa of the caecum (arrows). BOUIN, GRIMELIUS, X 1000
Fig. 10. Oval or spindle-shaped cells were seen clearly in the rectal portion. BOUIN, GRIMELIUS, X 500
Fig. 11. Many argyrophil cells in the lower half portion of the fundic mucosa. BOUIN, HELLERSTRÖM-HELLMAN, X 500
Fig. 12. Long-process cells in the pyloric mucosa. BOUIN, HELLERSTTÖM-HELLMAN, X 500
Fig. 13. Numerous reacting cells concentrated at the base of the duodenal mucosa just behind the pylorus, BOUIN, HELLERSTRÖM-HELLMAN, X 500
Fig. 14. The most numerous positive cells at the base of the center of the ansa sigmoid. BOUIN, HELLERSTÖM-HELLMAN, X 1000

Plate III

Fig. 15. Four black-colored cells in the mucosa of the caudal duodenal flexure. BOUIN, HELLERSTRÖM-HELLMAN, X 1000
Fig. 16. Argyrophil cell (arrow) in the glands of the caecum. BOUIN, HELLERSTRÖM-HELLMAN, X 1000
Fig. 17. Oval cells in the mucosa of the rectum (arrow). BOUIN, HELLERSTRÖM-HELLMAN, X 1000
Fig. 18. Some silver reacting cells at the base of the fundic glands. formalin, SEVIER-MUNGER, X 500
Fig. 19. A few positive cells in the pyloric mucosa. formalin, SEVIER-MUNGER, X 500
Fig. 20. Many argyrophil cells concentrated strongly at the base of the glands just behind the pylorus. formalin, SEVIER-MUNGER, X 500

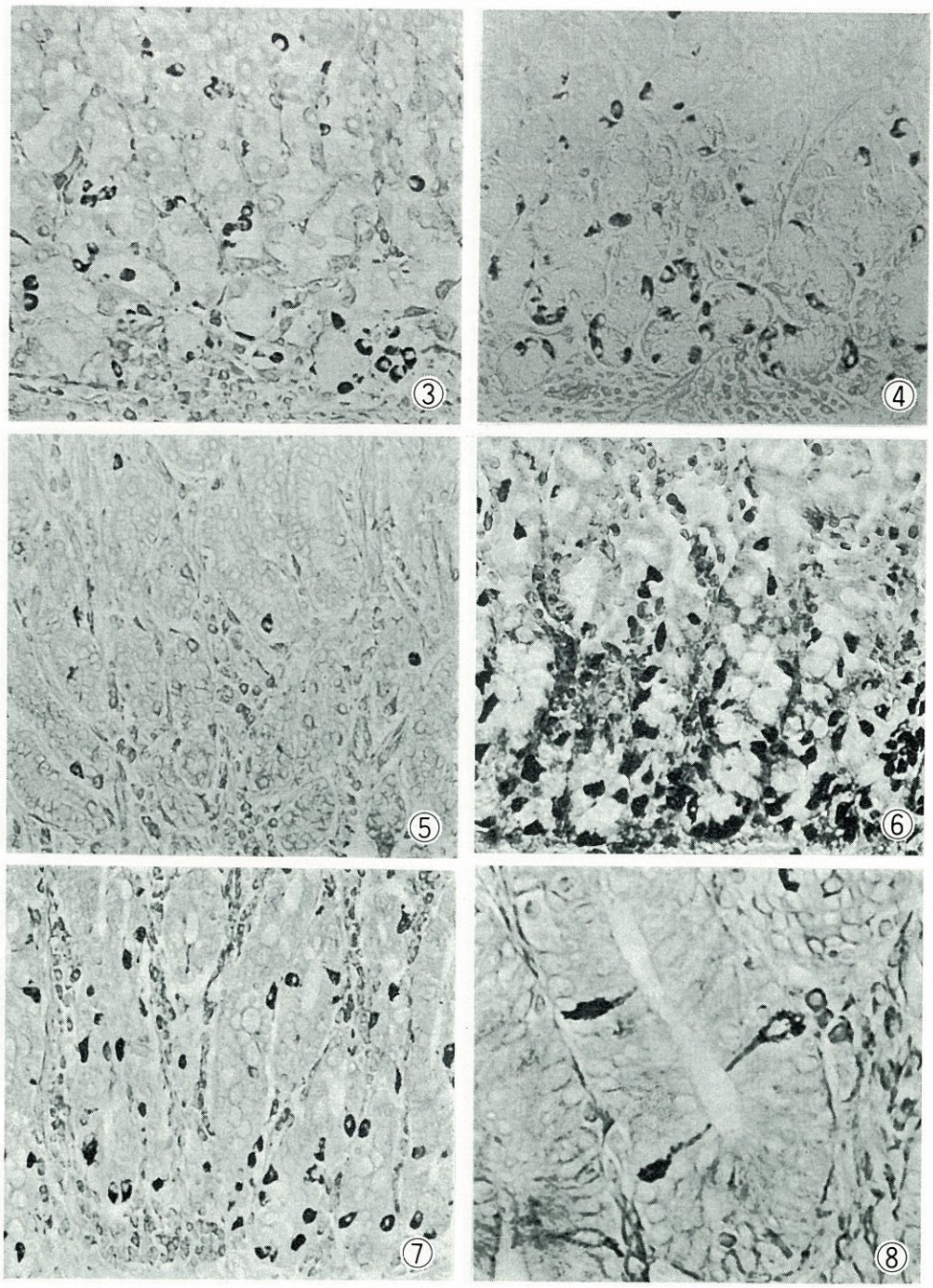
Plate IV

Fig. 21. Black-colored cells in the lower half portion of the center of the ansa sigmoid. formalin, SEVIER-MUNGER, X 500
Fig. 22. Two argyrophil cells in the mucosa of the ileum (arrows). formalin, SEVIER-MUNGER, X 1000
Fig. 23. Several oval cells in the rectal mucosa (arrows). formalin, SEVIER-MUNGER, X 1000
Fig. 24. Two argentaffin cells in the fundic mucosa, were rare or showed weak reaction (arrows). formalin, MASSON-HAMPERL, X 1000
Fig. 25. Argentaffin cells showed the highest frequency in the center of the ansa sigmoid. formalin, MASSON-HAMPERL, X 500
Fig. 26. Black-colored cells in the lower half portion of the jejunum. formalin, MASSON-HAMPERL, X 500

Plate V

Fig. 27. Several enterochromaffin cells in the rectal mucosa. formalin, MASSON-HAMPERL, X 1000
Fig. 28. Many positive cells in the lower half portion of the fundic gland. formalin, Leadhaematoxylin, X 500
Fig. 29. Many blue-colored cells in the pyloric gland. formalin, Lead-haematoxylin, X 500
Fig. 30. Numerous positive cells at the base of the duodenal mucosa just behind the pylorus. formalin, Lead-haematoxylin, X 500
Fig. 31. Lead-haematoxylin positive cells appeared in the basal portion of the gland of the ansa sigmoid. formalin, Lead-haematoxylin, X 500
Fig. 32. Oval or spindle-shaped cells in the base of the jejunual mucosa. formalin, Lead-haematoxylin, X 1000

Plate I



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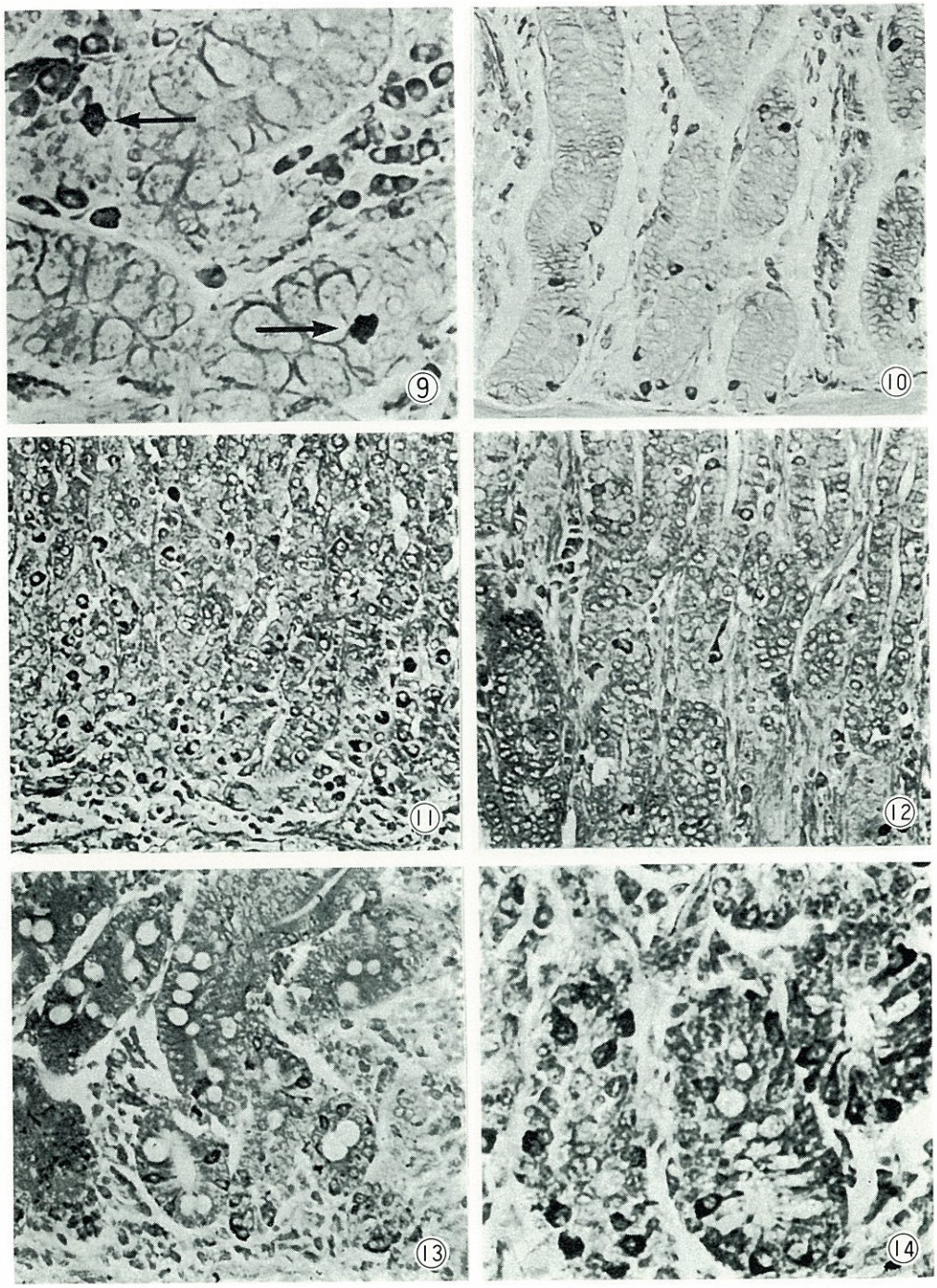

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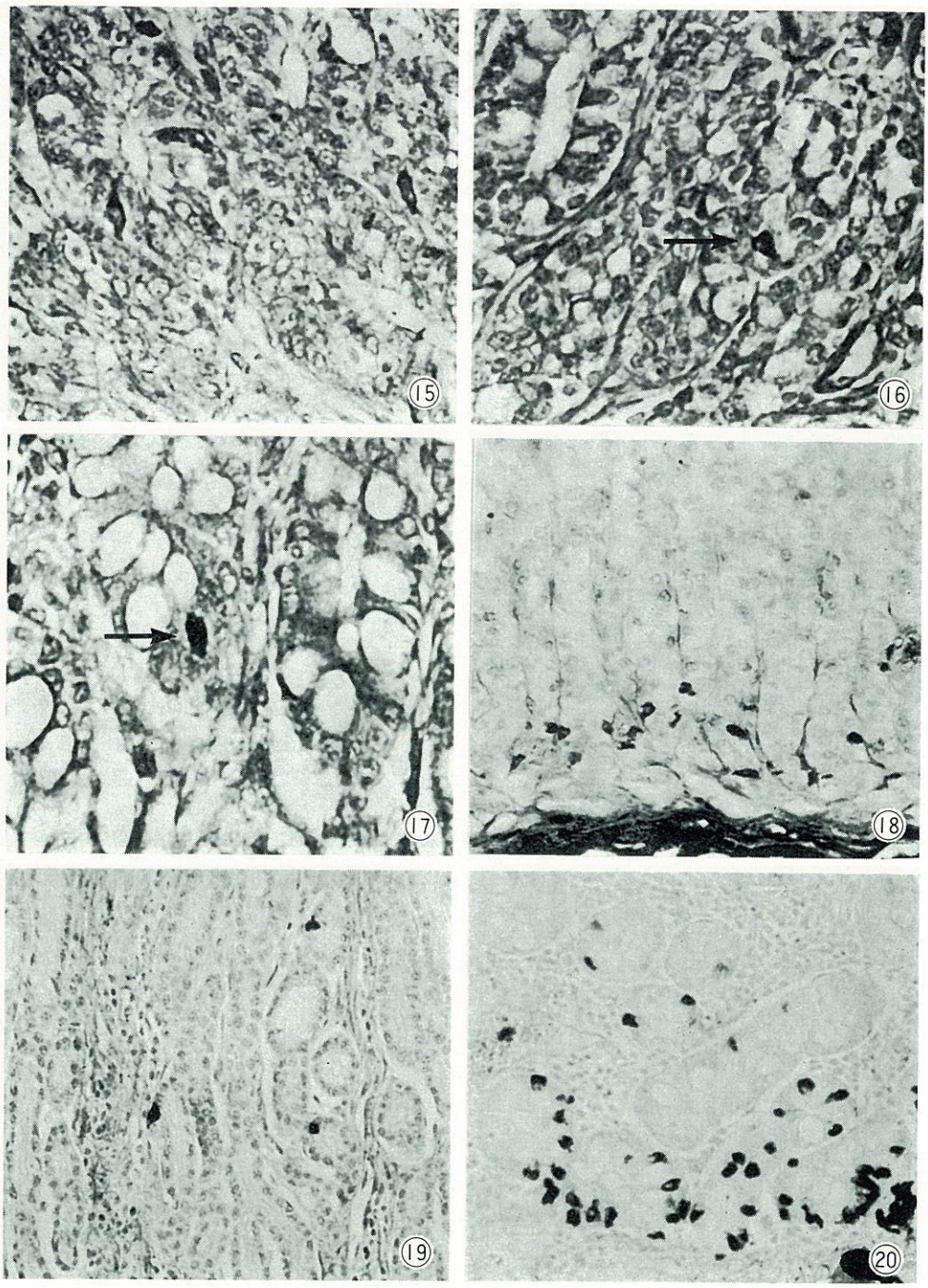

Plate IV

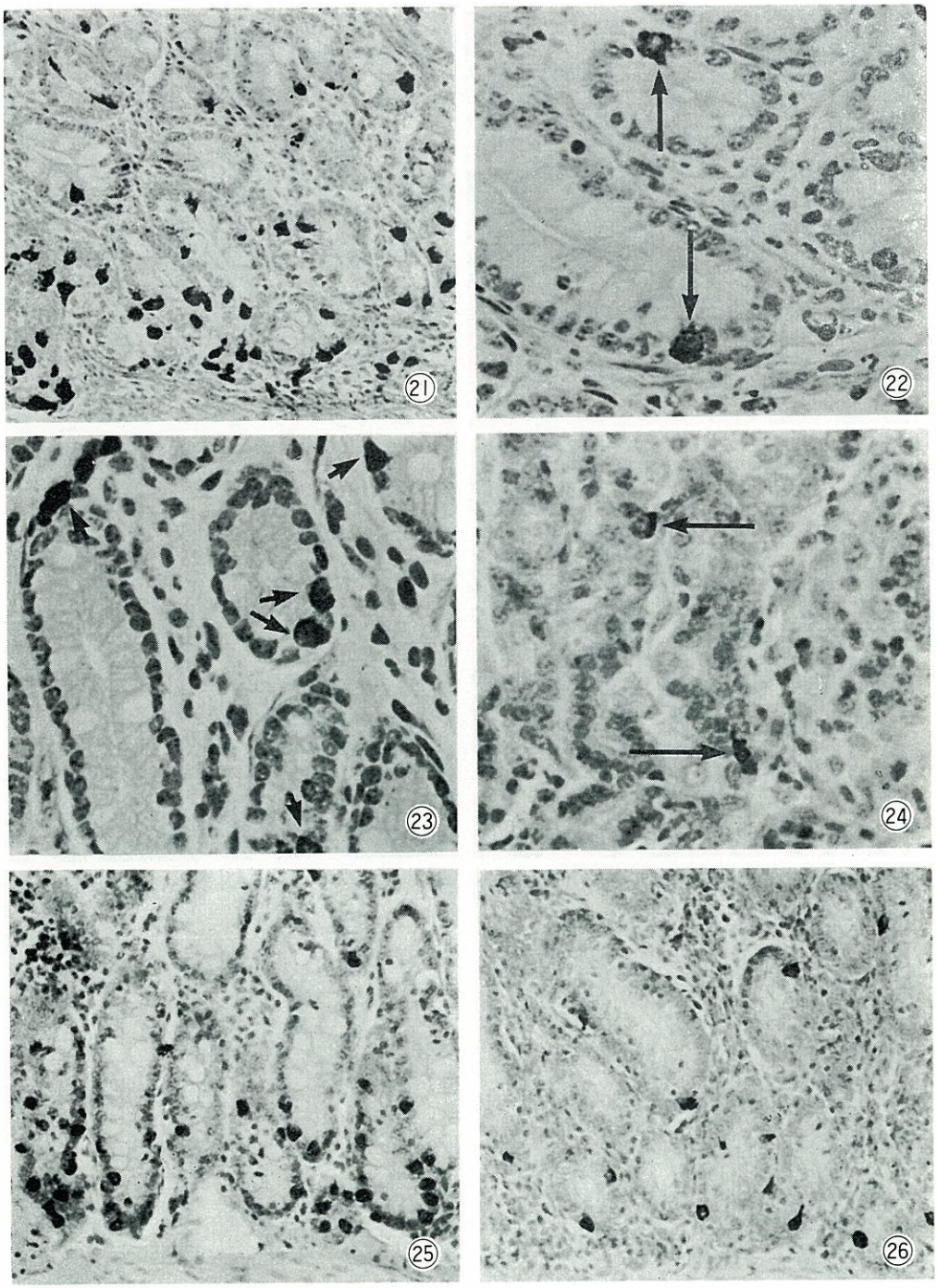

Plate V