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Distribution of Endocrine Cells in Duck Digestive Tracts

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(Figure. 1 ; Plate 1)

In the mammalian gastrointestinal mucosa, several types of endocrine cells have been recently described by certain histological and electron microsopic methods[1~13].

In domestic fowl, enterocromaffin cells or argentaffin cells were observed in the intestine by light microscopy[14,15] and by electron microscopy[16,17]. Furthermore argyrophil cells were observed in the proventriculus and gizzard by light microscopy[18]. In the proventricular mucosa of the finch, two types of endocrine cells have been also distinguished in electron microscopy[19]. On the distribution of the endocrine cells in the digestive tract of domestic birds, however, there are no informations available to the auther's knowledge.

The present paper intends to describe the presence and distribution of the endocrine cells along the whole length of the duck digestive tracts and to discuss these data with reference to the dense distribution of the endocrine cells in the psyloric regions of the gizzard.

MATERIALS AND METHODS

Ten white Pekin ducks were used as materials. The specimens were obtained from ten different regions of the duck digestive tracts; oesophagus, proventriculus, isthmus, central part of the gizzard, pyloric region of the gizzard, duodenum, jejunum, ileum, caecum and colo-rectum. The specimens were fixed in 3% glutaraldehyde or 10% formalin. Then, they were embedded in paraffin, and sectioned at 6–8μ in thickness. Staining methods and procedures used are as follows: lead-hematoxylin according to SOLCIA *et al.*[20] HCl-toluidine blue method for endocrine cells according to SOLCIA *et al.*[21], Bielschowsky argyrophil method as modified by SEVIER and MUNGER[22], Masson's ammoniacal silver method as modified for staining argentaffin cells according to SINGH ,[23] and hematoxyin-eosin.

The frequency of the endocrine cells were calculated in four to seven cases of each observed region *i.e.* the number of the cells stained with each staining method was counted in 3mm^2 and more of the mucosal area per a case and then the mean number of cells per 1mm^2 was estimated. The obtained data were analysed by various analysis.

RESULTS

1. Staining property and presence of endocrine cells.

In the oesophageal mucosa of the duck, from the upper part of the crop to the end of the oesophagus, no cells were stained with the methods applied for endocrine cells.

In the proventriculus, cells stained deep blue purple with lead-hematoxylin, deep blue with HC1-toluidine blue or darkened by the argyrophil methods of Sevier and Munger were observed in the mucosa and the proventricular glands (Pl.1, 1–5). They were found in great number in the glands, but lesser in the mucosa. No cells stained with Masson's method were observed in the proventriculus of the duck.

In the proventricular glands, the cells stained with lead-hematoxylin and with HC1-toluidine blue were scattered in the lobules in great quantity, and they were oval or spindle shaped (Pl. I, 1 and 2). While the cells stained by Sevier and Munger's argyrophil method were only oval in shape (Pl. I, 3). The oval shaped cells stained with each of the three methods above were larger (long diameter about from 12μ to 15μ and short diameter about 7.5μ) than the spindle shaped cells (long diameter about 10μ and short about 5μ). The former were rather more located around the primary or secondary ducts, while the latter were more located in the periphery of the lobules.

In the proventricular mucosa, the endocine cells were usually located in the base of simple tubular glands, oval in shape and larger than epitheliar cells of the mucosa (Pl. I, 4 and 5).

In the mucosa of the isthmus, the shape, the location and the frequency of the cells which were stained with the above mentioned three methods were similar to them in the proventricular mucosa (Pl. I, 6).

In the section of gizzard central part, a small number of the cells stained with only lead-hematoxylin and HC1-toluidine blue were found between the epithelial cells of simple branched tubular glands (Pl. I, 7), but no obvious cells stained with the Sevier and Munger's method could be found.

It was worthy to note that in the restricted part of the gizzard mucosa adjacent to the duodenum, cells stained with lead-hematoxylin or with HC1-toluidine blue were more concentrated than in any other region of the duck digestive tracts observed in this investigation, although there were no cells stained with Masson's and Sevier and Munger's methods. This restricted part is called the pyloric region of the gizzard mucosa in this paper. The pyloric region was about 5mm in wide and distinguished from the duodenum by the absence of villus. A narrow mucosal fold was observed between the pyloric region and the duodenum on the longitudinal section of the gizzard-duodenal junction. In the pyloric region, the horny layer was thinner and the gland was somewhat more alveolar than in the central part of gizzard. The definite boundary between the pyloric region and the central part of the gizzard, however, was indistinct, because the frequency of the endocrine cells gradually decreased as the thickness of the horny layer towards the central part of gizzard increased.

The shapes of the cells in the pyloric region were oval (long diameter about 10μ and

short diameter about 7.5μ), spindle (long diameter about 13–15μ and short diameter about 7.5μ) or more slender spindle (long about 10μ and short about 5μ) (Pl. I, 8 and 9).

In the intestine of the duck, five regions, the duodenum, the jejunum, the ileum, the caecum, and the colo-rectum, were examined. Cells stained with any of the methods used in this investigation were found in the epithelium of both the villi and the crypts of the above mentioned five regions in the same way (Pl. I, 10–13). In other words, Masson's ammoniacal silver reactive cells, argentaffin cells, were observed only in the intestinal mucosa among the duck digestive tracts (Pl. I, 13). Shapes of these cells in five regions of the intestine, were oval in the crypts and spindle in the villi. The long diameter of the oval shape cells was from 10μ to 15μ and their short diameter was about 7.5μ, while the long one of spindle was about 25μ and the short one was from 5μ to 7.5μ..

2. Distribution of endocrine cells.

The frequency and distribution of the endocrine cells in the different regions are shown in the Fig. 1. The cells stained with lead-hematoxylin and HCl-toluidine blue

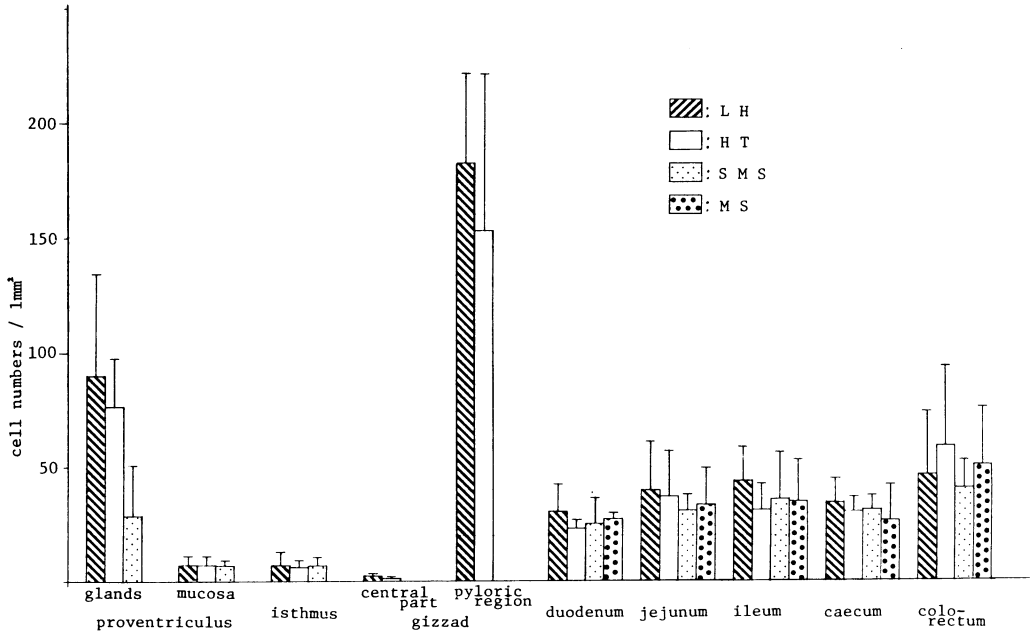

Fig. 1 Frequency of endocrine cells in the different region of the duck digestive tract. LH, HT, SMS and MS show number of cells stained with lead-hematoxylin, HCl-toludine blue, Sevier and Munger's argyrophil method and Masson's ammoniacal silver method respectively. The bar indicates standard deviation.

were most numerous in the pyloric region of the gizzard and secondly in the glandular area of the proventriculus. In the intestine, a moderate number of endocrine cells were observed, and no significant difference was observed among the different regions of the intestine. Only a few endocrine cells occurred in the proventricular and isthmus mucosa, and the fewest in the central part of the gizzard. It was note worthy that Masson's argentaffin method reactive cells were present only in the intestine, and Sevier and

Munger's argylophil method and Masson's method stained cells were absent in the pyloric region.

The frequency of the lead-hematoxylin stained cells did not differ from the HCl-toluidine blue reactive cells in all the regions from the proventriculus to the colo-rectum. In the proventricular glands, however, the mean number of Sevier and Munger's silver method reactive cells per 1mm^2 was about one third of lead-hematoxylin or HCl-doluidine blue. In the intestinal mucosa, there was no significant difference among the mean number of the four methods reactive cells.

DISCUSSION

Lead-hematoxylin and HCl-toluidine blue have been known selectively to stain many kinds of enterochromaffin and nonenterochromaffin, endocrine cells of the gastrointestinal mucosa[3,4,9,20,21] . By means of these two methods in addition to the argyrophil and argentaffin method, the distributions of almost endocrine cells in the duck digestive tracts have been able to be obtain in this paper. In consequence, it seems to indicate that the frequency of the endocrine cells in the duck digestive tracts were highest in the pyloric region of gizzard mucosa, next in the proventricular glands, equally about half the frequency of the pyloric region in the five regions of intestine, one tenth of the proventricular glands in the proventricular and isthmus mucosa, and fewest in the central part of gizzard mucosa.

The gastrointestinal endocrine cells amounted to ten types in humans[5,8,10,12] and so some mammals, for example, five types in the cat[4] six types in the rabbit[3] eight in the pig[9], by means of electron microscopic observation in addition to some histological methods. From the results of this paper, there are at least two types of endocrine cells, argyrophil cells and nonargyrophil cells in the proventriculus. In the pyloric region of the gizzard mucosa, the existence of an other type of cells, nonargentaffin and nonargyrophil cells, is also suggested concerning its shape and staining properties. Moreover, another type of cells, the argentaffin cell, exists in the intestinal mucosa. Particularly, the existence of argentaffin cells seems to point to that of enterocromaffin (EC) cells only in the intestine in reference to the histochemical character of EC cell. The observation of the existence of argyrophil cells in the proventriculus and of argentaffin cells in the intestine of the duck has been consistent with the same observation in the fowl[15,18]. DAWSON and MOYER [15] also observed argyrophil cells in the fowl gizzard. But the existence of these cells in the duck gizzard could not be made evident in this paper. The question whether argyrophil cells are present or not and the more detailed distribution of endocrine cells in the duck gizzard mucosa must be a matter for future investigation. That is to say, the present investigation appears to show the existence of four and more types of endocrine cells in the duck digestive tract. For identification of the endocrine cells types in the duck gastrointestinal mucosa, therefore, electron microscopic investigation should be follows.

FARNER[24], quoted from a paper by SWENANDER, referred that in cormorants,

grebes, many waders, ducks and geese, there is a pyloric stomach which is a separate or partially separated chamber between the gizzard and intestine. The portion which has been called the pyloric region in this paper nearly corresponded to the pyloric stomach mentioned above locally but was distinctly a part of the gizzard under the light microscope. Histilogical structure of the pyloric region of the gizzard mucosa in this observation was similar to the structure in about 0.5cm anterior to the fold separating the gizzard from the intestine of the fowl[25]. It was particularly interesting to find that the endocrine cells were densely present in the pyloric region.

SUMMARY

The four histological methods, previously known to be useful in selective detection of endocrine cells, were applied to the duck digestive tracts, from oesophagus to colorectum. Cells stained with lead-hematoxylin and HCl-toluidine blue were observed in all regions of the duck digestive tracts with the exception of the oesophagus. Argyrophil cells were observed in proventricular mucosa and glands, and in the intestine. Argentaffin cells were observed only in the intestine.

The frequency of endocrine cells in the duck digestive tracts was highest in the restricted region of gizzard mucosa where was called the pyloric region in this paper, next in the proventricular glands, equally about half of the frequency in the pyloric region in the five regions of the intestine, one tenth of that of the proventricular glands in the proventricular and isthmus mucosa, and the smallest frequency was noted in the central part of gizzard mucosa.

The pyloric region was about 5mm anterior to the narrow mucosal fold separating the gizzard from the intestine. It was an interesting to find that the endocrine cells which were nonargentaffin and nonargyrophil were densely present in this region.

From the staining properties and the distribution of the endocrine cells, the possibility of existence of four and more types of endocrine cells in the duck digestive tracts was discussed.

REFERENCES

1) SOLCIA, E and SAMPIETRO, R.: *Z. Zellforsch.*, **68** 689–698 (1965)
2) SOLCIA, E., VASSALLO, G. and SAMPIETRO, R.: *Z. Zellforsch.*, **81**, 478–486 (1967)
3) CAPELLA, C., SOLCIA, E. and VASSALLO, G.: *Arch. histol. jap.*, **30**, 479–495 (1969)
4) VASSALLO, G., SOLCIA, E.: *Z. Zellforsch.*, **98**, 333–356 (1969)
5) PEARSE, A. G. E., COULLING, I., WEAVERS, B. and FRIESEN, S.: *Gut*, **11**, 649–658 (1970)
6) KOBAYASHI, S., FUJITA, T. and SASAGAWA, T.: *Arch. histol. jap.*, **31**, 477–494 (1970)
7) SASAGAWA, T., KOBAYASHI, S.and FUJITA, T.: *Arch. histol. jap.*, **32** 275–288 (1970)
8) KOBAYASHI, S., FUJITA, T. and SASAGAWA, T.: *Arch. histol. jap.*, **32**, 429–444 (1971)
9) CAPELLA, C. and SOLCIA, E.: *Arch. histol. jap.*, **35**, 1–29 (1972)
10) OSAKA , M., SASAGAWA , T., KOBAYASHI , S. and FUJITA , T.: *Arch. histol. jap.*, **33**, 247–260 (1971)

11) OSAKA, M., SASAGAWA, T. and FUJITA, T.: *Arch. histol. jap.*, **35**, 235–248 (1973)
12) SASAGAWA, T., KOBAYASHI, S. and FUJITA, T.: *in* "Gastro-entero-pancreatic endocrine system. A cell-biological approach."
(FUJITA, T. ed.) pp. 17–38, Igaku Shoin, Tokyo (1973)
13) OSAKA, M., SASAGAWA, T. and FUJITA, T.: *Arch. histol. jap.*, 37, 73–94 (1974)
14) MONESI, V.: *Acta anat.*, **41**, 97–114 (1960)
15) AITKEN, R. N. C.: *J. Anat.*, **92**, 453–469 (1958)
16) TONER, P. G. : *Z. Zellforsch.*, **63**, 830–839 (1964)
17) PENTTILÄ, A.:*Z. Zellforsch.*, **91**, 380–390 (1968)
18) DAWSON, A. B. and MOYER, S. L.: *Anat. Rec.*, **100**, 493–515 (1948)
19) KATAOKA, K.: *Arch. histol. jap.*, **36**, 391–400 (1974)
20) SOLCIA, E., CAPELLA, C. and VASSALLO, G.: *Histochemie*, **20**, 116–126 (1969)
21) SOLCIA, E., VASSALLO, G. and CAPELLA, C.: *Stain Technol.*, **43**, 257–263 (1968)
22) SEVIER, A. C. and MUNGER, B. L.: *J. Neuropathol. exp. Neurol.*, **24**, 130–135 (1965)
23) SINGH, I.: *Anat. Anz.*, **115**, 81–82 (1964)
24) FARNER, D. S.: in "Biology and comparative physiology of birds."
(MARSHALL, A. J. ed.), Vol. 1, 435pp., Academic Press, New York and London (1960)
25) HODGES, R. D.: The histology of the fowl, pp. 63–64, Academic Press, London New York San Francisco (1974)

アヒル消化管における内分泌細胞の分布

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食道から直腸末端までのアヒル消化管における内分泌細胞の存在と分布を，鉛ヘマトキシリン，塩酸トルイジンブルー，Sevier and Munger の鍍銀法および Masson の銀親和反応を用いて検索した。

鉛ヘマトキシリンと塩酸トルイジンブルーで染まる細胞は食道を除く消化管の全部位でみられ，好銀性細胞は腺胃粘膜，腺胃腺および腸管の全部位に，銀親和性細胞は腸管にのみみられた。

内分泌細胞の分布密度は，筋胃幽門部が最も多く，次が腺胃腺であり，腸は十二指腸，空腸，回腸，盲腸，結直腸の5部位とも同程度で幽門部の約½，腺胃粘膜および胃峡部は腺胃腺の1/10，筋胃中央部は前2部位よりも少なく最少であった。

筋胃幽門部は，筋胃と十二指腸を分けるわずかな粘膜ひだから筋胃側5㎜位の部分であり，この部に非銀親和性で非好銀性の内分泌細胞が高密度に存在することは興味ある所見である。

これら内分泌細胞の染色性および分布から，アヒル消化管における4種類以上の内分泌細胞の存在が推測された。

Explanation of Plates

Plate I.

All figures show duck digestive tracts. The magnifying force of all figures are x 550.

1 : Proventricular glands. Lead-hematoxylin. Five spindle shaped endocrine cells stained deep blue purple are observed between the glandular epithelium lining the alveoli (a).

2 : Proventricular glands. HCl-toluidine blue. Similar to 1, four spindle shaped cells stained deep blue are observed.

3 : Proventricular glands. Sevier and Munger's argyrophil method. Two typical oval shaped cells darkened are observed. Upper cell is located around the secondary ducts (s).

4 : Proventricular mucosa. Lead-hematoxylin. Two endocrine cells are located in the base of simple tubular gland and oval in shape.

5 : Proventricular mucosa. Sevier and Munger's argyrophil method. Only one typical oval endocrine cell is located in the base of the gland.

6 : Isthmus. HCl-toluidine blue. Two oval endocrine cells are observed at the bottom of this figure. L, lumen.

7 : Central part of gizzard. Lead-hematoxylin. Only one oval endocrine cell is observed between the epithelial cells of gizzard glands.

8 : Pyloric region of the gizzard. Lead-hematoxylin. Oval, spindle or more slender spindle shaped cell are counted nine.

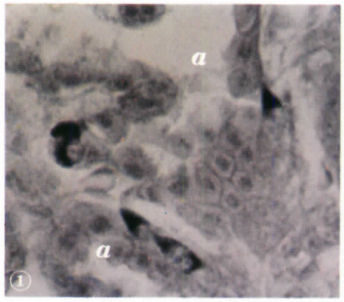
9 : Pyloric region of the gizzard. HCl-toluidine blue. Various shaped fifteen endocrine cells are observed.

10 : Jejunum. Lead-hematoxylin. Three spindle shaped cells are observed between the villous epithelial cells. ℓ, lumen between villi.

11 : Duodenum. HCl-toluidine blue. Between the epithelial cells of crypts, five oval endocrine cells are observed.

12 : Jejunum. Sevier and Munger's argyrophil method. Two typical spindle shaped cells are observed between the epithelial cells of villus. ℓ, lumen between villi.

13 : Jejunum. Masson's ammoniacal silver method. Two typical spindle shaped endocrine cells reacted dark brown are observed alike 10 and 12. ℓ, lumen between villi.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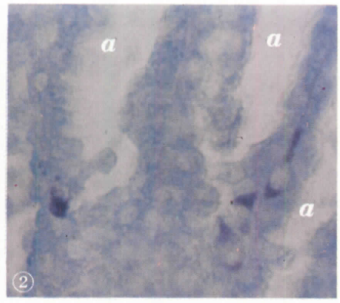
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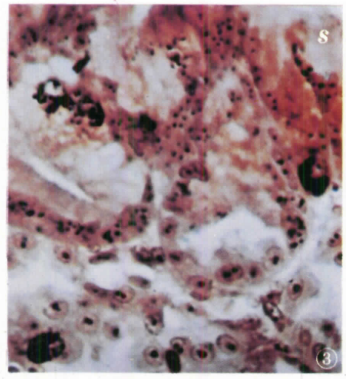
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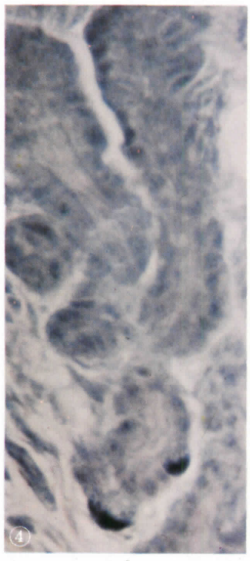

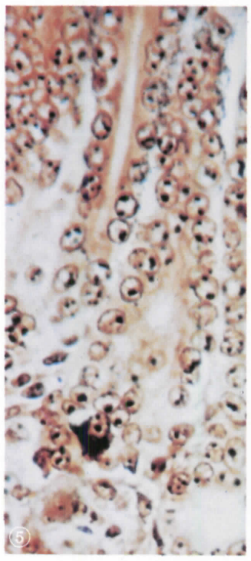

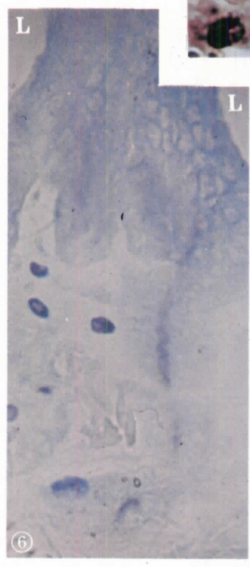
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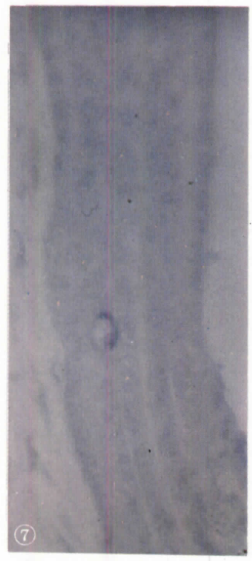

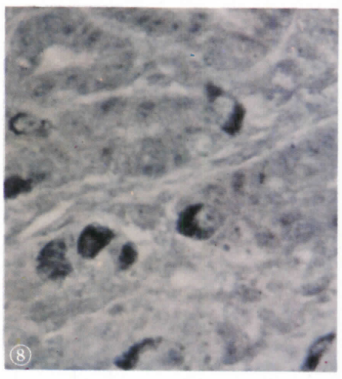

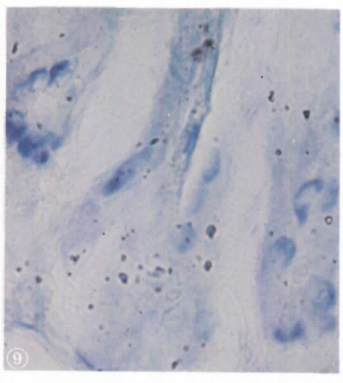

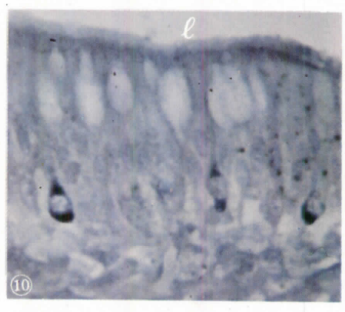
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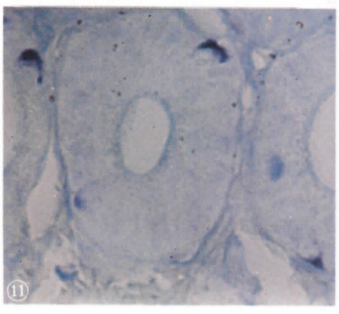

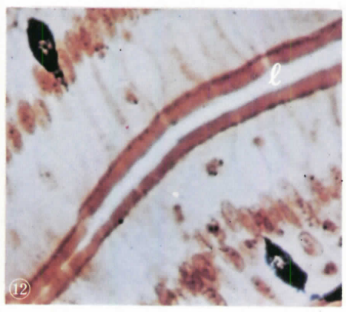
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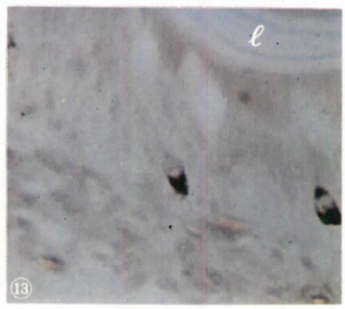
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