Do Enterochromaffin (EC) Cells Contain Motilin ?*

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Summary. Identity of motilin-immunoreactive cells (motilin cells) and enterochromaffin (EC) cells was investigated. Human and dog gastro-entero-pancreatic tissues were examined using conventional stain technologies, indirect immunofluorescence and immunoperoxidase methods and electron microscopy. Enterochromaffin cells were identified by Masson-Hamperl's silver impregnation method. Results obtained were summarized as follows.

1. Motilin-immunoreactive cells were dispersed in the epithelium of the small intestine with a vague tendency to be concentrated in the deeper portion of the crypt.

They were most frequent in the duodenal and jejunal mucosa; a few of them were also seen in the ileal mucosa. No motilin cells were found in the stomach, colon, rectum and pancreas.

2. In contrast, enterochromaffin cells were found in the stomach and pancreas as well as in the whole intestine.

3. Observation of the same or adjacent sections after immunofluorescence and argentaffin reaction demonstrated that motilin-reactive cells were non-argentaffin. Different techniques, including immunoperoxidase method at the electron microscope level, indicated that the enterochromaffin cells were totally different from the cells which contained motilin.

4. In the human fetus (16 to 24-week-old), Segi's cap, i.e., aggregated basal-granulated cells on the top of the villus discovered by SEGI (1935), was seen in the duodenum and upper jejunum. The majority of basal-granulated cells forming this body were argyrophil, 30-50% of them were argentaffin (i.e., enterochromaffin), and less than 10% of them were motilin-immunoreactive.

Motilin isolated from porcine duodenal extracts is a 22-amino acid polypeptide with the amino acid sequence $\text{NH}_2\text{-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-COOH}$ (BROWN, COOK and DRYBURGH, 1973; SCHUBERT and BROWN, 1974). An intravenous injection of motilin induces an increased motor activity in the transplanted pouches of the stomach in the dog

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(BROWN, MUTT and DRYBURGH, 1971) similar to that observed after alkalization of the duodenum (BROWN, JOHNSON and MAGEE, 1966). ITOH and co-workers (1976) suggested that motilin stimulates interdigestive motility of the gut to produce so-called "hunger" or "housekeeper" contractions in the dog.

With regard to the cellular origin, PEARSE and co-workers (1974), POLAK and co-workers (1975), FORSSMANN and co-workers (1976), TOBE and co-workers (1976), GRUBE and co-workers (1977), HEITZ and co-workers (1978), SUNDLER and co-workers (1978), HELMSTAEDTER and co-workers (1979) and SEINO and co-workers (1979) have succeeded in localizing motilin in basal-granulated cells of the gut. There seems to have been general agreement in that, in either human or dog, immunocytoologically motilin-containing cells (called hereafter motilin cells) are abundant in the duodenum and jejunum, and are present in the ileum, but are absent in the stomach and large intestine. However, the coincidence between motilin cells and enterochromaffin (EC) cells is controversial. PEARSE and co-workers (1974), POLAK and co-workers (1975), TOBE and co-workers (1976) and HEITZ and co-workers (1978) stated that the motilin cell is a kind of enterochromaffin cell which is responsible for production of serotonin (5-hydroxytryptamine), whereas FORSSMANN and co-workers (1976), GRUBE and co-workers (1977), SUNDLER and co-workers (1978) and HELMSTAEDTER and co-workers (1979) recognized the motilin cell as differing from any cell of the enterochromaffin category.

We have examined the occurrence and distribution of motilin cells and enterochromaffin cells in both human and dog materials. The present communication describes our data on the cellular origin of motilin in the gastro-entero-pancreatic (GEP) tissues of the human and dog.

In the human fetus older than 5 months of age, basal-granulated cells form a cluster on the top of the intestinal villus. As this peculiar structure was first described by SEGI (1935, 1936) we recently proposed to call it SEGI's cap (KOBAYASHI et al., 1980). The present report also includes some findings on the SEGI's cap with special reference to the occurrence of motilin cells and enterochromaffin cells in it.

MATERIALS AND METHODS

Tissues. Tissues examined were as follows: 1) The fundic and pyloric portions of the stomach, duodenum, jejunum, ileum, colon, rectum and pancreas of adult mongrel dogs. 2) Adult human duodenal mucosa obtained by biopsy. 3) Fundic and pyloric portions of the stomach, duodenum and pancreas of the human fetus (5 fetuses, 16–24 weeks old). The jejunum, ileum, colon and rectum from the same human fetuses.

Fixation and embedding. Tissues were fixed for 2 to 8 hrs in Bouin's fluid or in 10% formalin at room temperature. Freeze-dried and diethylpyrocarbonate (DEPC)-treated tissues were also used. Sections of 4–6 μ m thickness were cut on a microtome after paraffin embedding.

Anti-motilin sera. Two anti-motilin sera (YANAIHARA et al., 1980: R1104, R1105) were used. These sera were obtained from two rabbits immunized with synthetic porcine motilin. Data concerning the specificities of these two sera have been published

elsewhere (YANAIHARA et al., 1980).

Immunohistochemistry. Identification of motilin cells at the light microscopic level was performed by the technique of indirect immunofluorescence (COONS, LEDUC and CONNOLLY, 1955) or immunoperoxidase methods (NAKANE and PIERCE, 1966) using above-mentioned antisera at a dilution of 1:40.

Specificity tests of the immunohistochemical reactions were performed as follows: 1) Incubating the sections with normal rabbit serum as the first layer. 2) Incubating the sections with antigen-inactivated anti-sera (10 μ g synthetic motilin per ml diluted serum).

Electron microscopic observation. Bouin-fixed tissue-sections of 20 μ m thickness were mounted on a glass slide. Indirect immunoperoxidase reaction was performed. After osmication overnight in a 1.0% osmium tetroxide solution at 4°C, the tissue sections were detached from the glass slide with a razor blade and embedded in Araldite. Ultrathin sections were examined in the electron microscope after staining with uranyl acetate and Reynold's lead.

Identification of enterochromaffin cells. Enterochromaffin cells were identified using Masson-Hamperl's argentaffin method (SINGH, 1964).

Argyrophil reaction was also examined using the technique of GRIMELIUS (1968).

RESULTS

Motilin cells

Identical results were obtained by the use of two different anti-motilin sera, R1104 and R1105, with regard to the distribution of motilin cells in the gastro-entero-pancreatic tissues. Immunohistochemical reaction was not demonstrated by the use of anti-motilin sera pre-treated with an excess amount of motilin.

In the adult human duodenum obtained by biopsy and in the dog small intestine, motilin cells were localized predominantly in the lower portion of the crypts (Fig. 1). A few of the motilin cells were situated on the villi. Motilin-immunoreactivity was not seen within any cells in the lamina propria, tela submucosa, tunica muscularis and tunica serosa in all segments of the gastro-enteric tract.

Motilin cells were basal-granulated cells with a thin cytoplasmic process reaching the gut lumen. The immunoreactivity was intense in the basal-portion of the cell whereas the neck portion showed a weakly positive reaction (Fig. 2).

Distribution of motilin cells and enterochromaffin cells

Table 1 shows the distribution of motilin cells, enterochromaffin cells and argyrophil cells in the dog gastro-entero-pancreatic tissues. The motilin cells were predominantly found in the duodenum and jejunum. A few of them were also seen in the ileal mucosa. No positively immunoreacting cells were demonstrated in any region of the stomach and large intestine. Enterochromaffin cells were found throughout the whole length of the dog gastro-enteric mucosa. They were most numerous in the duodenum. A considerable number of them were present in the stomach and large intestine.

Comparison of the distribution of the enterochromaffin cells demonstrated by

Fig. 1. Motilin-immunoreactive basal-granulated cells in the dog duodenum. They were situated in the epithelial tissue of both crypt and villus. Motilin cells were demonstrated by the indirect immunoperoxidase method using R1104 antiserum. Bouin fixation. $\times 400$

Fig. 2. A closer view of a cross sectioned crypt in the dog duodenum. Three motilin-immunoreactive cells are seen. The thickened basal cytoplasm contains strong motilin-immunoreactivity. $\times 1,400$

Table 1. Distribution of motilin cells, enterochromaffin cells and argyrophil cells in the gastro-entero-pancreatic tissues of the dog

Cell kind	Argyrophil cell	Enterochromaffin cell (Argentaffin cell)	Motilin cell
Technique	Grimelius' method	Masson-Hamperl's method	Indirect immunoperoxidase method
Stomach	22,000/mm ³	2,200/mm ³	0/mm ³
Duodenum	13,000	5,700	680
Jejunum	12,000	2,300	570
Ileum	13,000	1,800	70
Colon	8,900	3,200	0
Pancreas	Many	A few	None

The number of cells contained in each section was counted under the light microscope, whereas the volume of the mucosa was calculated from the area and thickness of the section.

Concentration of cells was expressed by the number per 1 mm³.

The accuracy of the value of each figure should not be overestimated since the shrinkage and deformation of tissues was neglected and it was frequently difficult to decide whether a profile of a cell represented a single cell. Motilin cells were demonstrated in 4 μ m thick paraffin sections of Bouin-fixed tissues by indirect immunoperoxidase method using R1104 anti-motilin serum.

the argentaffin reaction and the motilin cells revealed by the immunoperoxidase method gave the following results:

- 1) In the dog no motilin cells were found in either the stomach or large intestine, whereas many enterochromaffin cells were present there.
- 2) Motilin cell to enterochromaffin cell ratio in the canine duodenum, jejunum and ileum was 1:8, 1:4 and 1:25, respectively.

Argyrophil cells

All the enterochromaffin (=argentaffin) cells were argyrophil, but only a part of the argyrophil cells were argentaffin. In the human duodenum obtained by biopsy, argyrophil cells were demonstrated in the villus and crypt. They were conspicuously larger in number than the enterochromaffin cells.

In the dog, argyrophil cells were seen throughout the gastro-enteric tract, i. e., from the cardia of the stomach to the rectum. In the gastric corpus, they were abundant in the bottom of the pits. A few of them were also seen in the neck portion of the fundic gland. In the fundic gland argyrophil cells (as well as other types of basal-granulated cells) were usually closed in type. Sometimes they extended considerably long cytoplasmic processes along the basement membrane.

In the pyloric antrum of the dog, argyrophil cells were abundant in the neck portion of the pyloric gland frequently forming a cluster. They were open in type with a cytoplasmic process reaching the gut lumen. In addition to this luminal process they extended a cytoplasmic process containing secretory granules towards the epithelial base and sometimes along the basement membrane.

In the small intestine of the dog, argyrophil cells were located in both villus and crypt and were open in type. In the large intestine argyrophil cells were open

Fig. 3. SEG1's cap on the duodenal villus of a 6-month human fetus as demonstrated in three $4\mu\text{m}$ thick serial sections (**a**, **b** and **c**). Motilin-cells (**a**), enterochromaffin(argentaffin) cells (**b**) and argyrophil cells (**c**) are illustrated. Bouin fixation. $\times 900$. **a.** Indirect immunoperoxidase method using anti-motilin serum, R1104. **b.** Masson-Hamperl's silver impregnation. **c.** Grimelius' silver impregnation.

in type and were distributed in the crypt.

The distribution of argyrophil cells in the dog gastro-enteric tract is shown in Table 1. Argyrophil cells were present throughout the whole length of the gut. They were 8 times as frequent as argentaffin cells in the stomach; 13 times in the pyloric antrum; 2 times in the duodenum; 5 times in the jejunum; 7 times in the ileum; and 3 times in the large intestine.

Basal-granulated cells in Segr's cap

In the human fetuses examined, enterochromaffin cells and argyrophil cells were distributed throughout the whole length of the gastro-enteric tract. Motilin cells were demonstrated in the small intestine.

One of the most striking features of the fetal gut was the formation of a large aggregation of basal-granulated cells on or near the apex of the intestinal villus (Fig. 3), which exactly corresponded to what SEGI (1935) reported and which we proposed to call Segr's cap (Kobayashi et al., 1980). When Segr's cap existed the intestinal villus was swollen and its club-shaped apex often hollowed out to form a deep concavity (Fig. 3). The cap consisted of hundreds of basal-granulated cells and a few goblet cells. These cells, as a whole, formed either a flat cushion-shaped, a shallow dish-shaped or a deep cup-shaped structure. Although nuclei of the basal-granulated cells in the Segr's cap were disposed at different levels of the epithelial tissue, it seemed reasonable to suggest that all the cells in the Segr's cap rested on the basement membrane and extended to the luminal surface with a thin cytoplasmic process.

As shown in Figure 3 most of the basal-granulated cells in the Segr's cap were argyrophil, i.e., GRIMELIUS positive. Enterochromaffin cells occupied 30-50% of the

Fig. 4. **a** and **b**. Motilin cells and enterochromaffin cells in the duodenum of a 6-month human fetus. The same section, first treated for the immunofluorescence demonstration of motilin (**a**) is silver-impregnated by Masson-Hamperl's technique (**b**). In **a** a motilin cell (long arrow) is strongly fluorescent in yellowish green. This cell is non-argentaffin in **b**. Cells with a weak yellowish fluorescence in **a** are argentaffin in **b** so that they are enterochromaffin cells (short arrows). Fixation in Bouin's fluid. $\times 700$

Fig. 5. **a** and **b.** Black-and-white pictures of the same section of the duodenum of a 6-month human fetus. After indirect immunofluorescence procedure for motilin (**a**), enterochromaffin cells were identified by Masson-Hamperl's silver-impregnation technique (**b**). In **a**, yellowish autofluorescence of the enterochromaffin cell (short arrows) cannot be distinguished from green fluorescence of the motilin cell (long arrow). Fixation in Bouin's fluid. $\times 900$

total population of the basal-granulated cells forming the cap. Motilin cells were found but they were noteworthy small in number occupying less than 10% of the whole population of the aggregated basal-granulated cells.

Motilin cells versus enterochromaffin cells

To further investigate the identity of motilin cells, argentaffin reaction was performed on the same section which had been examined by immunofluorescence for localization of motilin. The following points were made clear:

1) Argentaffin reaction was positive in cells completely different from the cells which showed a motilin immunoreactivity. This indicates that the motilin cells

Fig. 6. Autofluorescent cells (a), motilin cells (b), and enterochromaffin cells (c) demonstrated in a single section of the Segr's cap on the top of the duodenal villus of a 6-month human fetus. $\times 1,100$. After short fixation (1 hr) in formalin many cells in the Segr's cap are autofluorescent (a). Motilin-cells (asterisks) were demonstrated by the indirect immunofluorescence method (b). Then, the same section was silver impregnated by the method of Masson-Hamperl (c). Motilin cells (b) are non-autofluorescent (a) and non-argentaflin (c). Autofluorescent cells (a) and argentaflin cells (c) are identical.

Fig. 7. a. and b. Electron micrographs of a motilin cell. Secretory granules of the motilin cell are rounded in shape, hence they are distinguishable from the pleomorphic granules of an enterochromaffin cell. a: $\times 7,000$; b: $\times 40,000$

are not identical with the enterochromaffin cells (Fig. 4–6).

2) Enterochromaffin cells in the Bouin- or formalin-fixed material exhibited orange or yellowish fluorescence under the fluorescence microscope. This fluorescence was weak, but distinct (Fig. 4 a, 5 a, 6 a).

3) This fluorescence of the enterochromaffin cells was not related to motilin, since it existed prior to the immunohistochemical procedure (Fig. 6 a).

Motilin cells versus argyrophil cells

To examine the argyrophil properties of the motilin cells, sections were silver-impregnated by the method of GRIMELIUS (1968) after immunofluorescence procedure for motilin.

All the motilin cells were argyrophil, but only a portion of the argyrophil cells were motilin-containing. Counting in the dog showed that the motilin cell to argyrophil cell ratio was 1:19 in the duodenum, 1:21 in the jejunum and 1:180 in the ileum (see Table 1).

Electron microscopy of the motilin cell

Bouin-fixed and paraffin-embedded duodenal mucosa of a 6-month human fetus was examined in the electron microscope after immunoperoxidase procedure and post-osmication.

Motilin cells possessed rounded secretory granules with a profile diameter 200 to 400 nm. They were distinguishable from polymorphous secretory granules of the enterochromaffin (EC) cells on which no motilin immunoreactivity was demonstrated.

DISCUSSION

By immersing the fundic mucosa of the stomach in potassium dichromate and teasing the tissue with needles, HEIDENHAIN (1870) described that the fundic gland contained small, yellow/brown stained cells in addition to pepsinogen-producing chief cells and acid-producing parietal cells. For a long time since this discovery of the basal-granulated cells, it was accepted that various staining properties of the basal-granulated cells, e.g., the chromaffin reaction, argentaffin reaction, argyrophil reaction and diazonium reaction, might be explained by different stages in the life cycle of a single type of enterochromaffin or argentaffin cells which were regarded as serotonin-producing cells. With the progress mainly in the field of electron microscopy, this single-cell-type theory had to be replaced by a multiple-cell-type concept, according to which over ten independent types of basal-granulated cells corresponded to different hormones (For review see: FUJITA and KOBAYASHI, 1973). The serotonin producing enterochromaffin cells were then designated as EC cells and thought to represent one of the numerous cell types.

With the progress of this concept, possible production of both serotonin and a biologically active polypeptide in the EC cells was proposed by FUJITA and KOBAYASHI (1973). PEARSE and co-workers (1974) then reported that motilin was the polypeptide in the EC cells. Thus, these authors are of the view that the EC cell produces motilin plus serotonin.

In agreement with FORSSMANN and co-workers (1976; vide infra), however, we reached the opinion that the EC cells are not the source of motilin. The findings on which this concept is based will now be contrasted against the descriptions by PEARSE and his group.

PEARSE and co-workers (1974) stated that 85% of motilin-producing cells were argyrophil and argentaffin, and the rest were argyrophil and non-argentaffin. Our present result demonstrated that motilin cells were argyrophil but non-argentaffin.

PEARSE and co-workers (1974) wrote that EC cells were non-fluorescent after short fixation in formalin or after diethylpyrocarbonate (DEPC) treatment. In our study, however, EC cells, probably because of the presence of serotonin, showed faint but distinct yellowish fluorescence even after short fixation in formalin. Similar fluorescence was also seen in the Bouin-fixed materials.

It should be pointed out that PEARSE and co-workers (1974) published only black-and-white pictures and did not mention the fluorescence color of the EC cell. We noticed that, in black-and-white pictures, it is difficult to distinguish the motilin-specific green fluorescence of FITC and yellowish non-motilin-specific fluorescence

of the cell. The conclusion of PEARSE and co-workers (1974), thus, may possibly be based not on careful observations of the tissue sections in the fluorescence microscope but on judgment from photomicrographs.

By applying the Falck-Hillarp method for biogenic monoamines and the indirect immunofluorescence method to the same tissue section, FORSSMANN and co-workers (1976) concluded that motilin cells contained no serotonin, whereas enterochromaffin cells possessed no motilin-immunoreactivity. POLAK and BUCHAN (1979) explained the discrepancy between PEARSE and co-workers (1974) and FORSSMANN and co-workers (1976) with regard to the cellular origin of motilin by possible heterogeneity of motilin molecules. Amino acid sequence of motilin molecules could be different from animal to animal and/or from tissue to tissue. Furthermore, multiplicity of anti-motilin sera should also be considered. PEARSE and co-workers (1974) used anti-natural porcine motilin sera, whereas FORSSMANN and co-workers (1976) used anti-synthetic motilin sera. However, the results of the present study suggested that the "motilin immuno-fluorescence in the EC cells" reported by PEARSE and co-workers (1974) could have been indistinguishable from the non-immune fluorescence of the cell.

According to the "Lausanne classification" of the GEP endocrine cells, enterochromaffin cells were classified into subtypes, i.e., EC₁, EC₂ and EC_n (SOLCIA et al., 1978). EC₁ represents enterochromaffin cells which produce both serotonin and substance P, whereas EC₂ means those which secrete serotonin and motilin. EC_n cells are unrelated to either substance P or motilin. However, the present result does not support the view that motilin cells comprise a subpopulation of EC cells. No cells can be called EC when they are not chromaffin or argentaffin in nature, i.e., when they are not serotonin-containing.

Large aggregations of basal-granulated cells on the fetal intestinal villus which were found by SEGI (1935) were reinvestigated in the present study. We found EC cells as well as motilin cells in this characteristic structure whose embryological and physiological implications are still far-reaching. A more detailed immunohistochemical study of SEGI's cap is published elsewhere (IWANAGA et al., 1980).

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腸クロム親和細胞はモチリンを含むか

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腸クロム親和細胞とモチリン免疫活性細胞（モチリン細胞）の異同について、ヒトとイヌの胃、腸、脾の組織で検討した。通常の組織学的方法のほかに、蛍光抗体間接法と酵素抗体間接法、および電子顕微鏡の手法を用いた。腸クロム親和細胞は Masson-Hamperl の鍍銀法で同定した。結果は以下の4項目に要約される。

1. モチリン細胞は基底果粒細胞の一種で、小腸粘膜に散在する。陰窩の深部に集中する傾向が認められる。

2. モチリン細胞は十二指腸と空腸に多い。回腸にも認められる。胃、結腸、直腸および脾にはみられない。

3. 腸クロム親和細胞はモチリン免疫活性を示さない。モチリン細胞は非銀親和性である。したがって腸クロム親和細胞とモチリン細胞は異なると結論された。

4. 瀬木 (1935) が発見した基底果粒細胞の巨大な集団を、ヒト胎児 (16~24週齢) の十二指腸の絨毛先端部に観察した。瀬木の帽子と呼ばれるこの基底果粒細胞の集団の大部分が銀好性、30~50%が銀親和性、そして10%以下がモチリン免疫活性を示した。

REFERENCES

- Brown, J. C., M. A. Cook and J. R. Dryburgh:** Motilin, a gastric motor activity stimulating polypeptide: the complete amino acid sequence. *Canad. J. Biochem.* 51: 533-537 (1973).
- Brown, J. C., L. P. Johnson and D. F. Magee:** Effect of duodenal alkalization on gastric motility. *Gastroenterology* 50: 333-339 (1966).
- Brown, J. C., V. Mutt and J. R. Dryburgh:** The further purification of motilin, a gastric motor activity stimulating polypeptide from the mucosa of the small intestine of hogs. *Canad. J. Physiol. Pharmacol.* 49: 399-405 (1971).
- Coons, A. H., E. H. Leduc and J. M. Connolly:** Studies on antibody production. 1. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. exp. Med.* 102: 49-63 (1955).
- Forssmann, W. G., N. Yanaihara, V. Helmstaedter and D. Grube:** Differential demonstration of the motilin-cell and the enterochromaffin cell. *Scand. J. Gastroenterol.* 11 (Suppl. 39): 43-45 (1976).
- Fujita, T. and S. Kobayashi:** The cells and hormones of the GEP endocrine system—The current of studies. In: (ed. by) T. FUJITA: Gastro-entero-pancreatic endocrine system. A cell-biological approach. Igaku-Shoin, Tokyo, 1973 (p. 1-16).
- Grimelius, L.:** A silver nitrate stain for α_2 cells in human pancreatic islets. *Acta Soc. Med. Upsal.* 73: 243-270 (1968).
- Grube, D., V. Helmstaedter, G. Feurle, N. Yanaihara and W. G. Forssmann:** Die enteroendokrinen Zellen des Spitzehörnchen. *Verh. Anat. Ges.* 71: 1137-1143 (1977).
- Heidenhain, R.:** Untersuchungen über den Bau der Labdrüsen. *Arch. mikrosk. Anat.* 6: 368-406 (1870).
- Heitz, P. U., M. Kasper, G. Krey, J. M. Polak and A. G. E. Pearse:** Immunoelectron cytochemical localization of motilin in human duodenal enterochromaffin cells. *Gastroenterology* 74: 713-717 (1978).
- Helmstaedter, V., W. Kreppein, W. Domschke, P. Metznegg, N. Yanaihara, E. Wunsch and W. G. Forssmann:** Immunohistochemical localization of motilin in endocrine non-enterochromaffin cells of the small intestine of humans and monkey. *Gastroenterology* 76: 897-902 (1979).
- Itoh, Z., R. Honda, K. Hiwatashi, S. Takeuchi, I. Aizawa, R. Takayanagi and B. F. Couch:** Motilin-induced mechanical activity in the canine alimentary tract. *Scand. J. Gastroenterol.* 11 (Suppl. 39): 93-110 (1976).
- Iwanaga, T., S. Kobayashi, T. Fujita and N. Yanaihara:** Immunocytochemistry and ultrastructure of SEG's cap. *Biomed. Res.* 1: 117-129 (1980).
- Kobayashi, S., T. Iwanaga and T. Fujita:** SEG's cap: Huge aggregation of basal-granulated cells discovered by SEG (1935) on the intestinal villi of human fetus. *Arch. histol. jap.* 43:

- 79-83 (1980).
- Nakane, P. K. and G. B. Pierce, Jr.:** Enzyme-labelled antibodies: preparation and application for the localization of antigens. *J. Histochem. Cytochem.* 14: 929-931 (1966).
- Pearse, A. G. E., J. M. Polak, S. R. Bloom, C. Adams, J. R. Dryburgh and J. C. Brown:** Enterochromaffin cells of the mammalian small intestine as the source of motilin. *Virchows Arch. B Cell Pathol.* 16: 111-120 (1974).
- Polak, J. M. and A. M. J. Buchan:** Motilin. Immunocytochemical localization indicates possible molecular heterogeneity or the existence of a motilin family. *Gastroenterology* 76: 1065-1066 (1979).
- Polak, J. M., A. G. E. Pearse and C. M. Heath:** Complete identification of endocrine cells in the gastrointestinal tract using semithin-thin sections to identify motilin cells in human and animal intestine. *Gut* 16: 225-229 (1975).
- Schubert, H. E. and J. C. Brown:** Correction to the amino acid sequence of porcine motilin. *Canad. J. Biochem.* 52: 7-8 (1974).
- Segi, M.:** Über eine aus Chromaffine-Darmzellen bestehende Struktur auf den Zotten von Menschenembryo. (In Japanese with German summary). *Acta anat. nippon. (Kaibogaku Zasshi) (Tokyo)* 8: 276-280 (1935).
- : Über die Entwicklung der verschiedenen Granulazellen im Darmepithel des Menschenembryo. (In Japanese with German summary). *Acta anat. nippon. (Kaibogaku Zasshi) (Tokyo)* 9: 850-937 (1936).
- Seino, Y., D. Porte, N. Yanaihara and P. H. Smith:** Immunocytochemical localization of motilin-containing cells in the intestines of several vertebrate species and a comparison of antisera against natural and synthetic motilin. *Gen. comp. Endocrinol.* 38: 234-237 (1979).
- Singh, I.:** A modification of the Masson-Hamperl method for staining of argentaffin cells. *Anat. Anz.* 115: 81-82 (1964).
- Solcia, E., et al.:** Lausanne 1977 classification of gastroenteropancreatic endocrine cells. In: (ed. by) S. R. Bloom: *Gut hormones*. Churchill Livingstone, Edinburgh-London-New York, 1978 (p. 40-48).
- Sundler, F., J. Alumets, R. Håkanson and K. Sjölund:** Motilin and carcinoid tumours. *Lancet*: 1101 (1978).
- Tobe, T., S. T. Chen, H. Yajima, Y. Kai and H. Kawatani:** Localization of motilin in human and dog gastrointestinal tract by immunofluorescence. In: (ed. by) T. Fujita: *Endocrine gut and pancreas*. Elsevier, Amsterdam, 1976 (p. 113-118).
- Yanaihara, N., Ch. Yanaihara, K. Nagai, H. Sato, F. Shimizu, K. Yamaguchi and K. Abe:** Motilin-like immunoreactivity in porcine, canine, human and rat tissue. *Biomed. Res.* 1: 76-83 (1980).

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