An Immunohistochemical and Ultrastructural Study of Segi's Cap, a Large Aggregation of Gut Endocrine Cells, in Bovine Fetuses

Hirofumi KURAMOTO,* Junzo YAMADA, Nobuo KITAMURA, Tadayuki YAMASHITA1 and Noboru YANAIHARA2

Department of Veterinary Anatomy (Prof. T. YAMASHITA),1 Obihiro University of Agriculture and Veterinary Medicine, Obihiro; and Laboratory of Bio-organic Chemistry (Prof. N. YANAIHARA),2 Shizuoka College of Pharmacy, Shizuoka, Japan

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Summary. Segi's cap, a large aggregation of basal-granulated cells at the top of the intestinal villus, was studied in the proximal small intestine of bovine fetuses by histological, immunohistochemical and ultrastructural techniques. 1) Typical Segi's caps were seen in the duodenum and proximal jejunum in bovine fetuses. 2) Smaller groups of basal-granulated cells were found in the villous and partly also in the cryptal epithelium, as well as in the subepithelial lamina propria. The possible mechanism for their occurrence was discussed in connection with the fate of the cap. 3) Segi's caps were present in a neonatal calf before the suckling stage, but not in 3 or 4 week-old calves. The process of and reason for this abrupt disappearance of the caps are unknown. 4) The Segi's cap in bovine fetuses consisted mainly of argyrophil cells as demonstrated by Grimelius' and Hellerstrom-Hellman's silver methods. Only a few argentaffin cells were found in fetal caps using a modified Masson-Hamperl's silver method. 5) Immunohistochemically, somatostatin-, gastrin-, motilin- and secretin-immunoreactive cells were identified. 6) Four different endocrine cell types could be distinguished in the bovine Segi's cap on the basis of the ultrastructural appearance of their secretory granules.

SEGI (1935, 1936) first reported on a huge aggregation of basal-granulated cells at the top of the intestinal villus in human fetuses. This excellent description of SEGI, however, has attracted little attention from researchers for a long time. Recently KOBAYASHI et al. (1980) confirmed the occurrence of precisely what SEGI depicted 45 years ago and proposed the name "Segi's cap" to designate this peculiar structure. Furthermore, Iwanaga et al. (1980) investigated the cells forming the Segi's cap in the human fetus both immunohistochemically and ultrastructurally. They identified somatostatin-, gastrin- and motilin-immunoreactive cells by immunohistochemistry and EC, D and S cells on the basis of the ultrastructure of their secretory granules.

However, explorations into the mechanism of the formation and the significance of the structure of the Segi's cap have been hindered mainly because no study has been available on the occurrence of the Segi's cap in the gut of species other than the

*Present address: Department of Anatomy, Niigata University School of Medicine, Niigata, Japan
human. Our preliminary study reported that Segi's cap occurred not only in human fetuses but also in bovine and porcine fetuses (YAMADA et al., 1981). It seems worthwhile to compare the terms of occurrence and the cellular construction of the Segi's cap in bovine with those in humans, since the length of gestation is the same. The present study deals with the immunohistochemistry and ultrastructure of the Segi's cap in the gut of bovine fetuses, with some calves included.

MATERIALS AND METHODS

Sixty-two bovine fetuses ranging from 6 to 94 cm crown-rump length (CRL), a neonatal calf before the suckling stage, and a 3 week-old and a 4 week-old suckling calf were used in this study. In 35 fetuses and 3 calves, the cardiac, fundic and pyloric portions of the stomach, duodenum, jejunum, ileum, cecum, colon and rectum were investigated, but in the other 27 fetuses, only the duodenum was examined. The tissues were fixed in Bouin's fluid or 10% neutral buffered formalin and embedded in paraffin. Sections of 3-6 μm thickness were cut and mounted on glass slides coated with gelatin.

The argyrophil cells were stained by treating the sections with Grimelius' (GRIMELIUS, 1968) or Hellerstrom-Hellman's (HELLERSTROM and HELLMAN, 1960) silver impregnation method. To examine the argentaffin cells, Masson-Hamperl's method modified by SINGH (1964) was applied.

Immunohistochemical staining was performed to localize peptide hormones in the cells of Segi's cap, using the unlabelled antibody enzyme method with purified anti-peroxidase serum (bridge method) or peroxidase-antiperoxidase complex (PAP method) (STERNBERGER, 1979). The following antisera were used as the primary layer: 1) rabbit anti-synthetic somatostatin serum (Japan Immunoresearch Laboratories, Takasaki, JG-1, diluted at 1:2,000), 2) rabbit anti-synthetic porcine motilin serum raised by one of the authors (N. Y.) (R-1104, specific for the C-terminal portion, 1:2,000), 3) rabbit anti-synthetic porcine secretin serum (N. Y., R-801, 1:1,000), 4) guinea-pig anti-synthetic human gastrin serum (N. Y., GP-1304, 1:1,000), 5) rabbit anti-synthetic bovine neurotensin serum (N. Y., R-3501, 1:1,000), 6) rabbit anti-CCK serum (provided by Prof. D. GRUBE, Hannover, Germany; recognizing the part 11-20 of CCK and showing no cross reaction with gastrin, 1:1,000), 7) rabbit anti-synthetic porcine glicentin serum (N. Y., R-4804, recognizing the C-terminal portion, 1:2,000), 8) rabbit anti-synthetic porcine VIP serum (N. Y., R-502, 1:3,000), 9) rabbit anti-synthetic porcine substance P serum (N. Y., R-2404, 1:2,000). The specificity of the immunohistochemical reaction was checked as recommended by STERNBERGER (1979), including the use of the sections with corresponding antigen-inactivated antisera.

For electron microscopy, 8 bovine fetuses were used. Small tissue pieces were obtained from the duodenal mucosa, fixed in 2.7% glutaraldehyde buffered with 0.1 M phosphate with pH 7.4 for 2 hrs and post-fixed in 1.0% OsO₄ in the same buffer for 1 hr. After dehydration in a series of graded ethanol, the pieces were embedded in Spurr's resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate and observed with a Hitachi HU-125D-S electron microscope.
RESULTS

General view of Segi's cap

The Segi's cap in bovine corresponded in its essential structure to that in humans which was depicted by Segi (1935, 1936) and confirmed by Kobayashi et al. (1980), and appearing as follows: The top of the intestinal villus was generally swollen more or less and concave in its luminal surface, and a major part of this invagination was lined by numerous basal-granulated cells. The caps in bovine fetuses were found in the duodenum and proximal jejunum, more numerously in the former than in the latter.

The bovine Segi's cap contained numerous argyrophil cells as demonstrated by the Grimelius' (Fig. 1a) and Hellerström-Hellman's (Fig. 1b) silver impregnation, and only a few argentaffin cells as demonstrated by a modified Masson-Hamperl's method (Fig. 2a). Some of the argyrophil cells in the cap were "open" in type, having a direct con-

![Fig. 1. Argyrophil cells in Segi's caps demonstrated by Grimelius' method in a bovine fetus of 50 cm CRL (a) and Hellerström-Hellman's method in a 71 cm fetus (b). Note the numerous argyrophil cells in the caps. ×360](image1)

![Fig. 2. a. Argentaffin cells in a Segi's cap demonstrated by Masson-Hamperl's method. Only a few argentaffin cells (arrows) are present. b. Argentaffin cells in duodenal crypts demonstrated by the same procedure. Note that the reaction here is stronger than in the cap. Both from a 68 cm fetus. ×480](image2)
nection with the lumen by their apical cytoplasm. Cells located in the lower part of the cap seemed to have no connection with the lumen ("closed" type).

In the series of bovine fetuses examined by hematoxylin-eosin staining and silver impregnation techniques, the Segi's cap was first detected in a fetus with a CRL of 25 cm. It thereafter increased in size and frequency of occurrence as the CRL increased up to 90 cm in prenatal fetuses. It was also present in a newborn calf taken about one hour after birth and before starting to suckle, although the caps were recognized in the upper small intestine at a very low frequency of occurrence; no Segi's cap was found in any portion of the intestine in the 3 week-old or 4 week-old calves.

In contrast to the argyrophil cells which could be detected in the fetus with 25 cm CRL, argentaffin cells were demonstrable in fetuses larger than 33 cm CRL. The argentaffin reaction within the cap was generally weaker than in argentaffin cells in the epithelium outside the cap (Fig. 2b).

**Aggregations of argyrophil cells outside Segi's cap**

In the fetuses with 20–27 cm CRL, small cell groups containing argyrophil cells were frequently found in the duodenal epithelium, apart from the site of the Segi's cap. Most of them were found in the villi, but a few were in the crypts. When, in the fetuses with a CRL of 30–39 cm, the argyrophil cells significantly increased in number in the epithelium inside and outside the Segi's cap, these small groups of argyrophil cells suddenly increased in number in both the villi and crypts. In the fetuses with a CRL of 41–70 cm, small groups containing five or more argyrophil cells occurred not only in the epithelium but also in the lamina propria. Some of them in the lamina propria were connected with the cap and others were isolated from it. They were

![Fig. 3. a–e. Serial sections of 6 μm thickness stained by Grimelius' method. Note that groups of argyrophil cells (arrows) are seen in the lamina propria of the duodenal villi without a connection to the epithelium. From a 94 cm fetus. ×360](image-url)
mostly found in the upper part of the villi, and much less in the crypts; the lower part of the villi contained still fewer numbers of them. At this stage, the Segi's caps increased in number.

In fetuses with a CRL of more than 70 cm, argyrophil cell groups gradually decreased in number. The cell groups at this stage tended to protrude from the epithelium deeply into the lamina propria of the villi. They were sometimes observed surrounded by the latter tissue. It was confirmed in the serial sections that some of these cell groups had no connection with the epithelium (Fig. 3 a–e). No argyrophil cell group was found in the duodenal mucosae of the calves after birth.

**Immunohistochemistry in the Segi's cap**

Immunohistochemically, somatostatin-, motilin-, gastrin- and secretin-immunoreactive cells were demonstrated in bovine Segi's cap (Fig. 4 a–b). In the Segi's cap, somatostatin-immunoreactive cells occupied about 50% of the total population of cells, motilin-immunoreactive cells about 20%, secretin-immunoreactive cells about 10% and gastrin-immunoreactive cells less than 10%. Caps whose major part was occupied by somatostatin-immunoreactive cells were frequently observed (Fig. 5a). Somatostatin-immunoreactive cells were most numerous also in the epithelium outside the cap, where they tended to form small aggregations. These aggregations were located not only within the epithelium but also frequently in the propria mucosae closely beneath the epithelium (Fig. 5b) and occasionally in the lamina propria of the duodenal villus (Fig. 5c). However, other kinds of immunoreactive cells were found solitarily among the epithelial cells and did not form aggregations.

![Fig. 4. Segi's caps stained with antisera against somatostatin (a), gastrin (b, arrow), motilin (c) and secretin (d). The specimens shown in a, b, c and d are from 43 cm, 75 cm, 43 cm and 45 cm fetuses, respectively. Somatostatin immunoreactive cells (a) are the most numerous among the four endocrine cells. PAP method. ×440](image)
In the Segi's cap, somatostatin- and motilin-immunoreactive cells were first found in a fetus with a CRL of 25 cm, while secretin- and gastrin-immunoreactive cells were first seen in fetuses with a CRL of 26 and 68 cm, respectively.

Although substance P-immunoreactive cells were demonstrated throughout the gut of the fetuses (Fig. 6a), they were located outside the cap. Neurotensin-immunoreactive cells were restricted to the distal jejunum and ileum (Fig. 6b). Glicentin-immunoreactive cells which occurred in the jejunum were not included in the cap (Fig. 6c). No CCK- or VIP-immunoreactive cells were found in the intestinal epithelium in the fetuses examined. All the immunohistochemical reactions in this study were negative in all of the controls.

**Fine structure of Segi's cap**

Electron microscopically, endocrine cells of the bovine Segi's cap were distinguished from adjacent epithelial cells by their secretory granules and light cytoplasmic matrix.
The cells located on the luminal side of the cap possessed microvilli on the surface and numerous microtubules in the apical cytoplasm (Fig. 7). Their structures essentially corresponded to those of other endocrine cells outside the cap. However, no "finger-like cytoplasmic process" protruding into the lumen in the human cap (IWANAGA et al., 1980) was observed in the bovine cap. Cytoplasmic microfilaments and lysosomes were scanty and inconspicuous, and the "large bodies" in the human cap (IWANAGA et al., 1980), which are considered to be lipid droplets, could not be found in the bovine cap. Degenerative cells were seldom seen in the cap. Some endocrine cells of the cap seemed to extend their processes on the basal surfaces and to the intercellular spaces of the aggregated cells.

At least four types of endocrine cells were identified in the bovine Segi's cap based on the ultrastructural profiles of their secretory granules. They were tentatively named type I, II, III and IV cells, because a classification of the endocrine cells in bovine gut at the ultrastructural level has still not been established. The secretory granules of type I cells ranged from 300 to 400 nm in diameter. They were round or slightly irregular in shape and showed variable—low to high—electron densities (Fig. 8a). They seemed to be similar to those of the D cells. Type II cells were characterized by round granules about 250 nm in diameter and with moderate electron density (Fig. 8b). The granules of type III cells were smaller, measuring 150–180 nm in diameter. They were mostly round in shape and varied from low to medium in electron density (Fig. 8c). Type IV cells were characterized by small, rod-like or biconcave granules of high electron density, which were scattered in small numbers in the cytoplasm (Fig. 8d). The granules of type IV cells (60–180 nm in diameter) resembled those of EC (entero-
chromaffin) cells, although they were much smaller than the granules of the EC cells (250–300 nm in diameter) located in the crypts of the duodenum (Fig. 8e).

Of the four types of endocrine cells in the bovine Segi’s cap, type I cells were the most numerous. Type IV cells were next in frequency, while type II and III cells were scarce. Type I cells often showed aggregations of varied sizes in the cap. Sometimes type IV cells were also observed to form a small cluster in the cap. Type II and III cells showed no aggregation. The correlation between the electron-microscopically defined cell types and the histologically and immunohistochemically detected peptides will be dealt with in the Discussion, which follows.

**DISCUSSION**

Segi’s cap in humans has been reported to occur first at five months of gestation (Segi,
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1936; IWANAGA et al., 1980), while the present study in bovine revealed the occurrence of the cap in a fetus with 25 cm CRL, which was regarded as a four-month fetus by EVANS and SACK (1973). Taking into consideration that the fetal period is the same in humans and bovine, it seems likely that the bovine Segi's cap appears earlier than its human counterpart. Segi's caps were well-developed during the later half of pregnancy in bovine as well as in humans. Moreover, it was clear that Segi's caps were also existent in the neonatal calf before suckling milk. Thus, it seems that Segi's caps decrease suddenly just after birth and thereafter disappear, although the cause for this disappearance is yet unknown.

IWANAGA et al. (1980) reported that argentaffin cells corresponded to about half the number of the argyrophil cells forming the human cap. In bovine, however, only a few argentaffin cells were found in the cap, and the reactivity of the cells was weaker than that of argentaffin cells outside the Segi's cap. On the other hand, in the ultrastructural observation, the type IV cells seen frequently in the bovine cap seem to be a kind of EC cells, because the granules closely resemble in shape those of the gastric type EC cells reported in humans by SOLCIA et al. (1976). The granules of type IV cells were small in number in the cytoplasm and were much smaller in size than the granules of EC cells outside the cap in bovine. Thus, we consider that type IV cells, due to such characteristics of their granules, show weaker argentaffinity than EC cells outside the cap and consequently the bovine cap differs from the human cap in its population of argentaffin cells.

Immunohistochemically, secretin-immunoreactive cells were detected in the bovine cap in addition to somatostatin-, gastrin- and motilin-immunoreactive cells, which have already been reported in the human cap (IWANAGA et al., 1980). In the human cap, each of the somatostatin-, gastrin- and motilin-immunoreactive cells accounted for less than 10% of the total population of basal-granulated cells (IWANAGA et al., 1980). In the bovine cap, however, somatostatin-immunoreactive cells alone occupied about 50% of the total population of basal-granulated cells. Occasionally, the somatostatin-immunoreactive cells solely formed very large clusters in the bovine cap. These large clusters of somatostatin-immunoreactive cells, which have not been reported in any gut region in mammals, seem to be based on the particularly large population of somatostatin-immunoreactive cells in the upper small intestine of bovine.

Moreover, somatostatin-immunoreactive cells are characterized by a tendency to form small aggregations within the epithelium and in the subepithelial propria mucosae. Thus, when one considers that these cell aggregations might later participate in the formation of the cap, they may consequently contribute to the large clusters of somatostatin-immunoreactive cells in the cap.

Type I cells were the most numerous among the four types of the cells in the cap and showed aggregations of varied sizes. These cells showed the same frequency of occurrence as the somatostatin-immunoreactive cells demonstrated immunohistochemically. In addition to this, the granules of type I cells correspond, in electron density and size, to those of D cells, which are somatostatin producing cells (GRUBE and FÖRSSMANN, 1979). For this reason, type I cells seem to be regarded as somatostatin cells.

In this study, typical G cells with round granules containing obscure contents could not be identified, although gastrin-immunoreactive cells were found to occupy less than 10% of endocrine cells in the bovine cap. IWANAGA et al. (1980) also failed to detect G cells ultrastructurally in the human cap containing gastrin-immunoreactive cells. They explained this discrepancy by referring to the possible presence of immature cells having no characteristic G cell granules. BUCHAN et al. (1979) reported that
intestinal gastrin cells contained small, round, electron dense granules, measuring 200±20 nm in diameter, differing from the granules of antral gastrin cells. This may also explain the above mentioned discrepancy.

Considering the findings of Buchan et al. (1979), it is possible that type II cells in the bovine cap, having granules about 250 nm in diameter and with moderate electron density, correspond to the intestinal G cells. Naturally, this identification of the type II cells with gastrin containing cells only by the ultrastructural profiles of their granules needs further support by other methods, especially by immunohistochemistry at the electron microscope level.

In the lamina propria of the intestinal villi, we found some small groups of argyrophil cells isolated from the epithelium. Osaka (1975) reported a similar finding in the duodenum of the human fetus and suggested that these basal-granulated cells might possibly migrate from connective tissue into the epithelium. On the other hand, Kataoka (1980) also found the same structure in the proventriculus of finch embryos and suggested that endocrine cells might migrate from the epithelium to the connective tissue. Although the present study does not exclude the possibility of such a migration, it seems more reasonable to presume that, in this case, groups of argyrophil cells may have migrated from the epithelium to the lamina propria. We propose the possibility that the cell groups in the epithelium participate in the cap formation and thereafter move into the lamina propria of villi to there give rise to argyrophil cell group.

As to the possible factors of the formation of the bovine cap, two explanations have been proposed. One is based on the life span of endocrine cells being longer than that of enterocyte or mucous cells (Iwanaga et al., 1980), and the other gives importance to the endocrine cells' affinity to the basal lamina and to undifferentiated smooth muscles in the core of the villi (Tsubouchi, 1981). We are inclined to believe that a combination of these two explanations might cause the peculiar structure of the cap. Thus, it can be considered that different kinds of endocrine cells are originated at the cryptal base and ascend the villus (Cheng and Leblond, 1974) as single or grouped cells, and finally are aggregated at the top of the villus as the Segi's cap by combination of the above mentioned factors—which might cause this movement of the endocrine cells.

Little information on the mechanism of the disappearance of Segi's cap is available at the present time. As discussed above, we conceive of a possibility that the developed Segi's cap discharges groups of endocrine cells into the lamina propria of villi, resulting in the vanishing of the Segi's cap.

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