Histological and Immunohistochemical Studies of the Segi's Cap, a Large Aggregation of Endocrine Cells on the Intestinal Villi of Porcine Fetuses and Neonates

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Summary. Segi's cap, a large aggregation of endocrine cells on the top of intestinal villi, was studied in porcine fetuses and neonates by histological and immunohistochemical methods. The following observations were made: 1) Segi's caps were found in the proximal small intestine in all fetuses larger than 17 cm (beyond 10 weeks of gestation), in neonates before suckling and in 1-4 day-old piglets (suckling neonates); they were not found in a 1 week-old animal. 2) Segi's caps were seen more frequently in the distal duodenum and proximal jejunum than in the proximal and middle duodenum. 3) The Segi's cap consisted mainly of numerous argyrophil cells as demonstrated by Grimelius' method and a few argentaffin cells as identified by a modified Masson-Hamperl's method. 4) Immunohistochemically, ten kinds of immunoreactive cells were dispersed in the mucosal epithelium, outside of Segi's caps, in the proximal small intestine of fetuses: 5-hydroxytryptamine (5-HT)-, gastrin-, bovine pancreatic polypeptide (BPP)-, secretin-, somatostatin-, cholecystokinin-, gastric inhibitory polypeptide (GIP)-, motilin-, leucine-enkephalin- and neurotensin-immunoreactive cells. Except for neurotensin-immunoreactive cells, all of these cells were detected also in the caps. 5) Regional differences were noted in the distribution of cells in the caps; gastrin-, BPP- and secretin-immunoreactive cells were dominant in the caps in the proximal duodenum, while 5-HT-immunoreactive cells were most numerous in those in the proximal jejunum.

Segi (1935, 1936) discovered a huge aggregation of basal-granulated cells on the top of almost every villus in the duodenum and jejunum of human fetuses beyond 5 months of gestation. These reports by Segi were long neglected. Recently, Kobayashi et al. (1980a) confirmed these early findings by Segi and named the structure the "Segi's cap." Iwanaga et al. (1980) investigated the cellular population of the human Segi's cap and reported the presence of somatostatin-, gastrin- and motilin-immunoreactive cells immunocytochemically, and of EC, D and S cells ultrastructurally. Furthermore,
YAMADA et al. (1981a, b) reported that the Segi's cap occurred also in bovine and porcine fetuses. With regards to bovine fetuses, KURAMOTO et al. (1983) reported that the cap included somatostatin-, gastrin-, motilin- and secretin-immunoreactive cells and four different endocrine cell types ultrastructurally. They also confirmed the presence of the caps in a neonatal calf before suckling.

In order to comprehend the possible function of Segi's cap, it is important to study this peculiar structure in various animals. There has not yet been any detailed investigation of the porcine Segi's cap. The present histological and immunohistochemical study aims, therefore, to determine the times of the appearance and disappearance, distribution, frequency of occurrence and the cellular population of the porcine Segi's cap.

MATERIALS AND METHODS

Twenty porcine fetuses ranging from 9 to 26 cm crown-rump length (CRL) (between 7 and 14 weeks of gestation, estimated according to EVANS and SACK, 1973), six neonates before suckling and eight piglets (suckling neonates) of either sex were used in this study. The tissues were obtained from the proximal, middle and distal duodenum, proximal and middle jejunum, ileum, cecum, spiral colon and rectum. After fixation in Bouin's fluid, the specimens were dehydrated in alcohol, cleared in xylene and embedded in paraffin. Sections were serially cut at 2–6 μm in thickness, and stained with Masson-Hamperl's argentaffin method modified by SINGH (1964) and Grimelius' argyrophil method (GRIMELIUS, 1968).

For immunohistochemical investigation, sections were treated with the unlabeled antibody enzyme method using the bridge (MASON et al., 1969) or peroxidase-antiperoxidase (PAP) method (Sternberger, 1979). Details of the specific antisera used are shown in Table 1. The specificity of the immunohistochemical reaction was checked as recommended by STERNBERGER (1979), including preabsorption tests with appropriate antigens. All the immunohistochemical reactions in this study were negative in all of the controls. After the immunohistochemical staining, the sections were lightly counter-stained with Mayer's hematoxylin.

The frequency of the occurrence of Segi's cap in each portion was expressed as the mean percentage of the number of the villi having the cap against the total number of villi hit at their top in the sections. These frequencies were estimated for two groups, divided according to the CRL of fetuses; one group consisted of younger fetuses (18–22 cm CRL) and the other of older ones (23–26 cm CRL). The relative frequency of immunoreactive cells in the mucosal epithelium outside the cap, in each intestinal portion of the fetuses, was subjectively divided into five grades. The frequency of immunoreactive cells in the Segi's cap in each intestinal portion of fetuses was graded according to the mean percentage of the number of immunoreactive cells against the number of cells constituting the cap in the sections. The statistical significance of the results obtained was evaluated by Student's t-test.

RESULTS

Histological study
The top of the intestinal villus having a Segi's cap exhibited a shallow concave surface.
Porcine Segi’s Cap

Under this concavity, numerous endocrine cells appeared in single, pseudostratified or stratified arrangements and formed a special colony. Thus, Segi’s cap was easily recognized by its large aggregation of endocrine cells and characteristic concavity (Fig. 1).

Segi’s cap contained a predominant population of argyrophil cells in Grimelius stained sections (Fig. 1–3). Some of the argyrophil cells in the cap reached the intestinal lumen with their apical cytoplasm (Fig. 2). Only a few cells were argentaffin in modified Masson-Hamperl reaction (Fig. 4).

Segi’s caps were first found in the distal duodenum and proximal jejunum in a 17 cm fetus (10 weeks gestation). The caps were detected in the proximal small intestine in all fetuses larger than 17 cm. Furthermore, Segi’s caps were also found in the proximal small intestine of all neonates before suckling (Fig. 4) and of 1 (Fig. 2) and 2 day-old piglets as frequently as in the fetuses. In 3 (Fig. 3) and 4 day-old piglets, the caps were rarely seen in only the distal duodenum and proximal jejunum; none of them was detected in any portion of the intestine in a 1 week-old animal. Segi’s caps were not seen in the middle jejunum, ileum and large intestine in any animals examined.

The frequency of occurrence of Segi’s caps in each portion of the proximal small intestine of the fetuses is shown in Figure 5. In both the younger (18–22 cm) and older (23–26 cm) groups, the frequencies of the caps were low in the proximal and middle duodenum, but tended to become higher in the distal duodenum and proximal jejunum. The frequency was the highest in the proximal jejunum of the younger group (43 ±8.3%). There were significant differences (p<0.01) in the frequency between the

| Table 1. Antisera used in this study |
|-------------------------------------|
| Antiserum | Code     | Source                     | Specificity                                                                 | Dilution |
| 5-HT      | Lot. 16302 | Immuno Nuclear Corp., Stillwater | —                                                                           | 1:10,000 |
| Somatostatin | —        | S. Ito, Niigata            | —                                                                           | 1:3,000  |
| Gastrin   | GP-1304   | N. YANAIHARA, Shizuoka     | Does not cross-react with CCK-8                                            | 1:8,000  |
| Secretin  | R–801     | N. YANAIHARA               | Reacts with the C- and N-terminals                                         | 1:1,000  |
| Motilin   | R–1104    | N. YANAIHARA               | Reacts with the C-terminal                                                 | 1:1,000  |
| Neurotensin | R–3501   | N. YANAIHARA               | —                                                                           | 1:1,000  |
| Glicentin | R–4804    | N. YANAIHARA               | Reacts with the C-terminal                                                 | 1:2,000  |
| Glucagon  | GL–5      | N. YANAIHARA               | Reacts with pancreatic glucagon                                            | 1:3,000  |
| VIP       | R–502     | N. YANAIHARA               | Reacts against entire molecule                                             | 1:2,000  |
| Substance P | R–2404   | N. YANAIHARA               | Does not cross-react with gastrin releasing polypeptide                     | 1:1,000  |
| GRP       | R–6902    | N. YANAIHARA               | Reacts primarily with the C-terminal; Does not cross-react with substance P | 1:1,000  |
| CCK       | —         | D. Grube, Hanover          | Reacts with CCK 11–20; Does not cross-react with gastrin                   | 1:3,000  |
| Leu-enkephalin | 1671 | UCB, Bruxelles             | —                                                                           | 1:80,000 |
| GIP       | G/R/34–IIID | Guildhay, Surrey         | Does not cross-react with glucagon                                         | 1:8,000  |
| BPP       | Lot. 615–R–110–146–17 | E. Chance, Indianapolis | Cross-reacts with human pancreatic polypeptide                             | 1:10,000 |

All antisera were raised in rabbits except for gastrin which was raised in guinea pigs.
former two portions and the latter two in either age group. However, there was no significant difference in the frequency between the two groups.

**Immunohistochemical study**

In the youngest fetus (8 cm) studied, twelve kinds of immunoreactive cells: bovine pancreatic polypeptide (BPP)-, cholecystokinin (CCK)-, leucine (leu)-enkephalin-, secretin-, motilin-, gastric inhibitory polypeptide (GIP)-, glucagon-, neurotensin-, gastrin-, somatostatin-, glicentin- and 5-hydroxytryptamine (5-HT)-immunoreactive cells, were identified in the small intestine; the last four cell types were found also in the large intestine. Neurotensin- and BPP-immunoreactive cells in the large intestine were first detected in 13 cm fetuses. No vasoactive intestinal polypeptide (VIP)-, gastrin releasing polypeptide (GRP)- or substance P-immunoreactive cells were found anywhere the gut portions in all animals examined. The distribution and relative frequency of each kind of immunoreactive cell in the intestinal epithelium of the fetuses having Segi’s caps are summarized in Table 2. Since Segi’s cap did not appear
in the middle jejunum, ileum or large intestine, further immunohistochemical studies were performed on the proximal small intestine.

As shown in Table 2, ten kinds of immunoreactive cells were dispersed to some degree in the mucosal epithelium of the proximal small intestine outside the cap. All of these cells except for neurotensin-immunoreactive cells were detected in the Segi’s caps (Fig. 6-14). These cells were found singly or in aggregations within the caps.

Eight types of immunoreactive cells: 5-HT-, CCK-, somatostatin-, gastrin-, motilin-,
secretin-, leu-enkephalin- and GIP-immunoreactive cells were found first in the caps of the distal duodenum and proximal jejunum of the 17 cm fetus, while BPP-immunoreactive cells were first detected in the caps of the proximal and middle duodenum of the 18 cm fetuses.

The frequency of each kind of immunoreactive cells in the Segi’s cap was found to vary with the intestinal portion of the fetuses (Table 3). In the caps of the proximal duodenum, gastrin- (Fig. 6), BPP- (Fig. 8) and secretin-immunoreactive cells were numerous, while somatostatin-immunoreactive cells were moderate in number (Fig. 7). In the proximal jejunum, 5-HT-immunoreactive cells were numerous (Fig. 9) and smaller numbers of gastrin-, secretin-, somatostatin-, CCK- and GIP- (Fig. 13) immuno-

Fig. 6. Gastrin-immunoreactive cells in two caps from the proximal duodenum of a 23 cm fetus (12 weeks). Bridge method. × 170

Fig. 7. Somatostatin-immunoreactive cells in a cap from the proximal duodenum of a 23 cm fetus (12 weeks). PAP method. × 260

Fig. 8. BPP-immunoreactive cells in two caps from the proximal duodenum of a 23 cm fetus (12 weeks). PAP method. × 170

Fig. 9. 5-HT-immunoreactive cells in a cap from the proximal jejunum of a 23 cm fetus (12 weeks). PAP method. × 230

Fig. 10. A motilin-immunoreactive cell in a cap from the distal duodenum of a 18 cm fetus (10 weeks). PAP method. × 230
Porcine Segi's Cap

reactive cells were constantly detected. BPP-immunoreactive cells were seen only rarely. Although a few argentaffin cells were demonstrated histologically in the Segi's cap by the modified Masson-Hamperl's method, 5-HT-immunoreactive cells were more numerously found through the immunohistochemical investigation. Motilin- and leu-enkephalin-immunoreactive cells in the caps were rarely encountered in the proximal small intestine (Fig. 10, 14). Each kind of immunoreactive cell in the caps never showed any immunoreactivities for other peptides listed in Table 1, although any evidence for the coexistence of these immunoreactivities was carefully sought in the serial sections.

The relative frequency of each kind of immunoreactive cell in the cap and that in the mucosal epithelium was approximately equal in each portion except for BPP-immunoreactive cells in the proximal and middle duodenum (compare Tables 2 and 3). Although BPP-immunoreactive cells were numerous and frequently formed aggregations (Fig. 8) in the caps in these portions, they were relatively few in the mucosal epithelium.

Neurotensin-immunoreactive cells were detected very rarely in the distal duodenum.
and proximal jejunum of a few fetuses. In the present study, neurotensin-immunoreactive cells were not found in the Segi’s cap.

Some of the cells constituting the caps did not react to any antiserum used in this study. These non-immunoreactive cells were relatively numerous in the distal duodenum and proximal jejunum.

**DISCUSSION**

To date, Segi’s caps have been found in the proximal small intestine of human (Segi, 1935, 1936; Kobayashi et al., 1980a, b; Iwanaga et al., 1980), bovine (Yamada et al., 1981a, b; Kuramoto et al., 1983), porcine (Yamada et al., 1981a, b) and simian fetuses (Kobayashi, 1981). The occurrence of Segi’s caps was confirmed in porcine fetuses in the latter half of the gestation period, as in human and bovine fetuses. They were basically similar in shape, though the porcine caps were smaller, and their concavity and swelling on the top of the villi were not as prominent as found in human and bovine caps.

Although Segi (1935) reported in human fetuses that the human caps occurred on the top of almost every villus in the duodenum and jejunum, the porcine caps could be found on about 40% of the villi in the proximal jejunum which was the site where the caps were seen most numerous. In bovine fetuses, the caps were seen most frequently in the proximal duodenum (Yamada et al., 1981a, b; Kuramoto et al., 1983). Hence, species differences can be noted concerning the frequency of occurrence of Segi’s caps.

Iwanaga et al. (1980) reported the presence of somatostatin-, gastrin- and motilin-immunoreactive cells in human Segi’s caps. They also detected CCK-, secretin-, BPP- and glucagon-like immunoreactivity (GLI)-immunoreactive cells in the mucosal epithelium of the upper small intestine, but not in the caps. Somatostatin-, gastrin-, motilin- and secretin-immunoreactive cells were identified in the bovine caps (Yamada et al., 1981a, b; Kuramoto et al., 1983). Further investigation demonstrated the presence of 5-HT- and substance P-immunoreactive cells in the bovine caps (Yamada

|            | Proximal duodenum | Middle duodenum | Distal duodenum | Proximal jejunum |
|------------|-------------------|-----------------|-----------------|------------------|
| Somatostatin | ++                | ++              | +               | +                |
| Gastrin     | ###               | ###             | ###             | +                |
| BPP         | ###               | ###             | ±               | ±                |
| Secretin    | +                 | +               | +               | +                |
| CCK         | ±                 | ±               | ±               | ±                |
| Motilin     | ±                 | ±               | ±               | ±                |
| Leu-enkephalin | ±            | ±               | ±               | ±                |
| GIP         | ±                 | ±               | ±               | ±                |
| 5-HT        | +                 | +               | +               | #                |
| Neurotensin | –                 | –               | –               | –                |

--; absent (not detected), ±; less than 3%, +; 3-10%, ++; 10-20%, ###; 20-30%, ###; more than 30%.

These grades are based upon the percentage of the number of immunoreactive cells against the number of cells constituting the cap in the sections.
et al., 1983). In the present study, ten kinds of immunoreactive cells were seen in the mucosal epithelium of the proximal small intestine, and all of these cells, except for neurotensin-immunoreactive cells, were found also within the Segi's caps. Neurotensin-immunoreactive cells were detected very rarely in the mucosal epithelium of the distal duodenum and proximal jejunum of a few fetuses; it might be reasonable to conjecture that the chance of these cells being hit in the caps would be extremely rare. Thus, essentially all kinds of endocrine cells detected in the mucosal epithelium of the proximal small intestine should be also found in the Segi's caps.

Furthermore, the proportion of the cells constituting the porcine caps showed clear regional differences; gastrin-, BPP- and secretin-immunoreactive cells were dominant in the proximal duodenum, while 5-HT-immunoreactive cells were most numerous in the proximal jejunum. This regional difference has been described neither for human nor for bovine caps.

In the human Segi's cap, argentaffin cells comprised about half of the number of the argyrophil cells forming the cap (Iwanaga et al., 1980), while in the bovine cap only a few argentaffin cells were found (Kuramoto et al., 1983). Although argentaffin cells demonstrated in the porcine caps by the modified Masson-Hamperl's method were also scarce, 5-HT-immunoreactive cells detected by the immunohistochemical method were more numerous, especially in the caps of the proximal jejunum. Recently, Inokuchi et al. (1983) indicated that the population of EC cells identified by the PAP method was larger than those identified by conventional techniques. They pointed out that a certain part of the EC cells detectable by immunohistochemistry was neither argentaffin nor argyrophil. Furthermore, the difference between the low frequency of argentaffin cells in the caps and the high frequency of 5-HT-immunoreactive cells in the caps seems to be due to a difference in the sensitivities of the techniques used in this study. However, it is not yet clear whether the difference in staining of the nonargentaffin 5-HT-immunoreactive cells and the argentaffin 5-HT-immunoreactive cells is dependent upon the amount of 5-HT stored in the cells or the chemical components of the secretory granules. In the duodenum of adult and infant pigs, Nihei et al. (1983) reported that approximately 60% of the 5-HT storing EC cells were also reactive to the anti-met-enkephalin-8 serum. In the present study, however, 5-HT-immunoreactive cells showing leu-enkephalin immunoreactivity could not be detected in serial sections. Although the reason for this discrepancy is not yet clear, it might be explained by the differences in the developmental stages of the EC cells. Our material was obtained from fetal and neonatal pigs, while that of Nihei et al. (1983) was from adult and infant animals.

As discussed above, in the porcine caps, four types of immunoreactive cells—BPP-, CCK-, GIP- and leu-enkephalin-immunoreactive cells—were newly identified in addition to the cell types known in the bovine caps; substance P-immunoreactive cells, which are contained in bovine caps, were not detected in any portion of the fetal porcine gut. Some of the cells constituting caps did not react to any antisera used in this study. They can be considered to be degenerating enterocytes, immature or degenerating endocrine cells or cells which contain unknown products. Further studies are necessary to identify these cells.

The exact time of disappearance for the Segi's cap has not been studied in detail, although Kuramoto et al. (1983) reported the presence of the caps in a neonatal calf before suckling. In the present study, Segi's caps were found in all neonates before suckling and in 1 and 2 day-old piglets at numbers equaling the fetuses. In 3 and 4 day-old piglets, the caps were occasionally seen, but in a 1 week-old animal, they were
not detected. Based on these results, it is likely that the porcine caps disappear within a few days after suckling colostrum.

In bovine fetuses, YAMADA et al. (1981b) and KURAMOTO et al. (1983) found small aggregations of argyrophil cells isolated from the epithelium in the lamina propria of the villi, and proposed that they might have been discharged from the caps. However, such aggregations were not seen in this study. It seems reasonable to presume that, in pigs, the endocrine cells of the caps are exfoliated from the top of the villi into the intestinal lumen. The process of exfoliation of the endocrine cells could not be recognized probably because this happens in a very short time (TSUBOUCHI, 1981).

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Note added in proof. After the acceptance of this report, we found a brief description about porcine Segi’s caps. ALUMETS et al. (1983) reported that the Segi’s caps were found in the upper small intestine of the fetuses during weeks 15–17 of gestation and the endocrine cells forming the caps were somatostatin, CCK and GIP cells.

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