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Correlative Immunocytochemical and Electron Microscopic Studies: Identification of (Entero)glucagon- Somatostatin- and Pancreatic Polypeptide-like-Containing Cells in the Human Colon*

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Summary. Correlative immunocytochemical and electron microscopic studies, using the semi thin-thin technic, were performed to identify the (entero) glucagon, somatostatin and pancreatic polypeptide-like immunoreactive cells of the human colonic mucosa. Mean granule diameter for each cell type was estimated according to two methods and histograms showing the granule size distribution were constructed. A total of 139 immunostained cells identified at the ultrastructural level were analyzed. Mean granule diameter for (entero)glucagon-containing cells was 318 ± 11 nm but a reduction of granule size with age was noteworthy: granules were larger in the fetus (mean diameter 350 ± 15) than in adults (mean diameter 310 ± 10 nm). Somatostatin-containing cells, very rare in adults, were present in the fetal distal colon. Their general mean granule diameter was 354 ± 18 nm but many cells had a mean granule diameter of more than 400 nm. A pancreatic polypeptide-like immunoreactivity was found only in (entero)glucagon-containing cells, pointing out the possible occurrence of both peptides (or of similar sequences) in the same cells. Previous ultrastructural studies dealing with a tentative classification of the human colonic endocrine cells were compared with the present data.

Introduction

Several studies have been published which suggest that various hormones and other polypeptides are present in the human colon and rectum. A glucagon-like bioactivity and immunoreactivity was first demonstrated in the human colon by Markman and Sutherland (1964) and by Samols et al. (1966). Subsequently, cells containing a glucagon-like polypeptide were revealed by histoimmunological methods in the human gut including the colon (Polak et al. 1971; Knudsen et al. 1975; Grimelius et al. 1976; Cristina et al. 1978b). Multiple immunoreac-

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tive forms of vasoactive intestinal peptide (VIP) were recently reported in the human colonic mucosa (Dimaline and Dockray 1978) and a VIP-like, as well as a pancreatic polypeptide (PP)-like immunoreactive material, was immunocytochemically demonstrated in colonic endocrine cells (Buffa et al. 1978). In the human fetal colon, in addition to (entero)glucagon and PP immunoreactive cells, we have also described the presence of somatostatin (SRIF) and substance P immunoreactive cells (Lehy and Cristina 1979).

There have been two recent articles dealing with the presence at the ultrastructural level, and tentative classification of the human colonic endocrine cells (Cristina et al. 1978c; Buffa et al. 1978). Both described at least four types of endocrine-like cells. In addition to EC cells, Cristina et al. (1978c) suggested the existence of 3 types of cells containing generally rounded granules: type III, very rare, with small sometimes haloed granules (mean diameter of the largest granules at about 165 nm) and types II and IV both with granules often highly electron-dense, similar morphologically, but classified on the criterion of the mean size of their largest granules (diameter frequency peak at 200–300 nm and at 300–400 nm, respectively). Type IV was proposed – in adult human – as identical to L cells and corresponding to the glucagon-like immunoreactive (GLI) cells. Buffa et al. (1978) described EC cells, H cells (which certainly are identical to the above type III and are thought to be perhaps responsible for a secretion of a VIP-like peptide), L cells with round homogeneous dense granules (diameter about 235 nm, or 300 nm after correction) and F cells containing irregular granules of variable density whose maximum diameter was about 250 nm (340 nm after correction). The authors proposed F cells as being responsible for the PP-like peptide secretion. The presence of rare D cells, corresponding to SRIF immunoreactive cells, was noted by both groups. Very recently Polak and Buchan (1979) identifying GLI cells at the ultrastructural level in human ileum, colon and rectum found the mean GLI granule diameter to be about 200 nm. Furthermore, in the human fetus as in children, a fifth cell type was observed containing large round granules morphologically not very different from type II and type IV granules but with a diameter frequency peak at 400–500 nm (Cristina et al. 1978a, b).

It is apparent that the literature contains discrepancies with regard to the morphological aspects of peptide-containing endocrine cells in the human colon. The aim of the present paper is to tentatively explain such discrepancies by identifying ultrastructurally the cellular origin of three peptides – (entero)glucagon, PP-like and SRIF – using a reliable immunocytochemical semithin-thin technic (Polak et al. 1975b) in both adult and fetal human colonic mucosa.

Material and Methods

Tissues: Biopsies of rectal mucosa were removed during recto-colonoscopy from 4 adult men, free of digestive disease; samples of descending colon and rectosigmoid were obtained from a 17 week old fetus by therapeutic abortion. Fresh tissue samples were quickly immersed in the following fixatives:

– 1% glutaraldehyde + 3% formaldehyde mixture in phosphate buffered saline (PBS 0.1 M, pH 7.2) at 4° C for 20 min or 2 h, then rinsed overnight in PBS;

– 1% glutaraldehyde + 3% paraformaldehyde solution in PBS for 2 h, then in some cases, postfixed overnight in 4% paraformaldehyde only in PBS.

After rinsing, specimens were dehydrated and embedded in Epon 812.

Blocks were cut in such a way as to obtain alternatively serial adjacent semithin (700–800 nm) and ultrathin (60 nm) sections. The ultrathin sections were placed on a 200 mesh copper grid, stained by lead citrate-uranyl acetate and examined under a Siemens Elmiskop 1 A microscope. The semithin sections were mounted on glass slides, then Epon was removed by immersion in absolute ethanol saturated with NaOH (Lane and Europa 1965) before staining by the immunoperoxidase method.

Immunohistochemical Procedures: Rabbit anti-bovine PP (BPP) serum, N 615-R 110-146-10, as well as the homologous antigen were kindly supplied by Dr. E.E. Chance (Lilly Research Laboratory Indianapolis).

Antibodies against synthetic somatostatin (SRIF, gift of J. Rivier and R. Guillemin) coupled with carbodiimide (Mc Guigan 1968), glucagon (Sigma, more than 99% pure, extracted from a mixture of bovine and porcine pancreases) coupled with PP'-difluoro-m, m'dinitro-diphenyl-sulfone (FNPS, Sigma) (McEvoy et al. 1977) were raised in female New Zealand rabbits by repeated injection of an emulsion of the antigen in Freunds complete adjuvant.

Cross reactivity of each of the antisera against BPP, SRIF, pancreatic glucagon, gastric inhibitory polypeptide (GIP), VIP and secretin was tested. None of them showed any appreciable binding to the heterologous peptides. Antisera were used at 1:40 dilution (entero)glucagon and at 1:20 (SRIF) in the indirect immunoperoxidase reaction according to Piris and Whitehead (1974). The peroxidase-antiperoxidase (PAP) procedure of Sternberger (1974) was also used with a 36-h incubation at 4° C in the diluted antisera-dilution of 1:4,000 for (entero)glucagon antiserum, 1:1,000 for SRIF antiserum, 1:2,000 for BPP antiserum. The soluble PAP complex (Medac, Hamburg, RFA) was used at 1:100 dilution. Peroxidase activity was revealed by diaminobenzidine in tris-buffered saline followed in some cases by the post-osmification step.

The specificity of the immunocytological reaction was tested by the following controls including a) the omission of the first layer, b) the use of normal rabbit serum instead of the primary antiserum, c) the use of aliquots of diluted antisera which had been preincubated overnight at 4° C with an excess from 5 to 100 μg/ml diluted serum of either pancreatic glucagon, SRIF, BPP; results were negative when sera were inactivated by the corresponding antigen.

Quantitative and Morphometric Studies: Immunostained cells found in each semithin section were identified ultrastructurally in the serial ultrathin section. Each cell thus identified was photographed. The microphotographs were used to quantify the size of storage granules of SRIF, glucagon-like or BPP-like polypeptide according to two methods: a) first in each nucleated cell, all granules were measured at a magnification of ×54,000 and the mean diameter of these granules (d) was calculated and corrected for sectioning artifact with the formula $D_c = (4/\pi) \cdot d$ (Baetens et al. 1976); histograms of all granule diameters from all nucleated cells were constructed with a 50 nm frequency; b) secondly, in each cell, whether nucleated or not, the largest granule profiles were selected (Cristina et al. 1978c): at least 20 to 40 granules per cell were examined (×54,000). Histograms of granule diameters were constructed also with the same frequency as for (a). For each cell the mean diameter of the largest granules was indicated by D_L. Statistical analysis was performed using the Student's *t* test.

Results

Both the best immunocytochemical staining of endocrine cells, as well as a relativity well-preserved ultrastructure, were obtained with the first fixative (1% glutaraldehyde + 3% formaldehyde solution in PBS) used for 2 h. The most noticeable fixation artifacts at the fine structural level were increases in width of intercellular spaces within the colonic epithelium. The fetal tissue, being very fragile, was the most damaged; nevertheless it was used successfully in

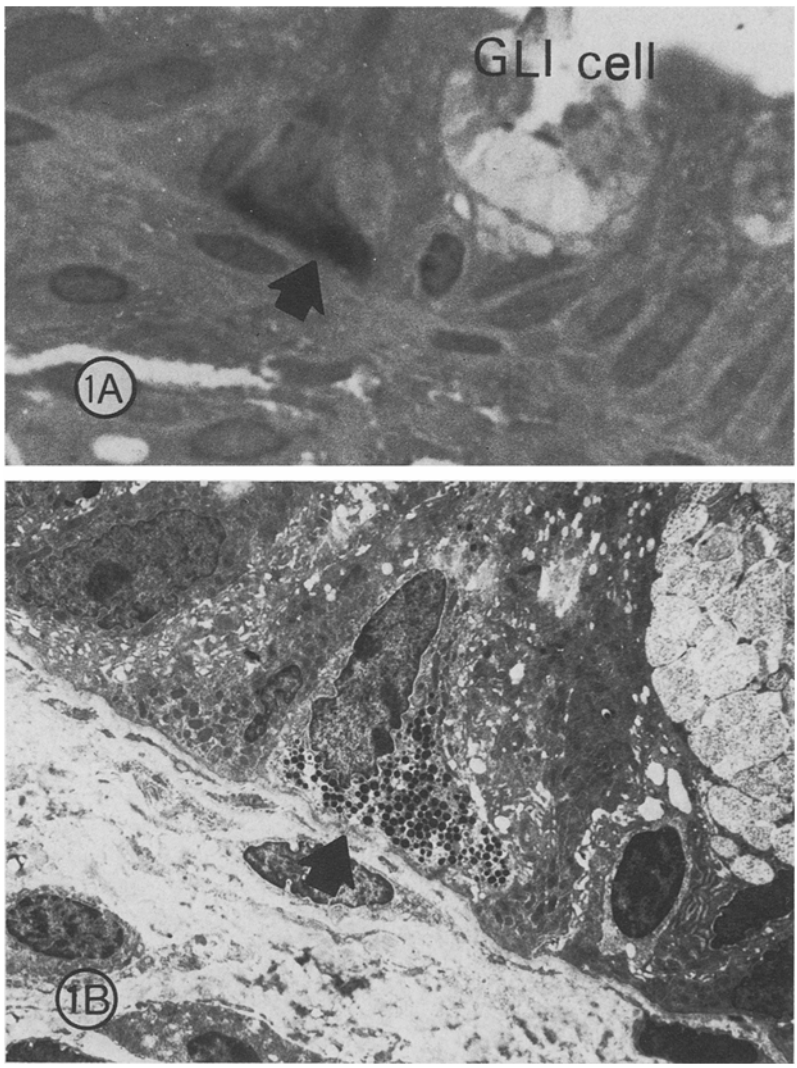

Fig. 1 A and B. Human rectal mucosa; adult patient no. 1. **A** Immunostaining of a nucleated GLI cell on a semi-thin section; indirect immunoperoxidase reaction with GLI serum used at a dilution of 1:40. ×2,025. **B** Electron micrograph of this GLI cell identified on the consecutive thin section; storage granules are relatively dense, mainly round, sometimes angular; the mean diameter of the largest granule profiles D_L is 352 nm; the mean diameter of all granule profiles d is 272 nm, and after correction of d, D_e is 346 nm. ×3,200

the semi thin-thin technic, and it was noted, especially, that the peptide secretory granules were not altered.

Colonic endocrine cells, scattered in the crypts, were specifically stained by the SRIF, BPP, (entero)glucagon-antisera and among these cells 139 were identified at the ultrastructural level on the consecutive thin sections: 76 GLI cells (Fig. 1, 2), 49 immunoreactive BPP cells (Fig. 3) and 14 immunoreactive

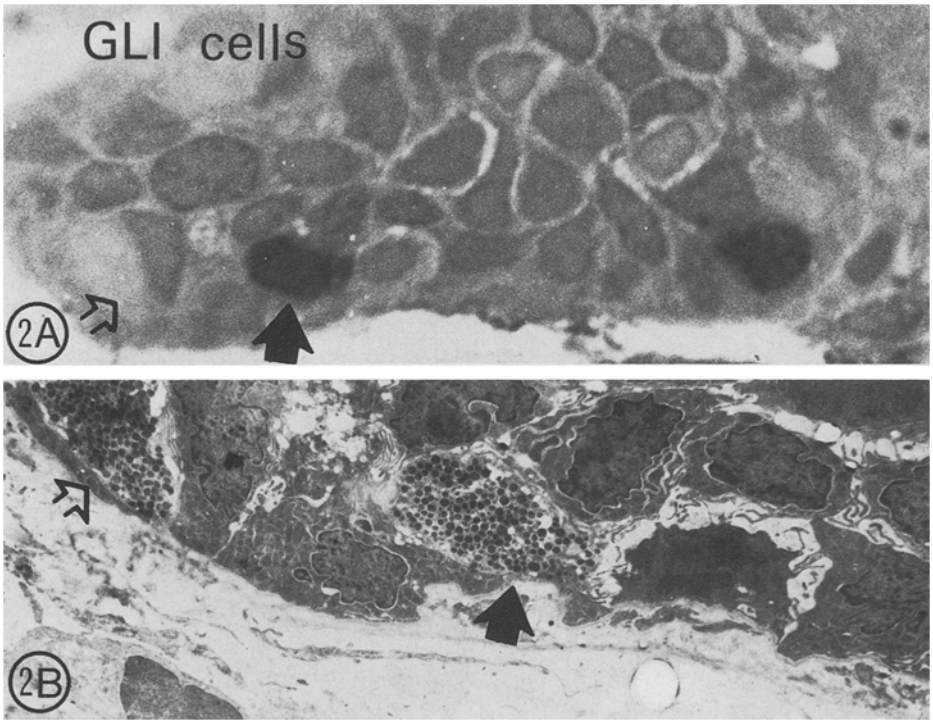

Fig. 2A and B. Human rectal mucosa; adult patient no. 1. **A** Immunostaining of two GLI cells on a semi-thin section. Indirect immunoperoxidase reaction with GLI serum used at a dilution of 1:40. ×2,025. **B** One GLI cell is identified on the serial thin section (black arrow), $D_L = 330$ nm. Another endocrine cell not stained by the antiserum (empty arrow) shows rounded granules with a D_L of the same order = 336 nm. ×3,500

SRIF cells (Fig. 4). By using paired serial semithin sections adjacent to the ultrathin section to perform immunoperoxidase reactions with glucagon and BPP antisera, 28 of these cells were found to react with both antisera (Fig. 5).

The granule size distribution for the whole of each cell type is shown in Fig. 6 in histogram I (all granules measured in nucleated cells only) and in histogram II (20 to 40 largest granules measured in all cells, whether nucleated or not). It is evident that the diameter frequency peak is the same for GLI cells and BPP cells: it is located at 200–300 nm (histogram I) and at 250–300nm (histogram II). For somatostatin cells, even though the peak value for diameter is located also at 200–300 nm in histogram I, we observed that a noticeable part of this histogram extended towards the largest values; thereupon the diameter frequency peak for histogram II is seen at 300–400 nm, a larger value than for GLI and BPP cells.

Tables 1 and 2 show the mean diameter of granules in identified nucleated GLI and SRIF cells respectively, calculated in each patient's colonic or rectal mucosa according to two methods. It should be observed that whatever the method used the values obtained (D_L and D_c) were always similar. Interindividual

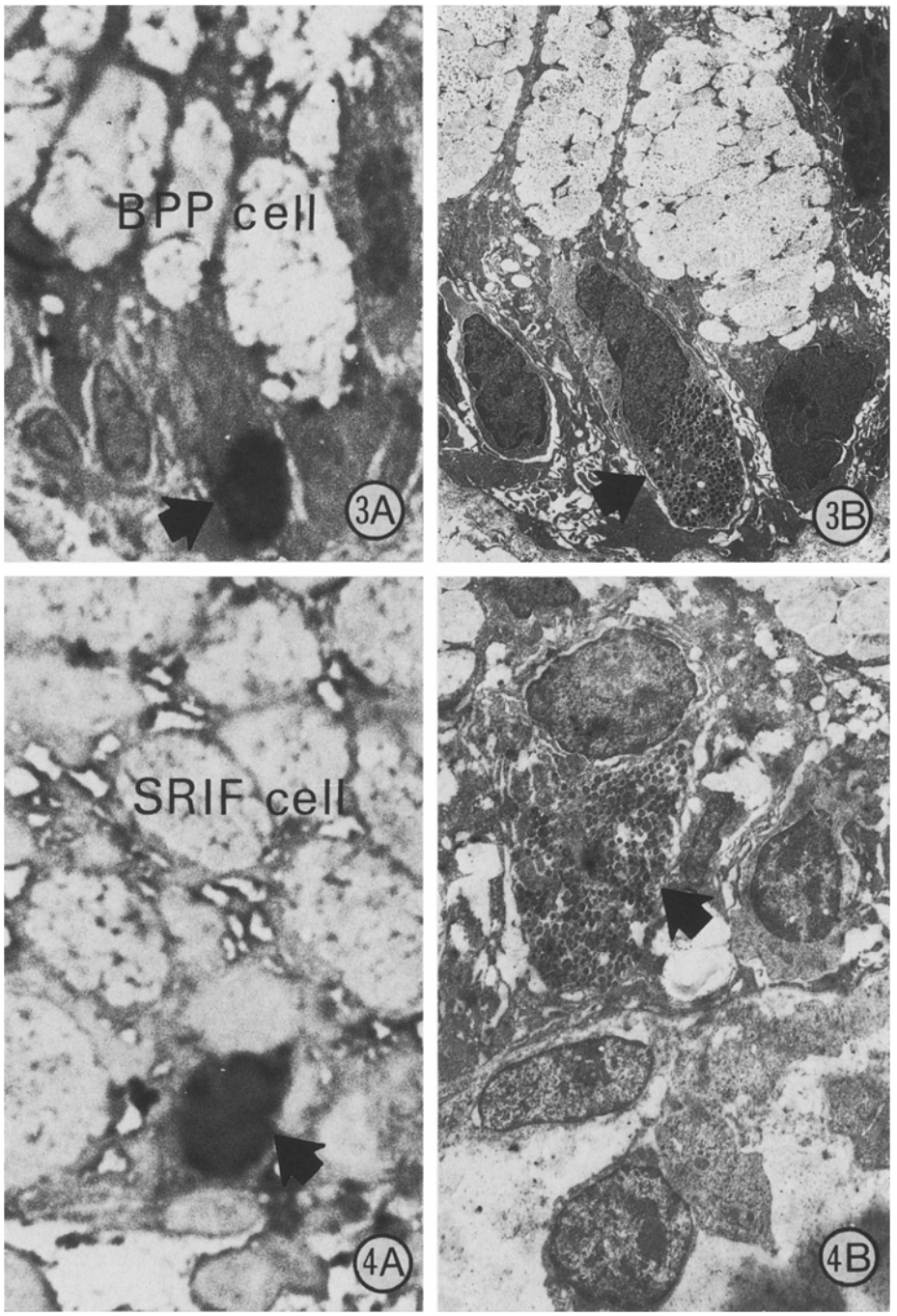

Fig. 3A and B. Rectal human mucosa; adult patient no. 2. **A** One nucleated BPP immunoreactive cell is seen on a semi-thin section. PAP technic with BPP serum used at a dilution of 1:2,000. ×2,200. **B** The BPP cell, identified on the consecutive thin section, shows rounded granules whose $D_L = 285$ nm, $d = 228$ nm, and after correction $D_c = 289$ nm. ×2,650

Fig. 4A and B. 15 week old human fetus; rectosigmoid mucosa. Fixation artifacts are striking but the morphological aspect of the epithelium is recognizable and the fine structure of granules is preserved, i.e. the granules are not swollen and the membrane is attached to the matrix. **A** Immunostaining of a somatostatin cell; PAP procedure with the somatostatin antiserum used at a dilution of 1:1,000. ×2,000. **B** The somatostatin cell identified on the consecutive adjacent thin section shows rounded granules closely packed with a medium electron density. $D_L = 324$ nm; $d = 240$ nm and $D_c = 305$. ×3,300

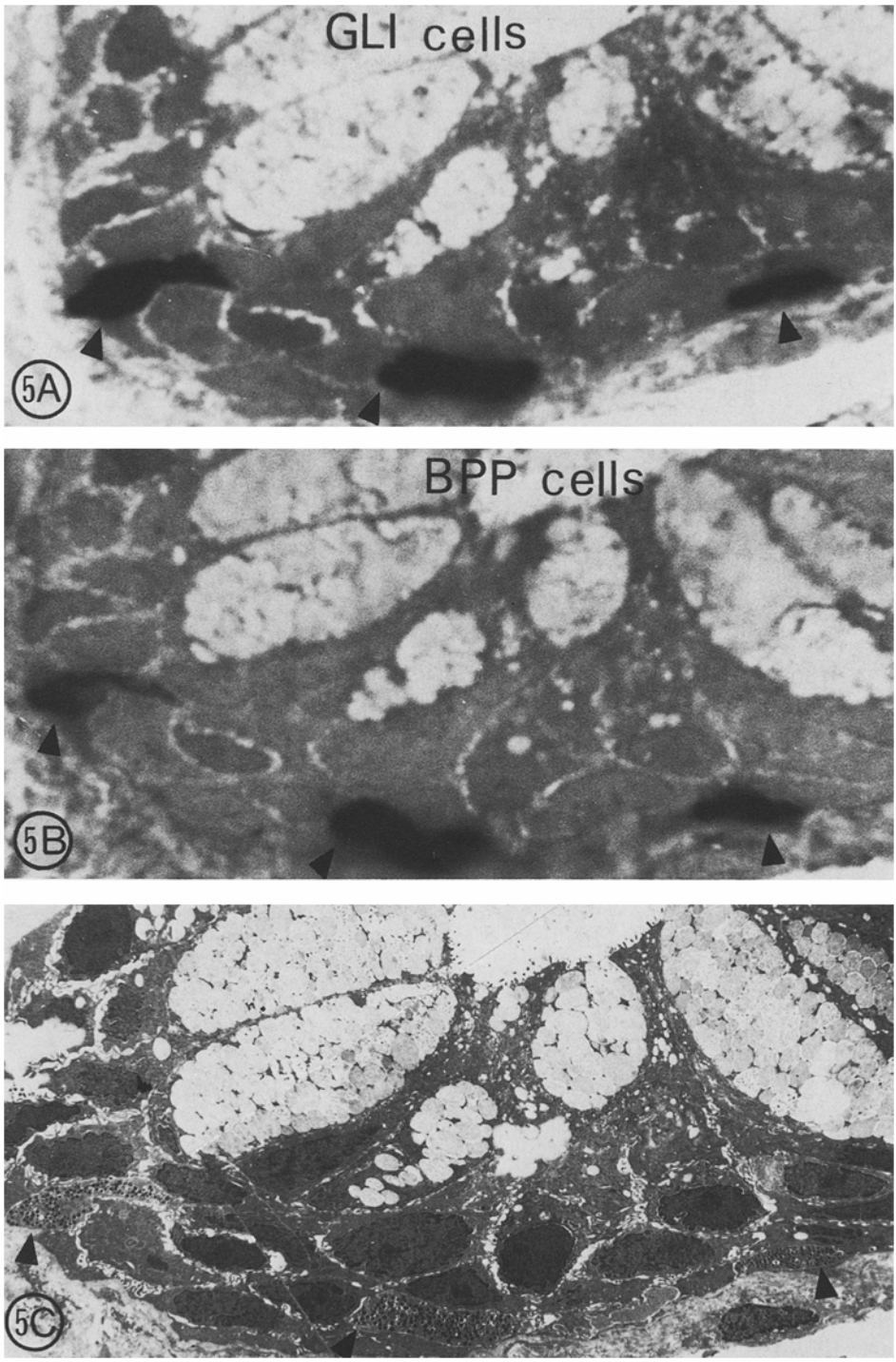

Fig. 5A–C. Rectal mucosa; patient no. 2. **A and B** Immunoperoxidase reactions with GLI antiserum (at a dilution of 1:4,000) and BPP antiserum (at a dilution of 1:2,000) respectively on two paired serial semi-thin sections. PAP procedure. The same cells (arrows) are stained by the two sera. ×2,025. **C** Ultrathin section adjacent to the B semi-thin section. The 3 cells are identified (arrows) and show rounded granules whose mean diameters DL are 261 nm, 300 nm and 235 nm, respectively, from the left to the right side of the photograph. ×2,200

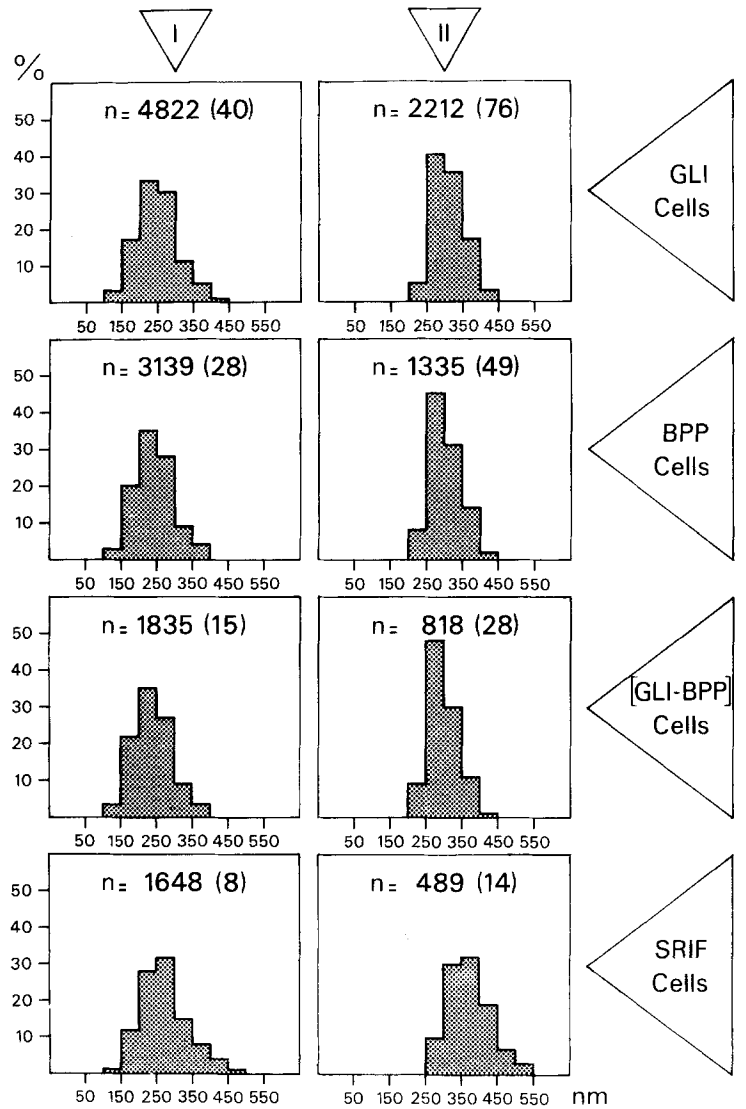

Fig. 6. Histograms showing the granule size distribution for each cell type with a frequency per class of 50 nm: 1) in nucleated cells all granules were measured (in their maximal diameter) and the corresponding histogram is shown in I; 2) in nucleated and non-nucleated cells the 20 to 40 largest granule profiles were measured (Cristina et al. 1978c) and the corresponding histogram is shown in II. Data from adult patients and fetus were mixed. n denotes the number of granules measured for each histogram; the number between parentheses corresponds to the number of identified cells from which granules were measured. The granule size distribution is the same for the BPP and the GLI immunoreactive cells. When only the granule diameters from cells identified as being immunoreactive with both GLI and BPP antisera were taken in consideration, the same histogram profile was obtained; for the whole of granules the frequency peak is seen at 200–300 nm and for the largest granules the frequency peak is seen at 250–350 nm. For the somatostatin immunoreactive cells the frequency peak for the whole of granules is also seen at 200–300 nm with an extension towards larger granules. For the largest granules (histogram 2) it is seen at 300–400 nm

Table 1. GLI cells: comparison of 2 methods for estimating the mean size (±1 SEM) of the secretory granules in the nucleated cells. Comparison of individual values

	d [nm][a]	D_e [nm][b]	D_L [nm][c]
Patient no. 1	254±10 (823 granules from 5 cells)	322±12	315±16
Patient no. 2	244±2 (454 granules from 3 cells)	310±9	311±6
Patient no. 3	256±18 (531 granules from 4 cells)	327±23	326±20
Patient no. 4	222±12 (851 granules from 8 cells)	282±15	291±16
Fetus (17 W. old)	276±12 (864 granules from 7 cells)	350±15	340±10
General mean	250±9	318±11	317±8

[a] d = mean diameter of secretory granules when all granule profiles were measured in each nucleated cell

[b] $D_e = (4/\pi) \cdot d$ (Baetens et al. 1976)

[c] D_L = corresponding mean diameter of the 20–40 largest granules in the same nucleated cells (Cristina et al. 1978c)

For each patient the mean size of the secretory granules given in this table was obtained by averaging mean values calculated for each identified cell

Table 2. SRIF cells: comparison of 2 methods for estimating the mean size (±1 SEM) of the secretory granules in the nucleated cells

	d [nm][a]	D_e [nm][b]	D_L [nm][c]
Adult patient no. 1	255±23 (402 granules from 2 cells)	323±29	325±40
Fetus (17 W. old)	286±17 (1,265 granules from 6 cells)	364±22	368±20
General mean	278±14	354±18	357±18

[a] d = mean diameter of secretory granules when all granule profiles were measured in each nucleated cell

[b] $D_e = (4/\pi) \cdot d$ (Beatens et al. 1976)

[c] D_L = corresponding mean diameter of the 20–40 largest granules in the same nucleated cells (Cristina et al. 1978c)

For each patient the mean size of the secretory granules given in this table was obtained by averaging mean values calculated for each identified cell

variations were revealed. Immunoreactive GLI cells contained larger granules in the fetus than in adults (diameter frequency peak, histogram II, in fetus: 300–350 nm). The smallest GLI granules were observed in patient No. 4 where 6 of the 8 nucleated cells examined have a mean granule diameter D_c far less than 300 nm. The difference between the mean GLI granule diameter values estimated in the fetus and in adult No. 4 (Table 1) was highly significant ($P < 0.01$). When averaging all granule diameters for each subject, values obtained in adult No. 4 (290±2 nm) were also found to be significantly different from those of adult No. 1 (323±2 nm) ($P < 0.001$).

Noticeable intraindividual variations can exist also within a single patient. Thus, mean GLI cell granule diameter D_c varied from 221±6 nm to 348±8 nm between two cells of adult No. 4 ($P < 0.001$) and from 296±6 nm to 364±7 nm between two cells of the fetal rectosigmoid mucosa ($P < 0.001$). Variations with the same magnitude were seen with SRIF cells in the fetus.

The staining of the same cells by BPP and (entero)glucagon antisera raised the problem of atypical results. Absorption of each antiserum with its specific antigen (10 μg/ml diluted antiserum) eliminated staining. Absorption of glucagon antiserum by BPP (up to 100 μg/ml diluted serum) did not affect the staining of GLI cells. By contrast, absorption of anti-BPP serum with 20 μg of pancreatic glucagon did not affect, or affected very slightly, the BPP cell staining but with 100 μg of glucagon/ml diluted serum there was either a definite although incomplete reduction in BPP cell staining, or even a failure of cells to react at all. However, when ^{125}I-pancreatic glucagon was incubated with the anti-BPP serum, diluted 1:250,000, no binding was found (0.2% compared with 59% of binding when ^{125}I-BPP was incubated with the same antiserum used at the same dilution). Moreover, in 3 patients (Nos. 2, 3, 4) where immunostainings with anti-BPP and antiglucagon sera were done on paired serial semi-thin sections, not a single cell was found to react uniquely with the anti-BPP serum. In patient No. 1 where BPP cells were studied independently from GLI cells, the mean granule diameter for BPP cells was $d = 244 \pm 6$ nm (1,305 granules from 13 nucleated cells); $D_c = 310 \pm 6$ nm; $D_L = 303 \pm 6$ nm. This was not different from values calculated for GLI cells in the same patient (compare with Table 1).

At the ultrastructural level the various rectal endocrine cells, except enterochromaffin cells, were not as easy to recognize and differentiate as in the upper gut. GLI cells contained rather highly electron dense granules, mostly round but sometimes ovoid or angular. SRIF cells had rounded, sometimes irregular granules of variable electron density, often as dense as GLI cell granules.

Discussion

A comparison of the results given in Tables 1 and 2, concerning the mean granule diameter of an endocrine cell type, brings out that whatever the method of estimation (Beatens et al. 1976; or Cristina et al. 1978c), the values found for D_c or D_L are quite identical; however D_L was obtained more quickly. In addition these tables indicate that for the same type of cell some interindividual variations in granule diameter exist. Because of these variations, and also intraindividual variations, the examination of several subjects and of many cells is recommended in order to obtain accurate and precise data.

In previous electron microscopic studies, we had described different human colonic endocrine cell types and examined their relative distribution along the length of the colon (Cristina et al. 1978a, b, c). *In the adult,* three types containing rounded granules were observed: type III, very rare, with small granules (mean size about 165 nm) and two other types which have granules morphologically similar except for the mean size, about 254 nm for type II cell and about 336 nm for type IV cell. Type II cells are well represented in all colonic segments with a higher density in the rectum where they are about twice as numerous as type IV cells; these were observed only in the distal colon. *In fetuses as well as in children,* in contrast to the adult, type II cells are present in very small percentage especially in the distal colon (3 to 4%) where type IV cells have their highest frequency (21 to 28% of the total endocrine cell population).

Type IV cells have been thought to correspond mostly to GLI- and also partly to SRIF-containing cells (Cristina et al. 1978a, b, c). An additional type V cell, with still larger granules – mean size about 450 nm – was found to be essentially localized in the distal colon. In young subjects the granules appeared to be larger than in adults.

GLI Cells

Histogram II of Fig. 6 shows that the largest GLI granules have their diameter frequency peak at 250–350 nm, i.e. intermediate between that of type II cell (peak at 200–300 nm) and that of type IV cell (peak at 300–400 nm) (Cristina et al. 1978c). The corrected mean size of granule calculated for each GLI cell in adult patients'rectal mucosa ranges from 220 to 350 nm. Thus it seems evident that there are two populations of GLI cells, including type IV (larger granules) and also type II (smaller granules). Although the L cells described by Buffa et al. (1978) correspond to GLI cells, it appears that these authors excluded a number of the latter containing large granules. In the fetal colonic and rectal mucosae, GLI cells correspond almost exclusively to type IV. Indeed, there is a good correlation between our previous ultrastructural studies and the present data. These clearly point out that, with age, the majority of the GLI cell population is subjected to a morphological change characterized by a reduction of the granule size.

In the human gut, Polak and Buchan (1979) indicated that the mean granule diameter of GLI cells was 200 nm but they did not mention whether this value was corrected for artifacts introduced by the plane of section. If we consider 200 nm as d, D_c is about 254 nm, which is still low compared with our data. It is however possible that size of GLI granules also varies along the digestive tract, being smaller in the upper gut than in the colon. This assumption would be supported by the fact that type IV cells – corresponding to the GLI cells with the larger granules in the adult human – are essentially found in the distal and not in the proximal colon.

SRIF Cells

These cells have been described as being very rare in adult (Buffa et al. 1978; Cristina et al. 1978c) and well represented, although less numerous than GLI cells, in the distal colonic mucosa of children and fetus (Cristina et al. 1978b; Lehy and Cristina 1979).

In the present work they were difficult to find in adult but not in fetal colonic tissue. Histogram II and table 2 data indicate that most SRIF cells belong also to type IV as indicated by Cristina et al. (1978a, b, c). However, in the fetus some of them have a mean granule diameter greater that 400 nm and thus in fact correspond to the type V cell. The secretion of SRIF was previously attributed to the ultrastructurally defined *D* cells (Polak et al. 1975a) which contain numerous round cytoplasmic granules displaying a varying but often low electron density and a finely granular structure (Caneze and Bussolati 1974; Alumets et al. 1977). The SRIF cells identified here did not exhibit all

the typical characteristics of *D* cells; nevertheless, they did show rounded granules which sometimes had a lesser electron density than those of GLI cell and a granule size distribution (histogram I, Fig. 6) like that of granules of both human pancreatic and gastric *D* cells (peak of diameter frequency at 200–250 nm) (Alumets et al. 1977).

BPP Cells

In previous studies these cells have been described in adult (Buffa et al. 1978) as well as in fetal (Lehy and Cristina 1979) human colonic mucosa. The staining of the same cells with anti-BPP and antiglucagon sera leads us to dispute the specificities of these antisera. Recently Rawdon and Andrew (1979) described staining of glucagon-containing A cells in the pancreas of the chick by an anti-avian-PP(APP)serum. Similar findings were also reported by Van Noorden and Patent (1978) and by Klein and Van Noorden (1979) in the pancreas of teleost fishes using the same anti-APP serum as the former authors. In these cases absorption of anti-APP serum by glucagon and of antiglucagon serum by APP reduced or even abolished the atypical staining and revealed two very different cell categories. Very recently Cheng Kaung and Elde (1980) observed the presence of glucagon and PP in the same islet cells in the frog pancreas. The origin of their anti-BPP serum was the same as ours. Absorption of anti-glucagon or anti-BPP sera by the heterologous antigen did not abolish the positive staining obtained with each antiserum.

In the present work, staining of human colonic cells by anti (entero)glucagon serum was not modified in presence of an excess of BPP (up to 100 μg/ml of diluted serum). However, staining of cells by the anti-BPP serum was obviously reduced or even abolished in the presence of the same excess (100 μg/ml) of pancreatic glucagon, although a lesser quantity of this hormone (20 μg/ml) had little or no effect. On the other hand, in a radioimmunoassay no binding of the anti-BPP serum was found with pancreatic glucagon. Moreover no cell was stained only by anti-BPP serum or anti-(entero)glucagon serum when paired serial semi-thin sections were examined. To consider the staining of GLI cells by the anti-BPP serum as an artifact is problematical; more likely is the proposal that some PP-like peptide or sequence, rather than PP itself, is present in intestinal cells, as suggested by Buffa et al. (1978). This PP-like sequence might have a certain number of amino acids in common with (entero)glucagon, which would allow it to be recognized by both (entero)glucagon and BPP antisera. Finally in the staining of the same cells by (entero)glucagon and BPP antisera, the hypotheses of an artifact should not be totally rejected. Indeed nonspecific staining of certain types of endocrine cells, i.e. G cells, have been described by Grube and Weber (1980) and Grube (1980): the staining of these cells should result in the binding of immunoglubulins to endocrine cell granules by non-specific ionic interactions.

In the human pancreas PP cells are always found to contain small granules, i.e. mean diameter d 120–170 nm, range 100–260 nm, in all reports (Heitz et al. 1976; Larsson et al. 1976; Baetens et al. 1977; Bergstrom et al. 1977; Pelletier and Leclerc 1977; Paulin and Dubois 1978). In the dog, the PP cell, identified

as corresponding to the F cell, contains larger granules than in man: 140–200 nm in their long axis (Forssmann et al. 1977), 200–250 nm (Larsson et al. 1976) or 300–400 nm (Greider et al. 1978). Buffa et al. (1978) noted that, in the human colon, their F-like cell resembled the F cell of dog pancreas and they postulated that intestinal F-like cells are possibly identical to BPP-immunoreactive cells. Moreover, they reported that sometimes both granules resembling those of F-like cells and granules resembling those of L cells were found in the same cell and hypothetized that such a cell might store both GLI and PP-like sequences. In the present study we have not distinguished F-like cells from L cells ultrastructurally, but we have pointed out above that GLI cell granules are sometimes irregular, ovoid and angular. It seems therefore that F-like cells of Buffa et al. might correspond to our GLI cell population containing large granules (type IV).

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