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Emiocytotic Granule Release Without Intraluminal Stimulus in Human Colonic Endocrine Cells of Fetuses, Children and Adults

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Summary. In endocrine cells of the colon of adults, children and fetuses, exocytotic granule release without any specific stimulation is reported. Omega-invaginations are observed on both the lateral and basal surfaces of all types of colonic endocrine cells. Several explanations for the phenomenon are suggested: 1) emiocytosis is probably more frequent in the colon than in the proximal gut, this allows its observation without requiring an exogenous stimulus, 2) since most of the exocytotic figures are from anaesthetized subjects it is also assumed that contraction of the muscular layer induced by anaesthetics and the resulting increase in intraluminal pressure were the possible causes of granule release, 3) in non-anaesthetized subjects release may have taken place in response to a normal endogenous physiological stimulus, or to the dilation of colon during colonoscopy. Less likely is an effect associated with the preparation for colonoscopy. Certain figures on lateral surfaces between endocrine and adjacent cells i.e., bulges of parallel plasma membranes surrounding a secretory granule, were observed. Their significance is unknown.

Key words: Emiocytosis – Endocrine cells – Human embryo, child and adult – Large intestine – Electron microscopy.

Introduction

Recent ultrastructural studies have demonstrated that granulated cells scattered throughout the gut are the source of gastro-enteric hormones. These endocrine cells extend an apical process covered with a peculiar tuft of microvilli into the intestinal lumen. This structure is the morphological basis of the hypothesis of Funita and Kobayashi (1973) according to which the gut endocrine cell is a "receptor-secretory" cell which recognizes adequate luminal information (i.e., is sensitive to pH and/or chemical substances or physical stimuli) via the apical process. Such a cell is able, via an "unknown mechanism of intracellu-

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lar transmission" to control its own secretory activity (release from the basal part of the cell). It is also assumed that gut endocrine cells may receive humoral stimuli through the blood stream which influence granule release.

Two main theories concerning the release mechanism of secretory granules from gut endocrine cells have been postulated: (a) diacrine and (b) exocytotic or emiocytotic. According to the first theory, granule contents are extruded from the vesicle and the cytoplasm only when a specific stimulus makes the plasma membrane permeable. This mechanism has been advocated by Forssmann and Orci (1969) following their electron microscopic findings in the gastrin cells of the cat under various feeding conditions. The second theory postulates emiocytosis, i.e., granule membranes fuse with the cell's surface membrane after stimulation and thus enter the extracellular space directly. This mechanism was proposed by the Japanese group (Fujita and Kobayashi, 1971; Fujita et al., 1974; Kobayashi and Fujita, 1973; Kobayashi and Sasagawa, 1976; Osaka et al., 1974) for antral and duodenal endocrine cells stimulated at their apices. Recently this release mechanism was also demonstrated for gastrin granules in man, after selective vagotomy with pyloroplasty (Miyagami et al., 1977).

Previous reports have concerned release of secretory material from the upper gut and pancreatic endocrine cells. In the present study we describe exocytotic figures in human colonic endocrine cells that we examined during morphological studies in adults (Cristina et al., 1978a), children (Cristina et al., 1978b) and fetuses (Cristina et al., 1978c). All these studies were carried out without any specific stimulation.

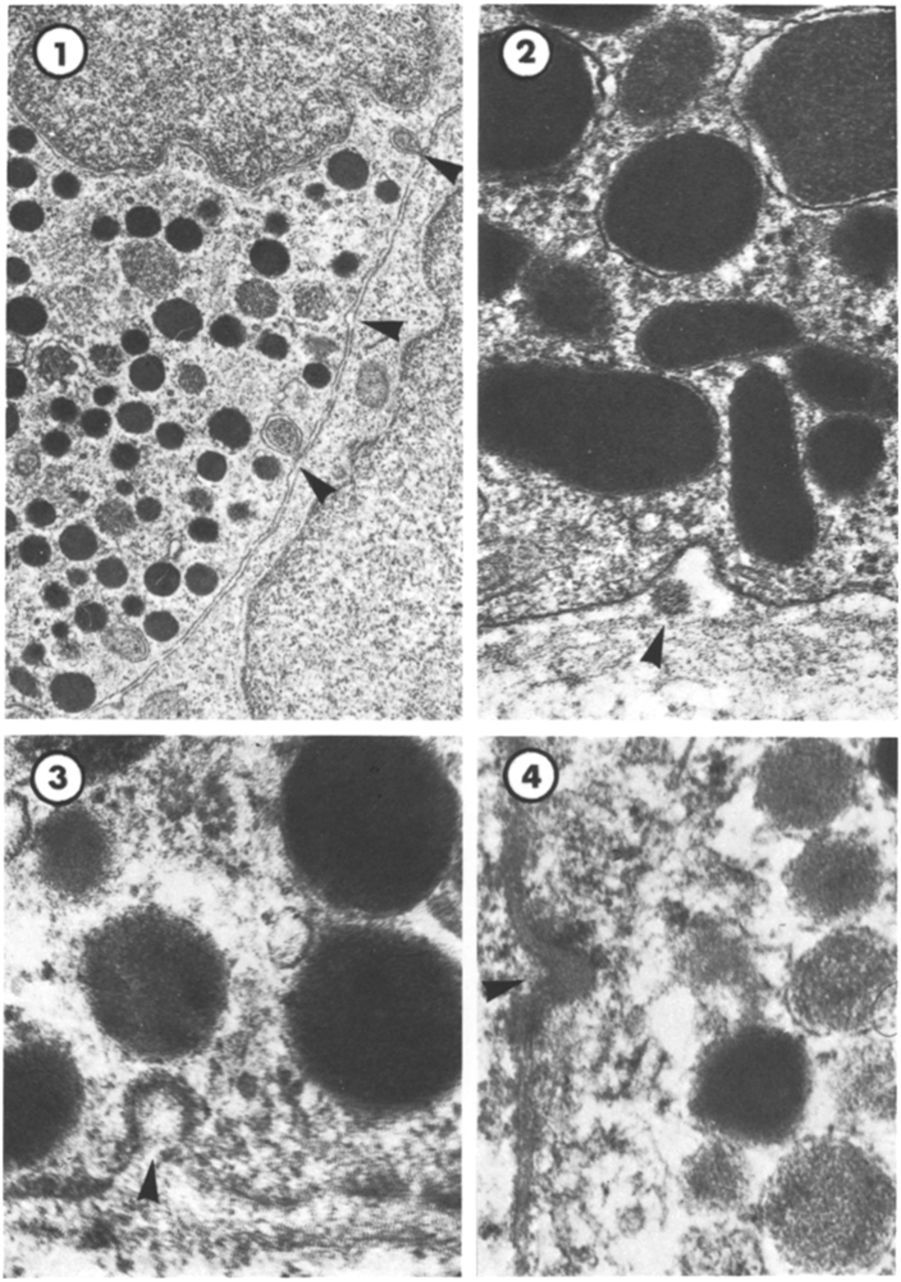
Material and Methods

Electron microscopic studies were done on biopsies obtained from 3 groups of subjects: (a) 39 biopsies from 16 normal adults performed during colonoscopy (except for one surgical case); (b) 27 biopsies obtained during surgical operations from 9 children one month to three years old (5 controls and 4 congenital megacolons); (c) 43 biopsies from a series of 11 fetuses obtained from legal or spontaneous abortions; in this group 7 mothers had been exposed to anaesthetics. In groups b and c, the anaesthetics used in combination included pentotal, phenoperidine and pavulon. In two cases of voluntary abortion pentotal was used alone.

Tissue samples were fixed for 2 h in 2.5% glutaraldehyde in a phosphate buffer (pH 7.2; 0.1 M) and post-fixed for 1 h with 1% OsO_4 solution in the same buffer; after dehydration and embedding in Epon, sections stained by uranyl acetate and lead citreate were examined with a Siemens Elmiskop 1 A microscope.

Results

In view of the great number of nucleated endocrine cells examined (about 2,500 in the 3 groups of subjects), exocytotic figures are evidently very rare; only 60 were observed: 12 in adults, 23 in children and 25 in fetuses. Following the Japanese classification of such figures (Kobayashi and Sasagawa, 1976) our data are summarized in Table 1. Signs of granule release were seen both on lateral and basal surfaces. This is true for all defined endocrine cell types in the human colon (Cristina et al., 1978a, b, c), i.e., EC cell and four



Figs. 1 to 4. Exocytotic figures of granules release in human colonic endocrine cells (*arrows*)

Fig. 1. Type II cell in rectum of normal adult (biopsy obtained during rectoscopy): bounding membranes of granules attached to lateral membrane of cell. ×23,000

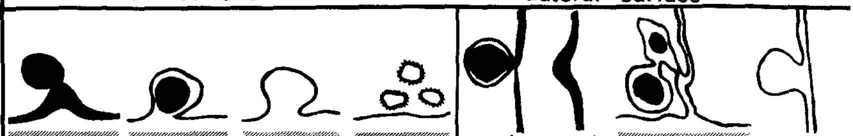
Fig. 2. EC cell in ascending colon of adult (surgical biopsy). Exocytotic figure with a granule outside cell. ×65,000

Fig. 3. Distal colonic region of 5–6-week old fetus (induced abortion; mother had received pentotal as anaesthetic, which is able to cross placenta). Type V cell (mean diameter of secretory granule 400–500 nm) specific of fetuses and young children. Omega-shaped invagination in basal part of cell close to basal lamina. ×80,000

Fig. 4. Part of D cell in rectum of normal child, one month old. The granule is apparently fusing with the lateral plasma membrane of the cell which was omega-shaped. ×48,000

Table 1. The different electron microscopic figures observed on our material; it is suggested that they are related to the emiocytotic process of secretory granule release from colonic endocrine cells. They were seen on the basal as well as the lateral surfaces of the cells. Successive events: a) the membrane encasing the granule fuses with the plasma membrane (more or less omega-shaped) and ruptures, b) the granule is liberated into the extracellular space where it dissolves, c) only the omega-shaped membrane is visible, d) small coated vesicles inside the endocrine cell (on our material they are seen only on basal surface). These exocytotic figures were found in similar number in the EC, type II and type IV cells (most frequent endocrine cell types in the distal human colon)

Emiocytotic Figures of Human Colonic Mucosa



Cell Type	Basal surface				Lateral surface		
EC	3	3	8				3
II 200–300nm	2	1	4		6	1	3
III = D_1 150–200nm						1	
IV 300–400nm D	3			1	2		1
IV 300–400nm L	2	1	7				1
V 400–500nm	1		4			1	1

other cell types containing rounded granules, that we have named types II to V and that correspond, according to the revised classification of Lausanne to D_1 (type III), L and D cells (type IV). Two cell types do not as yet have any place in this classification: type II (dense granules, mean diameter 200–300 nm) and type V (granules of variable density, mean diameter 400–500 nm) which is specific for fetuses and children and which is not seen in adults. Most of the exocytotic figures are found in subjects submitted to anaesthetics. Only 8 are seen in 5 adults that had not been anaesthetized.

Successive stages were observed in the exocytotic figures. Firstly the granule sac, still containing the granule, was seen closely attached to the plasma membrane (Fig. 1); secondly the granule sac appeared to fuse with the plasma membrane and was more or less omega-shaped (Figs. 2, 4). The invagination produced either contains granular material (Fig. 2) or is empty (Fig. 3), or may be accompanied by coated vesicles.

Special configurations, not included in Table 1, were found between endocrine and their adjacent cells, the latter being undifferentiated and rich in ribosomes. They appeared as bulges of the two parallel plasma membranes (not

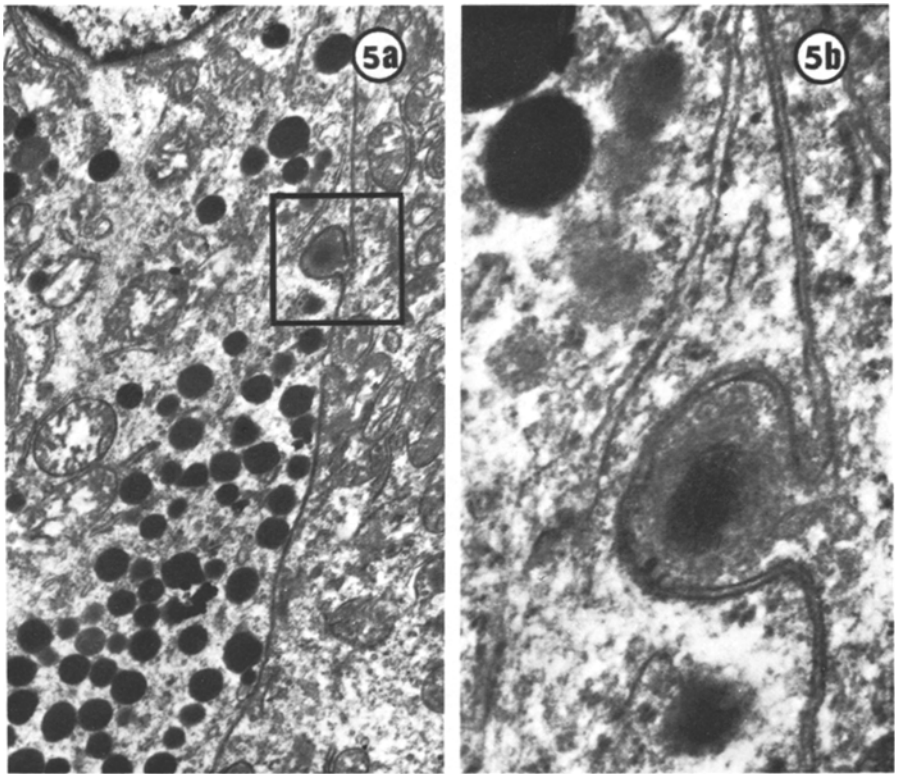
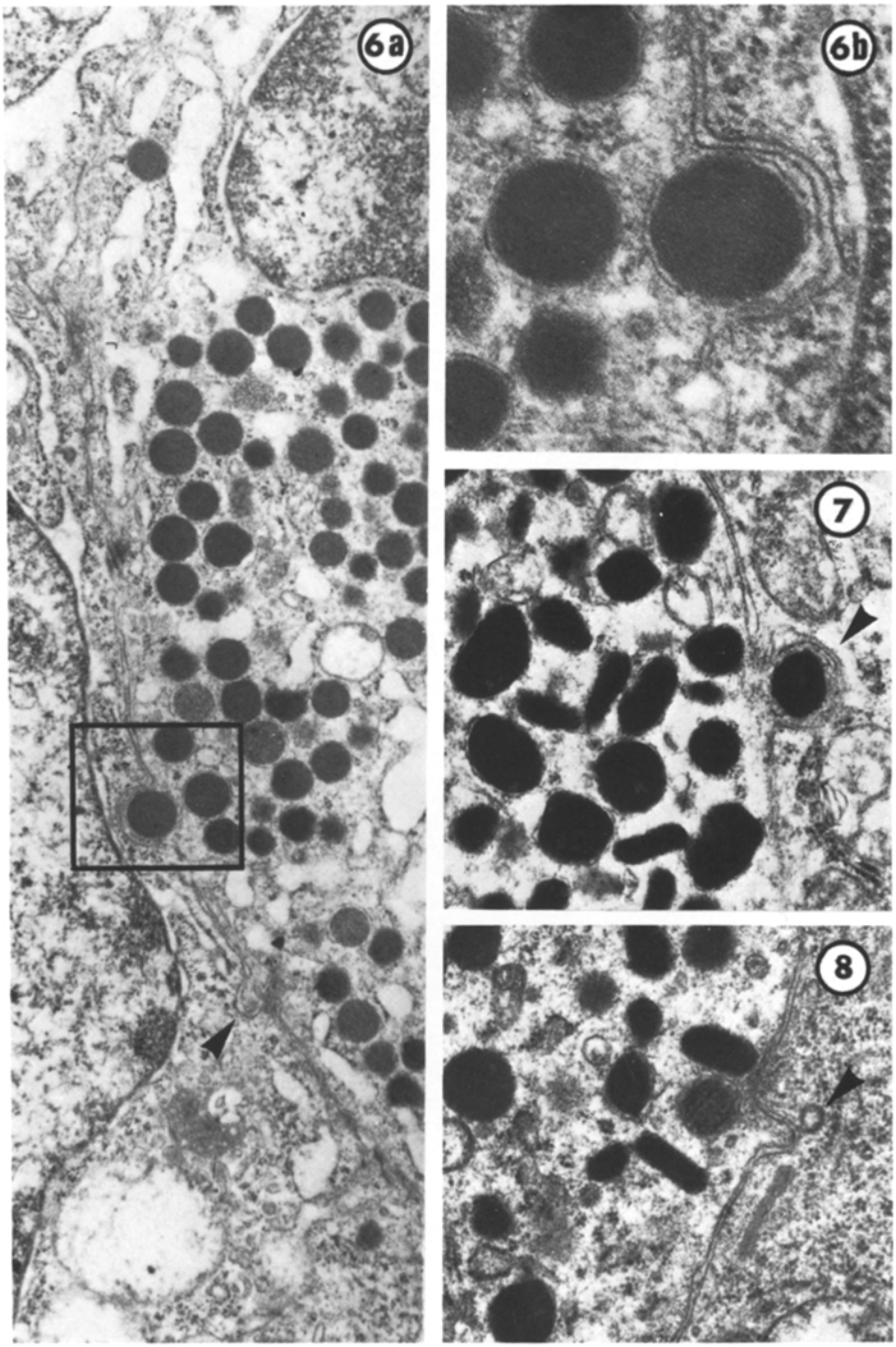


Fig. 5. Part of L cell in the rectum of a normal child, one month old. **a** Figures seen on the lateral surface. Invagination of two parallel plasma membranes i.e., the endocrine cell membrane and the adjacent cell membrane. This invagination contains a dense granular material which could perhaps be directly transfered from the endocrine cell into the undifferentiated adjacent cell, elsewhere devoid of secretory granule. ×10,800. **b** Detail of this configuration. ×54,000

one as in the case of "true" omega-figure) often surrounding a secretory granule. Both lateral surfaces were seen bulging either into the endocrine cell (Fig. 5) or into the adjacent cell (Figs. 6–8). We never have observed such figures between two adjacent endocrine cells. These configurations may be interdigitation of adjacent cells or may indicate something else, i.e., some of them (see Fig. 5) suggest a passage of granules through two adjacent cell membranes, with release into the adjacent cell, rather than the extracellular space.

Discussion

The present report furnishes additional electron microscopic observations related to the emiocytotic release of secretory granules from gut endocrine cells. What


6a
6b
7
8

is new is the site where these observations were made, i.e., the human colon and rectum; previous reports have dealt with the upper gut endocrine cells. The fact that such exocytotic figures were also seen in the fetal colon is interesting, as is the evidence that they occured without specific intraluminal stimulation. Exocytotic peptide granule release from gut endocrine cells has been difficult to demonstrate because of the rare moments suitable for electron microscopic demonstration, especially in unstimulated material. In our material, after a careful examination of photographs, exocytotic figures were found in colonic endocrine cells on their basal and/or lateral surfaces. Their frequency was probably much lower than might be expected under conditions of stimulation.

Whether exocytosis is a real phenomenon and not a morphological entity unrelated to physiological cell function is uncertain. Recently some papers and reviews have attempted to elucidate the subcellular events associated with the secretory functions of basal granulated cells (Lacy, 1975; Kobayashi and Sasagawa, 1976; Norman, 1976; Nagasawa, 1977). The data gathered are in favour of exocytosis as a real phenomenon (see, for example, studies concerning pancreatic insulin cells: Orci et al., 1973; Lacy, 1975; Orci and Perrelet, 1976).

There are several possible explanations of our own observations.

a) If, as suggested by Lacy (1975) the release of secretory granules by emiocytosis is continuous, one could suppose that such a phenomenon is more frequent in colon than in upper gut. It could also be assumed that release is more frequent in man than in animals (most of the works which describe granule release mechanism in gut endocrine cells were conducted in dog or rabbit). Thus, the action of additional and specific exogenous stimulus would not be necessary to increase the frequency of observation of emiocytotic figures.

b) Most of the exocytotic figures were found in surgical material from patients submitted to anaesthetics; one could suppose that these substances themselves may act as stimulants of the endocrine cells. In fetuses this presumed stimulus would have to be capable of transplacental transmission. The drugs used are able to cross the placenta except for pavulon (a non-histaminogenic substance) which only crosses in very small amounts. If such drugs are involved it appears probable that they act rather indirectly, for instance, it is known that, in man anaesthetics cause the contraction of the muscularis of the gut.

Figs. 6 to 8. Particular figures, i.e., bulges of parallel plasma membranes, seen on lateral surface between endocrine cell and undifferentiated adjacent cells which are in all three cases, rich in ribosomes. These figures could be interpreted as "bud evaginations" on the endocrine cell's cytoplasm bulbing into the adjacent cell (interdigitations?)

Fig. 6. a L cells in the rectum of a normal child, one month old, showing two bulges of adjacent parallel plasma membranes on the lateral surface with one containing a granule. ×16,000. **b** Detail of the latter figure. ×54,000

Fig. 7. EC cell in the rectum of the same child showing a bud evagination of its cytoplasm bulging into the adjacent cell and containing a dense granule. ×24,000

Fig. 8. Rectum of a child with megacolon, five months old. Bud evagination of an EC cell accompanied by a coated vesicle (*arrow*) bulging into the adjacent cell particularly rich in ribosomes. ×24,000

It is possible that the contraction of the muscular layer and the resulting increase in intramural pressure provokes granule release in colonic endocrine cells.

c) The exocytotic figures seen in adults from biopsies obtained during colonoscopy without anaesthetic, may occur in three ways: (1) release may have taken place in response to normal endogenous physiological stimuli, as suggested in (a), or (2) following inflation of the colon with air (immediate response to mechanical distension) or (3) following intraluminal treatment usually given several hours before colonoscopy. The latter explanation seems highly improbably since in the literature exocytotic figures were observed at short intervals after the application of an intraluminal stimulus (Kobayashi and Sasagawa, 1976).

Whatever the mechanism of stimulation may be, it is noteworthy that exocytotic figures observed are not specific for one endocrine cell type; about the same number were seen in EC, type II and type IV cells which are the most frequent in the distal colon (Table 1). This fact is rather in favour of a general phenomenon.

Exocytotic figures, mainly represented by omega-invaginations, have been found in all types of glandular cells (endocrine and exocrine), neurosecretory cells, other neurons and paraneurons (Nagasawa, 1977) as well as in mast cells (Lawson et al., 1977). In our study almost of the sequential stages postulated by Douglas et al. (1971) were seen, i.e., omega figures corresponding to proper exocytosis and small coated vesicles and packed arrays of smooth vesicles away from the cell periphery, corresponding to the vesiculation process.

Although the physiological role of colonic endocrine cells is not clear they release their granules in the same way as all the other gut endocrine cells. Even in fetuses they seem to be capable of being stimulated, it remains to be demonstrated that they are really physiologically active during gestation.

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