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Increased populations of endocrine cells in Crohn's ileitis

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Summary. Hyperplasia of nerves has been described previously in Crohn's disease. To determine whether similar alteration of the enteric endocrine system occurs, endocrine cells of the ileal epithelium were quantified in typical cases of the disease. In the ileum from patients with Crohn's disease, there was an increase in the endocrine cell population, as visualised by immunostaining of chromogranin. Quantification of endocrine cell numbers showed significant increases in both macroscopically uninvolved (i.e. histologically normal) (35.0 ± 3.8, cells per unit length of muscularis mucosae mean $\pm$SEM, $P<0.05$) and involved (44.5 ± 5.5, $P<0.01$) Crohn's disease samples, compared with normal controls (23.7 ± 3.4). Although individual types of endocrine cell showed slight increases in Crohn's samples, only the enterochromaffin cells in abnormal bowel showed a significantly greater population (normal controls 10.5 ± 2.3; involved Crohn's 21.3 ± 4.4, $P<0.05$).

Key words: Crohn's disease – Immunocytochemistry – Endocrine cells

Introduction

In Crohn's disease, an increased level of innervation, or so-called neuromatous hyperplasia, has been known for some time to be present in affected areas of bowel (Davis et al. 1955; Morson and Dawson 1972; Whitehead 1975). This hyperplasia noted in conventional histological preparations was later shown by immunocytochemical techniques to be due largely to an abnormally dense population of vasoactive intestinal polypeptide (VIP)-containing nerves (Bishop et al. 1980; Sjoklund et al. 1983; O'Morain et al. 1984; Spaepen et al. 1984; Schmitz-Reinhardt et al. 1985; Bishop et al. 1986). The pathogenesis and functional significance of these morphologically abnormal peptide-producing nerves are not yet known.

Altered numbers of endocrine cells have been associated with several diseases of the gastrointestinal tract, often those affecting the mucosa. Such diseases include coeliac disease (Polak et al. 1975; Sjoklund et al. 1979, 1982; Pietroletti et al. 1986), graft versus host disease (Lampert et al. 1985) and the major inflammatory bowel disease, ulcerative colitis (Morson and Pang 1967; Miller and Hatton 1982; Gledhill et al. 1986). In the case of the latter condition, the increased population of endocrine cells was suggested to be a feature of the general reactive hyperplasia of the epithelium in response to mucosal damage. It has been postulated (Gledhill et al. 1986) that, as certain gastrointestinal hormones are trophic to the gut (Johnson 1979), the hyperplastic endocrine cells may produce an excess of peptides which are contributing factors to the high frequency of tumour formation associated with ulcerative colitis. Unlike ulcerative colitis, there is no evidence for any change in mucosal cell production rate in Crohn's disease and an association of the disease with carcinogenesis is disputed (Cantwell et al. 1968; Sheil et al. 1968; Lennard-Jones 1969; Morson and Bussey 1970; Farmer et al. 1971; Darke et al. 1973; Weedon et al. 1973; Savage et al. 1975; Valdes-Dapena et al. 1976; Nesbit et al. 1976).

No investigation has been made of the endocrine component of the epithelium in Crohn's disease. Recently, antibodies have become available to a family of proteins grouped together under the general name of chromogranin (Wilson and Lloyd 1984). Immunostaining with these antibodies had

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proved to be a means for demonstration of all types of gastrointestinal endocrine cells (Facer et al. 1985). Thus, the aim of the present study was to establish whether any alteration in gut endocrine cell numbers occurs in typical cases of Crohn's ileitis by immunostaining for chromogranin and specific antigens known to be present in these cells, such as serotonin (5-hydroxytryptamine, 5-HT), glucagon, somatostatin, neurotensin and peptide with tyrosine (PYY).

Materials and methods

Fresh specimens of adult human ileum were taken at surgery from patients with chronic Crohn's disease ($n=10$) or, as controls, carcinoma ($n=5$). Representative samples, measuring approximately 1×0.5 cm, were taken from both involved and macroscopically normal areas from the clear cut cases of Crohn's disease and from the controls, as distant from the tumour as possible.

All tissues were fixed by immersion for 8 h in Bouin's solution, followed by overnight washing in 50% alcohol. Fixed tissues were dehydrated through graded alcohols to xylene and vacuum-embedded in paraffin wax. Sections of 5 μm were collected on poly-L-lysine-coated glass slides (Huang et al. 1983) allowed to air-dry and stained with haematoxylin and eosin or immunostained using the peroxidase anti-peroxidase (PAP) method (Sternberger 1979). After de-waxing, endogenous peroxidase activity and nonspecific staining due to the bridging antisera were blocked by incubation for 30 min in 0.03% hydrogen peroxide followed by a further 30 min in normal goat serum (dilution 1:30). The primary antibodies were applied at appropriate dilutions (Table 1) and incubated for 16–20 h at 4° C in a moist atmosphere. After thorough rinsing in buffer, those sections incubated with a primary antibody from mouse (anti-chromogranin) received an excess (1:100 dilution) of goat anti-mouse immunoglobulin G, and those that had a rabbit primary antiserum (anti-peptide or amine) were incubated with an excess (1:200 dilution) of goat anti-rabbit immunoglobulin G. After further rinses in buffer, the sections were incubated with PAP complex. To localize mouse- and rabbit-derived reagents, mouse monoclonal PAP (clono-PAP, Sternberger-Meyer, dilution 1:200) and rabbit PAP (Miles Laboratories, Inc., Elkhart Ind., dilution 1:500) were used, respectively. All incubations were for 30 min at room temperature. Visualization of the PAP complex was achieved by the diaminobenzidine method of Graham and Karnovsky (1966). When developed, the sections were dehydrated through graded alcohols to xylene, mounted in DPX, and examined under a transmitted light microscope. Photographs were taken using Technical Pan black and white film (speed 150 ASA, Kodak Ltd.).

Controls for immunostaining included replacement of the first layer with nonimmune serum from the same donor species or omission of the first and second layers. These gave no immunostaining. In addition, successful quenching of the immunostaining was obtained by prior absorption of each antiserum with its respective antigen (Table 1).

To quantitate the endocrine cell populations in the sections, the number of cells overlying a uniform length (0.4 mm) of muscularis mucosae was counted in each section (Pietroletti et al. 1986). Only portions of mucosa with continuous epithelium were counted. For each immunostain, a minimum of 10 units was counted in perpendicularly-orientated sections. Data were assessed using analysis of variance.

Table 1. Details of antibodies

Antibodies to	Region specificity	Dilution	Concentration of corresponding antigens for absorption (nmol/ml) diluted antiserum
Mouse monoclonal			
Chromogranin	NK	1:200	10.0
Rabbit polyclonal			
Glucagon	N-terminal	1: 5,000	1.0
Neurotensin	Whole	1:16,000	1.0
PYY	Whole	1: 4,000	1.0
Serotonin	NK	1:10,000	1.0
Somatostatin	Whole	1:10,000	1.0

NK = Not known

Results

Histology

No pathological changes could be detected in either the control samples taken at resection for carcinoma or in the macroscopically normal regions of specimens removed from patients with Crohn's disease.

Samples taken from involved parts of the Crohn's disease specimens showed typical features of the disease to varying extents. All but 2 cases had transmural inflammation and in all cases the submucosa was widened by oedema. Classical fissuring ulceration was observed in half of the specimens. Few crypt abscesses were seen and granulomata could be detected in only 4 of the 10 cases. Pyloric gland metaplasia was seen in one case.

Immunocytochemistry

Immunostaining for the general endocrine cell marker chromogranin revealed large numbers of endocrine cells in the mucosal epithelium of all specimens. The changes ranged from small increases in cells, which showed normal distribution, to some areas of epithelium in which the endocrine cells were densely packed, with few non-endocrine cells being distinguishable (Fig. 1). Five types of endocrine cell were identified by individual peptide immunostains. The predominant cell type was the enterochromaffin cell, visualised by immunostaining using antibodies to the amine serotonin (5-hydroxytryptamine) (Facer et al. 1979). Cells containing glucagon-, neurotensin- or somatostatin-immunoreactive material formed smaller popula-

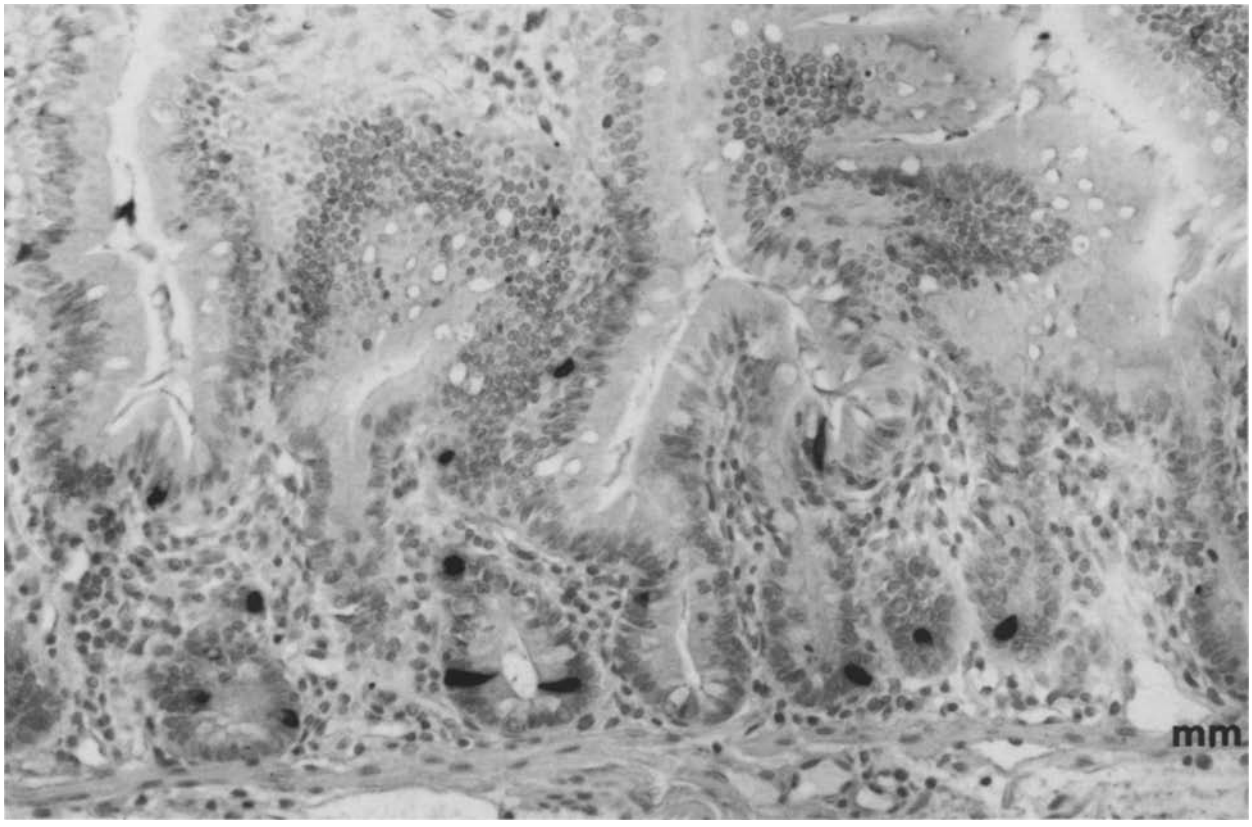

Fig. 1. Histologically normal ileum from a carcinoma resection immunostained with antibodies to chromogranin. Numerous endocrine cells can be seen in the epithelium, mainly towards the base of the mucosa. *mm* = muscularis mucosae Mag. ×250

Table 2. Results of quantification of endocrine cells

Antibodies immunostained	No. of immunoreactive cells/units muscularis mucosae [mean ± SEM]		
	Controls	Crohn's-involved	Crohn's-non-involved
Chromogranin	23.7 ± 3.4*, **	44.5 ± 5.5*	35.0 ± 3.8**
Serotonin	10.5 ± 2.3**	21.3 ± 4.4**	16.2 ± 2.0
Glucagon	7.2 ± 1.8	13.5 ± 2.8	11.0 ± 1.8
Neurotensin	9.7 ± 1.2	5.7 ± 0.8	8.6 ± 1.1
Somatostatin	2.3 ± 0.5	2.1 ± 0.4	2.5 ± 0.6

* $P < 0.01$, $P < 0.05$

tions. Only a very small number of cells were found to contain peptide with tyrosine (PYY).

The results of quantitation of the cell populations are displayed in Table 2, except for PYY-containing cells which were too few in number. The data for abnormal and histologically normal Crohn's specimens were compared separately with the normal controls. The whole endocrine cell population, as revealed by chromogranin immunoreactivity, was found to be increased significantly in Crohn's disease, both in abnormal ($P < 0.01$) and macroscopically and histologically normal ($P < 0.05$) areas, in comparison with normal controls. There was no significant difference between the results obtained for involved and non-involved areas of the Crohn's samples, although there were slight increases in all types of endocrine cells. The only individual cells which showed a significant alteration were the enterochromaffin cells which were abnormally numerous in only the involved areas of the Crohn's disease samples ($P < 0.05$).

Discussion

The results of this study reveal an alteration of mucosal endocrine cells in Crohn's ileitis. The general population of endocrine cells, as demonstrated by immunostaining of chromogranin, was significantly greater in both involved and macroscopically and histologically normal areas of resection specimens from patients with Crohn's ileitis, compared with normal controls. Individual endocrine cell types also tended to be increased in number in the Crohn's disease tissues but only the serotonin-containing, enterochromaffin cells showed a statistically significant change. The low number of

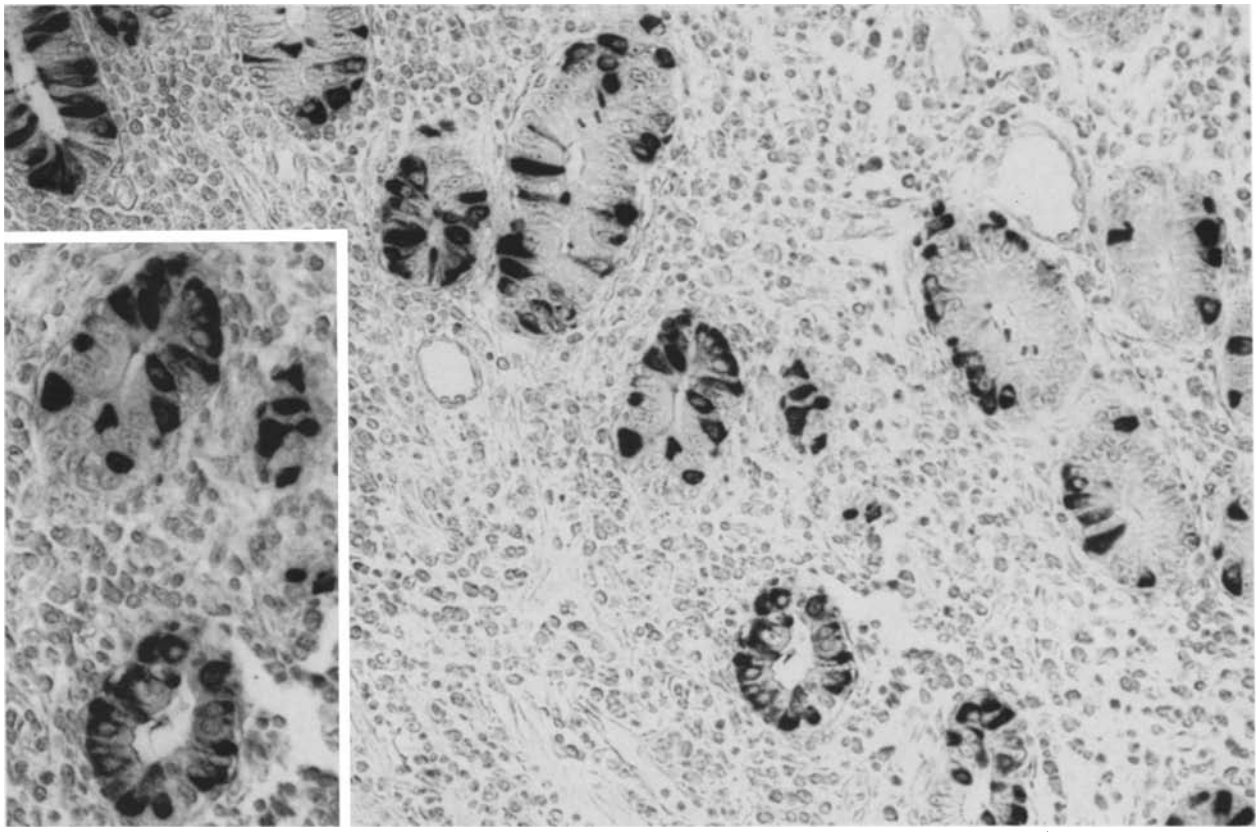

Fig. 2. Section of involved ileum from a patient with Crohn's disease immunostained with antibodies to chromogranin. Large numbers of endocrine cells are packed in the epithelium. *Insert:* high magnification of one area of the field. Mag. main picture ×220; *insert* ×350

PYY cells found in this study corresponded with the normal distribution of this peptide which is present mainly in distal bowel, its concentration in the ileum being only one sixth of that in the rectum (Adrian et al. 1985). Although substance P has been reported to occur in the enterochromaffin cells in certain mammals (Pearse and Polak 1975; Heitz et al. 1975; Sundler et al. 1977; Nilsson et al. 1986) this was not the case in the human material studied here, as has been found previously (Sokolski and Lechago 1984).

Endocrine cell hyperplasia has been observed in other gastrointestinal diseases, with ulcerative colitis being the most relevant to this study (Morson and Pang 1967; Miller and Hatton 1982; Gledhill et al. 1986). As ulcerative colitis is primarily a mucosal disease, with defined increases in crypt cell proliferation (Bleiberg et al. 1970; Eastwood et al. 1973; Lipkin 1974; Serafini et al. 1981), such a hyperplastic response of one component of the epithelium is not a surprising finding. In contrast, Crohn's disease is a panenteritis with primarily submucosal involvement. Although the histogenesis of Crohn's disease remains unclear, it is widely considered to be mainly a disease of lymphoid tissue (Morson and Dawson 1982). It is thought that primary accumulation of lymphocytes under the epithelium leads to degeneration of the proliferating basal cells with subsequent ulceration. Enterochromaffin cells have been proposed to be end-stage and non-proliferating (Chang and Leblond 1971a, b; Cheng and Leblond 1974), although some endocrine cells may possess a small capacity to proliferate (Pradal et al. 1973; Lehy and Williams 1975; Fujimoto et al. 1979). If the epithelial degeneration seen in Crohn's disease is confined to the rapidly proliferating non-endocrine epithelial cells, the enterochromaffin and perhaps other endocrine cells may not be affected, leading to the apparent increase in their number. A mechanism like this has been postulated for graft versus host disease where there is far greater destruction of the epithelium (Lampert et al. 1985). The non-proliferative enterochromaffin cells were often found clumped where the colonic epithelium had been destroyed, possibly being spared by the disease process just as it fails to affect other non-proliferating tissues like nerve and muscle (Billingham 1971).

It should be borne in mind, however, that the abnormally large numbers of endocrine cells seen in Crohn's disease may be due to a functional change with increased content of product within cells allowing more to be visualised by immunocytochemistry.

The relevance of the increased endocrine cell population to the histogenesis of Crohn's disease cannot be defined from the results of this study. Serotonin, the hormone produced by the only cell type found to be increased to a statistically significant level, has a vasodilatory effect (Biber et al. 1973) which may contribute to the vascular changes associated with the disease. VIP is also a powerful vasodilator (Said 1978) and a functional relationship between VIP nerves and enterochromaffin cells has been established in reflex hyperaemia in the bowel (Eklund et al. 1980). It is therefore tempting to suggest that a relationship exists between the hyperplastic VIP nerves observed previously in Crohn's disease (Bishop et al. 1980; O'Morain et al. 1984; Schmitz-Reinhardt et al. 1986) and the abnormally large numbers of endocrine cells found in the present study, although these morphological studies alone cannot determine whether either neuroendocrine component is functioning. As crypt cell proliferation rate does not appear to be altered in Crohn's disease, it is unlikely that the trophic action of serotonin (Tutton 1974) is fully exerted, unless a specific trophic effect on endocrine cells and VIP nerves is postulated. No previous publications, however, support this view. Furthermore, although autonomic nerves can affect epithelial cell turnover (Dupont et al. 1965; Tutton 1974; Lachat and Goncalves 1978; Tutton 1980), there is no evidence that VIP plays any part in epithelial growth (Johnson 1977).

In conclusion, our finding of an altered population of endocrine cells in Crohn's ileitis suggests an hitherto unsuspected change in epithelial cell turnover.

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