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Ultrastructural study of inflammatory bowel disease

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Summary. Ultrastructural changes that occurred in chronic active ulcerative colitis and Crohn's disease were investigated and compared to normal as well as to higher grades of dysplasia in adenomas and carcinomas. A greater number of immature absorptive cells, undifferentiated and intermediate cells were seen as compared to normal. One case of Crohn's and two cases of chronic ulcerative colitis including one with coexisting carcinoma showed increased number of vesicles and electron-dense bodies (EDB) in the absorptive cells and increased heterogeneity of mucin droplets in goblet cells and presence of atypical secretory cells (ASC). Higher grades of dysplasia characterised by large numbers of atypical secretory cells were not seen in the present series and provide no relationship between the atypical ultrastructural features and increased risk of malignancy. However, the number of cases investigated is too small and a large series is required to clarify the significance of observations such as increased number of electron-dense bodies and vesicles in the apical cytoplasm and presence of atypical secretory cells.

Key words: Ultrastructure, Ulcerative colitis, Chron's disease

Abbreviations used: EDB = electron-dense bodies, ASC = atypical secretory cells

Introduction

It is generally accepted that patients with ulcerative colitis have an increased risk of developing carcinoma and that this risk is related to the extent and duration of the disease. The morphological criteria for risk is the presence of dysplasia. In a few cases reliance of dysplasia may not indicate the development of carcinoma (Filipe et al., 1980; Ehsanullah et al., 1985). Carcinogenesis is a stepwise process from normal through dysplasia to carcinoma, and therefore investigation of the preneoplastic tissue may identify abnormal features which will indicate increased individual susceptability to malignant change. Several attempts have been made to establish the criteria for determining the precancerous changes in ulcerative colitis but no precise marker exists as yet for assessing malignant potential (Fozard et al., 1986; Ahrnen et al., 1987; Filipe et al., 1988; Thomas et al., 1989). In previous studies we have reported ultrastructural changes in the mucosa adjacent to carcinoma and in adenomas which appeared to be related to the degree of dysplasia (Filipe et al., 1980; Mughal and Filipe, 1978; Mughal et al., 1981).

This study was conducted to determine whether ultrastructure of the colonic epithelium could help in assessing the risk of malignant change in long-standing ulcerative colitis and Crohn's disease as compared with normal and with previous findings in carcinoma-bearing colonic mucosa and adenomas.

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Materials and methods

The following material was included in this study: (a) seven specimens from ulcerative colitis patients, two of which were resected for carcinoma; and (b) three of Chron's colitis. There were 5 males and 2 females in the ulcerative colitis group, and all were females in the Crohn group, with an age range of 23-51 years and 29-35 years respectively. From each resected specimen fragments of involved and apparently normal mucosa were taken from various regions of colon; control rectal biopsies were obtained from 6 male patients with an age range of 25-72, mean 58 years, with no known gastrointestinal disease.

All tissues were collected fresh and small fragments about 1 mm^3 were immediately fixed in 0.1M Millonig phosphate buffer (pH 7.2) containing sucrose and 1 mM $CaCl_2$. They were then fixed in 1% buffered OsO_4. The

tissues were then dehydrated in a graded series of ethanol and embedded in araldite. On average, 20 tissue blocks were examined from each resected specimen and all biopsy material was embedded and examined. 1 μm sections were cut and stained with toluidine blue, and the properly oriented mucosa showing longitudinally open crypts were selected for ultrathin sectioning. The sections were cut with glass and diamond knives on LKB III, IV and V ultramicrotomes (LKB Instruments Inc., Rockville M.D.), and mounted on G75 formvar-carbon-coated grids. The sections were then stained with uranyl acetate in 50% ethanol and Reynold's lead citrate. The grids were checked in Hitachi U12 (Hitachi Ltd, Tokyo, Japan), Jeol 100 CX and Jeol 1200 EX (Jeol Ltd, Tokyo, Japan) at 80 KV. The micrographs were taken on Kodak 4489 thick base films (Eastman Kodak Company, USA).

Results

The criteria for defining and grading epithelial changes at ultrastructural level were based on the following features, as described earlier in familial polyposis coli (Mughal and Filipe, 1978):

1. Vesiculation and an increase in the number of electron-dense bodies (EDB) and lysosomes in the mature and immature absorptive cells.
2. Presence of immature and undifferentiated cells.
3. Reduction in the number of microvilli.
4. Variation in the secretory granules of goblet cells and the appearance of atypical secretory cells.
5. Nuclear changes.

Chronic active ulcerative colitis

Mucosa from ulcerative colitis presented a great spectrum of changes. The number of electron-dense bodies and vesicles in the mature and immature absorptive cells had increased (Fig. 1). These vesicles were delimited by membrane. A few lysosomes were also present. There was an increase in the number of immature absorptive cells (Fig. 2), undifferentiated cells with clear cytoplasm (Fig. 3) and intermediate cells.

Immature absorptive cells showed an increased number of mitochondria and polyribosomes. Some mitochondria showed myelin figures in their matrix. Presence of centrioles in the apical region of the cytoplasm was a prominent and consistent feature (Fig. 2). Some cell organelles were mixed with mucin droplets. There were many desmosomes.

The intermediate cells resembled absorptive cells in being columnar in shape with some microvilli, yet revealed secretory droplets of varying size similar to those of mucin droplets of goblet cells in the apical cytoplasm (Fig. 4).

Microvilli were irregularly spaced and pleomorphic.

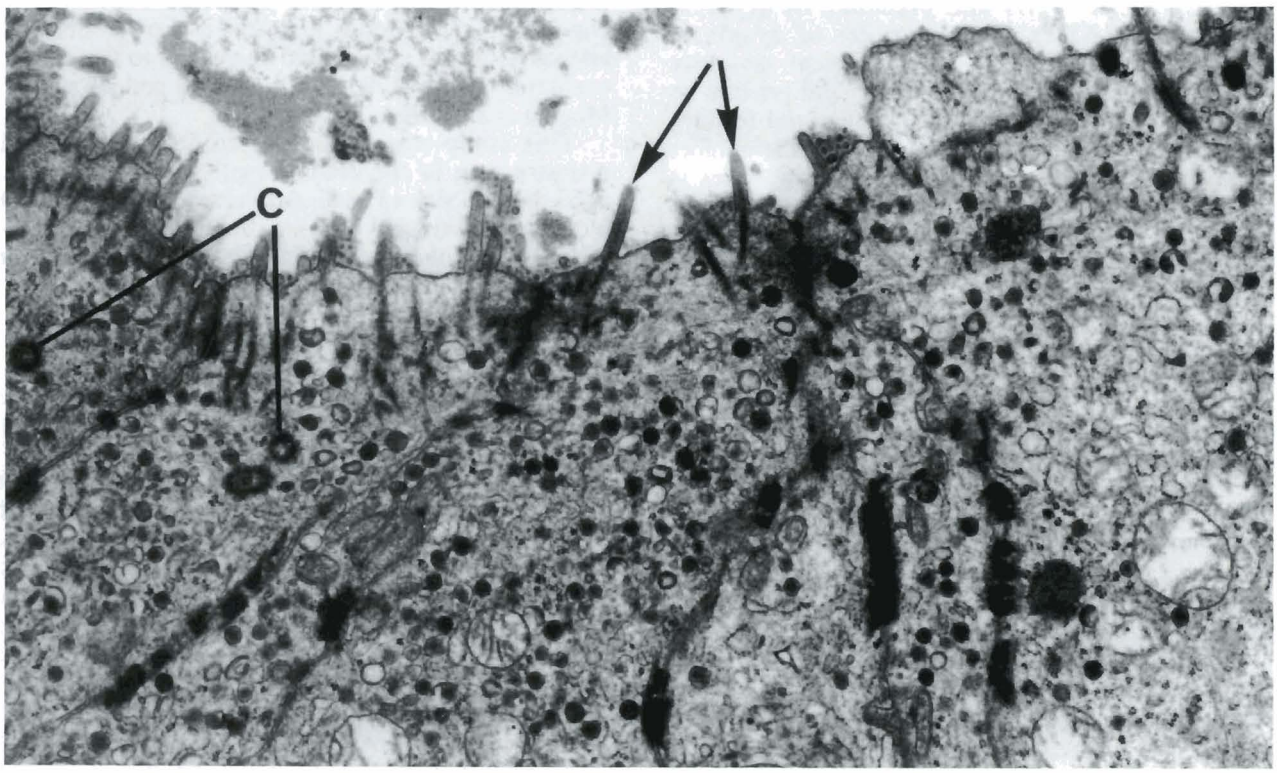

Fig. 1. Epithelium from ulcerative colitis patient shows moderate number of vesicles, EDB and the presence of centrioles (C) in the absorptive type of cells. Microvilli are irregular, some with deep roots (arrows). Glycocalyceal bodies are present and a fuzzy coat is prominent. x 16,000

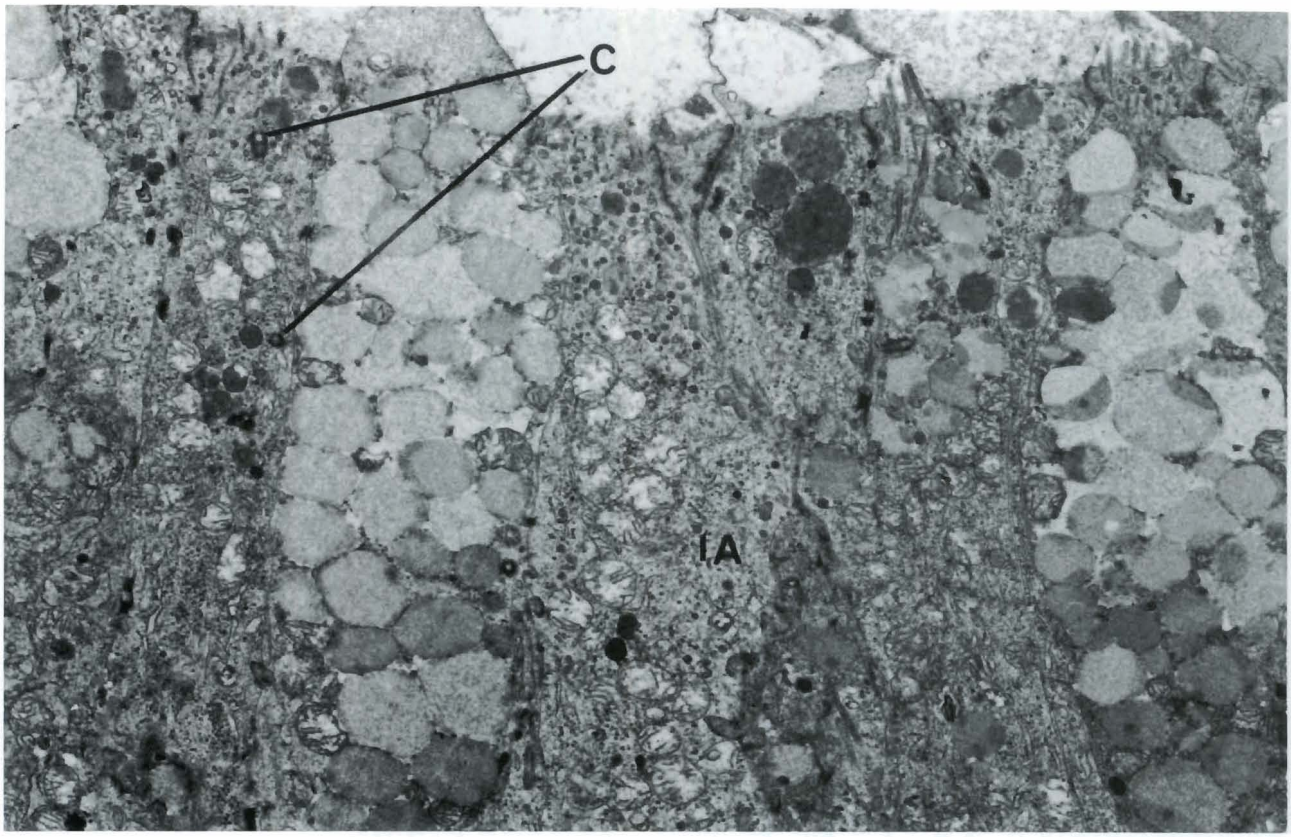

Fig. 2. Epithelium reveals an increase in immature absorptive-type cells (1A) and the presence of centrioles (C). Goblet cells show variable density of mucin droplets. There is reduction in the number of microvilli (A case of ulcerative colitis). x 7,200

They varied in shape and size, some being short and sparse or sometimes were absent. Others were tall with deeply penetrating rootlets (Fig. 5) and some were arranged in tufts. Glycocalyceal bodies and a fuzzy coat were prominent.

There was an increase in the number of goblet cells (Fig. 6). They often emptied their contents, including cytoplasmic organelles, into the lumen in an apocrine manner. Goblet cells showed increased heterogeneity of mucin droplets which were pleomorphic (Fig. 7), and atypical secretory cells were also present. The number of these cells was considerably increased in one case.

Nuclei were irregular in shape and at different heights (Fig. 8) with one to two nucleoli and loosely-arranged nucleolemma.

Ulcerative colitis with coexisting carcinoma

The ultrastructural features were similar to those seen in some cases of chronic active ulcerative colitis, having an increased number of electron-dense bodies and vesicles in the apical portion of the cytoplasm, pleomorphic microvilli, prominence or a fuzzy coat, goblet cells with increased heterogeneity of their mucin droplets and atypical secretory cells (Fig. 9). Nuclei

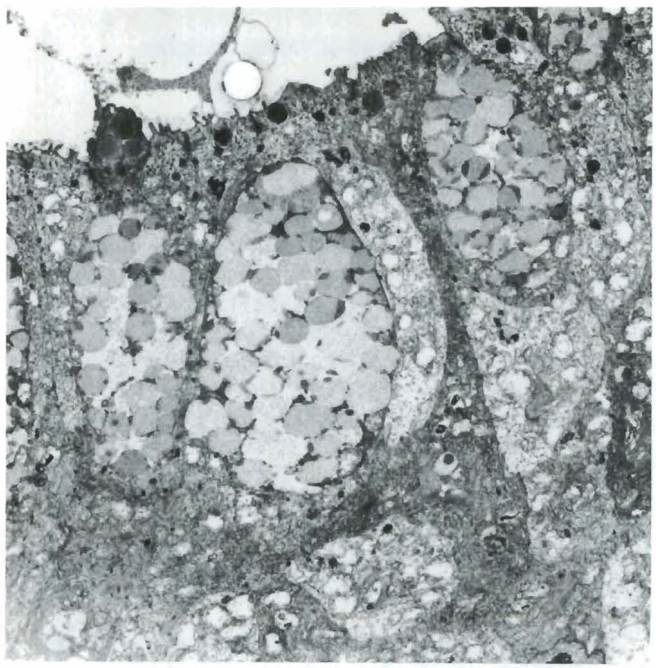

Fig. 3. Epithelium shows undifferentiated cells with clear cytoplasm. Goblet cells show slight heterogeneity of mucin droplets (A case of ulcerative colitis). x 3,100

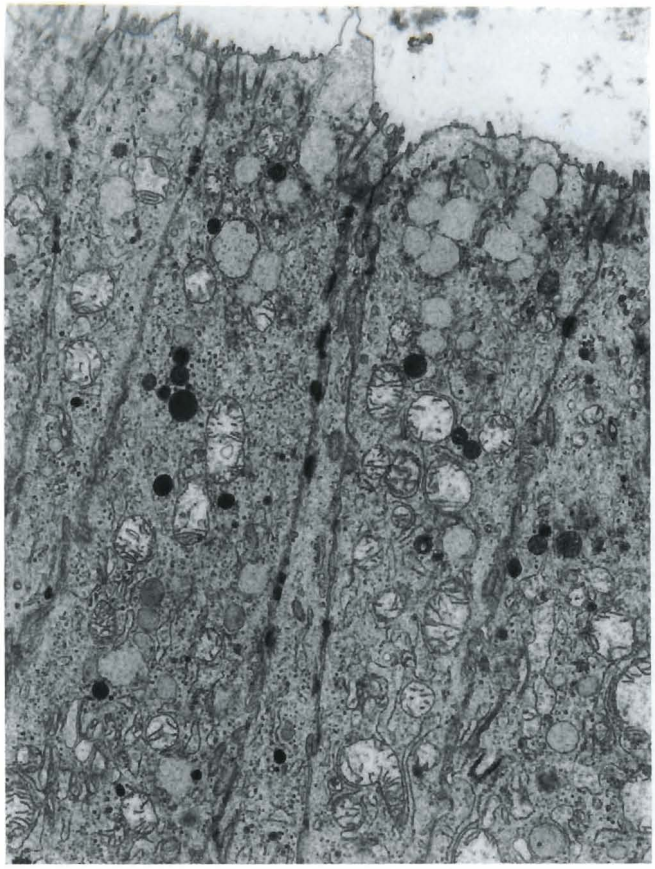

Fig. 4. Undifferentiated cells with mucin-like secretory granules are present in the apical cytoplasm. (A case of ulcerative colitis). x 6,500

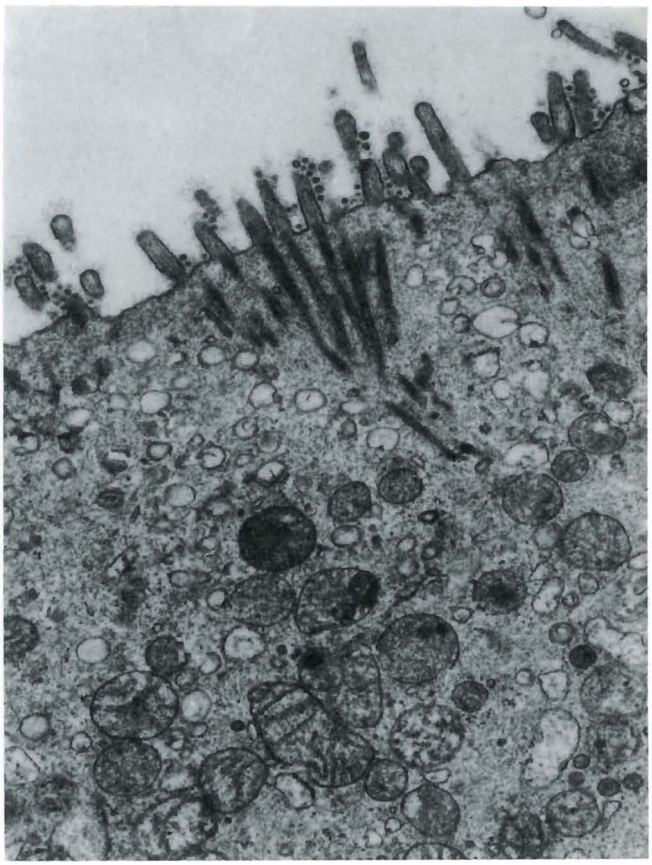

Fig. 5. Epithelium shows microvilli with very deep rootlets and glycocalyceal bodies. (A case of ulcerative colitis). x 20,000

were irregular in shape and at various heights.

Crohn's disease

Sections from various segments of colonic mucosa showed remarkably similar changes in all the three cases studied. The number of vesicles and electron-dense bodies in the supranuclear cytoplasm of the mature and immature absorptive cells varied from very few to moderate. However, in one case there was a considerable increase in the number of electron-dense bodies. There was an increase in the immature absorptive cells with many polyribosomes and mitochondria. The mitochondria varied in shape and size, being spherical, elongated with flattened ends or curved. Some showed myelin figures. Few lysosomes, multivesicular bodies and many desmosomes were present. Presence of centrioles was a consistent feature.

Few undifferentiated cells with light cytoplasm, large pale nuclei and with prominent nucleoli were present (Fig. 10).

Goblet cells were mostly homogeneous with only a few showing atypical secretory cells (ASC). Some immature goblet cells were also observed.

Nucleoli showed loosely-arranged nucleolonema in most of the cases.

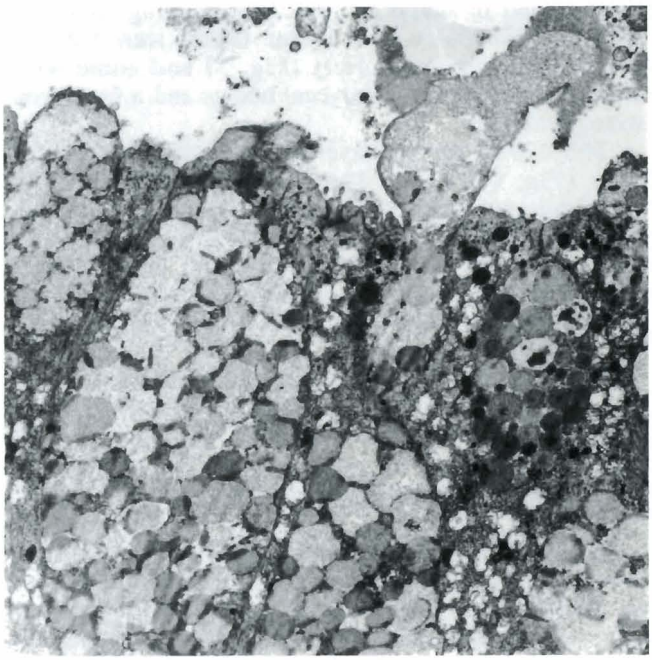

Fig. 6. Epithelium with increased number of goblet cells. Note the heterogeneity of mucin droplets and loss of microvilli. (Ulcerative colitis). x 4,000

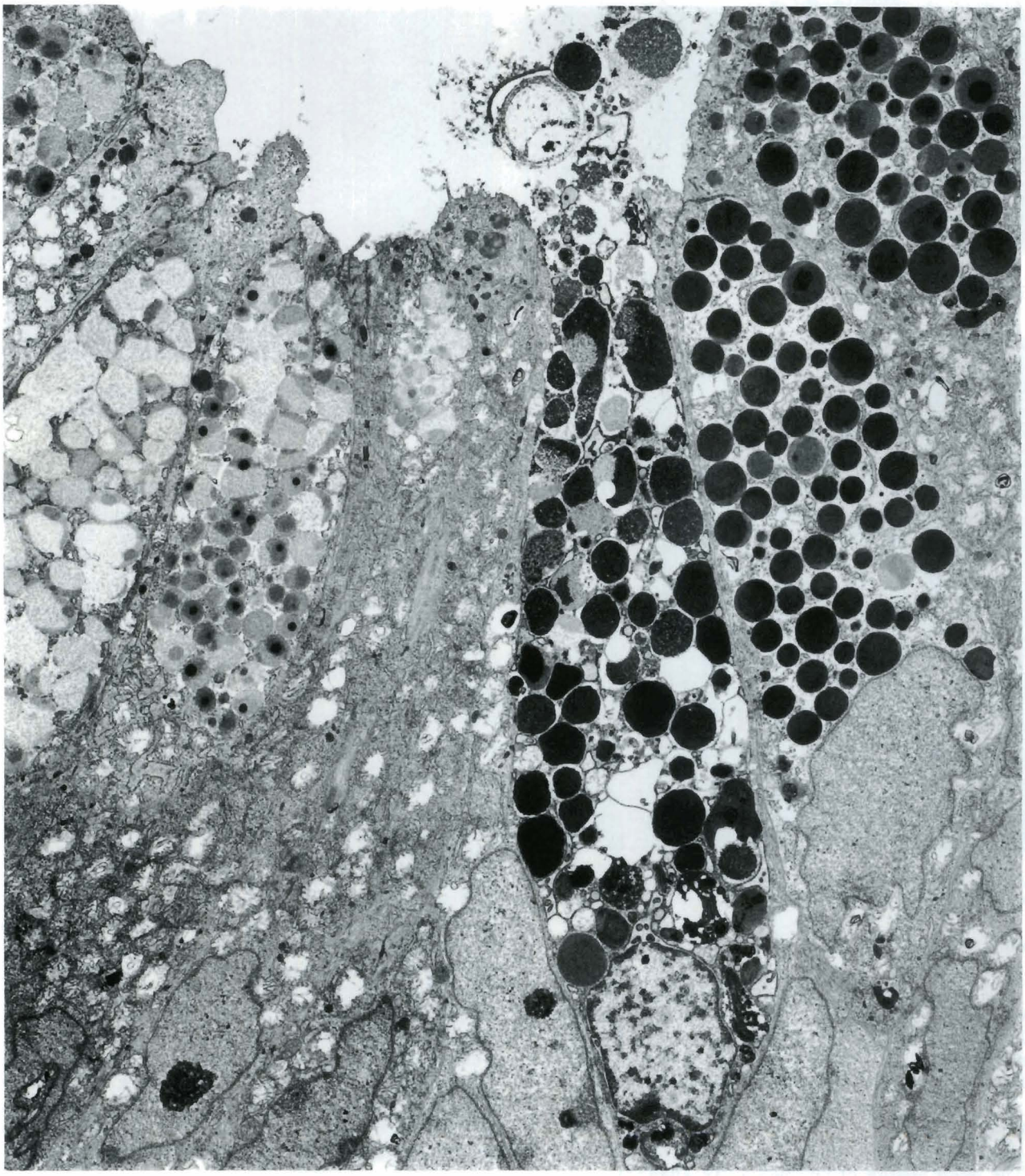

Fig. 7. Goblet cells with pleomorphic mucin droplets and atypical goblet cells. (Ulcerative colitis). x 5,650

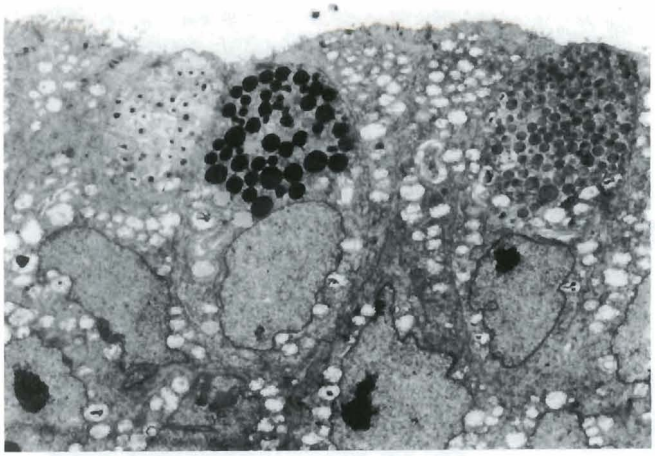

Fig. 8. Nuclei are irregular in shape and at different heights. Epithelium from ulcerative colitis patient. x 3,700

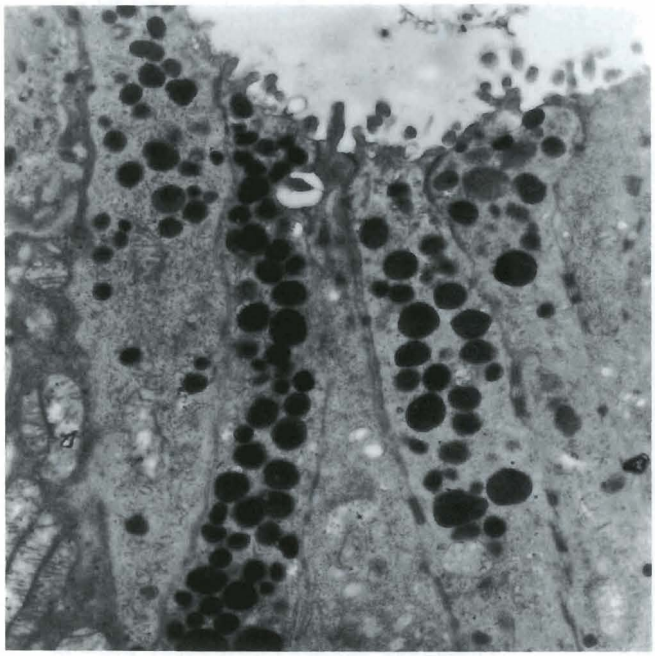

Fig. 9. Epithelium from ulcerative colitis with coexistent carcinoma shows atypical secretory cells. x 10,500

Normal controls

Very few electron-dense bodies were present in the apical region of mature absorptive cells. Immature and undifferentiated cells were absent. A fuzzy coat was slightly present. Goblet cells were mostly homogeneous or showed only slight heterogeneity of mucin droplets.

Discussion

The main feature of chronic active ulcerative colitis and Crohn's disease was the increase in the number of immature absorptive cells with large number of

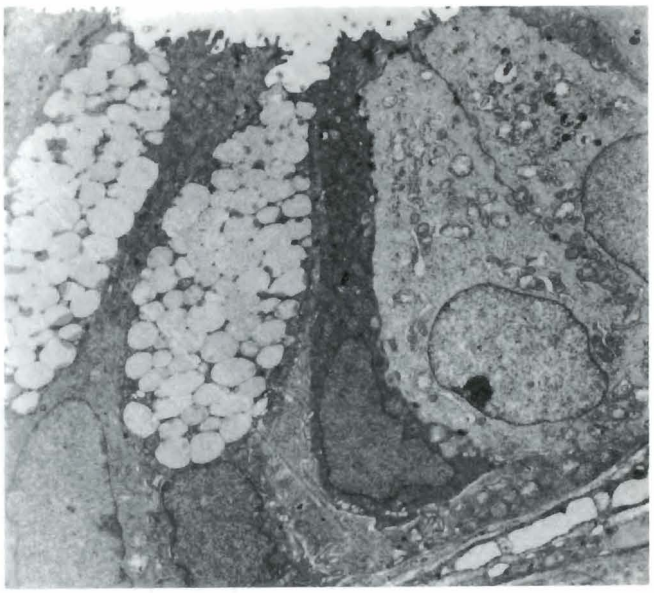

Fig. 10. Epithelium from Chron's disease shows undifferentiated cells with clear cytoplasm and large pale nuclei. Goblet cells are homogeneous. x 3,600

polyribosomes, mitochondria and centrioles. Greater number of undifferentiated cells and intermediate cells were also seen, as compared to normal. One case of Crohn's and all the cases of chronic active ulcerative colitis, including those with synchronous carcinoma, showed an increased number of vesicles and electron-dense bodies in the absorptive cells, heterogeneity of mucin droplets of goblet cells and presence of ASC. These features were similar to those described earlier in familial polyposis coli (Mughal and Filipe, 1978) and in non-familial adenomatous polyps (Mughal et al., 1981).

Increased numbers of immature and intermediate cells have been observed in hyperplastic polyps (Kaye et al., 1973; Mughal et al., 1981), transitional colonic epithelium bordering carcinoma (Dawson and Filipe, 1976) and adenomas (Dawson et al., 1977; Mughal and Filipe, 1978a,b; Mughal et al., 1981; Kaye et al., 1973). Intermediate cells show features of both an absorptive cell and a secretory cell and they are probably maturing ultimately into either a goblet or an absorptive cell. Their presence may be related to a growth and differentiation disorder and are found in various conditions, including ulcerative colitis and Crohn's disease as a non-specific response to injury. On the other hand, the demonstration of atypical secretory granules, including the small vesicles and EDB, in columnar absorptive type of cells appeared to be related to an increasing degree of cellular atypia in familial polyposis coli (FPC) (Mughal and Filipe, 1978). Since these atypical secretory droplets were more frequently observed in adenomas from high risk patients, either with FPC or with a synchronous carcinoma, it was suggested that these structures could be indicators of early malignant change.

In the present study, however, these structures, (ASC) albeit fewer and infrequent were observed in 2 cases of ulcerative colitis, one in the presence and the other in absence of carcinoma, and occasionally in Crohn's disease, a pattern comparable to grade 3-4 described in low-grade dysplasia in adenomas (Mughal and Filipe, 1978; Mughal et al., 1981). Higher grades of dysplasia in adenomas and carcinomas characterized by large numbers of atypical secretory cells as well as predominance of immature and undifferentiated cells were not seen in the present series.

Of interest are the ultrastructural findings reported by Mathan and Mathan (1985), in the rectal epithelium of healthy volunteers in Southern India. These authors observed increased numbers of EDB and vesicles in undifferentiated crypt cells in the rectal mucosa, shorter and irregular microvilli, evidence of cell immaturity and changes in the goblet cell mucus, similar to those described above in ulcerative colitis and in the mucosa adjacent to colonic carcinoma (Dawson and Filipe, 1976; Dawson et al., 1977). These observations suggest that increased EDB and vesicles in the upper cytoplasm of columnar cells, altered goblet cell secretion, increased number of intermediate cells and irregular nuclear polarity, may be a response to persistent injury. Atypical secretory cells were not reported in rectal epithelium from Indian subjects. The value and clinical significance of these features are not known. One can only speculate that a persistent cycle of injury, damage and repair may lead to impaired maturation and aberrant differentiation of the colonic crypt cells, expressed in a large population of immature and intermediate cells and atypical secretory cells. However, the number of cases investigated in each group was too small, and a large series including various control groups could help to clarify the significance of these observations.

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