Virchows Arch. A Path. Anat. and Histol. 387, 193–205 (1980)

Virchows Archiv A

Electron-Microscopic Study of the Villous Adenoma of the Colon

Márta Balázs

Department of Pathology, János Hospital of Budapest Council, H-1125 Budapest, Diósárok ut l, Hungary

Summary. The author studied the electron-microscopic peculiarities of villous adenoma as illustrated by 12 cases. She found that the dominant cells of villous adenoma are undifferentiated epithelial cells. Electron-microscopic signs of malignant transformation were revealed by nuclear changes, by the changes in the cytoplasmic membrane and by the immaturity of cells. Leuchtenberger's inclusion bodies are assumed to develop by herniation of cytoplasmic processes of immature tumour cells.

In one tumour associated with electrolyte loss, atypical cells were observed with an electronmicroscopic picture indicative of an intense secretory activity. However, the secretion drops differed from the normal mucus drops of the mature goblet cells of the colonic mucosa. The author believes it to be possible that the tumour cells of atypical morphological appearance secrete mucus of abnormal composition which could be responsible for the syndrome of electrolyte loss.

Key words: Villous adenoma – Absorptive cell – Goblet cell – Leuchtenberger's inclusion body.

Introduction

Several clinical and pathological reports have been published on the villous adenoma of the colon. It differs in importance from adenomatous and hyperplastic polyps in that the frequency of malignant transformation is estimated at between 30 and 74 per cent (Horn, 1971; Jahadi et al., 1975; Kurzon et al., 1974; Muto et al., 1975; Welch et al., 1976; Wiebecke et al., 1974). The tumour is, in some cases, associated with a syndrome of fluid-electrolyte loss. Light-microscopically, there is no detectable difference between tumours associated or not associated with electrolyte loss.

Few reports, containing contradictory data on the origin of epithelial cells, have been exclusively concerned with the electron-microscopic characteristics

0340-1227/80/0387/0193/$02.60

of the cells of villous adenoma (Imai et al., 1963; 1965; Ioachim et al., 1974). No electron-microscopic studies are available on the cells of tumours associated with the syndrome of electrolyte loss.

In the present paper, the electron-microscopic picture of 12 cases of villous adenoma have been studied. In four of them, signs of light-microscopically observable malignant transformation were apparent. In one case showing a malignant tendency, the tumour was associated with fluid- and electrolyte loss. We investigated the following questions:

1. Can the malignant potential of the tumour be inferred from the electron-microscopic picture of the epithelial cells?

2. Is there an ultrastructural difference between the cells of tumours associated with the electrolyte loss syndrome and those of tumours not associated with it?

Material and Methods

The study material was derived from 12 pure villous adenomas, from patients between 33 and 77 years of age. There were 8 males and 4 females and histories varied between one month to two years duration. The main symptoms were: mucous diarrhoea in 6 cases, periodic rectal bleeding in 4 cases, loss of weight and weakness in 2 cases. Definite fluid and electrolyte loss was observed in one case with a serum potassium level of 3.1 mEq per liter, which even on potassium administration did not return to normal. All tumours were in the rectum or sigmoid colon.

Our material was chosen on the basis of light microscopy using the classification of Kurzon et al. (1974). Only pure villous adenomas were taken for the present study. Adeno-villous polyps were excluded and their electron microscopic features are described in another report.

In all cases the study material was obtained by rectoscopy and colonoscopy from the peripheral area of the tumors. In 9 cases biopsies were followed by operation. In 4 out of the 12 cases signs of malignant transformation were seen in the biopsy material. Dysplasia of the epithelial cells and infiltration of the branching connective tissue core were regarded as criteria of malignancy (Jahadi et al., 1975, Wiebecke et al., 1974).The diameter of the tumors varied from 3 to 10 cm. In the 4 cases where signs of malignant transformation of the tissues obtained by biopsy were seen, we observed infiltration of the muscular layer of the intestine in different parts of the tumors.

For electron microscopic studies 5 pieces of each tumor, of 1 mm^3 size, were fixed in 1 percent Palade buffered osmium tetroxide, then dehydrated in graded alcohols and embedded in araldite. The sections were prepared by a Reichert ultramicrotome and photographed by JEM 100 B electron microscope. For orientation 0.5 μm semi-thin sections were prepared stained by toluidine blue.

For control studies 14 highly differentiated pure adenomatous polyps were examined. All these were pedunculated lesions having smooth surface, ranging in size from 5 mm up to 20 mm.

For normal controls 14 intact colonic mucosa, prepared as above, were studied.

Results

Epithelial Cells of Villous Adenomas (8 Cases Without Malignant Transformation)

The dominant elements of the tumour were undifferentiated epithelial cells. The nuclei were oval or slightly irregular in shape, the chromatin was evenly distributed. The cytoplasm was narrow and poor in organelles. There were few mitochondria and little rough endoplasmic reticulum. There was also a

relatively large number of free ribosomes (Fig. 1). The lateral cell membrane was strikingly serpiginous with several interdigitations and irregular cytoplasmic processes (Fig. 2). The intercellular space was greatly widened in several places.

In some places, more differentiated absorptive columnar cells and occasionally, some goblet cells could be found. On the surface of the absorptive cells, there were few irregularly shaped microvilli (Fig. 3). The goblet cells contained mucin drops of varying density and atypical dense bodies.

Epithelial Cells of Villous Adenomas with Malignant Transformation (3 Cases)

The cells were less differentiated than those of the former group and the cytoplasmic organelles showed evidence of injury. On the cell surface, oedematous microvilli were found in several places. In the hyaloplasm there were occasional colliquative areas. Apically, there were several vesicula, vacuoles, and a substance resembling mucus (Fig. 4). The nuclei were strikingly large, in many places lobular and contained large nucleoli (Fig. 5). At the base of the cells cytoplasmic processes could be observed penetrating the basement membrane (Fig. 6).

It is to be noted that in these 3 cases differentiation of neither the absorptive nor of goblet cells could be seen, every region of the tumours was composed of undifferentiated epithelial cells.

Leuchtenberger's Inclusion Bodies

In each case, partly intracellularly and partly intercellularly, near the base of the cells, inclusion bodies of varying density surrounded by membrane could be found. These contained traces of cell organelles and an amorphous mass of great density (Fig. 7). It could be noted in several places that cytoplasmic processes on the lateral surface of the cells showed herniation and a gradual degeneration of organelles occurred (Fig. 8). Several transitory forms of degeneration and transformation of organelles into a dense mass were observed.

The frequency of inclusions showed a correlation with the degree of dedifferentiation of epithelial cells. They appeared in the greatest number in villous adenomas with a malignant potential.

Villous Adenoma Associated with Electrolyte Loss (1 Case)

The nuclei were irregular in shape and varied in size. The nuclear membrane was serpiginous. In the cytoplasm of the cells, there were extended Golgi zones and occasional vacuoles apparently empty or containing a small amount of granular substance. The vacuoles resembled the mucin drops of goblet cells with membrane only occasionally appearing around them (Fig. 9). Drops of secretion could be found in each cell, however, with no apparent fusion of

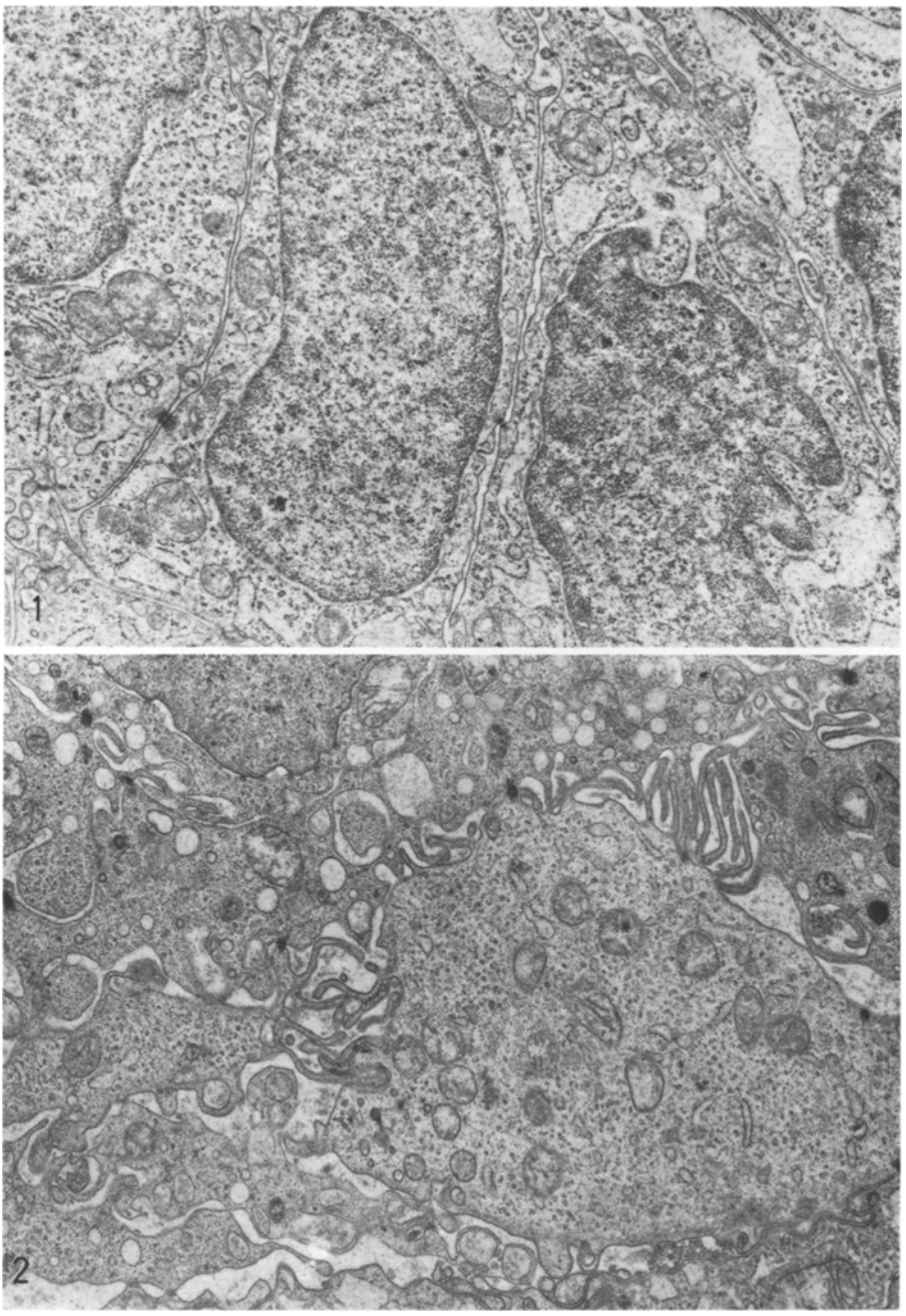

Fig. 1. The electron-microscopic picture shows the undifferentiated cells of villous adenoma. The nuclei are oval, the nuclear chromatin is evenly distributed. The cytoplasm is narrow and poor in organelles (× 15,000)

Fig. 2. The Figure shows details of several tumours. The several interdigitations of the lateral cell membrane and development of irregular processes are striking (× 15,000)

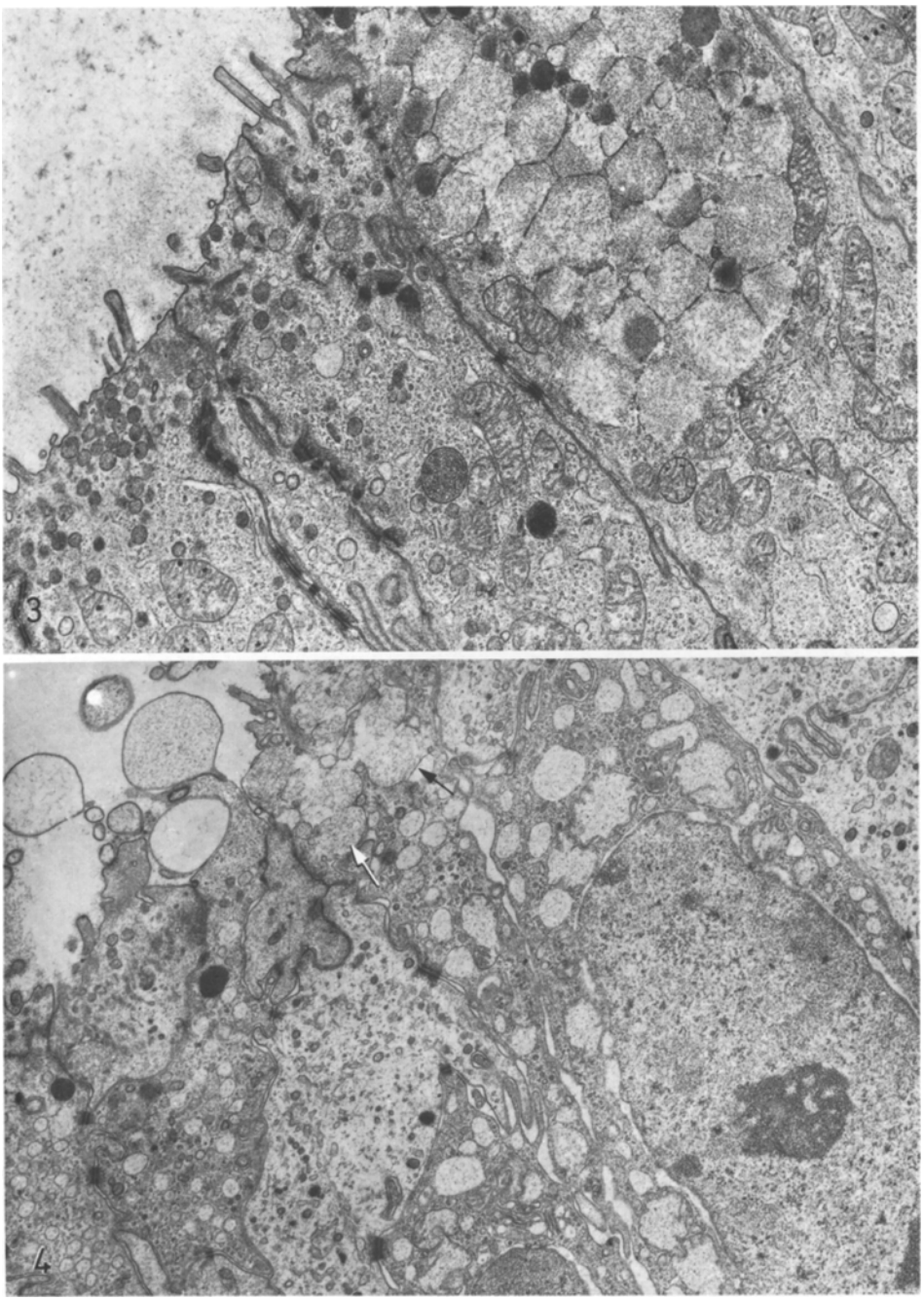

Fig. 3. Surface of 3 absorptive cells and a goblet cell. The absorptive cells have a few undeveloped microvilli on their surface. In the apical region of the cytoplasm there are numerous vacuoles. The cell-connecting structures are definite. In the goblet cell, beside mucin granules, there are also round dense bodies (× 12,500)

Fig. 4. Electron-microscopic picture of villous adenoma with malignant transformation. On the surface of the cells irregular occasionally oedematous microvilli can be seen. In the cytoplasm of the cells there are severely injured organelles, apically, there are vesicles and dense bodies. The region marked by an arrow contains a substance resembling mucin (× 15,000)

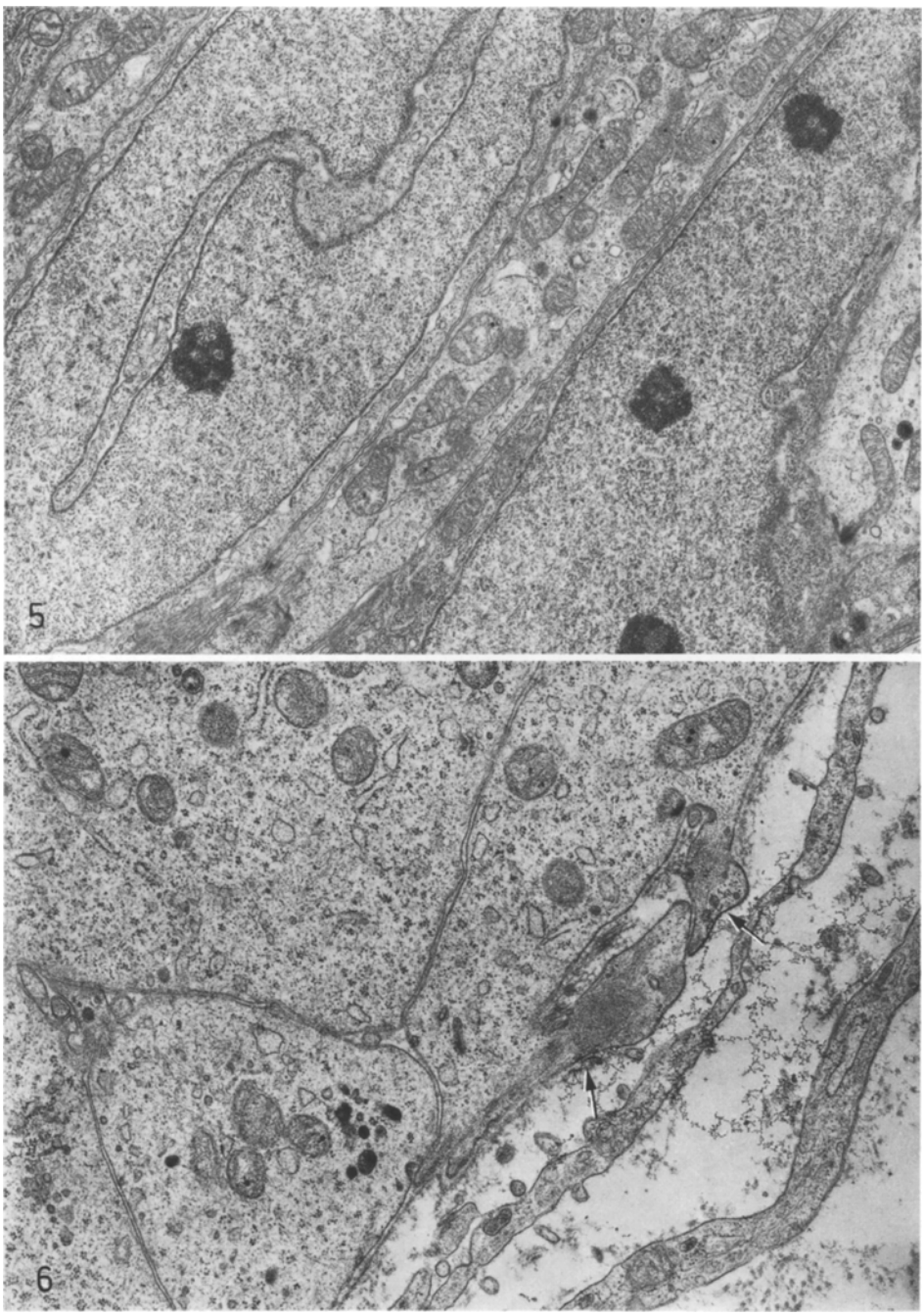

Fig. 5. Picture of another region of the former tumour. It shows large nuclei with striking nucleoli. The nucleus on the left is lobular (× 15,000)

Fig. 6. Villous adenoma with malignant transformation. At the base of the tumour cells, in the region marked by an arrow, there are cytoplasmic processes penetrating the basement membrane (× 22,500)

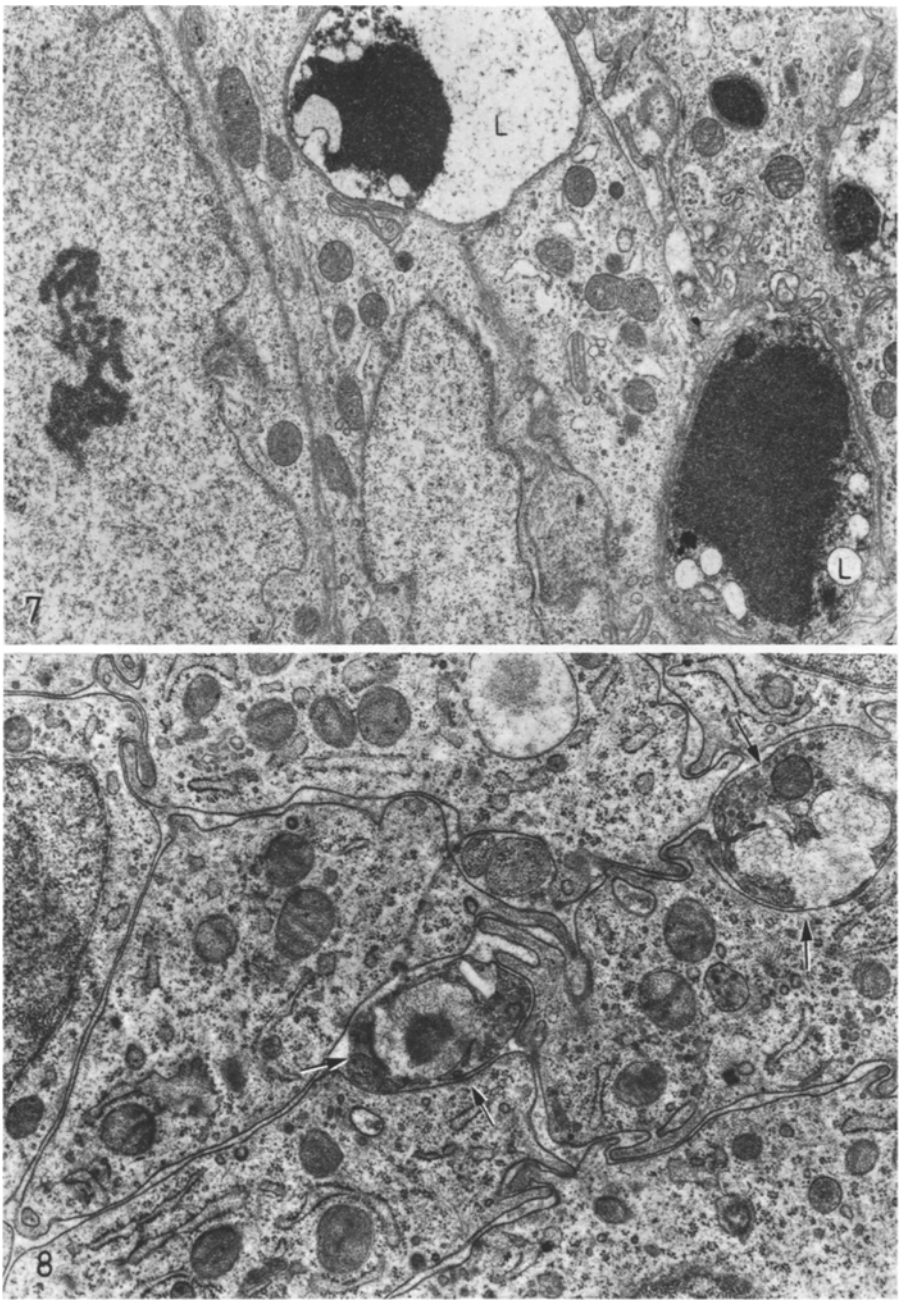

Fig. 7. Inclusion bodies (*L*) surrounded by membrane near the base (×8,200)

Fig. 8. In the regions marked by arrows, intercellularly herniated cytoplasmic portions are seen (×20,000)

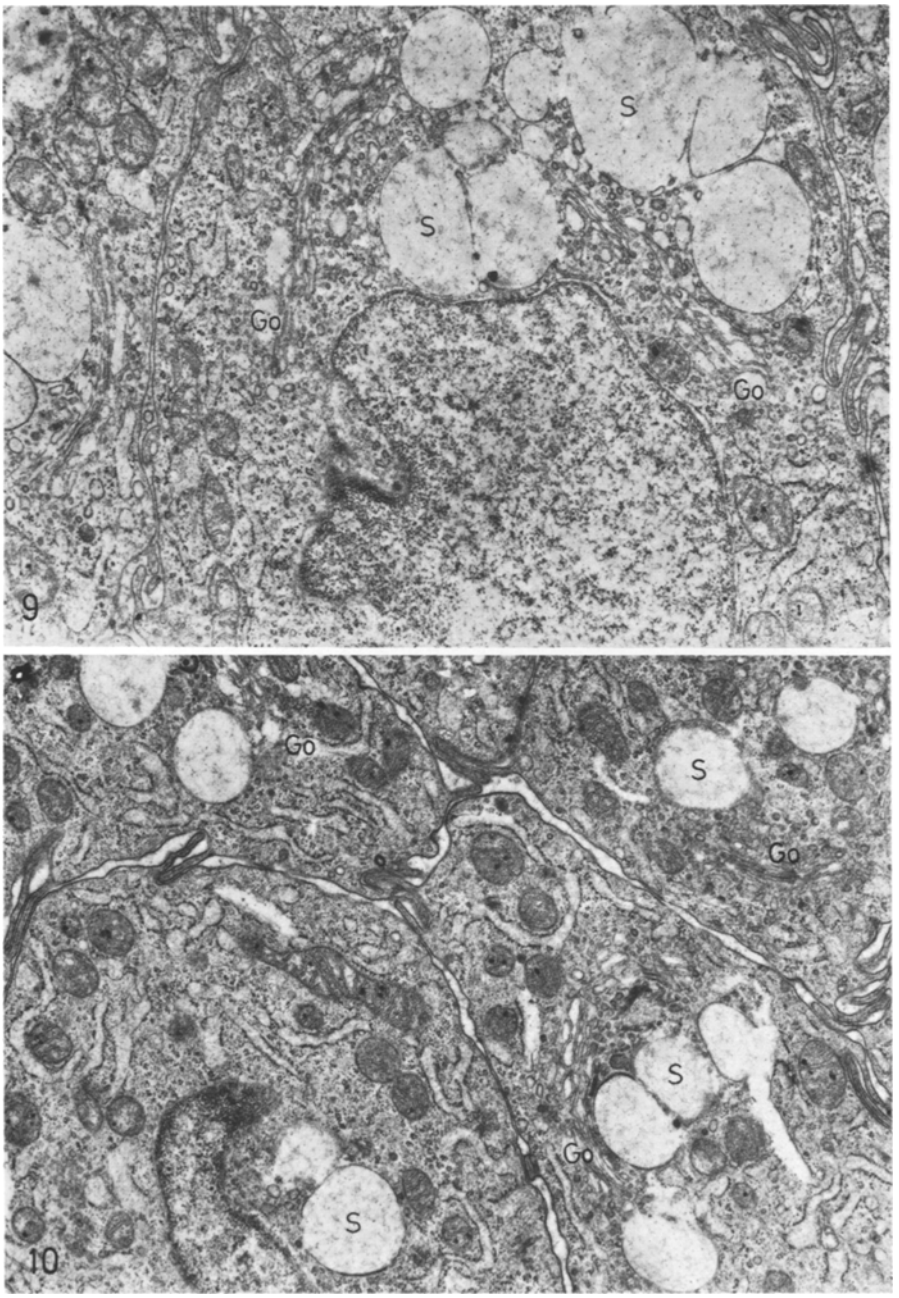

Fig. 9. Electron-microscopic picture of villous adenoma associated with electrolyte loss. The nucleus is irregular in shape. In the cytoplasm there are mitochondria, a small number of rough endoplasmic reticula, several free ribosome granules, an extensive Golgi zone (*Go*), and secretion droplets ($\times 15{,}000$)

Fig. 10. Another region of the former tumour. In each of the tangentially sectioned cells there are sporadic granules (*S*) and Golgi zones (*Go*) ($\times 15{,}000$)

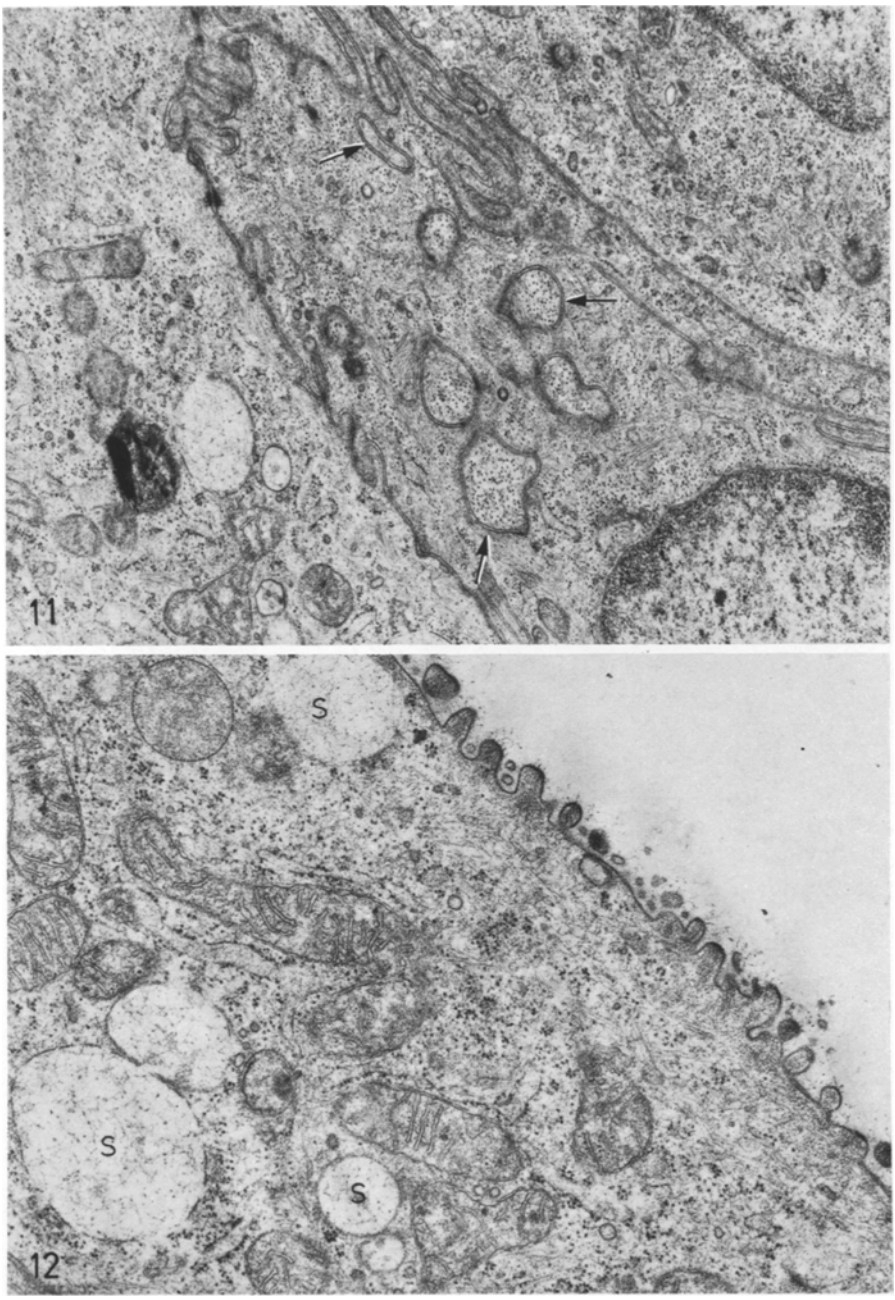

Fig. 11. In the central tumour cell there are several fine filaments. The lateral cell membrane is strongly serpiginous penetrating the cytoplasm of the cell in the region marked by an arrow (× 15,000)

Fig. 12. Detail of the tumour cell shows sporadic secretion granules (*S*) and along the surface several filaments. On the surface there are rudimentary microvilli (× 25,000)

vacuoles (Fig. 10). In the cytoplasm of some cells, there were several fine filaments. The lateral cell membrane was strongly serpiginous, sometimes penetrating the cytoplasm of the cells (Fig. 11). On the surface of the cells rudimentary microvilli were seen. Under the surface, the network of irregularly arranged filaments could be observed (Fig. 12).

Discussion

The electron-microscopic picture of the intact mucosa of the colon is well known (Donnellan, 1965; Lorenzson et al., 1968; Nagle et al., 1967; Pittman et al., 1966; Toner, 1971). Its dominant cells are the main or absorptive cells responsible for absorbing water and electrolytes, and goblet cells secreting sulphated mucoproteins (Forstner, 1978; Phillips, 1969; Rapp et al., 1979; Schrager et al., 1973). It is known from the studies of Lorenzson et al. (1968), that epithelial cells migrate in 5 or 6 days from the basal portions of the crypt to the surface, and meanwhile undergo maturation. At the base of the Lieberkühn's crypts undifferentiated cells can be found and are considered to be the precursors of both the absorptive and goblet cells (Kaye et al., 1973; Nagle et al., 1967).

In agreement with previous data in the literature (Imai et al., 1965; Ioachim et al., 1974), our examination reveals that the dominant cells of villous adenomas resemble the undifferentiated epithelial cells situated normally at the base of the crypts. In our benign cases the differentiation of a small number of cells into absorptive and goblet cells could be seen. In tumours with a malignant potential, signs of differentiation were missing. The cells differed in various essentials from the electron-microscopic appearance of mature of overdifferentiated cells of adenomatous, juvenile or hyperplastic polyps (Hayashi et al., 1974; Imai et al., 1963; Weller, 1966; Wiebecke et al., 1974).

Rudimentary development of microvilli on the surface of the cells and a great number of cytoplasmic processes on the lateral surface, were observed in each case. These cannot be regarded as specific changes because they have also been observed in adenomatous polyps and adenocarcinomas of the colon (Birbeck et al., 1963; Imai et al., 1963; Kaye et al., 1973; Spjut et al., 1967). In the malignant cases, at the basal surface of the cells there were cytoplasmic processes penetrating the basement membrane. This phenomenon is considered to be a sign of microinvasion (Ioachim et al., 1974). A further characteristic of malignant tumours was the lobulation of cell nuclei. A similar phenomenon has been described by Elias et al. (1978), in adenocarcinoma of the colon, who considered it a sign of amitosis in rapidly growing tumours. Electron-microscopic signs of malignant transformation in our study largely agreed with the data of Mughal et al. (1978) on the malignant transformation of familial polyposis.

In benign and malignant tumours of the colon, the dense inclusion bodies first described by Leuchtenberger (1954) can often be found. Subsequent examination showed that they occur not only in tumours but in inflammatory diseases of the colon, for example, diverticulitis, appendicitis and ulcerative colitis (Monis et al., 1961; Otto et al., 1975). Leuchtenberger (1954) believed the inclusions

to be of viral origin, an observation not confirmed by subsequent electron-microscopic examination (Fisher et al., 1962; Ioachim et al., 1974). The origin and importance of the inclusions is still unknown. Our examination disclosed that inclusions derive from the lateral cytoplasmic processes of the epithelial cells by herniation. In the herniated cytoplasmic portions a gradual degeneration of cell organelles was observed, appearing to be similar to the cytoplasmic degradation reported by Swift et al. (1964). It was found that the number of Leuchtenberger's bodies was largest in villous adenomas with a malignant potential. In our opinion this is due to the fact that the less differentiated the tumour cells, the more processes they have. Their cytoplasmic membrane is thus more likely to be injured.

In the case with electrolyte loss the tumour cells resembled immature or atypical goblet cells. The stages of the normal maturation of goblet cells are known from the study of Freeman (1966). During their differentiation mucus drops emerge from the vesicles and vacuoles of the Golgi apparatus. There is a simultaneous accumulation of rough endoplasmic reticulum probably forming the protein component of mucus (Forstner, 1978). In mature goblet cells the mucus drops almost fill the central and apical portions of the cytoplasm. Their content is granular. Mucus drops fuse and enter the gut lumen by an apocrine type of secretion. In our case the tumour cells contained extensive Golgi zones and secretory vacuoles, having hardly any optically detectable granular substance. The usual fusion of mucus drops was not observed. The unusually large microfilament-content of tumour cells was also striking, this was probably also due to abnormal mucus secretion (Forstner, 1978).

The cells of the tumour associated with electrolyte loss closely resembled the cells of the transitional mucous membrane surrounding the malignant tumours of the colon, mentioned in the literature of recent years (Dawson et al., 1976; Filipe et al., 1974; Riddell et al., 1977; Saffos et al., 1977). Rapp et al. (1979) demonstrated human intestinal goblet cell antigen in premalignant (transitional) and malignant gastrointestinal cells. Histochemical examination showed that in transitional mucosa adjacent to large bowel carcinoma, sialomucin accumulates and the amount of sulphomucin decreases or is missing altogether (Filipe et al., 1974; Forstner, 1978). Recently, electron-microscopic examinations proved that pathological mucus secretion is due to pathological epithelial cell differentiation (Riddell et al., 1977). Based on electron-microscopic similarity, it is believed to be possible that in the tumour associated with the syndrome of electrolyte loss, epithelial cells produce secretion of a pathological composition. Further studies are needed to prove our hypothesis.

References

Birbeck, M.S.C., Dukes, C.E.: Electron microscopy of rectal neoplasm. Proc. Roy. Soc. Med. **56**, 793–797 (1963)

Dawson, P.A., Filipe, M.I.: An ultrastructural and histochemical study of the mucous membrane adjacent to and remote from carcinoma of the colon. Cancer **37**, 2388–2398 (1976)

Donnellan, W.L.: The structure of the colonic mucosa. Gastroenterology **48**, 496–514 (1965)

Elias, H., Fong, B.B.: Nuclear fragmentation in colon carcinoma cells. Hum. Pathol. **9**, 679–684 (1978)

Filipe, M.I., Branfoot, A.C.: Abnormal patterns of mucous secretion in apparently normal mucosa of large intestine with carcinoma. Cancer **34**, 282–299 (1974)
Fisher, E.R., Sharke, D.A.: The ultrasturcture of colonic polyps and cancer with special reference to the epithelial inclusion bodies of Leuchtenberger. Cancer **15**, 160–170 (1962)
Forstner, J.F.: Intestinal mucins in health and disease. Digestion **17**, 234–263 (1978)
Freeman, J.A.: Goblet cell fine structure. Anat. Rec. **154**, 121–148 (1966)
Hayashi, T., Yatani, R., Apostol, J., Stemmermann, G.N.: Pathogenesis of hyperplastic polyps of the colon: a hypothesis based on ultrastructure and in vitro cell kinetics. Gastroenterology **66**, 347–356 (1974)
Horn, R.C.: Malignant potential of polypoid lesions of the colon and rectum. Cancer **28**, 146–152 (1971)
Imai, H., Stein, A.A.: Ultrastructure of adenocarcinoma of the colon. Gastroenterology **44**, 410–418 (1963)
Imai, H., Stein, A.A.: Ultrastructure of adenomatous polyps and villous adenomas of large intestine. Lab. Invest. **12**, 868–875 (1963)
Imai, H., Saito, S., Stein, A.A.: Ultrastructure of adenomatous polyps and villous adenoma of the large intestine. Gastroenterology **48**, 188–197 (1965)
Ioachim, N.J., Delaney, W.E., Madrazo, A.: Villous adenoma of the colon and rectum: an ultrastructural study. Cancer **34**, 586–596 (1974)
Jahadi, M.R., Baldwin, A.: Villous Adenomas of the Colon and Rectum. Am. J. Surg. **130**, 729–732 (1975)
Kaye, G.I., Fenoglio, C.M., Pascal, R.R., Lane, N.: Comparative electron microscopic features of normal, hyperplastic and adenomatous human colonic epithelium. Gastroenterology **64**, 926–945 (1973)
Kurzon, R.M., Ortega, R., Rywlin, A.M.: The significance of papillary features in polyps of the large intestine. Am. J. Clin. Pathol. **62**, 447–454 (1974)
Leuchtenberger, C.: Cytoplasmic "inclusion bodies" containing desoxyribose nucleic acid (DNA) in cells of human rectal polyps. Lab. Invest. **3**, 132–142 (1954)
Lorenzsonn, V., Trier, J.S.: The fine structure of human rectal mucosa. The epithelial lining of the base of the crypt. Gastroenterology **55**, 88–101 (1968)
Monis, B., Mendeloff, A.I.: Observation on inclusion bodies in normal and pathologic mucosa of the colon in man. J. Natl. Canc. Inst. **26**, 1429–1444 (1961)
Mughal, S., Filipe, M.E.: Ultrastructural study of the normal mucosa-adenoma-cancer sequence in the development of familial polyposis coli. J. Natl. Canc. Inst. **60**, 752–768 (1978)
Muto, T., Bussey, H.J.R., Morson, B.C.: The evolution of cancer of the colon and rectum. Cancer **36**, 2251–2270 (1975)
Nagle, G.J., Kurtz, S.M.: Electron microscopy of the human rectal mucosa. Am. J. Dig. Dis. **12**, 541–567 (1967)
Otto, H.F., Gebbers, J.O.: Epitheldysplasien bei Colitis ulcerosa. Langenbecks Arch. Chir. **341**, 99–110 (1976)
Otto, H.F., Gebbers, J.O., Müller-Wieland, K.M.: Untersuchungen zur Ultrastructurpathologie der Colitis Ulcerosa. Virchows Arch. A Path. Anat. and Histol. **367**, 113–130 (1975)
Phillips, S.F.: Absorption and secretion by the colon. Gastroenterology **56**, 966–971 (1969)
Pittman, F.E., Pittman, J.C.: An electron microscopic study of the epithelium of normal human sygmoid colonic mucosa. GUT **7**, 644–661 (1966)
Rapp, W., Windisch M., Peschke P., Wurster, K.: Purification of human intestinal goblet cell antigen (GOA), its immunohistological demonstration in the intestine and in mucus producing gastrointestinal adenocarcinomas. Virchows Arch. A Path. Anat. Histol. **382**, 163–177 (1979)
Riddell, R.H., Levin, B.: Ultrastructure of the "transitional" mucosa adjacent to large bowel carcinoma. Cancer **40**, 2509–2522 (1977)
Saffos, R.O., Rhatigan, R.M.: Benign (nonpolypoid) mucosal changes adjacent to carcinomas of the colon. Human Pathology **8**, 442–449 (1977)
Schrager, J., Oates, M.B.G.: A comparative study of the major glycoprotein isolated from normal and neoplastic mucosa. GUT **14**, 324–329 (1973)
Spjut, H.J., Smith, M.N.: A comparative electron microscope study of human and rat polyps and carcinomas. Exp. Molec. Pathol. **6**, 11–24 (1967)

Swift, H., Hruban, Z.: Focal degradation as a biological process. Fed. Proc. **23**, 1026–1036 (1964)
Toner, P.G., Carr, K.E., Wyburn, G.M.: The digestive system. An ultrastructural atlas and review. London: Butterworths 1971
Toner, P.G.: Cytology of intestinal epithelial cells. Int. Rev. Cytol. **24**, 233–343 (1968)
Toth, B., Malick, L., Shimizu, H.: Production of intestinal and other tumors by 1,2 – Dimethylhydrazine dihydrochloride in mice. Light and transmission electron microscopic study of colonic neoplasms. Am. J. Pathol. **84**, 69–86 (1976)
Welch, J.P., Welch, C.E.: Villous adenomas of the colorectum. Am. J. Surg. **131**, 185–191 (1976)
Weller, R.O., McColl, I.: Electron microscope appearances of juvenile and Peutz Jeghers polyps. GUT **7**, 265–270 (1966)
Wiebecke, B., Brandts, A., Eder, M.: Epithelial proliferation and morphogenesis of hyperplastic and villous polyps of the human colon. Virchows Arch. A Path. Anat. Histol. **364**, 35–49 (1974)

Accepted February 26, 1980