Mucus-secreting Colonic Carcinoid Tumors:

Light- and Electron-microscopic Study of Three Cases

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THE EXISTENCE of mixed carcinoid and mucus-secreting intestinal tumors is well documented in medical literature.[2, 6, 9–12, 15, 17] The coexistence of areas of carcinoid tumor and mucus-secreting adenocarcinoma within malignant gastrointestinal neoplasms has been regarded by most investigators as representing a variety of adenocarcinoma with proliferation of argentaffin cells rather than a composite type of neoplasm. From our departmental files we have selected three carcinoid tumors that showed mucus secretion and had the typical histologic features of carcinoid tumors. Two of the tumors (rectal carcinoids) were argentaffin-positive. The other, an appendiceal tumor, was argentaffin-negative; however, at the ultrastructural level, argentaffin granules could be demonstrated in the cytoplasm of the tumor cells.

Report of Three Cases

Patient 1: A 71-year-old Caucasian woman entered the hospital on January 24, 1970, with the chief complaint of rectal bleeding of two weeks' duration. Physical examination was unremarkable except for the presence of a palpable, smooth, spherical mass just inside the anus. Proctoscopic examination showed a spherical mass, covered by smooth intact mucosa, on the anterior wall of the rectum, just above the dentate margin. The tumor, which was resected through the anus, consisted of a rubbery brown-yellow submucosal mass, measuring 2.2 × 2.0 × 1.6 cm (Fig. 1). Histologically, it was made up of nests and cords of uniform cells with a moderate amount of eosinophilic cytoplasm and monotonous round hyperchromatic nuclei with no evidence of mitotic activity. For the most part the tumor cells were arranged in a trabecular fashion (Fig. 2), but in some areas there was a rosette-like pattern around centrally placed lumina. Mucus-secreting glands and small lakes of mucin between solid nests of tumor cells were demonstrated occasionally (Fig. 3). The tumor infiltrated the submucosa deeply and was covered by an intact mucosa. Fontana–Masson stain showed argentaffin granules in most of the cells of the tumor. A diagnosis of rectal carcinoid was made. To date, the patient remains free of symptoms.

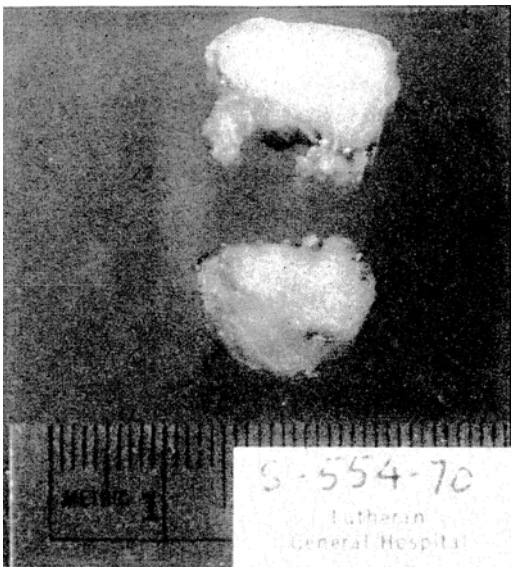

FIG. 1 (Patient 1). Carcinoid tumor with gray-yellowish cut surface *(above)*. It manifested as a well-circumscribed unencapsulated submucosal nodular mass, 2 cm in diameter, covered by an intact rectal mucosa *(below)* (×1.5).

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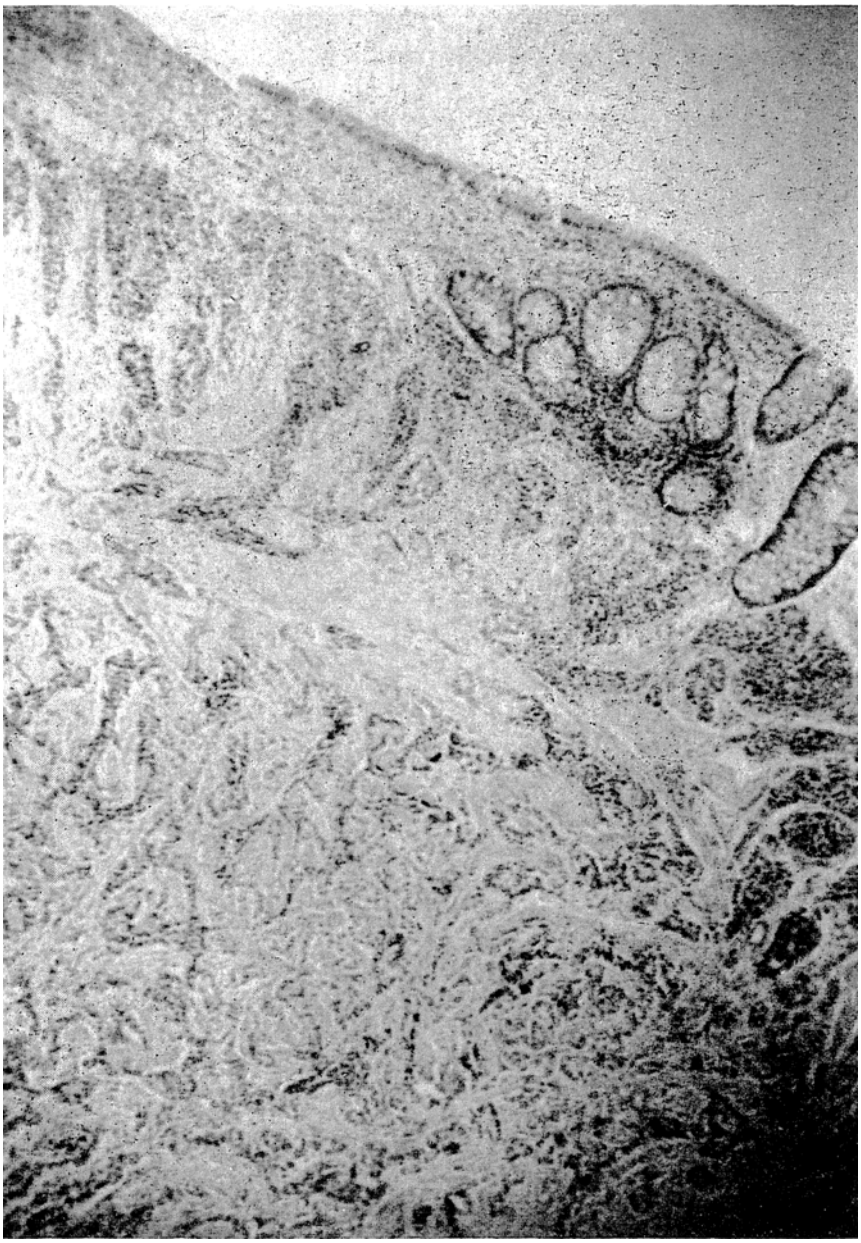

FIG. 2. The tumor infiltrated the submucosa and muscularis, showing the trabecular pattern typical of carcinoid tumors (hematoxylin and eosin, ×35).

Patient 2: An 80-year-old Caucasian man entered the hospital on August 5, 1970, complaining of severe abdominal pain and vomiting. Past history disclosed an appendectomy elsewhere five years prior to the present admission. About three weeks prior to this admission, he had begun having cramping lower abdominal pain, distention, and occasional vomiting. Physical examination revealed stable vital signs. Examination of the head, neck and thorax was negative. The abdomen was distended, tympanitic with hyperactive sounds. There was generalized tenderness but no rigidity. The rectal examination was negative. X-ray studies were consistent with a mechanical obstruction of the

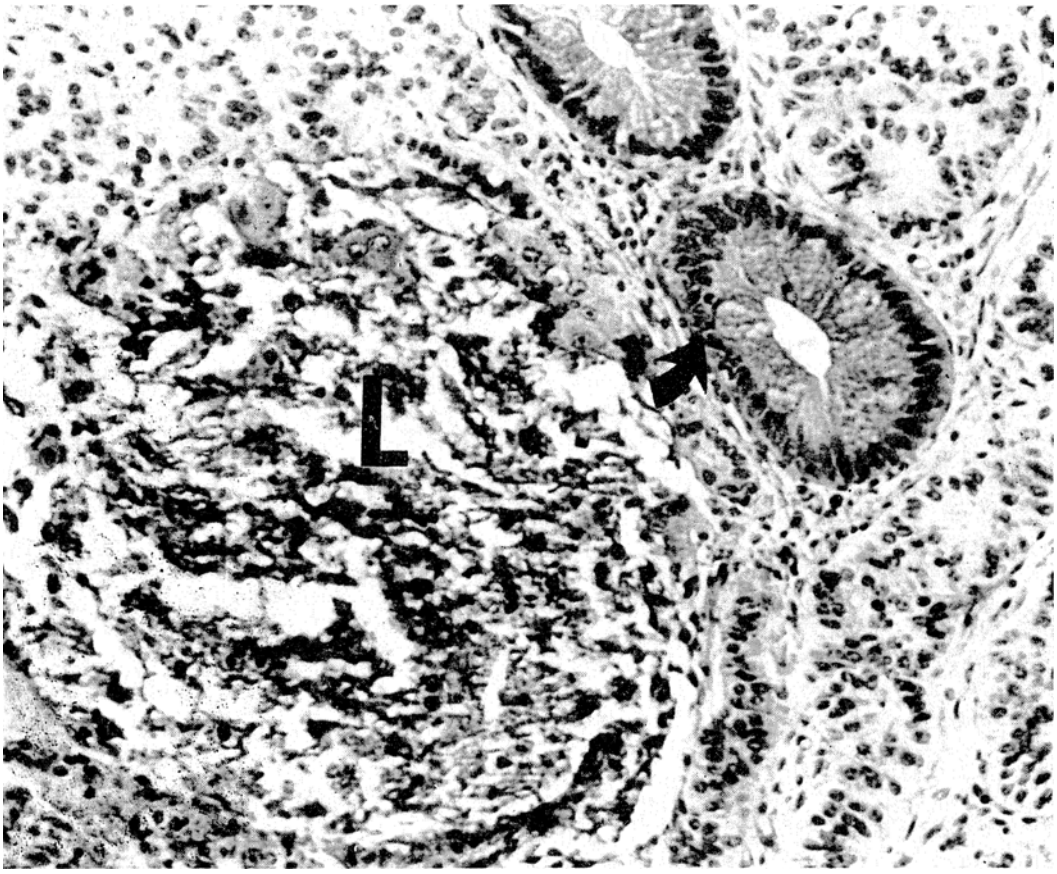

FIG. 3. In some areas the carcinoid tumor contained acini with goblet cells *(arrow)* and lakes of mucus *(L)* (hematoxylin and eosin, ×200).

distal small intestine. After conservative treatment for five days failed to improve the patient's condition, exploratory laparotomy was performed. Dense adhesions were found in the right lower quadrant, and the small intestine appeared distended down to the ileocecal valve. The cecum was small and firm; when it was freed from the adhesions, a firm mass involving its wall as well as the terminal ileum was found. A right hemicolectomy with end-to-end ileocolostomy was performed. The postoperative course was uneventful with the exception of scanty focal drainage from the seventh to the twelfth postoperative days. The patient was discharged on the fifteenth postoperative day.

The patient died two years later, in another hospital. Autopsy disclosed peritoneal seeding by a mucus-secreting adenocarcinoma with carcinoid tumor features. The surgical specimen consisted of a right hemicolectomy specimen having attached a segment of terminal ileum 11 cm long. Examination disclosed diffuse thickening with gray discoloration and flattening of the mucosal folds. It involved the area of the appendectomy stump and extended along the medial wall of the cecum, involving the ileocecal wall, which was constricted. Sections from this area showed a tumor which infiltrated the intestinal wall from the serosa to the submucosa. The mucosa was intact throughout the specimen. It was made up of cords and nests of uniform cells with a moderate amount of amphophilic cytoplasm and hyperchromatic nuclei with occasional prominent nucleoli. The tumor had a solid pattern, with a trabecular arrangement and closely compacted nests of uniform cells. In many areas signet-ring cells were present. In other areas the tumor was made up of small glandular structures. Mucicarmine stains revealed an intense positive reaction. The argentaffin stain was negative. Because of the peculiar infiltration of the intestinal wall, more consistent with a metastatic spread than with a primary tumor, slides from the appendectomy were obtained. They demonstrated an infiltrating mucus-secreting adenocarcinoma of the base of the appendix, with secondary acute suppurative

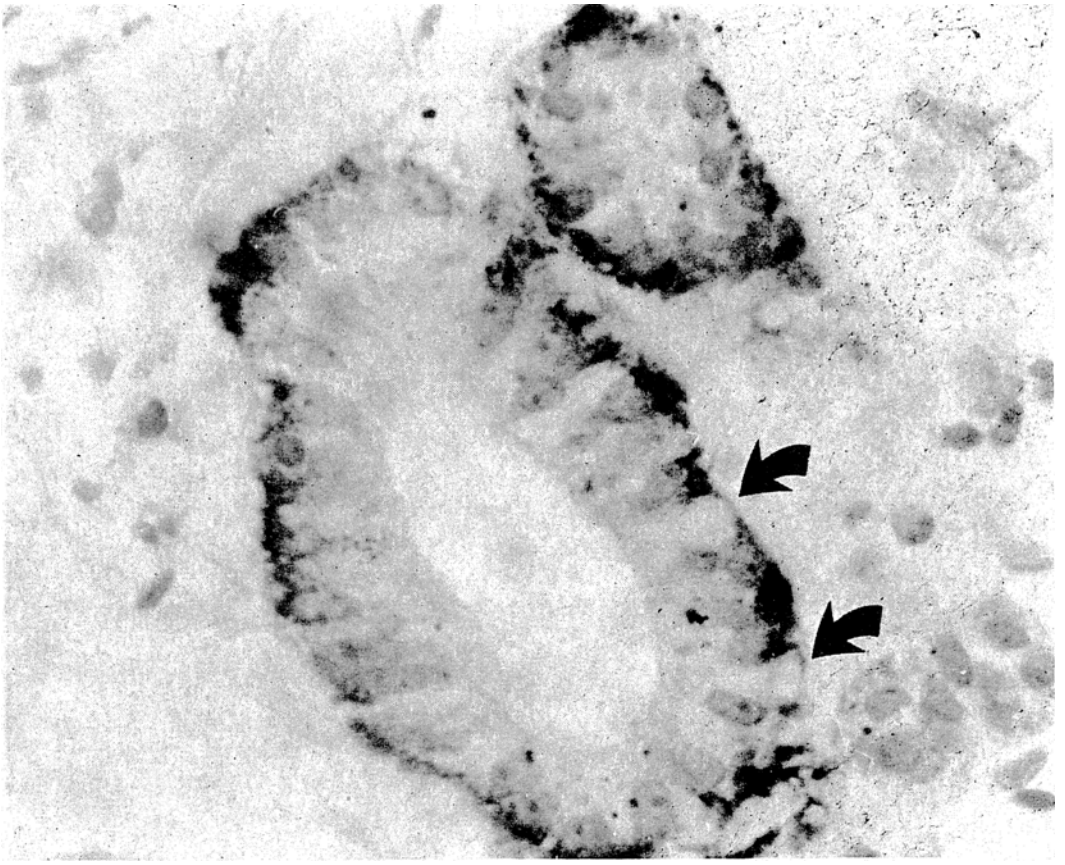

FIG. 4. Epithelial cells of acini selected for electron microscopic study contained argentaffin granules (basilar portion of cells) and apical and subnuclear intracytoplasmic vacuoles with mucicarmine and PAS-positive material *(arrows)* (Fontana–Masson, ×430).

appendicitis. The tumor showed a carcinoid pattern in some areas.

Patient 3: A 60-year-old Caucasian woman was found to have a red, sessile tumor in the rectal wall about 13 cm from the anus. Biopsy was performed through the anus. The submitted specimen, which appeared to the surgeon who performed the operation to include the entire lesion, consisted of an ovoid fragment of gray-white soft tissue, 0.8 × 0.6 × 0.4 cm. Microscopically it was covered by rectal mucosa with a rather dense inflammatory cell infiltrate composed chiefly of mononuclear cells but with occasional polymorphonuclear leukocytes. The underlying wall was infiltrated by a tumor forming nests of uniform tumor cells and small glandular structures lined by columnar epithelium with small uniform hyperchromatic nuclei. Fontana–Masson stain revealed argentaffin granules in the epithelial-cell linings of most of the glandular structures. Mucicarmine stain was positive. As of the date of preparation of this study, six months after operation, the patient remains asymptomatic and in good general condition.

Electron Microscopy

Tissue specimens from Patient 2 and Patient 3 had already been fixed for several hours in formaldehyde solution (10 per cent formalin). Blocks of tumor tissue from Patient 1 were placed directly in 3 per cent cacodylate-buffered glutaraldehyde at pH 7.2 for two hours. The formaldehyde-fixed tissue was washed for 24 hours in running tap water, then placed in the glutaraldehyde solution. After fixation it was washed in cacodylate (sucrose buffer) for an entire day. On the following day it was placed in Palade's fixative (1 per cent buffered osmium tetroxide) for an hour. The specimens were washed in cacodylated buffer at pH 7.2 and dehydrated in a

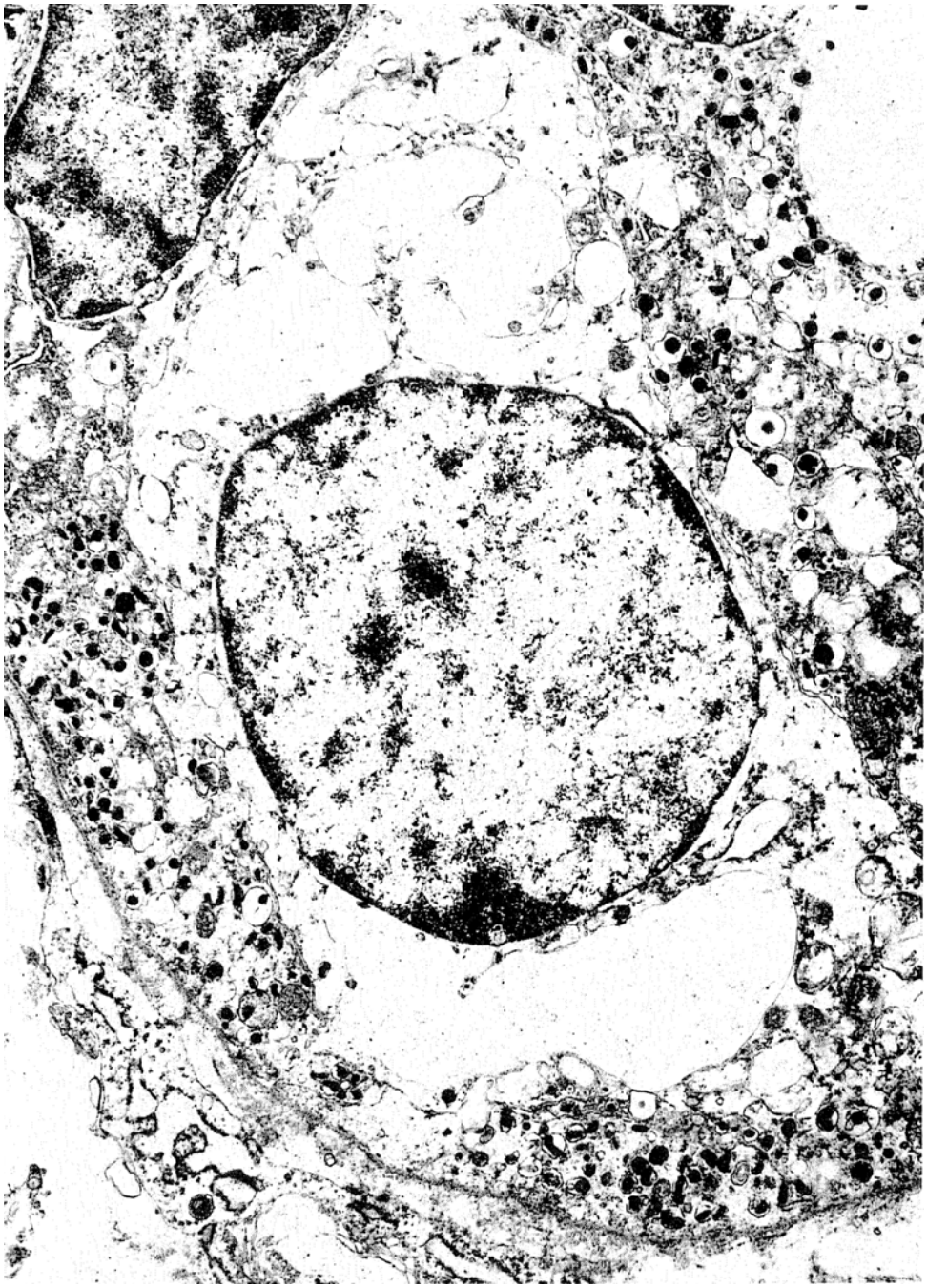

FIG. 5. At the ultrastructural level, the epithelial cells lining the acini of Figure 4 contained empty subnuclear and apical vacuoles compressing the argentaffin granules toward the periphery of the cytoplasm (uranyl nitrate–Reynold's lead citrate stain, ×12,000).

series of alcohols from 30 to 100 per cent, transferred to propylene oxide and infiltrated with a 1:1 mixture of propylene oxide and resin (Araldite) for 30 minutes, then kept in an oven at 60 C for 48 hours supported in gelatin capsules. After trim-

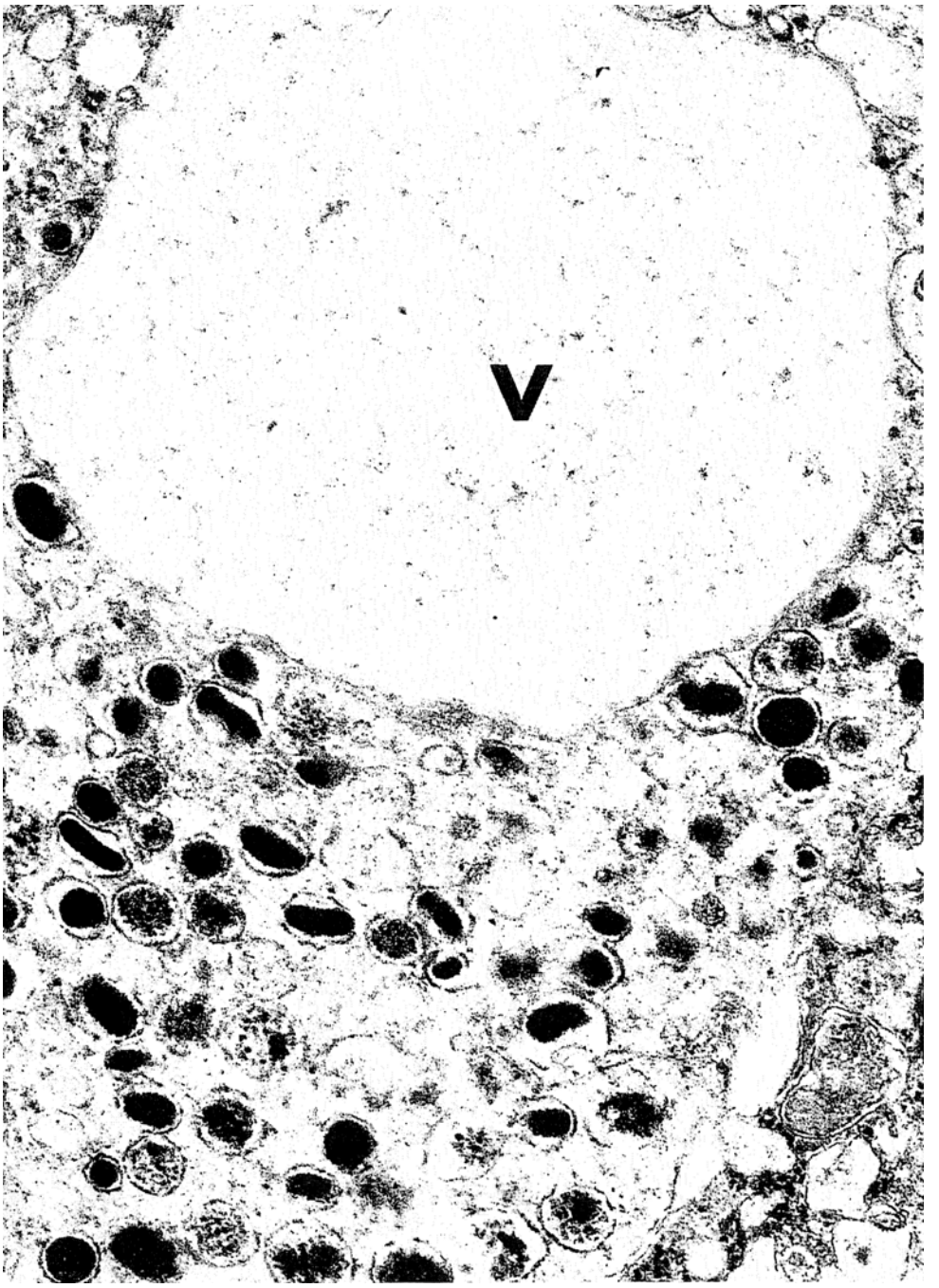

FIG. 6. Apical cytoplasmic vacuole *(V)* which contained mucin-positive material. Light microscopy (uranyl nitrate–Reynold's lead citrate stain, ×35,000).

ming, the tissue sections were correlated by the adjacent thick–thin method; sections 1–2μ thick were made with a microtome with a diamond knife. They were stained with a modified PAS–Fontana–Masson stain and Mayer's mucicarmine Fontana–Masson

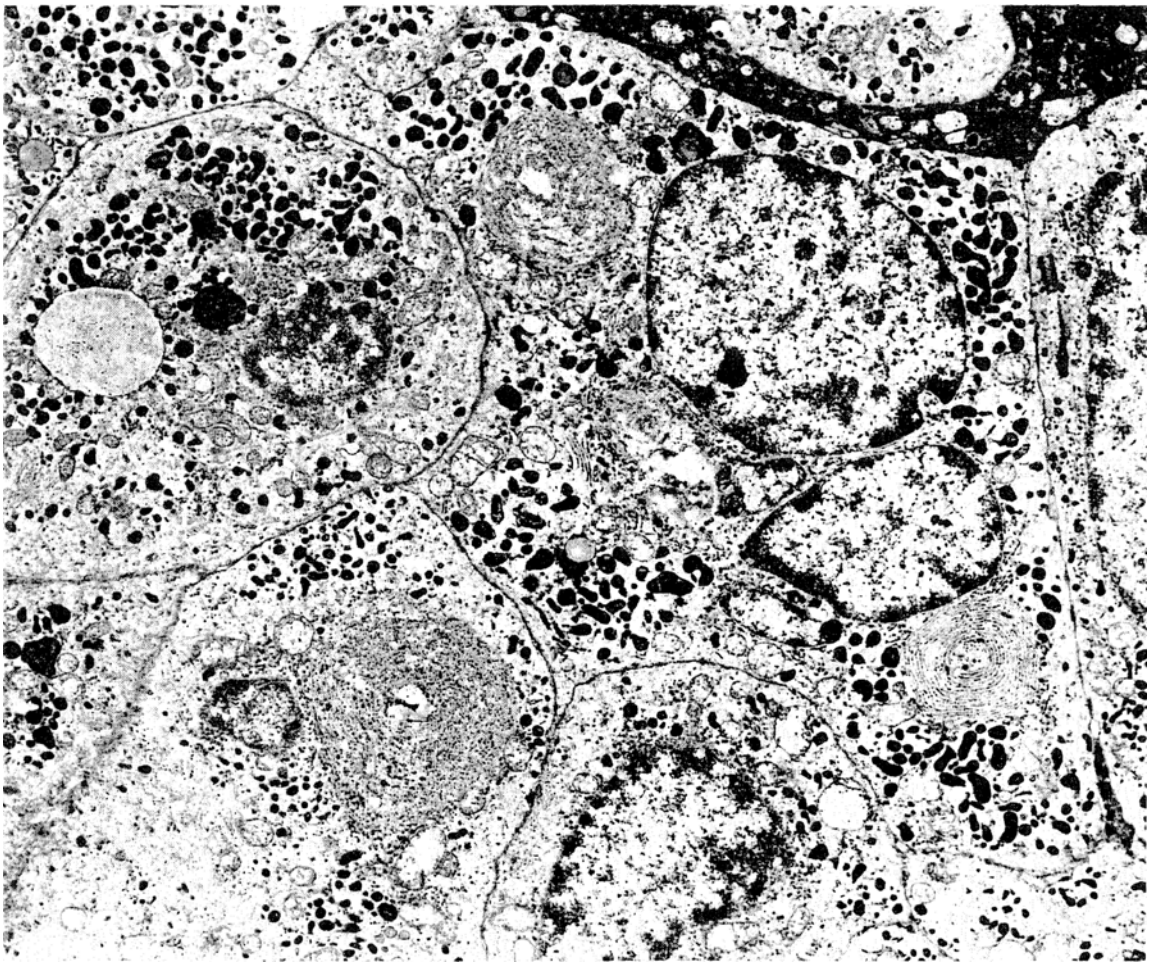

FIG. 7. Group of tumor cells with no evidence of intracytoplasmic vacuolization. Note the difference in size and morphology of the argentaffin granules and the conspicuous concentric lamellar bodies frequently encountered in carcinoid tumors (uranyl nitrate-Reynold's lead citrate stain, ×6,000).

stain, as well as Alcian blue stain. Blocks of sections with acini showing cells containing both argentaffin granules and PAS-positive droplets were selected for electron microscopic examination (Fig. 4). Thin sections (60–70 μ) from those blocks of tissue were mounted in uncoated copper grids and stained with 2 per cent uranyl nitrate and 50 per cent alcohol and Reynolds' lead citrate sodium hydroxide solution for 30 and 10 minutes, respectively. Sections were examined with an electron microscope. The specimens from Patients 1 and 3 were rather similar. The PAS-positive droplets disclosed by light microscopy corresponded at the ultrastructural level to numerous apical and perinuclear vacuoles that compressed the enterochromaffin granules toward the periphery of the cytoplasm (Fig. 5). In some of the cells the vacuoles and argentaffin granules were intimately related to the Golgi apparatus. The argentaffin granules were mostly found in subnuclear locations and, except for the coexistence with secretion vacuoles in the cytoplasm of the tumor cells, no definite morphologic relationship between these two cell products could be established (Fig. 6). Nests of tumor cells

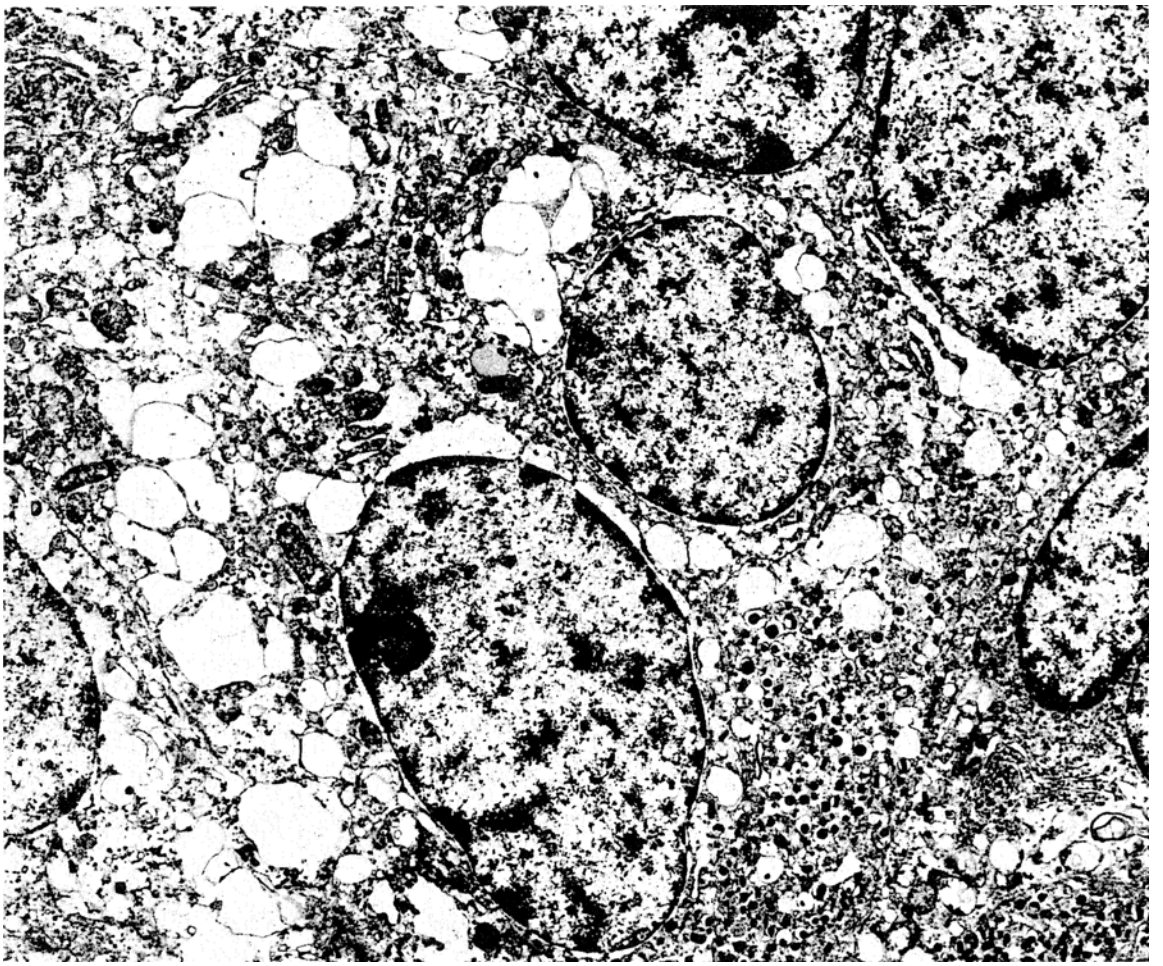

FIG. 8. Tumor cells containing vacuoles and argentaffin granules in the cytoplasm. Light microscopy disclosed that these cells contained mucin droplets (uranyl nitrate–Reynold's lead citrate stain, ×6,000).

with numerous argentaffin granules and no evidence of intracytoplasmic vacuoles were encountered in some areas (Fig. 7). The specimen from Patient 2 contained nests of carcinoid cells with a smaller number of argentaffin granules in the cytoplasm. They contained clusters of small vacuoles in one pole of the cells, usually opposite the aggregates of enterochromaffin granules (Fig. 8). The scattered intracytoplasmic mucin droplets shown by light microscopy to be associated with the argentaffin granules corresponded at the ultrastructural level to large vacuoles limited by a single membrane (Fig. 9).

Discussion

Argentaffin cells have been identified in polyps and adenocarcinomas of the intestines.[1, 3, 7, 13–15] Because the lesions have lacked the classic histologic pattern of carcinoid tumors, this phenomenon had been regarded as proliferation of this specialized type of cell in gastrointestinal neoplasms concomitant with growth of the neoplastic tissue, similar to the proliferation of Paneth

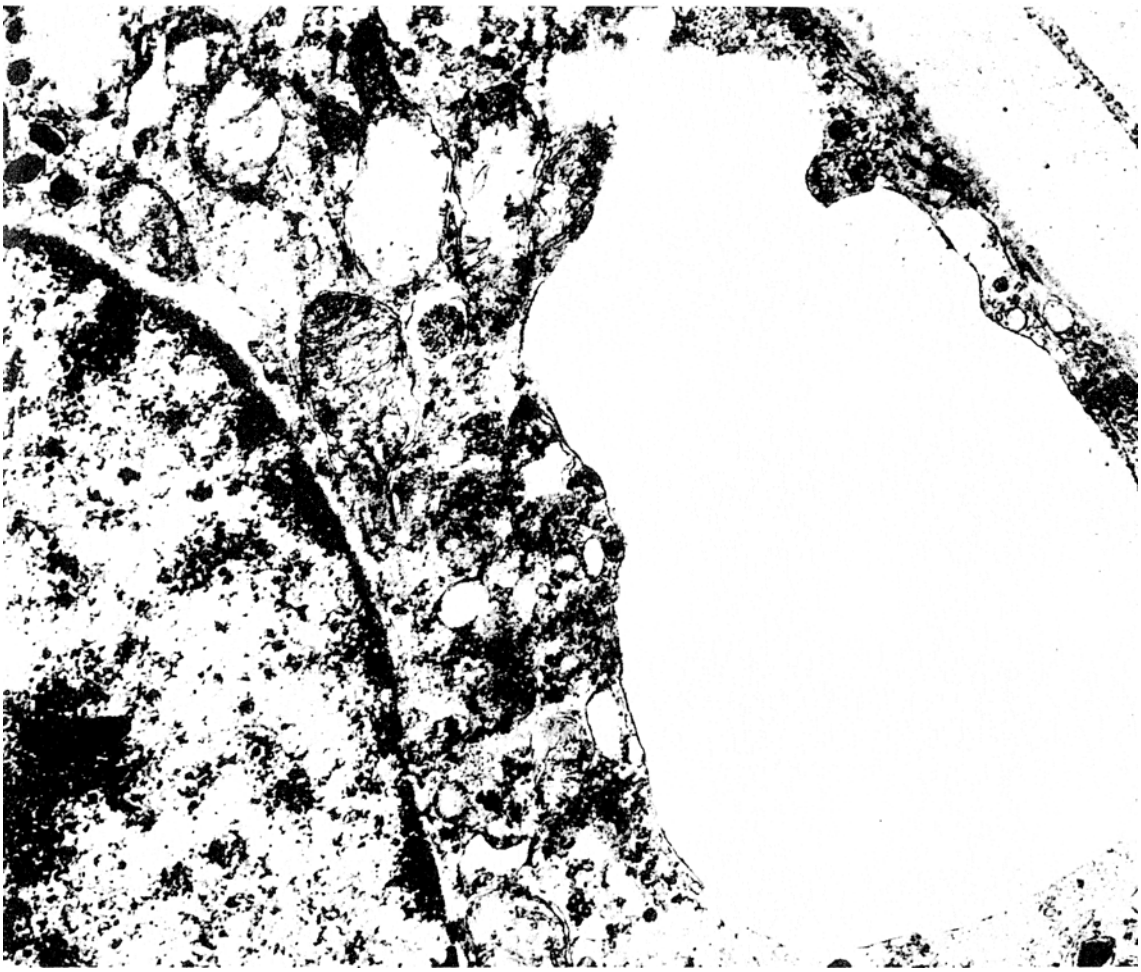

FIG. 9. Carcinoid tumor cell with subnuclear vacuole that corresponds to PAS-positive vacuole under light microscopy. Note perinuclear argentaffin granules (uranyl nitrate–Reynold's lead citrate stain ×24,000).

cells, also observed in intestinal tumors.[7] For the diagnosis of a mixed carcinoid and mucus-secreting intestinal tumor, it is important not only to find a considerable number of argentaffin-positive cells in the neoplasm, but also to find areas with the classic morphologic pattern of carcinoid tumors, such as arrangement in a trabecular fashion and solid nests of uniform polygonal cells with uniform rounded or ovoid nuclei. Glandular formation and mucin secretion in carcinoid tumors are not rare. In almost every case of carcinoid tumor that we have studied extensively, evidence of glandular formation and mucus secretion could be demonstrated in some areas. In a previous report,[10] we demonstrated transition from carcinoid cells showing a solid and trabecular pattern to acini with mucus secretion to frank adenocarcinoma. This indicated that the unique cell of origin of carcinoid tumors is not always recognized in practice. The mucus secretion by argentaffin cells is evidence for the close relationship of Kulchitsky's cells to mucus-secreting intestinal cells. The unitary hypothesis of the

derivation of carcinoid tumors from Kulchitsky's cells will not appear valid unless the argentaffin cells are one stage in the maturation cycle of goblet cells, as originally proposed by Popoff.[14] Clara[4] had shown that in the human embryo these cells are derived from undifferentiated intestinal epithelium, and Popoff[14] believed that during adult life they continued to be formed from the mucus-secreting cells. In 1924 and 1929, Cordier[5] and Siburg[16] described carcinoid tumors in which mucus-secreting cells were associated with glandular cells. Numerous publications in the literature of mucus-secreting carcinoid tumors support the viewpoint of Popoff[14] and the derivation of argentaffin cells and mucus-secreting cells from a common ancestor. Stout,[17] in 1942, made the interesting observation that if the basigranular cells (argentaffin cells) are formed from the goblet cells and only secondarily assume the pyramidal or wedge shape and acquire their granules, it is not so difficult to explain the variants seen in the tumor morphology. This explains the occasional occurrence of great variation in the histologic patterns of some carcinoid tumors, which at times are diagnosed as undifferentiated carcinomas. The authors had the opportunity to study two carcinomas of the stomach that had undifferentiated patterns with negative argentaffin staining and manifested the ultrastructural features of carcinoid tumors. By heterotransplantation of carcinoid tumor in cheek pouches of golden hamsters, Goldenberg and Fisher[8] obtained mucin-secreting cells of the signet-ring type; they showed evidence of transformation of a tumor with a carcinoid pattern into mucin-secreting adenocarcinoma. These observations are in consonance with the reported cases of carcinoid tumors showing gelatinous changes with numerous signet-ring cells[9, 10] similar to those of Patient 2 of this report. To our knowledge, mucus secretion in carcinoid tumors does not bear any relationship to the extent of malignancy; however, mucus-secreting adenocarcinomas evolving from carcinoid tumors behave like other colonic adenocarcinomas.

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