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The Carcinoembryonic Antigen (CEA) in Carcinomata of the Stomach*

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Summary. The localization of CEA in gastric carcinomata of different histological types was studied by indirect immunofluorescence. In well differentiated adenocarcinomata CEA was present on the surface of the tumor cells. CEA was absent in anaplastic cancers. Signet ring cells contained both CEA and mucus in their cytoplasm; this may indicate that signet ring cells are fairly well-differentiated tumor cells. In areas with intestinal metaplasia, CEA was detected on the luminal surface of the cells similar to a malignant tumor.

Introduction

CEA was originally isolated by Gold and Freedman (1965a) from adenocarcinomata of the large bowel and later extracted from cancers of the stomach, pancreas and liver (Gold and Freedman, 1965b; Krupey *et al.*, 1968; Martin and Martin, 1970, 1972). Its organ- and cancer-specificity, however, was disproved by several authors (von Kleist and Burtin, 1969; Burtin *et al.*, 1972a, 1972b; Martin and Martin, 1970, 1972; Rosai *et al.*, 1972; Abeyounis and Milgrom, 1972; Tappeiner *et al.*, in press). By immunofluorescence CEA was found in colonic carcinoma on the luminal surface of the tumor cells as well as in the lumina of the glands (von Kleist and Burtin, 1969; Denk *et al.*, 1972) requiring a certain degree of differentiation (Denk *et al.*, 1972). Well differentiated tumors contained more CEA than less well differentiated ones. In some pancreatic, gastric and colonic cancers CEA was demonstrated within the cytoplasm of some tumor cells which exhibited the morphological characteristics of signet ring cells (Denk *et al.*, 1972; Burtin, personal communication).

In the present study gastric carcinomata were studied in more detail by the indirect immunofluorescence technique. Antisera against CEA and a perchloric acid soluble antigen of normal large bowel mucosa (NC) were used. The latter antigen was shown to be present in intra- and extracellular mucus of normal and cancerous colonic mucosa (von Kleist and Burtin, 1969; Tappeiner *et al.*, in press) and may be used as marker for mucus of intestinal type. Gastric carcinomata tend to show considerable structural variations in one and the same tumor. Therefore, the localization and quantity of CEA were related to the different morphological patterns very often seen even closely mixed in one individual specimen.

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Material and Methods

48 stomachs surgically removed for carcinoma were studied. The specimens were obtained 2–6 hours after surgery, quick frozen in dry ice-isopentane and stored in a −80° C freezer. Indirect immunofluorescence (IIF) was performed as described previously (Denk *et al.*, 1972). Antisera against a perchloric acid extract of colonic carcinomata (anti-CEA serum) and a comparable extract of normal large bowel mucosa (anti-NC serum), respectively, were used (for preparation see Denk *et al.*, 1972). The globulins of the sera were isolated by ammonium sulphate precipitation and hence the term "serum" used in the paper refers to these globulin fractions. Anti-CEA serum was compared with a specific anti-CEA serum kindly provided by Dr. von Kleist and gave identical reactions with colonic carcinoma extract. Anti-CEA serum was absorbed with perchloric acid extract of normal large bowel mucosa (30 mg of dry lyophilized substance/ml serum), pooled human plasma (Hyland, 20 mg dry substance/ml serum), perchloric acid extract of normal human lung (30 mg dry lyophilized substance/ml serum) and in some instances with human AB erythrocytes. Anti-NC serum was absorbed with pooled human plasma (Hyland, 20 mg dry substance/ml serum) and with human AB erythrocytes. Commercial FITC-coupled anti-rabbit globulin serum of a goat (Hyland) was used in the second layer. The specifications of the antisera used in IIF in the present study were the same as described previously (Denk *et al.*, 1972).

Results

In parts of the *carcinoma with adenoid and adeno-papillary structures* CEA could be demonstrated in linear or granular form on the luminal surface of the tumor cells as well as within the glands if the glands appeared fairly well differentiated and were lined by columnar epithelium (Fig. 1). In some tumors with atypical irregular glandular structures with cuboidal or flat epithelium of low differentiation CEA was not demonstrable. NC was usually present in adenocarcinomata either as superficial lining or in cloudy fashion in mucous secretions irrespective of the differentiation. Goblet cell-like tumor cells were rare but, if present, specifically stained by anti-NC serum.

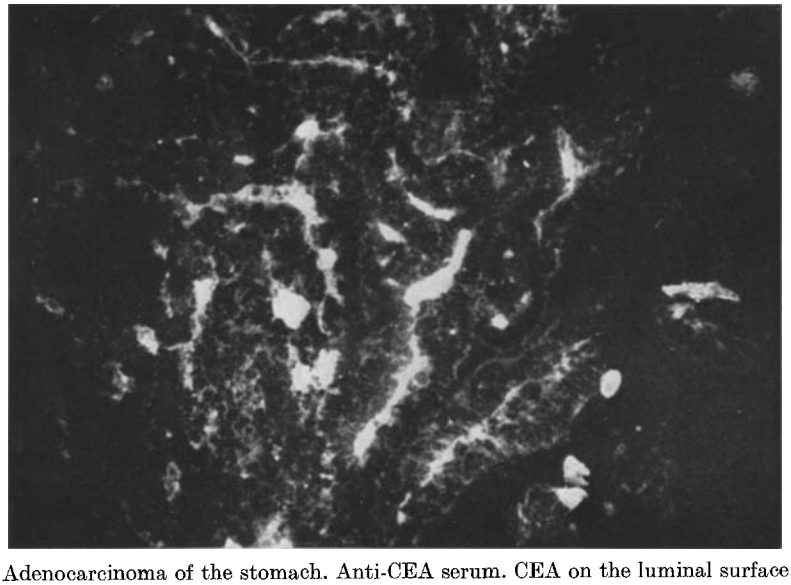

Fig. 1. Adenocarcinoma of the stomach. Anti-CEA serum. CEA on the luminal surface of the tumor cells. Magn. ×32

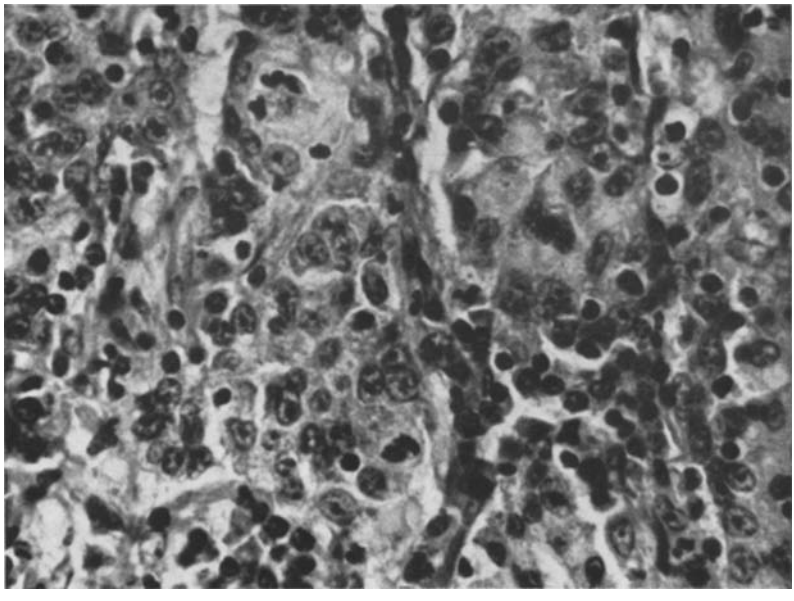

Fig. 2. Anaplastic carcinoma of the stomach. Hematoxylin-Eosin. Magn. ×80

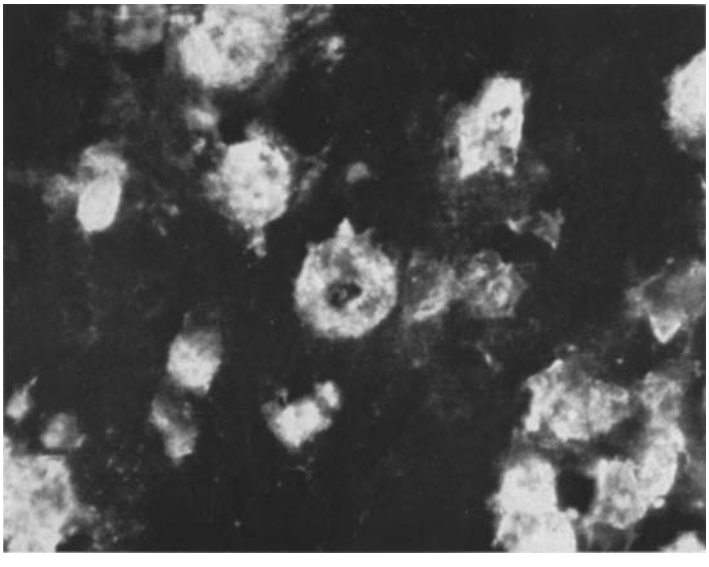

Fig. 3. Signet ring cells. Anti-CEA serum. Granular cytoplasmic staining. Magn. ×128

In *undifferentiated solid or scirrhous areas* of the tumor (Fig. 2) usually consisting of cells with darkly and homogenously staining cytoplasm (hematoxylin-eosin) CEA was invariably absent. In some of these tumors NC was present in small amounts as drops or lakes of specific fluorescence between the cells.

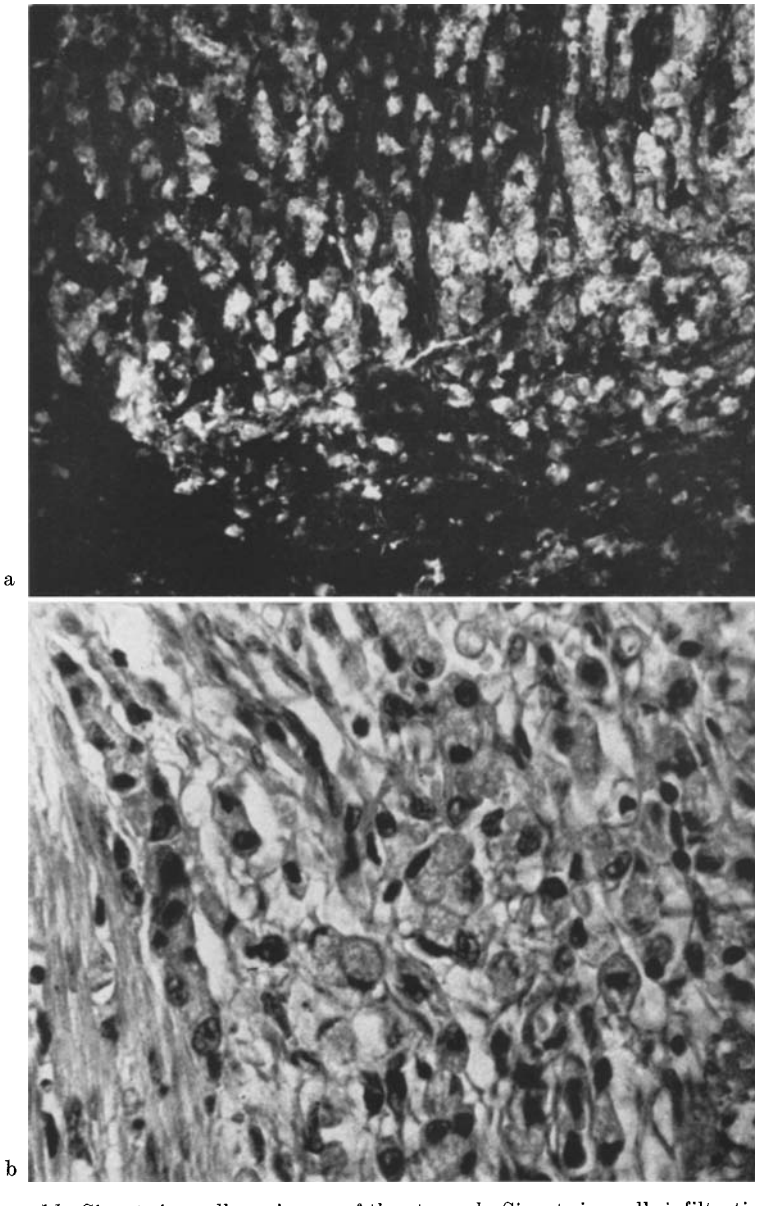

Fig. 4a and b. Signet ring cell carcinoma of the stomach. Signet ring cells infiltrating the wall of the stomach. a Anti-CEA serum. Magn. ×32. b Hematoxylin-Eosin (comparable area). Magn. ×80

In *signet ring cell* areas with or without abundant mucus surrounding the cells most of the signet ring cells showed a cytoplasmic granular fluorescence and, to some degree, also membrane staining with anti-CEA serum (Fig. 3). The specifically stained cells were either floating in the mucus or still in contact with each other infiltrating the wall of the stomach (Fig. 4a, b). Cells morphologically similar to

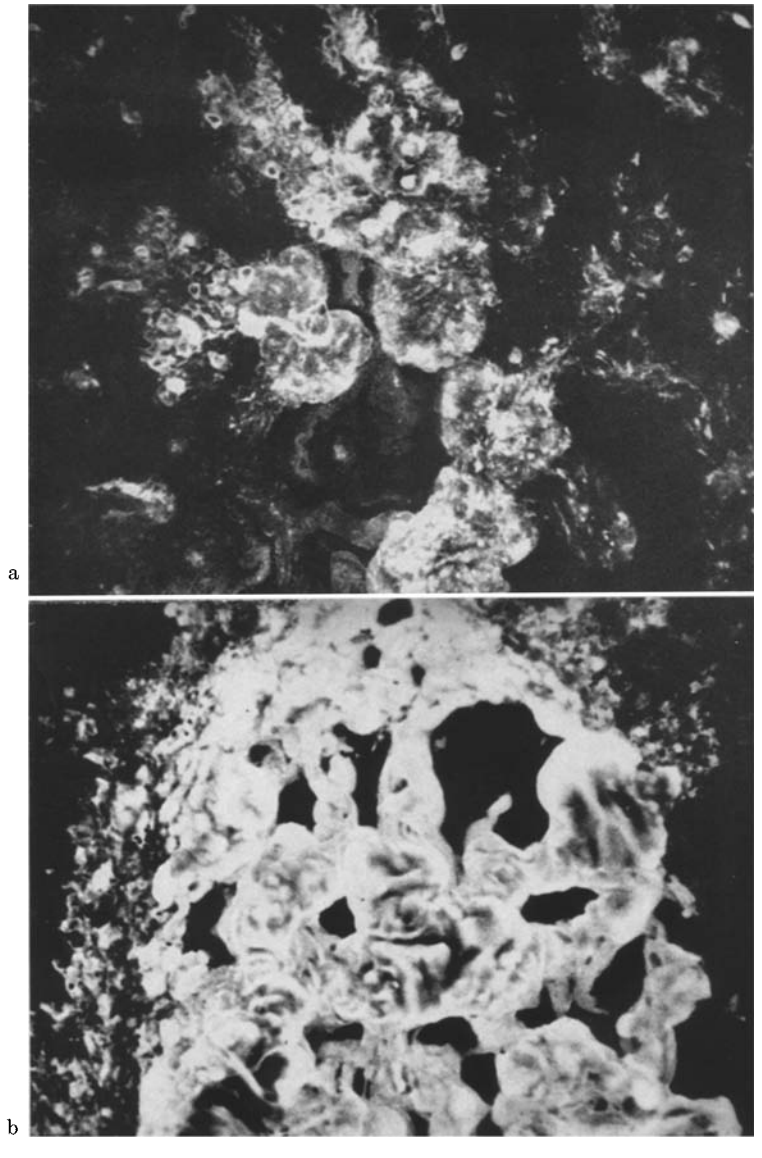

Fig. 5a and b. Mucoid carcinoma of the stomach with signet ring cells floating in mucus. Comparable areas stained with anti-CEA and anti-NC serum, respectively. Magn. ×32. a Anti-CEA serum. Granular specific fluorescence of the mucus. b Anti-NC serum. Diffuse cloudy staining of the mucus

signet ring cells but with dark homogenously stained cytoplasm (in hematoxylin-eosin stained sections) were negative in IIF. The mucus exhibited granular specific fluorescence with anti-CEA serum (Fig. 5a). Treatment of the tissue sections with anti-NC serum resulted in a quite different morphological image. Mucus was stained diffusely in a cloudy fashion (Fig. 5b). The signet ring cells were often

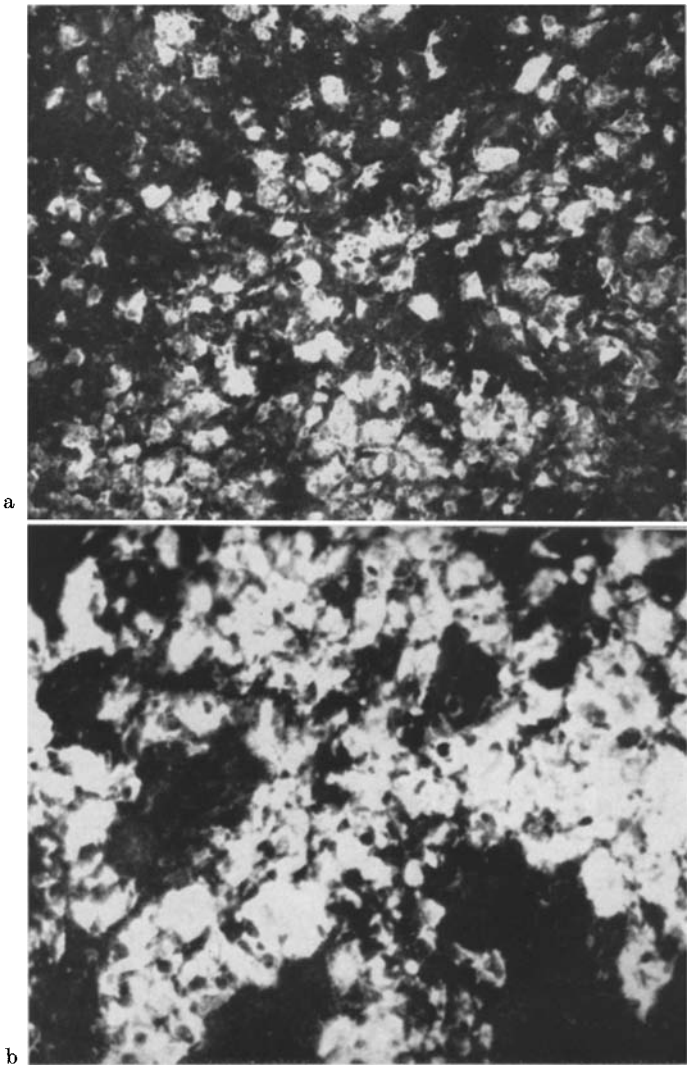

Fig. 6a and b. Signet ring cell carcinoma. Comparable areas. a Anti-CEA serum. Granular staining of the cytoplasm and of intercellular mucus. Magn. ×32. b Anti-NC serum. Specifically stained droplets of varying size in the cytoplasm and diffuse staining of mucus between the cells. Magn. ×80

hardly recognized within the mucus. They showed a cytoplasmic fluorescence due to intracellular mucus deposits of varying size. Contrary to the results obtained with anti-CEA serum, the immunmorphological picture was blurred by diffusely stained mucus between the cells (Fig. 6a, b).

In areas of the gastric mucosa with *intestinal metaplasia* the secretory parts of the goblet cells as well as the mucous secretions reacted specifically with anti-NC serum similar to the mucosa of the large bowel. In metaplastic areas without goblet cells the NC-specific fluorescence was confined to a small superficial line

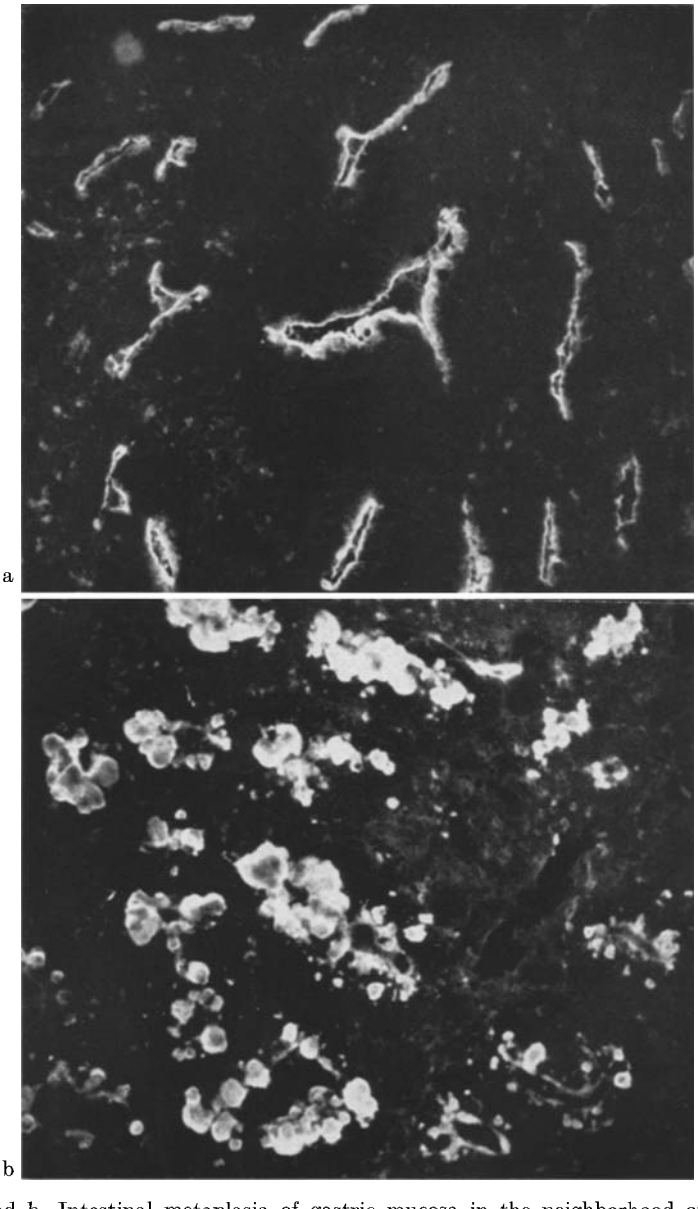

Fig. 7a and b. Intestinal metaplasia of gastric mucosa in the neighborhood of the tumor. Magn. ×32. a Anti-CEA serum. Specific fluorescence lining the lumina. b Anti-NC serum. Staining of the cytoplasm of the goblet cells

on the luminal surface. With anti-CEA serum somewhat variable results were obtained. In most of the cases a superficial line of specific fluorescence was observed on the luminal surface of the metaplastic mucosa irrespective of the presence or absence of goblet cells (Fig. 7a, b). In addition, the surface of non-metaplastic gastric mucosa in close vicinity of the cancer showed sometimes but

not regularly a weak superficial fluorescence after incubation with anti-CEA serum.

Independent of the degree of differentiation tumor areas revealed a diffuse, but weak cytoplasmic fluorescence with anti-CEA but not with anti-NC serum by which the tumor could be differentiated from the surrounding tissue. An identical observation was made in colonic cancers (Tappeiner *et al.*, in press).

Discussion

Adenocarcinomata of the stomach with or without mucus production contained CEA in a similar localization as seen in colonic cancers but in lesser amounts. The latter is consistent with the finding of Gold and Freedman (1965b) that the CEA content of intestinal epithelial malignancies decreases from aboral to oral in the GI-tract. Similar to colonic cancers, CEA-production and secretion in carcinomata of the stomach depended upon the differentiation of the tumor (Denk *et al.*, 1972). CEA was not detected with certainty, at least under our experimental conditions, in anaplastic carcinomata thus confirming the results of Martin and Martin (1970) obtained by an extraction method. However, the diffuse weak but possibly specific cytoplasmic staining of the whole tumor area independent of its differentiation both in gastric and in colonic carcinomata (Tappeiner *et al.*, in press) awaits further clarification.

Intracellular CEA together with mucin was a striking feature of signet ring cells in gastric cancers. These cells were morphologically similar to the CEA-containing cells seen in carcinomata of the pancreas (Denk *et al.*, 1972). With respect to signet ring cells of the stomach, similar results were obtained independently from us by Burtin (personal communication). The production of CEA and mucin by signet ring cells supports the concept that these cells represent to some degree the malignant counterparts of the goblet cells. It favours the assumption that signet ring cells are fairly well differentiated tumor cells which gained autonomy on the cellular level. In this context, the similarities between signet ring cells and cultured colonic carcinoma cells (Goldenberg *et al.*, 1972) are striking.

By IIF CEA was demonstrated in areas of intestinal metaplasia thus confirming the observations of von Kleist and Burtin (1969) with respect to peritumoral colonic mucosa and of Burtin (personal communication) with respect to gastric intestinal metaplasia. This finding again shows that CEA cannot be regarded as strictly cancer specific.

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