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Microscopic and Pulse Cytophotometric Investigation of a Carcinoma of the Jejunum in a Seven Year Old Child

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Summary. In a seven year old boy with acute abdominal pain and intestinal bleeding, laparotomy revealed an invaginated carcinoma adenomatosum cylindrocellulare in the distal part of the jejunum. Histological and pulse cytophotometric investigations support the diagnosis of a highly differentiated but locally destructive and malignant tumor.

Key words: Adenocarcinoma – Small intestine – Pulse cytophotometry.

Introduction

Cancer of the jejunum and ileum is very rare, being almost exclusively restricted to the elderly (mainly 5th and 6th decade).
In general the tumor becomes apparent in its advanced stages through non-specific tumor symptoms such as anemia (intestinal bleeding), loss of weight (Clark, 1949; Darling, 1959; Lick, 1966), or intestinal obstruction due either to the tumor's size or to invagination (Rabinowitsch, 1950; Shamiyeh, 1972). Thus the prognosis is usually bad since at the time of operation the tumor has already metastasized into the regional lymph nodes (Sheehy, 1964; Truelove, 1972).

We describe here a case of a child only 7 years of age in which an intestinal disturbance led to the diagnosis and surgical removal of a carcinoma of the distal jejunum. The diagnosis was supported by cytophotometric DNA-measurements.

Clinical Observations

M.E., born March 23, 1967 was admitted to the hospital in April 1974 with a pronounced anemia (HB 3.3 g%, erythrocytes 1.5 million), due to intestinal bleeding. On May 30, 1974, acute abdominal attacks were encountered. An immediate exploratory laparotomy revealed an invaginated tumor in the distal jejunum which was removed. Metastases were not detected. The postoperative convalescence proceeded without complication.

* Dedicated to my father, Dr. C.W. Büsing, on the occasion of his 65th birthday

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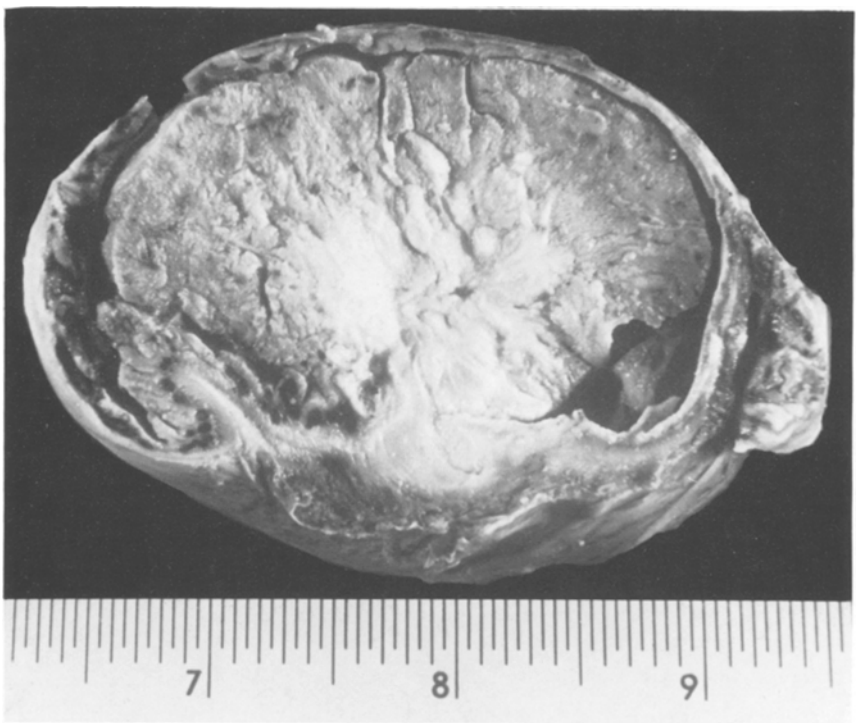

Fig. 1. Small intestine carcinoma: macroscopic section through the polypous-endophytic tumor with major obstruction of the lumen. Macroscopic 1:2

Patho-Anatomical Examination of the Specimen

(E.-Nr.: 19828/74, Institute of Pathology, University of Heidelberg, Director: Prof. Dr. W. Doerr) revealed a 50 cm long segment of jejunum bearing a polypoid tumor, which extended to the intestinal lumen measuring 7 cm in diameter (Fig. 1). An invagination was no longer present. The tumor, at its base, was densely interwoven with the adjoining intestinal wall.

Histological slides presented densly arranged adenoid formations lined by predominately tall columnar epithelia. The nuclei appeared relatively uniform with few mitoses, some goblet cells were found (Fig. 2). The submucosa and the tunica muscularis proper were infiltrated by epithelial formations, which nearly reached the serosa (Fig. 3). By staining with the PAS-reaction and with Alcian-blue in this region distinct mucus formation could also be seen. In some places the epithelial cells appeared "drowned" in the mucus (Fig. 2B). Small foci of necrotic epithelial cells were found, which had calcified in a way resembling psammoma granules. Both Fontana-Masson's staining for argentaffinity (Romeis, 1968) and formalin-fluorescence on unstained histological sections for the detection of 5-hydroxytryptamine (Enerbäck, 1973) were negative. So our diagnosis was a highly differentiated polypoid, in part mucinous carcinoma adenomatosum cylindrocellulare of the distal jejunum. The lymph nodes of the mesenteric adipose tissue were free from metastatic infiltrates. The normal jejunal mucosa exhibited tall folds with normal villi (mean value 0.45 mm of height) and normal crypts (mean value 0.185 mm).

Cytophotometric Investigations

For cytophotometric DNA-determination on the formalin fixed material a suspension of individual cell nuclei was prepared by mechanical homogenization with subsequent enzymatic digestion. The

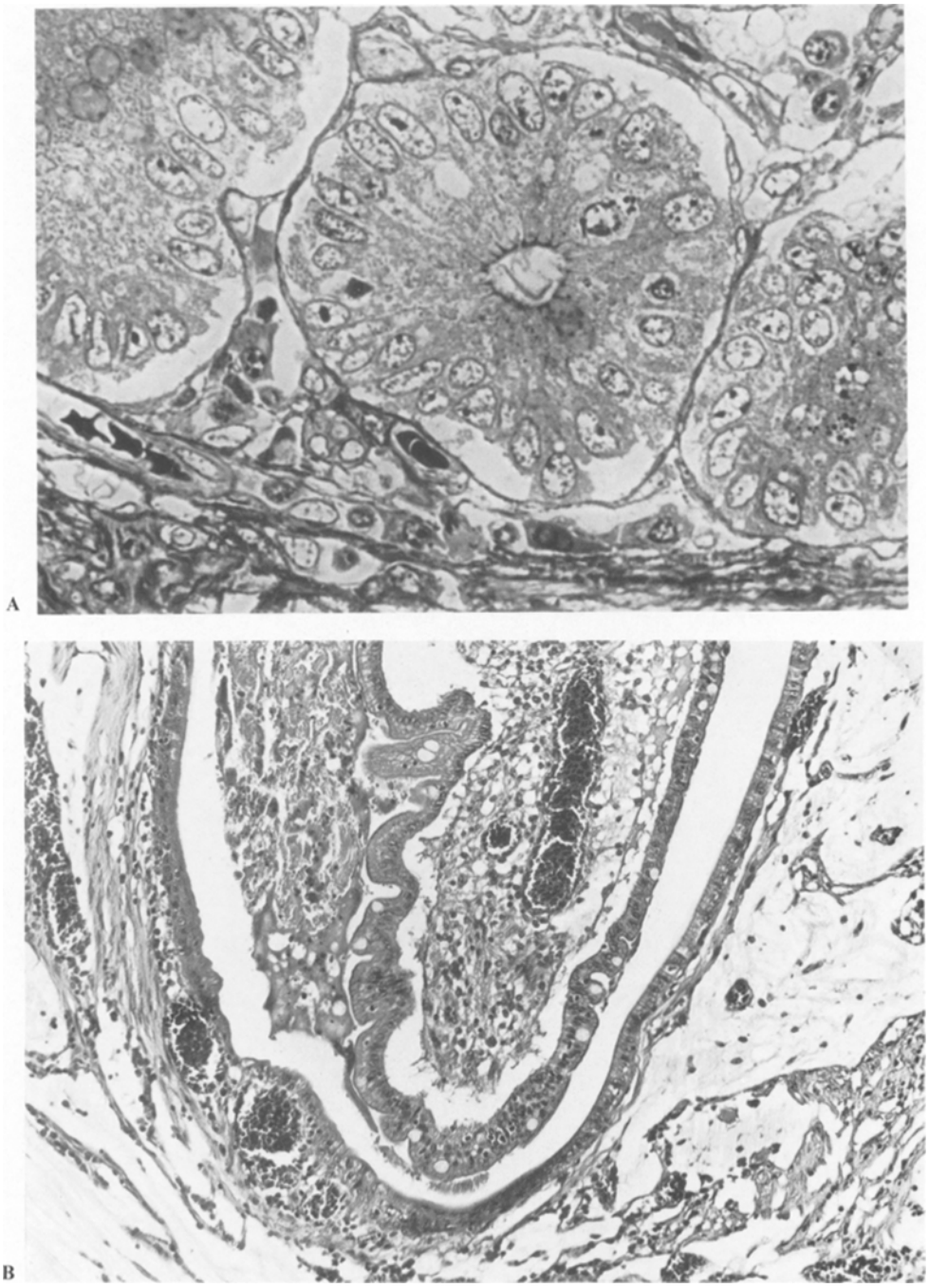

Fig. 2A and B. Small intestine carcinoma: invasive epithelial formations with only slight cellular and nuclear polymorphism. **A** Marked back to back placement of the epithelium. Formalin fixation, Araldit-embedded, semi-thin-section. Magn. 1:160 ×7,5. **B** Marked presence of mucus with a large mucoid patches. Formalin fixation, paraffin, haematoxilin-eosin. Magn. 1:280

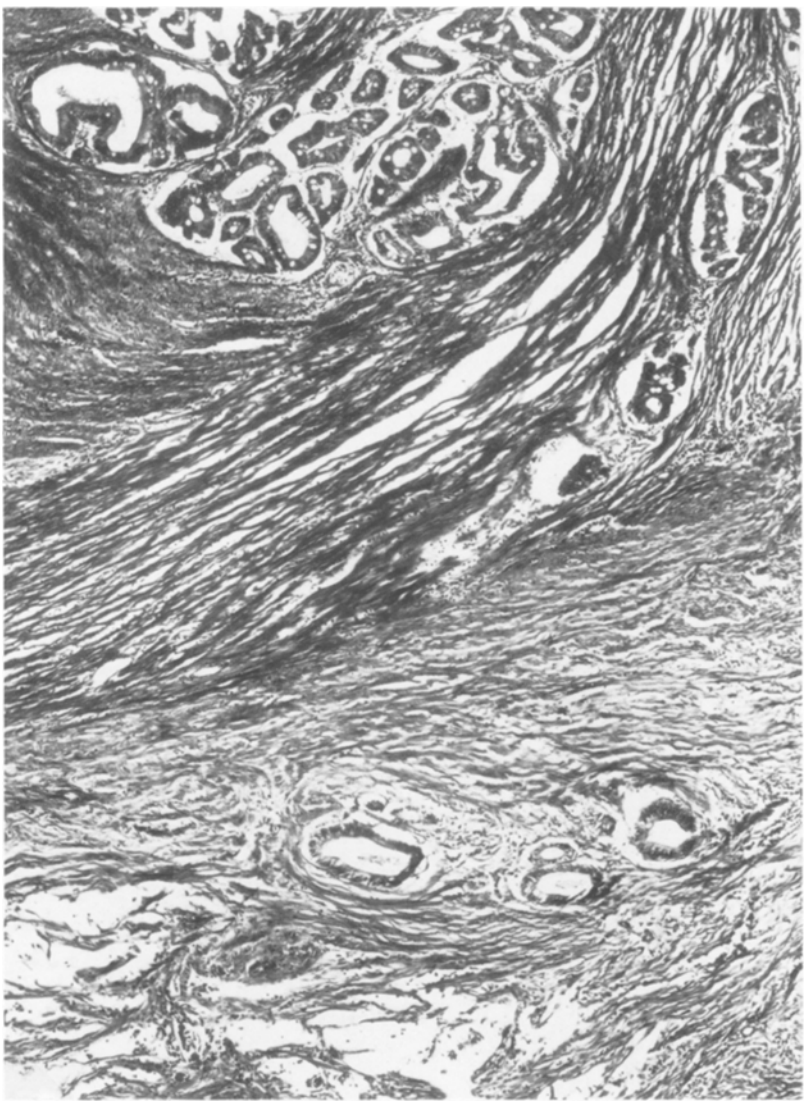

Fig. 3. Small intestine carcinoma: tumor cell complexes of mainly tubular arrangement with dos-à-dos-position of epithelial cells with only slight cellular and nuclear polymorphism and scattered mitoses. Relativly little stroma exhibiting only a poorly disseminated lymphocytic infiltration. Formalin fixation, paraffin, haematoxilin-eosin. Magn. 1:200

washed suspension was tested under the phase contrast microscope and proved to contain separated cell nuclei largely free from cytoplasm. The suspension was utilized in part for smear preparations while the cell nuclei of another part were stained with ethidium-bromide for pulse cytophotometry of nuclear DNA (Dittrich, 1969). We used the ICP 11 from the PhyWe Company (Göttingen, Germany) for the determination of DNA histograms and human lymphocytes as the standard-control. Single cell fluorescence measurements were performed with the microscope-photometer (MPM, C. Zeiss, Germany) according to the method of Böhm (1968) after the smears had been subjected to the Feulgen-reaction in Schiff-type acriflavine (Prenna, 1964).

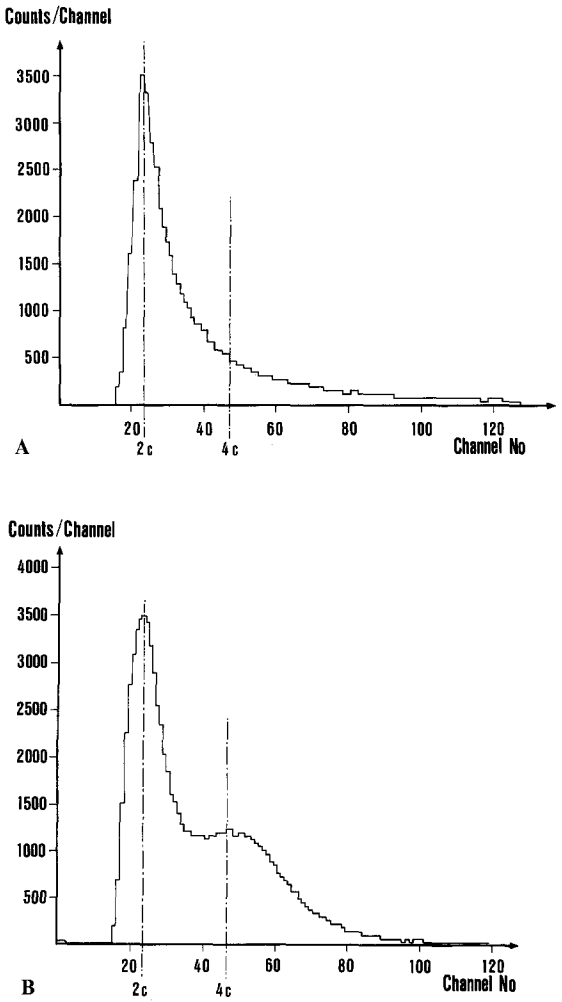

Fig. 4A and B. Pulse cytophotometric DNA measurements; ordinate: counts recorded in the different DNA-channels; abscissa: number of channels. The 2-C-region and 4-C-region is indicated by dashed lines. **A** Normal intestinal mucosa with unimodal histogram with typical hyperbolic decline. **B** Tumor nuclei with bimodal histogram revealing a second maximum with a wide distribution at the 4-C-region

Results

In the healthy part of the mucosa the pulse cytophotometric measurement yielded an unimodal histogram with a maximum in the 2-C-region, and a hyperbolic decline down to the 4-C-region (tetraploid DNA-content of the cell nuclei; Fig. 4A). In contrast the histogram of the tumor cell nuclei showed a distinct second maximum in the 4-C-region, with some values even exceeding this (Fig. 4B). In agreement with these findings measurements of single cells, performed on 200 cell nuclei, also showed a second maximum in the 4-C-region together with values above this for the nuclei of the tumor (Fig. 5A and B). By this procedure cell clusters with a high DNA-content in pulse cytophotometry could be excluded, through visual control.

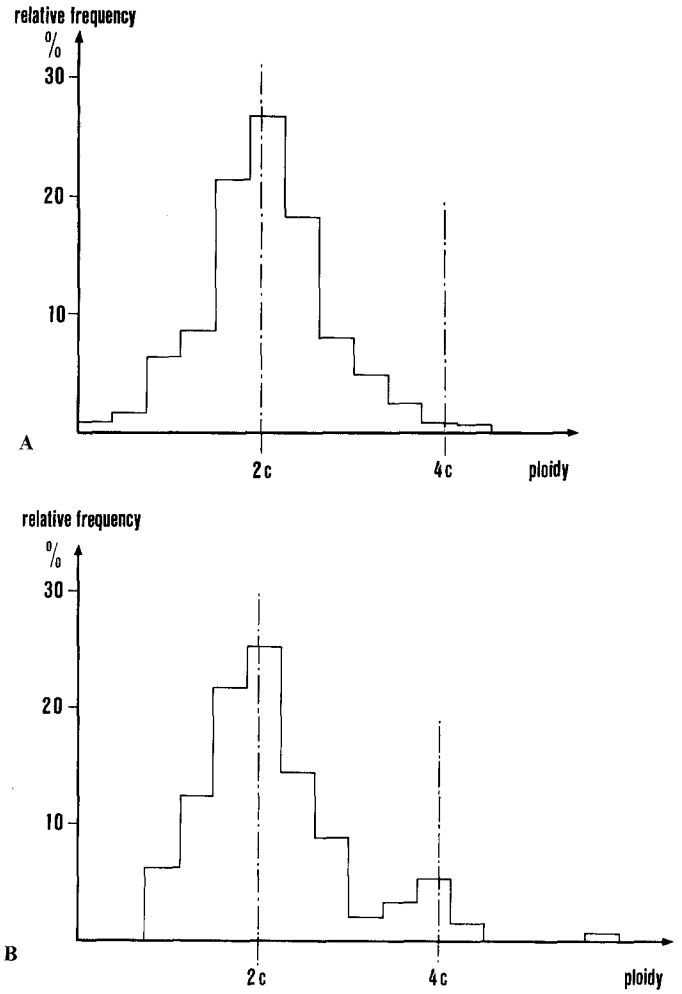

Fig. 5 A and B. Microfluoroscopic DNA-determination on 200 cell nuclei of both groups respectively; **A** Normal mucosa with a typical distribution of the relative frequency of the DNA-content with a maximum at 2-C-region. **B** Tumor nuclei: marked second peak at the 4-C-region with some nuclei revealing an even higher ploidy

Discussion

In the literature available to us, we were unable to find a case report comparable to our observation.[1] Since Sorlin's case report (1824) of a 49-year-old patient, most probably the earliest communication of a malignant (epithelial?) tumor of small intestine, numerous synopses and retrospective analyses have been presented. With regard to all malignant epithelial tumors of the gastrointestinal tract, the relative frequency of the jejunal carcinoma has been estimated to be 0.15%, with a slight male preponderance. The patients' ages ranged between 40 and 70 years (Lick, 1966; Bollag, 1933; Siris, 1949; Wittig, 1955; Merkel, 1956; Mc Comb, 1957; Diebold, 1962; Shamiyeh, 1972; Frommhold, 1973).

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The question of etiology and pathogenesis remains open. The etiological influence of bile acids (Shamiyeh, 1972), chronic inflammation (RHA, 1971) or of a dystopic gastric mucosa (Dietel, 1972) have been discussed. Rapid intestinal passage (Shamiyeh, 1972) and various local factors are said to be responsible for the relative infrequency of tumors in the more mobile section of the small intestine (Wibecke, 1973). In our case it is the youth of our patient and the polypoid endophytic growth of the tumor, that lead us to suspect the presence of some congenital or early acquired local process which favored the tumor development. A solitary polyp of the intestinal mucosa with secondary malignant degeneration (Truelove, 1972; Eder, 1974) might provide an explaination, however, there was no indication of a Gardner-syndrome or Peutz-Jeghers-syndrome. It is interesting to note the medical case history on the child's paternal side: a grandfather who died of stomach cancer, a grandmother who died of uterine cancer and a greatgrandmother who died of lung cancer.

The relatively uniform cellular picture with only moderately pronounced atypical cellular and nuclear features, led us to the question as to whether or not this highly differentiated tumor might be considered to rank on a relatively "low level of malignancy", a concept proposed by Roessle (1950; compare Doerr, 1961).

Aditional information was gained by cytophotometric examination, which revealed differences between the intact intestinal mucosa and the tumor. According to the stemline concept of aneuploid chromosomes (Makino and Kano, 1952; Levan and Hauschka, 1952) atypical DNA-content of nuclei is considered to be an emperical criterion of malignant tumors (Sandritter, 1952; Leuchtenberger, 1954). In the tumor cell histogram, the position of the first maximum corresponded very well with that found in the normal intestine. However, the second maximum (4-C) proves the existence of an additional tumor cell population with even greater amounts of DNA, ranging up to octoploid values (8-C-region).

These values are interpreted as an indication of malignant degeneration (Böhm, 1971). Nevertheless, Sprenger et al. (1974) demonstrated that the DNA-stemline by itself is not sufficient to identify a tumor cell population, and introduced additional criteria such as the ploidy-value (U) as well as the relative frequency (Z) of euploid and polyploid values to discriminate malignant processes. With respect to these criteria, the tumor histogram also differs from that of the normal intestinal mucosa. However, the well-nigh tetraploid stemline in the histogram shows the relatively high degree of differentiation of the tumor. This and the fact that no metastases could be detected in the regional lymph nodes, eventhough the layers in the intestinal wall were infiltrated, would support the assumption of a moderately agressive carcinoma, with a good post-operative prognosis.

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