MORPHOLOGY OF CARCINOMA OF THE LARGE INTESTINE IN ORGAN CULTURE

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In 1976, using a method of organ culture, Lucas [3] discovered correlation between sensitivity of a tumor to irradiation in tissue culture and the effect of radiotherapy. Wolff et al. [4] showed that organ culture in conjunction with embryonic tissue can be used to determine the rate of invasion, which correlates with the rate of spread of a tumor *in vivo*. Unlike tissue cultures, in organ cultures structures typical of a particular organ are formed more distinctly, so that this method can be used to study histogenesis of a tumor. The writers decided to use electron-microscopy to study the cell composition in the zone of growth of glandular structures of tumors. However, since there are only isolated references in the literature to organ culture of carcinoma of the large intestine and rectum [1, 2] and no detailed picture of the morphology of tumors of the large intestine grown in organ culture, the aim of the present investigation was to study the character of growth and to determine the functional activity of cells of carcinoma of the large intestine at different periods of culture. Synthesis and appearance of carcino-embryonic antigen (CEA) in the culture medium were chosen as indicators of functional activity.

EXPERIMENTAL METHOD

Three tumors of the large intestine removed at operation from patients aged 32, 42, and 72 years, respectively, were used as material for investigation. Histologically all three tumors had the structure of adenocarcinomas (Fig. 1a). The tumor tissue was cut into small pieces with sides measuring 1-2 mm and transferred to filters with a pore size of 40 μ (from "Synpor," Czechoslovakia). The composition of the nutrient medium was as follows: medium RPMI-1640 (70%), bovine serum (20%), embryonic extract (10%), 0.06 mg glucose, 0.03 mg ascorbic acid, 5 mg hydrocortisone, and kanamycin and gentamicin 50 μg/ml medium of each. The tissue fragments were cultured at 36.0-36.5°C in a Bruvo thermostat with 100% humidity and with a CO_2 concentration of about 10%. The nutrient medium was changed twice a week. In parallel experiments, the concentration of CEA in the incubation medium on the 6th, 13th, and 20th days was determined with the aid of standard kits from CIS (France) for the corresponding radioimmunologic procedures *in vitro*.

EXPERIMENTAL RESULTS

The morphological study on the 1st-2nd days of the experiment showed that the tumor fragments were insecurely fixed to the substrate and had irregular outlines. In some fragments there were zones of central necrosis. Around the periphery single groups of hyperchromic cancer cells and small cells with a round nucleus and poorly developed cytoplasm, resembling lymphocytes, could be identified. By the 3rd day the fragments had begun to be partially covered by cells resembling those of cylindrical epithelium (Fig. 1b). On the 5th day the wound surface where the section had been cut was no longer present because the whole fragment was covered by tumor epithelium (Fig. 1c). In some areas the epithelial cells were swollen and had become circular, so that that particular part began to protrude slightly. At the same time single outgrowths covered with epithelial cells could be seen (Fig. 1d). Mitoses were visible.

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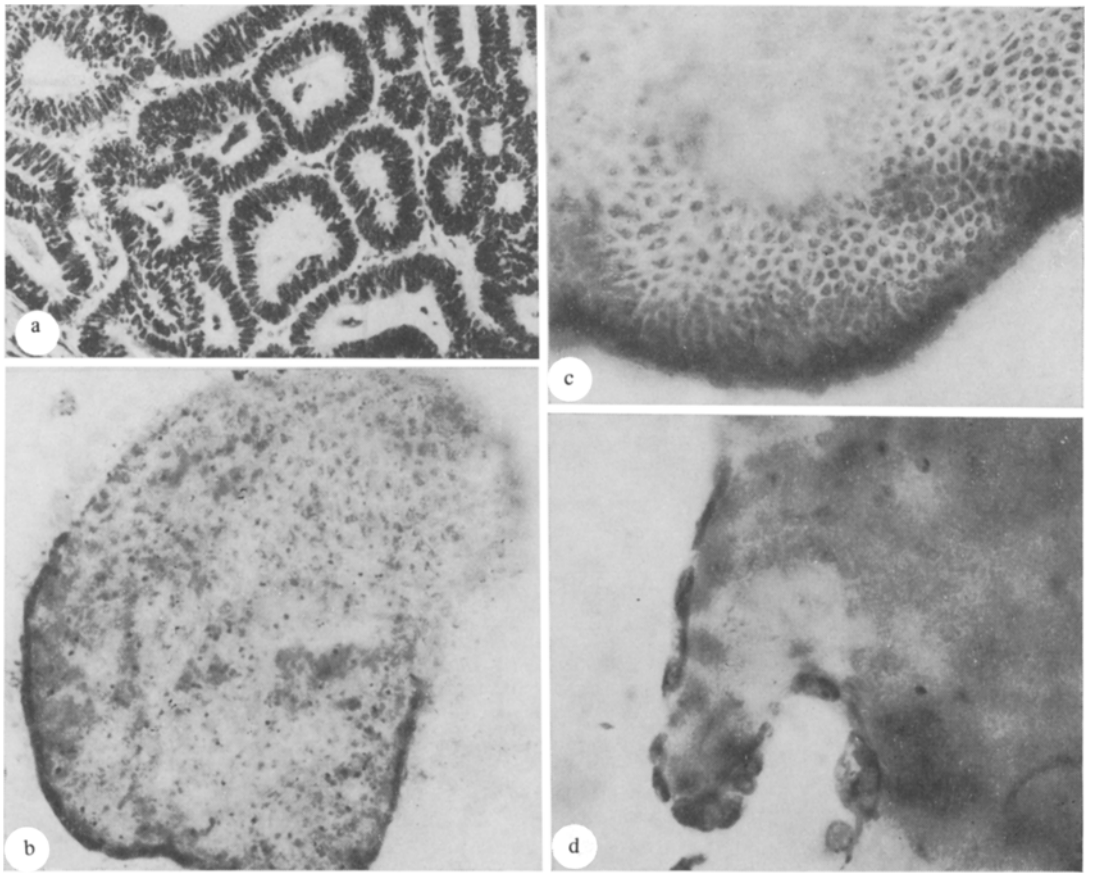

Fig. 1. Carcinoma of large intestine on 3rd-5th day of organ culture. a) Tumor has structure of highly differentiated adenocarcinoma, consisting of gland-like structures; b) 3rd day in culture: fragment partly covered by cells resembling cylindrical epithelium; c) 5th day in culture: entire fragment covered by epithelial cells; d) 5th day in culture: single outgrowths covered by epithelial cells are appearing. a) Hematoxylin and eosin, 120 ×; b, c, d) hematoxylin, 160 ×.

On the 7th day the tumor fragments were hemispherical in shape with numerous outgrowths of curious shape (Fig. 2a, b). The surface of the fragments was covered with epithelial cells with large hyperchromic nuclei, some of them containing visible nucleoli. At the end of some of the digitiform processes the epithelial cells proliferated to form gland-like structures, which became detached in places from the main fragment. At the periphery of the fragments solitary breaks could be seen in the epithelial cover, with cells escaping from the fragment. Unlike those outgrowths which remained in communication with the main fragment and formed gland-like structures, these groups of cancer cells were free-lying and did not form structures of any kind. Already glandular structures had begun to appear in the fragment itself. In transverse section through its center the fragment had the appearance of a hollow tube with a single layer of cylindrical or (less frequently) cubical tumor cells lining it (Fig. 2c), and which in places projected into the lumen of the hollow tube described above. Outgrowths from the fragment had a similar structure in transverse section.

On the 9th, 12th, 15th, 17th, and 20th days the changes remained the same. The fragments grew considerably in size and became curiously shaped. The outgrowths became larger, and collections of large, juicy cells could be seen at the ends of the digitiform processes. Meanwhile breaks could be seen in the peripheral covering of the fragment, together with collections of cancer cells which, whatever the time of culture, still remained as separate groups, without forming structures of any kind (Fig. 2c). On the 24th and 27th days, empty spaces appeared inside the larger fragments and intensive formation of gland-like structures was observed.

An organ culture of carcinoma of the human large intestine could be maintained in the medium described above for 27 days.

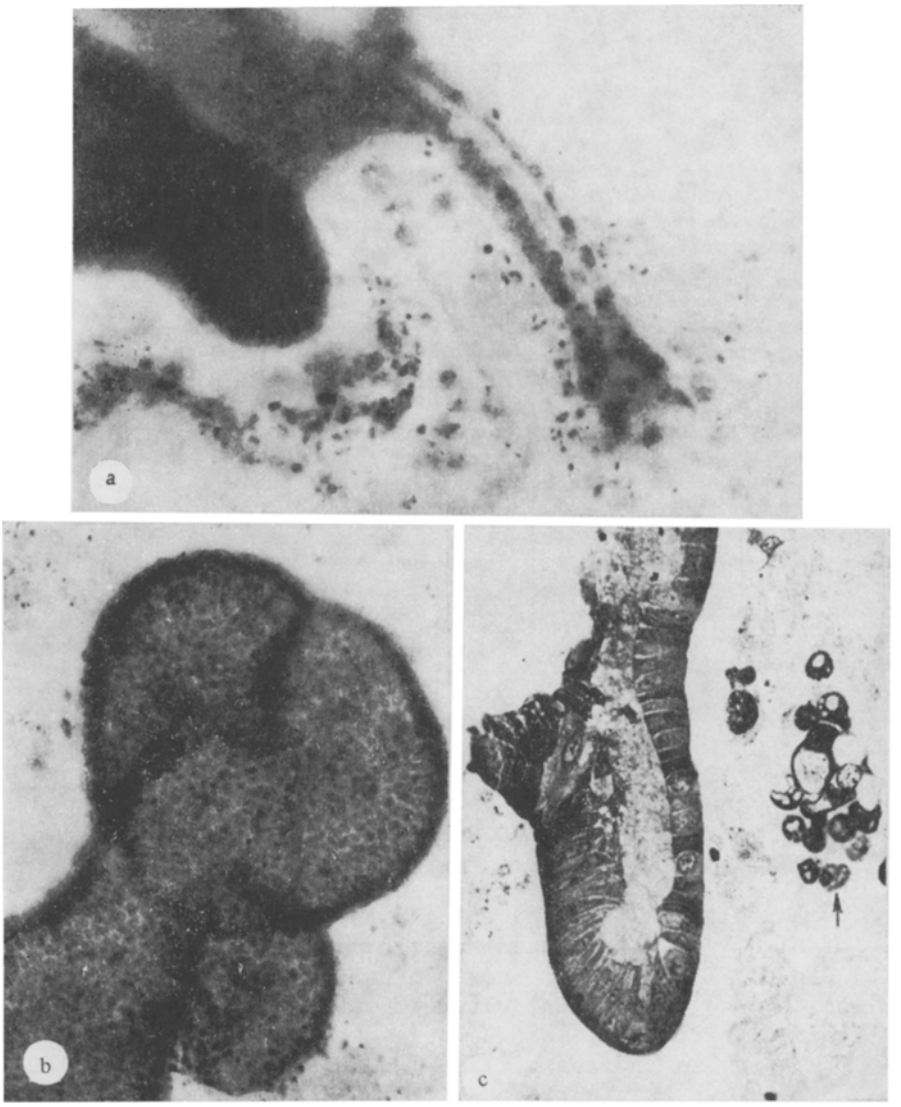

Fig. 2. Carcinoma of large intestine on 7th day of organ culture. a, b) Numerous curiously shaped processes consisting of epithelial cells, with groups of epithelial cells appearing at the end of some digitiform processes. Hematoxylin; c) semithin section: fragment in transverse section has the appearance of a hollow tube lined by a single layer of cylindrical and cubical tumor cells; groups of cancer cells can be seen lying freely and not forming structures of any kind (arrow). Stained with toluidine blue. Magnification: a) 200, b) 160, c) 250 ×.

Parallel with the morphological study, the concentration of CEA in the incubation medium also was investigated on the 6th, 13th, and 20th days of culture. CEA is synthesized *in vivo* by tumor cells arising mainly from epithelium of the gastrointestinal tract, and it is found in highest concentrations in carcinoma of the large intestine. The appearance of high concentrations of CEA in the medium indicates preservation of the synthetic capacity of the tumor cells, and this reflects their functional state. Medium prepared before the beginning of culture served as the control. The CEA concentration in this medium was between 3.7 and 6.9 µg/liter, which can be interpreted as a trace amount. In medium used for incubation of the tumor the CEA concentration was considerably higher, and on the 6th day of culture (this medium was used from the 4th to the 6th day) the CEA concentration was 53.5-81.1 µg/liter, rising on the 13th day (medium used from the 10th to the 13th day) to 50.4-300.0 µg/liter, and on the 20th day (medium used from the 18th to the 20th day) from the beginning of culture to 37.1-300.0 µg/liter. It must be recalled that during organ culture there is no direct contact between the cultured fragment and the medium, and CEA can enter the medium only through the filter because the cells cannot pass through pores 40µ in diameter.

The investigation thus showed that during culture of carcinoma of the human large intestine the typical structure of tumors of this kind is preserved, with the formation of glands and with the characteristic organ-specificity. The tumor cells, even in the late stages of culture, retain their ability to synthesize CEA, a specific protein for tumors arising from epithelium of the gastrointestinal tract. The tumor grows mainly at the expense of the fragment itself, with the formation of digitiform processes. However, besides tumor cells characterized by preservation of organ specificity, gland formation, and connection with the main fragment, there is also a group of cells which, from the beginning, disturbed the integrity of the epithelial cover of the fragment, left the main fragment and lost contact with it, and until the end of culture formed no organ-specific structures of any kind. Considering that groups of cells of both these types can be found in one cultured fragment, it can be tentatively suggested that they are two populations of tumor cells with different growth tendencies. More precise conclusions may perhaps be drawn after an electron-microscopic study.

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