AUTORADIOGRAPHIC INVESTIGATION OF SCHIRROUS CARCINOMA OF THE HUMAN STOMACH BY CULTURE IN DIFFUSION CHAMBERS

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When a schirrous carcinoma of the human stomach was cultured in diffusion chambers, growth of epithelial and connective-tissue cells was observed. Unlike the original tumor tissue in vitro when the connective-tissue cells did not incorporate thymidine-H^3, during growth in diffusion chambers they were labeled. The fraction of cells with labeled nuclei 1 h after injection of thymidine-H^3 in the zone of growth averaged 25.1%, much greater than the proportion of labeled cells determined after incubation of the original tumor tissue in vitro (6.6%). Under the conditions of culture, a rapidly proliferating subpopulation of cells with a mitotic cycle 14.8 h in duration was discovered.

KEY WORDS: schirrous carcinoma of the human stomach; culture in diffusion chambers; thymidine-H^3.

The method of tissue and cell culture in diffusion chambers, implanted in vivo [6], has provided new opportunities for tissue heterotransplanation both to study the histogenesis of tumors [2, 4] and to investigate certain principles governing the proliferation of tumor cells [10, 13].

The results of culture of a schirrous carcinoma of the human stomach in diffusion chambers are described below.

EXPERIMENTAL METHOD

Tumor tissue was obtained at operation (immediately after gastrectomy) on a patient 53 years old for carcinoma of the antral portion of the stomach infiltrating all layers of the stomach wall, and with metastases in the lymph glands of the greater and lesser omentum. The histological diagnosis was schirrous carcinoma.

Diffusion chambers were made from Millipore filters (Synpore, Czechoslovakia) with a pore diameter of 0.1-0.3 μ. Two or three pieces of tumor tissue measuring 0.5-1 mm were placed in each chamber. The chambers were implanted in the peritoneal cavity of C57BL mice weighing 20-23 g. On the 6th day after transplantation the mice received an injection of thymidine-H^3 (specific activity 11.2 Ci/mole) in a dose of 1 μCi/g body weight. The animals were killed after 1, 2, 4, 7, 10, 14, 18, 22, and 24 h. A group of mice received thymidine-H^3 every 4 h for 24 h and these animals were killed 1 h after the last injection. The filters were fixed in 96° ethanol, coated with type M photographic emulsion, and exposed for 1 week at 4°C. Subsequent treatment of the autoradiographs was carried out in the usual way. The parameters of proliferation were determined for all cells in the zone of growth together. The original tumor tissue also was studied autoradiographically in vitro by incubation of pieces of the tumor in medium containing thymidine-H^3 (1 μCi/ml medium) for 1 h at 37°C.

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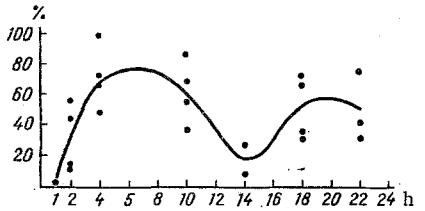

Fig. 1. Change in number of labeled mitoses with time. Abscissa, time after single injection of thymidine-H^3 (in h); ordinate, number of labeled mitoses (in %).

EXPERIMENTAL RESULTS

On the 6th day of culture a well-marked zone of growth, formed by epithelial and connective-tissue cells of fibroblast type with epithelial cells predominant, was present around most of the grafts. The epithelial cells were large in size, and their nuclei, with a delicate chromatin network, varied greatly in size. Lymphocytes and plasma cells also migrated from the graft. The mitotic index varied depending on the time of day from 15 to 21 $^0/_{00}$, with no marked rhythm. In some areas of the zone of growth the mitotic index varied from 3 to 34 $^0/_{00}$.

Autoradiographic investigations showed that the proportion of cells synthesizing DNA 1 h after the injection of thymidine-H^3 averaged 25.1% (18.8-35.1%). The index of labeling, determined in vitro, was much lower – it averaged 6.6% (6.5-7.1%). In the original tumor tissue, studied in vitro, only the cells of the tumor parenchyma were labeled, and no incorporation of thymidine-H^3 into connective-tissue cells was observed.

Not only the epithelial cells, but also cells of fibroblast type were labeled in the diffusion chambers. After repeated injection of thymidine-H^3 the fraction of labeled cells in the zone of growth averaged 77.6% (62.1-86.6%).

The duration of the phases of the cell cycle was determined by graphic analysis of the curve of labeled mitoses [11] (Fig. 1). The total duration of the mitotic cycle was 14.8 h, the period of DNA synthesis 8.2 h, the presynthetic period 3.8 h, and the premitotic period 2.8 h. The fact that the level of labeled mitoses did not reach 100% indicates heterogeneity of the population for parameters of the cell cycle. The resulting curve characterized only the most rapidly proliferating fraction of cells of the particular tumor.

The results of these experiments are closest of all to those obtained for carcinoma of the human cervix uteri, for which the duration of the mitotic cycle was 14.3-15.5 h, and the period of DNA synthesis 9.3-11.8 h [7]. For some other human solid tumors the duration of the mitotic cycle and the duration of the period of DNA synthesis, determined from the curve of labeled mitoses, were 21-44.5 and 5.3-24 h respectively [9, 12, 14]. The duration of the mitotic cycle in a monolayer culture of carcinoma of the human stomach after prolonged subculture was 41.2 h and the duration of the period of DNA synthesis 12.9 h [3]. A longer duration of the cycle (72 and 120 h) and the S-period (20 and 32 h) were observed in carcinoma of the stomach for a cell population kept in ascites fluid [8]. In that case the longer duration of the cell cycle is possibly explained by the fact that cells kept under evidently unfavorable conditions of existence were studied.

An intensively proliferating subpopulation of cells of the gastric carcinoma with a mitotic cycle of 14.8 h was thus revealed in the diffusion chambers. This may correspond to the intensity of proliferation of tumor cells that takes place in vivo. However, the possibility cannot be ruled out that tumor cells proliferating relatively slowly in vivo may pass through the cycle much more rapidly during growth in diffusion chambers and that many cells may leave the resting state and embark upon the cycle [5]. The increase in the number of cells taking part in the cycle during cultivation of some experimental tumors in diffusion chambers compared with the original tumor has been demonstrated [10, 13].

The results of culture of certain human tumors, notably schirrous carcinoma of the breast, in diffusion chambers have been described by Evgen'eva [1, 2]. She observed as a special feature of the behavior of this tumor the territorial separation of the epithelial and connective tissues and high mitotic activity of the connective-tissue cells.

No such separation of the connective and epithelial tissue was observed in the present experiments during culture of the schirrous carcinoma of the human stomach, possibly because of the shorter times of observation. Growth was mixed, and connective-tissue cells of fibroblast type in the chambers synthesized DNA, in agreement with Evgen'eva's observations of high mitotic activity of the connective-tissue cells of schirrous carcinoma of the breast. Proliferative activity of the zone of growth in the present experiments was analyzed as a whole. The parameters of the cell cycle as determined reflect mainly the behavior of epithelial cells, for they were more numerous in the zone of growth.

During cultivation of schirrous carcinoma of the human stomach in diffusion chambers an increase was found in the number of cells taking part in the cell cycle compared with the original tumor tissue. Unlike the original tissue, in which the stromal cells did not synthesize DNA, in the diffusion chambers connective-tissue cells of fibroblast type were labeled.

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