

STIMULATING ACTION OF SOME CHEMICAL CARCINOGENS ON ORGAN CULTURES OF THE STOMACH

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In numerous experiments on organotypical culture carried out in the writers' laboratory, practically all substances used had some effect or other on cultures of embryonic tissues of laboratory animals. However, known carcinogens had the most marked action, and under their influence, first, hyperplastic changes were observed in the explants, and benign tumors in the case of certain carcinogens [7] and second, stimulation of growth [6] took place. In all series of experiments the experimental cultures survived longer (about 30%) than the controls [1-3].

This paper gives the results of measurement of the relative increase in the zone around explants of embryonic rat stomach after transplacental exposure to the chemical carcinogens benz(a)pyrene (BP) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). It will be recalled that the latter induces tumors specifically in the stomach. A simultaneous morphological study was made of control and experimental explants.

EXPERIMENTAL METHOD

BP and MNNG were given to noninbred female rats starting from the 16th day of pregnancy. BP was injected as a single dose of 25 mg per rat intraperitoneally, whereas MNNG was given perorally 4 times in a total dose of 60 mg per rat. On the 19th-20th days of pregnancy the animals were killed and the stomachs of the embryos were explanted in organ culture. Organ cultures of embryonic stomach of intact noninbred rats at the same period of embryogenesis served as the controls to both series. The method of culture was that normally used in the writers' laboratory [2]. Millipore filters of the AUF5 type, with a pore size of 0.6-0.9 μ , were used as the supporting substrate, and five or six fragments of embryonic stomach were explanted on them. The filters were mounted on tantalum grids, placed in deep watch glasses, into which nutrient medium was poured. The duration of culture was 17 days. Explants were studied at different times of the experiment in total preparations made after fixation of the cultures in 70° alcohol and staining with hematoxylin.

The explants themselves and zones of growth around them were measured with an ocular micrometer. The relative increase in area of the zone of growth, in per cent, was calculated by the equation $(\Delta S/S_K) \cdot 100$, where ΔS is the area of the zone of growth and S_K the area of the transplant. This value, obtained at different times of culture, characterized the stimulating action of the carcinogens tested compared with the control. Altogether 76 control and 166 experimental explants (101 with MNNG and 65 with BP) were studied.

EXPERIMENTAL RESULTS

During the course of culture proliferation of cells and their spread from the explanted fragment over the surface of the Millipore filters were observed in the control explants of embryonic rat stomach. These cells formed sheets or membranes distinguished, as a rule, by the monolayer arrangement of the constituent cells. Among large cells with a round or oval nucleus, individual long cells could be distinguished. Starting from the 11th day of explantation necrotic zones appeared in the monolayers, and by the 17th day these occupied the greater part of individual explants. As a result, toward the end of the 2nd week of culture, 35 of the 76

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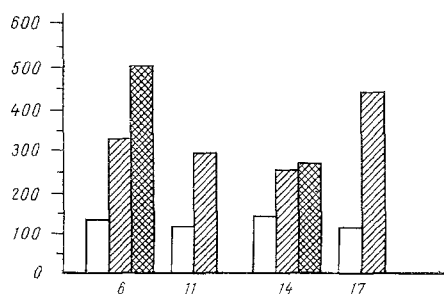


Fig. 1. Diagram of relative increase in area of zones around explants. Abscissa, times of culture (in days); ordinate, increase in area of zones (%). Unshaded columns — control, obliquely shaded — action of MNNG, cross-hatching — action of BP.

explants (46%) still survived. In the control explants growth of connective tissue was observed in only 11.8% of cases. This effect, according to Khlopin [4], is due to the fact that the proliferating epithelial cells cause death of the connective tissue a more or less short time after the beginning of explantation.

Investigation of explants exposed to the transplacental action of MNNG also revealed epithelial monolayers in them. In some cases their character was similar to that of the control, whereas in others the cells forming them were polymorphic in character: from small cells with a long or round nucleus, to larger cells. In these monolayers it was difficult to identify the predominant type of cells.

Further study of the explants showed that their survival rate was higher than in the control. In the control, for instance, 46% of explants formed a zone of growth consisting of sheets of epithelial cells, whereas in the experiment with MNNG the number was 65.3% ($P < 0.01$). The number of explants showing growth of connective tissue was practically indistinguishable from the control, namely 4.8% ($P > 0.05$).

In the experiments with BP wide zones of growth were observed in the explants, and their number also was higher than in the control, namely 64.6% ($P < 0.05$). By contrast with the control, a characteristic feature of the epithelial membranes was loosening of the structure of the peripheral areas of the zone of growth and the formation of cavities on its free border. Distinctive rosettes or nests, structures composed of paler cells with round nuclei, could be seen in the epithelial membranes formed from densely packed epithelial cells. At the periphery of the epithelial sheets growth of connective tissue was frequently (in 49.2%) observed ($P < 0.01$), whereas in the control the corresponding figure was 11.8%.

The results of the morphological study of cultures exposed to the transplacental action of MNNG and BP can be summarized by the statement that embryonic rat stomach tissue possesses high sensitivity to these carcinogens. This was manifested as a higher survival rate of the experimental cultures and characteristic morphological changes which were not found in the control.

The results of measurements made in the control on the 6th, 11th, 14th, and 17th days of explantation are given in Fig. 1. The diagram shows that the relative increase in area of the zones around the fragments in the control was at about the same level throughout the period of culture and varied only between 120 and 147%. Meanwhile, the relative increase in size in explants exposed to the transplacental action of MNNG was on average 2.5-3.5 times greater than the control measurements at the same times of culture, and in the case of transplacental exposure to BP the greatest increase of all was observed on the 6th day of culture, namely 503% ($P < 0.001$), compared with the control level of 129%.

The results of these measurements (Fig. 1) thus indicate a stimulating action of the test carcinogens on embryonic stomach tissue.

The quantitative results of the stimulating action of the carcinogens are in agreement with those of previous morphological studies [5, 9] and they are confirmed by later work with organotypical culture [7, 8]. The question of the stimulating action of chemical carcinogens requires further study. The use of the method of organotypical culture of different embryonic organs, including fragments of stomach, on Millipore filters followed by quantitative measurement may prove to be useful.

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