

CHANGES IN THE MORPHOLOGY OF CONNECTIVE TISSUE EXPLANTATES IN RATS TREATED WITH METHYLCHOLANTHRENE AND EXTRACT FROM A HUMAN LUNG TUMOR

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That malignant change may occur in tissues outside the body must be regarded as proven. Evidence of this is given by the work of Earle and his co-workers [20], A. D. Timofeevskii and his co-workers [14-18], and others. The question of the direct causes of malignant change in explantates, however, remains open. In A. D. Timofeevskii's opinion, malignant change of tissues regularly takes place in explantates only after the combined action of carcinogenic substances and tumor-producing viruses, which could be demonstrated experimentally [2-6, 14-18]. It was shown, moreover, that malignant change may arise after the action of an extract from certain tumors, the virus nature of which was not proven [6]. A similar effect was observed after the action of an extract of a human lung carcinoma on a connective tissue culture in rats [10]. The observations mentioned above, along with others, suggest that in certain tumors there is a virus-like principle which causes malignant change in the tissues. On this basis the problem of the virus nature of neoplasms, including tumors in man, could be considered.

According to certain reports [19, 21], in a number of human malignant tumors, globular corpuscles are found with considerable regularity, and revealing definite virus-like properties [1, 7, 8, 9, 11, 12, 13]. The study of the malignant effect of this agent on connective tissue explants is of definite interest.

In our experiments we investigated the effect of the combined action of methylcholanthrene and an extract of a human lung carcinoma on connective tissue explants of rats.

METHOD

For the experiments we used intensively growing connective tissue cultures from newborn rats of the "Wistar" line. Cultivation took place in Carrel flasks by the

usual method adopted in laboratories under the direction of A. D. Timofeevskii. During subculture, each explantate was cut in two, and the halves were placed in different flasks. In this way six groups of cultures were obtained, of which one grew without any form of interference in the ordinary nutrient medium. The second group was treated seven times with methylcholanthrene in horse serum in a dose of $1 \gamma / \text{ml}$ of nutrient medium. The third and fourth groups grew in a medium to which was added on two occasions (at intervals of one month) extracts from two different human lung tumors (in the third group, crude extracts, in the fourth group, extracts heated to 60° for 2 hours which, according to data in the literature, inactivates many viruses). As shown by investigations under the electron microscope, considerable numbers of globular virus-like corpuscles, measuring 50-70 μ , were found in both tumors. The fifth and sixth groups of cultures were preliminarily treated seven times with methylcholanthrene, and then treated twice with extracts of human lung carcinomas; in the first of these two groups crude extracts were added, and in the second, heated extracts. In our experiments we thus had one experimental group of cultures (methylcholanthrene and crude extract from a carcinoma of the lung) and five control groups. In the process of the investigation a morphological study was made of the experimental and control explantates. The cultures were fixed in copper acetate by Shabadash's method and stained with hematoxylin by Weigert's or Heidenhain's method.

RESULTS

The investigation of living cultures and stained to-tal preparations showed that the first two groups of cultures, grown in an ordinary medium and treated with methylcholanthrene, were almost morphologically indistinguishable from each other (Fig. 1). The effect of



Fig. 1. Zone of growth of a control culture of rat connective tissue (38th transplantation, 12 days). Stained with Weigert's hematoxylin. Magnification 130x.

methylcholanthrene was usually apparent in the first subculture after treatment. In this case growth of the cultures was slightly retarded and in the zone of growth were observed rounded cells and individual disintegrating cells. After transplantation of the cultures into fresh nutrient medium, growth of the explantates was restored, and the zone of growth acquired its usual appearance. After complete cessation of the action of the carcinogen, the cultures grew in the ordinary nutrient medium; their growth became slightly more intensive than that of the cultures not treated in any way. The zone of growth of the explantates became thicker, the cells were more closely packed together, and among them were occasionally observed multinuclear giant cells, although these discoveries were not constant (Fig. 2).

The third and fourth groups of cultures, treated with crude and heated extracts of human lung carcinomas, were morphologically indistinguishable, after observations lasting over 20 months from explantates grown in ordinary nutrient medium and untreated in any way.

Highly significant morphological changes were observed in the fifth and sixth groups of explantates, after the combined treatment with methylcholanthrene and extracts (crude and heated) of human lung tumors. In the first months after treatment the cultures were indistinguishable from the controls, but after four months these cultures showed a more intensive growth; their zone of growth became so thick that it was readily detected with the naked eye.

As a result of the close packing of the cells, the outline of the edge of the zone of growth was clearer and better defined. The individual cells appeared rounder and more flattened; some of them were without

processes and, for this reason, they acquired an epithelial-like form. At the edge of the zone of growth giant cells were found, with a homogeneous and, sometimes, foamy cytoplasm. In these cells could be observed a single, hypertrophied nucleus or 4-7 (or more) nuclei, usually not arranged in any definite order, but occasionally in palisade fashion. The nuclei appeared juicy, and regularly contained 3-5 large nucleoli. Most of the cells of the zone of growth showed signs of a considerable polymorphism. Figures of mitotic division were very often found in the cultures, and from time to time these were atypical. Cells in a state of direct division were often found.

At the 10th-12th month of observation, the morphological picture described became more constant and was found in nearly all the cultures. During further cultivation these changes became a regular feature. The zone of growth of certain cultures after growth for 20 months consisted of a collection of giant, multinuclear cells, separated from each other by thin bands of atypical fibroblast-like cells.

The combined action of methylcholanthrene and human lung carcinoma extract thus caused characteristic changes in rat connective tissue cultures, which gave evidence of malignant change in the cultures. The specificity of the morphological changes described was demonstrated by the earlier work of A. D. Timofeevskii, S. V. Benevolenskaya, and M. T. Mogila, showing that the inoculation of cultures with analogous morphological changes is accompanied, as a rule, by the development of tumors in the animals at the site of inoculation. Of some interest is the fact that malignant change occurred in the cultures after the action of heated as well as crude extract. The findings of Gross [21], however,

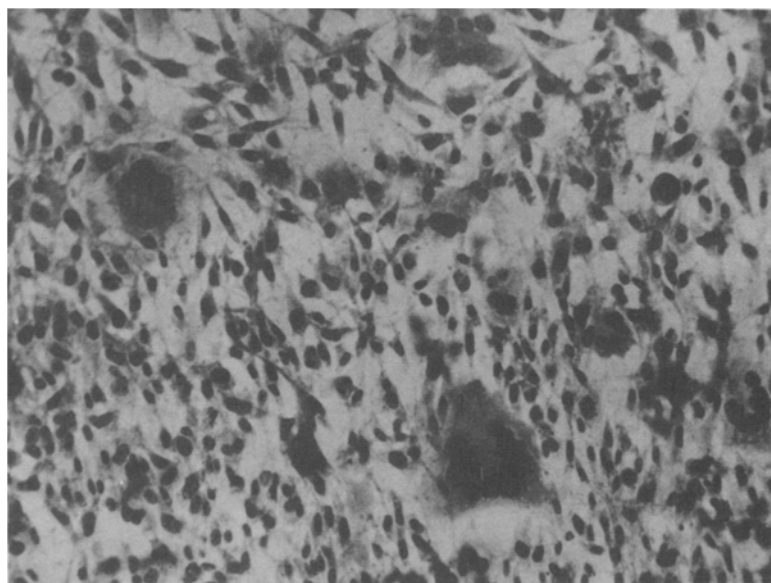


Fig. 2. Zone of growth of a culture of rat connective tissue, treated 20 months beforehand with methylcholanthrene and an extract of a human lung carcinoma. Stained with Weigert's hematoxylin. Magnification 130x.

show that not all viruses lose their activity even after heating at 68°. It may be supposed that, in our experiments, heating for two hours to 60° did not deprive the tumor extract of its biological properties. For this reason the experiments with crude and heated extracts could evidently be regarded as identical.

SUMMARY

The authors studied the morphology of connective tissue explants in rats subjected to the combined effect of methylcholanthrene and extract of cancer tissue of human lung.

In 4 months and more, stable changes of the growth zone structure were revealed, as well as a large number of multinuclear giant cells.

These changes were retained during culture for two years.

It is suggested that malignant change occurred in the connective tissue cultures of rats.

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