6. R. V. Petrov and R. M. Khaitov, Radiobiologiya, No. 1, 69 (1972).
7. G. De Moor, O. Steeno, M. Raskin, et al., Acta Endocrinol. (Copenhagen), 33, 297 (1960).
8. O. Vos, W. A. Buurman, and R. E. Ploemacher, Cell Tissue Kinet., 5, 467 (1972).

ORGAN CULTURES OF RAT EMBRYONIC BRAIN TISSUE (HIPPOCAMPAL REGION) IN THE STUDY OF THE TRANSPLACENTAL ACTION OF NITROSOETHYLUREA

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The transplacental action of nitrosoethylurea (NEU) was studied in organ cultures of embryonic rat brain tissue (from the region of the hippocampus). Brain tissue is distinguished by high sensitivity to NEU. This was manifested as a higher rate of survival of the experimental cultures compared with the control and as the appearance of foci of proliferation of the epithelium of the vascular plexus, in some cases resembling adenomas. The morphological changes observed in the experiments depended quantitatively on the dose of carcinogens.

KEY WORDS: transplacental carcinogenesis; organ cultures; nitrosoethylurea; hippocampus.

Ever-increasing attention is being paid to the study of transplacental carcinogenesis. On the one hand, it offers a method of studying fundamental problems in oncology, and on the other hand it may be of direct clinical importance, as observations starting in 1971 have shown [6]. In the writers' laboratory investigations into the nature of transplacental carcinogenesis both *in vivo* and *in vitro* in organ cultures have been in progress for many years [4].

The object of this paper is to describe the results of organ culture of embryonic rat brain tissue from the hippocampal region; it is in this part of the brain that tumors are found most frequently after transplacental exposure of the progeny to nitroso-compounds and, in particular, to nitrosoethylurea (NEU) [7].

EXPERIMENTAL METHOD

NEU was injected intravenously in doses of 30 and 60 mg/kg into noninbred female albino rats during the last week of pregnancy. The compound was synthesized by Candidate of Chemical Sciences O. A. Pan'shin, working in the writers' department. The animals were killed on the 20th day of pregnancy and the brain of the embryos, from the hippocampal region, was used for organ culture. The method of culture suggested by Chen [5] and modified by Adil'gireeva [1] was used. The nutrient medium consisted of two parts medium No. 199, one part calf serum, and one part chick embryonic extract, and contained 0.3% glucose. This concentration of glucose has the most favorable effect on nerve tissue cultures [8, 9]. The duration of culture in the present experiments was 18-20 days. Every 3-4 days some of the cultures were removed for fixation (in Bouin's fluid and 10% neutral formalin), and after further histological treatment serial sections of the explants were stained with hematoxylin-eosin and by Van Gieson's and the Bielschowsky–Gros methods. Altogether 196 control and 252 experimental explants were studied.

EXPERIMENTAL RESULTS

The morphological structure of the organ in control explants of embryonic brain remained throughout the period of culture characteristic of the original embryonic hippocampus and

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neighboring regions. For instance, both principal types of nerve cells were observed in the test cultures: granular and pyramidal cells, formed by the corresponding differentiated layers. The ependymal and subependymal cells were represented by large cell concentrations in the form of distinctive rosettes, in which mitoses were found. The so-called epithelial covering cells (according to Mikhailov [2], of neural origin) surrounded the developing vascular plexus. All the main components of the hippocampus were thus preserved in the explants and in their ordinary relationships.

In the course of culture the brain explants passed through a definite cycle of changes, which the writers described previously in papers on organotypical culture of embryonic kidney and lung tissue [4]. However, in the present investigations some differences from the previous patterns were observed. One difference was the absence of an epithelial or connective-tissue capsule surrounding explants. The absence of a capsule led in turn to changes in the shape of the cultures during explantation. For instance, instead of rounding of the fragments on account of the epithelial or connective-tissue capsule, which could be observed in cultures of kidney and lung tissue, the brain explants were flattened and increased in area. Instead of a capsule, at the periphery of the cultures there was a distinctive zone of connective-tissue cells, fibroblasts, and macrophages, loosely arranged in it.

By the end of the second and beginning of the third weeks of culture, many of the cells in the embryonic brain explants died, with the formation of an extensive zone of necrosis occupying their central part. By the end of the third week, of 196 control explants only about 52% still survived. The longest-living cells were ependymal. Pyl'dvere [3] describes the extraordinary endurance of old ependymal cultures, which could be kept alive without subculture for up to 3 months.

Having obtained the organ cultures of embryonic rat brain tissue from the region of the hippocampus, they could be used to study the transplacental action of NEU.

The results of these experiments showed that following exposure of the organ cultures of the hippocampus to NEU their rate of survival was higher than in the control. In control explants only 51.9% survived after three weeks of culture, compared with 82% in the experiments with NEU, which was significantly higher ($P < 0.001$).

Definite morphological changes, mainly starting from the 2nd week of culture, were observed in the experimental explants. They mainly affected the epithelium covering the vascular plexuses of the lateral ventricles and in close contact with the hippocampus. The epithelium showed foci of proliferation with the formation, initially of small, and later of larger, cysts. Papillary formations, projecting into the lumen, grew from the lining of the cysts. Later, adenomatous structures, in places papillary in character, formed from these foci of proliferation. Neoplasms similar to adenomas thus appeared (Fig. 1a, b) similar to those found, for example, in the kidneys and derived from the epithelium of the glomerular capsules.

Later, in the experimental explants the cells of the ependyma and subependymal layer sometimes proliferated to form characteristic layers and to obliterate the normal structure of the hippocampus. It can tentatively be suggested that cells of the ependyma and vascular plexuses were most sensitive to the carcinogenic action of NEU. These phenomena were not found in the control explants. Whereas in some places the covering cells of the vascular plexuses also began to proliferate, this was observed much less frequently (1% compared with 9-28%) and was less marked. The morphological changes observed in the experimental explants depended quantitatively on the dose of the carcinogen. For instance, after NEU in a dose of 31 mg/kg they were found in 11 of 120 explants, i.e., in 9.1% ($P < 0.01$), whereas with a dose of 60 mg/kg they were found in 37 of 132 explants, i.e., in 28% ($P < 0.001$).

To sum up the results of organotypical culture of embryonic rat hippocampal tissue in the study of the transplacental action of a carcinogenic nitroso-compound it must be emphasized that embryonic rat tissue is highly sensitive to NEU. This was manifested as a higher rate of survival of the experimental cultures compared with the control and as the appearance of distinctive foci of proliferation of the epithelium of the vascular plexuses, which, in some cases, resembled adenomas.

As the writers' previous investigations showed [4], organ cultures of the lungs and kidneys can be used with success for various investigations. The known stages of precancer can be studied in them, just as in experiments on animals. However, under explantation conditions these lesions arise after a shorter time than in adult animals. This result may depend

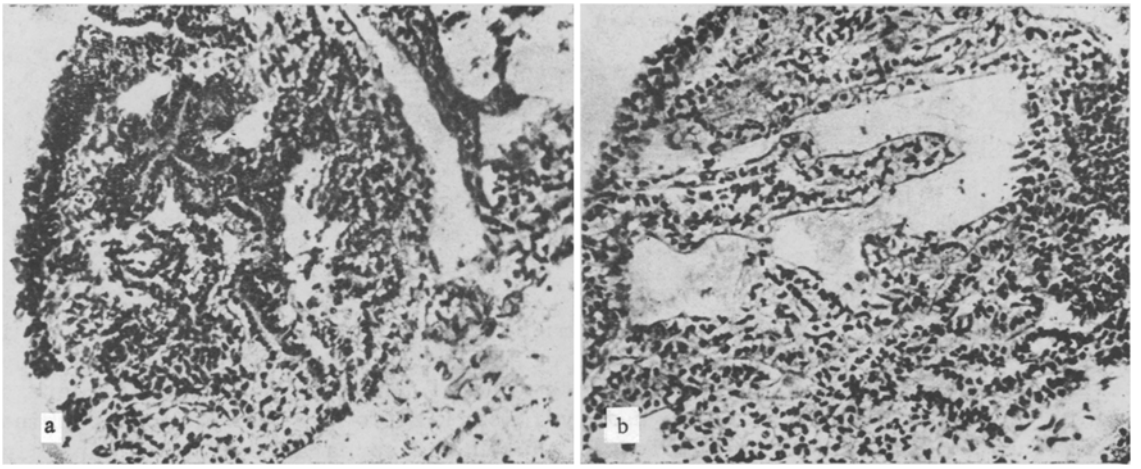

Fig. 1. Papillary-adenomatous foci of proliferation of epithelium of vascular plexus (rat receiving NEU, 60 mg/kg). Hematoxylin-eosin, 150 times.

both on the greater sensitivity of embryonic tissue to carcinogenic influences and on the fact that culture itself creates unique conditions for growth. To the list of experimental models containing organ cultures of embryonic lung and kidney tissue another can now be added, namely organotypical cultures of the embryonic hippocampus.

LITERATURE CITED

1. R. Kh. Adil'gireeva, Byull. Éksp. Biol. Med., No. 4, 115 (1964).
2. V. P. Mikhailov, Dokl. Akad. Nauk SSSR, 12, 121 (1938).
3. K. I. Pyl'dvere, Arkh. Anat., No. 9, 58 (1957).
4. L. M. Shabad, T. S. Kolesnichenko, and Yu. D. Sorokina, Transplacental Carcinogenesis and Organ Cultures [in Russian], Moscow (1975).
5. J. M. Chen, Exp. Cell Res., 7, 518 (1954).
6. A. L. Herbst et al., New Engl. J. Med., 284, 878 (1971).
7. S. Ivankovic, in: Transplacental Carcinogenesis, Lyon (1973), pp. 92-99.
8. C. M. Pomerat and J. Costero, Am. J. Anat., 99, 211 (1956).
9. O. Suyeoka, Exp. Cell Res., 47, 222 (1967).