ONCOLOGY

EXPERIMENTS ON HETEROTRANSPLANTATION OF HUMAN TUMORS INTO CORTISONE-TREATED RATS AND HAMSTERS

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Although attempts at transplantation of human tumors were begun a very long time ago, it is only very recently that repeated passage of such tumors through animal hosts has been successful. It is obvious that the availability of human tumor strains which have been transplanted from animal to animal for prolonged periods of time opens up wide research possibilities for workers in various fields of experimental oncology [2, 5]. It is only during the past few years that certain workers [6-8] succeeded in obtaining a few strains of human tumors which could be repeatedly passaged through animals, using cortisone to inhibit immune reaction of the host. Considerable difficulties were encountered in maintaining these strains. Thus H. Toolan [8] transplanted over a thousand human tumors into rats and hamsters, and only 5 strains emerged which could be passed repeatedly from animal to animal.

Such strains have not yet been reported in the Soviet literature. Those authors who performed heterotransplantation of human tumors into chick embryos and other animals were able to maintain the transplants for not more than a few passages [1, 3, 4].

The present paper describes the results of transplantation of 90 human tumors into rats and hamsters.

EXPERIMENTAL METHODS

Tumor tissues were obtained from the Clinical Division of the Institute of Experimental Pathology and Therapy of Cancer, AMN SSSR, and were inoculated within 30-40 minutes into the cheek pouches of golden hamsters, aged 1-2 months, or subcutaneously into 3- to 4-week-old rats. The tissue was first treated with penicillin (10,000-15,000 units per ml of physiological saline) for 10 minutes, and was then cut up into fragments with the aid of a pair of scissors, in a Petri dish. The hamsters were anesthetized with ether, the cheek pouches were turned inside out and exteriorized, a fold of mucosa was raised with the aid of a pair of forceps, cleaned with a sterile swab, and the tumor tissue suspension was injected submucosally. Cortisone was given subcutaneously. The cheek pouches were everted every 15-20 days and examined for growth of the transplant. The rats were inoculated with tumor tissue subcutaneously, in the usual way. Injections of 5 or 2.5 mg of cortisone were given to the rats and hamsters immediately after inoculation, and twice weekly thereafter.

EXPERIMENTAL RESULTS

The human tumor material transplanted into rats included the following: 31 sarcomas from various sites, 8 melanomas, 11 gastric carcinomas, 3 ovarian carcinomas, 2 mammary carcinomas, 3 seminomas, 3 laryngeal carcinomas, and 1 lung carcinoma. Slight growth was found in the first generation (during the first 10-12 days

after inoculation) for 10 sarcomas, 3 melanomas, 2 gastric carcinomas, 1 lung carcinoma, and 2 laryngeal carcinomas; in the second generation we found growth of 4 sarcomas, 2 gastric carcinomas, and 1 melanoma. Growth was, however, insignificant in all cases, and in no case could we achieve prolonged serial transmission of a tumor.

Human tumor tissues transplanted into hamsters included 13 sarcomas, 2 gastric carcinomas, 4 melanomas, 3 mammary carcinomas, 2 lung carcinomas, and 3 laryngeal carcinomas. As noted above, they were injected twice weekly with doses of 5 or 2.5 mg of cortisone.

In the first group (16 tumors, cortisone dosage level 5 mg) the hamsters died as a result of the toxic action of cortisone during the first 10 days after inoculation; in no case was any significant growth of tumor tissue observed. The dosage of cortisone was then reduced to 2.5 mg, and this resulted in a much longer survival time of the hamsters. We implanted 25 tumors at this dosage level. Slight growth of 1 of 2 melanomas and of 1 of 2 lung carcinomas was observed in the first generation; 14 carcinomas taken from different sites (including mammary, lung, and others) gave no marked growth.

The best results in this series were obtained with sarcomas. Considerable growth of 4 of 8 human sarcomas implanted into the cheek pouches was seen; one of them (spindle-cell uterine sarcoma) grew vigorously during the first 3 serial passages, but failed to grow thereafter. Two other tumors (chondrosarcoma and thymoma) survived to the 4th and 3rd generations, respectively; further passage of these tumors is in progress. One of the tumors has now been maintained by serial passage through hamsters for over 15 months, and is now in its 14th generation.

We shall give a more detailed description of the properties of this tumor, which we have designated as HT-67, as it was the 67th human tumor taken for our transplantation experiments.

A tumor, diagnosed clinically as a sarcoma of the abdominal wall, was removed surgically from the patient Sh., on May 23, 1956. Histological examination showed that it was an angiosarcoma which had infilitrated the muscle and connective tissue. The patient died 23 days after the operation. At autopsy a recurrence of the angiosarcoma was found in the anterior abdominal wall, with invasion into the abdominal wall and dissemination into the visceral peritoneum of the large and small intestines, and with metastatic deposits in the left lung.

The tumor, which was a soft, milk-white mass, was implanted immediately after removal into the cheek pouches of 5 hamsters and subcutaneously into 4 rats; all the animals were given cortisone. After a month the rats had tumors of up to 1 cm diameter; these tumors were transplanted into a fresh group of rats, but they failed to grow. Of the 5 hamsters, 3 died during 15 days after implantation, for a variety of reasons. Growth of the implant was seen in one of the survivors at the end of a month. On the 43rd day after implantation a tumor of about 1 cm diameter was present, and this was transplanted into 3 fresh hamsters; the implants all showed growth by the end of a month, and the tumor was passed serially through a succession of hamsters. The biological properties of the tumors (inoculability, rate of growth, etc.) varied fairly widely from passage to passage, in an irregular manner.

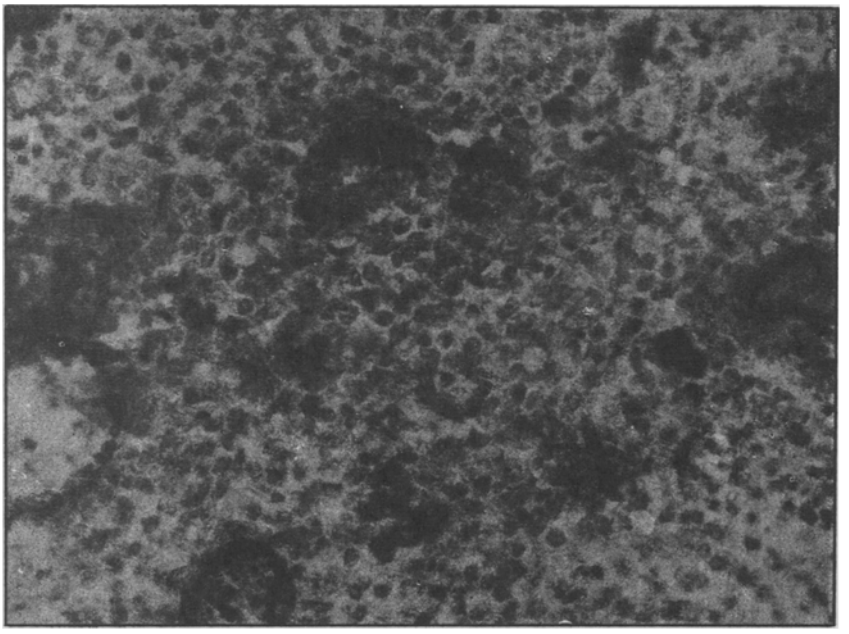
During the first 11 generations we found that 68.4% of the transplants gave rise to tumors (calculated for each cheek pouch separately). Tumors were usually taken for transplantation when they had achieved a diameter of 1-1.5 cm. In some generations this was achieved within 11-16 days, while in other animals there was a latent period of up to $2\frac{1}{2}$-3 months. The number of animals at our disposal was limited, and wastage due to various complications resulting from the toxic effects of cortisone was fairly considerable; for these reasons we have not yet been able to accumulate sufficient experimental data to justify any firm statements as to the final fate of the tumor-bearing hamsters. Individual observations showed, however, that growth of HT-67 tumor may proceed for a long time (up to 4-5 months) in the cheek pouches, and that the diameter of the tumors may be 2-3 cm or more.

We have hardly ever observed resorption of the tumors. In one case only did we find that a tenth generation tumor, which had reached a diameter of 1 cm at the end of the 3rd month of growth, underwent total resorption during the course of a few days.

Attempts at transplanting HT-67 tumor which had been passed through a series of hamsters into cortisone-treated rats were unsuccessful; tumor growth was not observed in any of the inoculated rats.

The histological structure of angiosarcoma HT-67 deviated very little from that of the original tumor

during its passages. The transplants grown in hamsters consisted of large, atypical connective tissue cells with large oval or round, faintly staining nuclei (see figure); the arrangement of these cells was in most cases a disorderly one, and only in certain parts of the tumor could we see any tendency toward formation of parallel rows of cells. In many, although not in all, of the transplanted tumors there were numerous sinusoidal dilatations of the capillaries, which were lined with atypical tumor cells; the lumina of these capillaries were filled with erythrocytes.



Histological structure of a human angiosarcoma (Strain HT-67) taken from its 12th passage through hamsters. Numerous sinusoidally dilated capillaries are to be seen. Photomicrograph. Magnification 300 ×.

Our experiments have revealed certain factors of importance in work on the heterotransplantation of tumors into cortisone-treated animals. Among such factors are, above all, the species of animal, and the site of implantation ; transplantation into the cheek pouches of hamsters gave a higher proportion of takes than did subcutaneous inoculation into rats. Moreover, even HT-67 tumor, which grew actively for a long time in the cheek pouches of hamsters, failed to grow when transplanted into rats. We cannot suggest any explanation of this difference, but it is evident that hamsters are more suitable than rats for the heterotransplantation of human tumors. The histological form of the neoplasm is also of importance; sarcomas appear to give active growth more frequently than do tumors of epithelial origin. The HT-67 tumor, which has been passed serially through hamsters, may be used for various purposes. As was noted above, this angiosarcoma retained its initial structure through numerous passages. The full elucidation of the effects of prolonged heterotransplantation on the properties of this human tumor must, however, await the results of special investigations, involving the application of cytological (study of chromosome complement), immunological, and tissue culture methods.

SUMMARY

Human malignant tumor tissues, taken from 90 patients, and including carcinomas, melanomas, and sarcomas from various sites, have been implanted into the cheek pouches of golden hamsters, and subcutaneously into rats, all cortisone-treated. Growth of the implants was found in a number of cases, but continued growth over a number of serial passages was achieved only with one tumor (an angiosarcoma) in hamsters. This tumor has been passed serially through 14 generations for over 15 months, over which time it has retained its original histological structure. It could not be transplanted from hamsters to rats.

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