

# PROTRACTED HETEROTRANSPLANTATION OF THE MOUSE TUMOR TO RATS

## ARTICLE 1: A STUDY OF THE BIOLOGICAL PROPERTIES OF MOUSE ASCITIC ADENOCARCINOMA SERIALLY TRANSPLANTED TO RATS

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(Presented by N. N. Zhukov-Verezhnikov, Active Member of the USSR Academy of Medical Sciences  
Received September 4, 1956.)

The importance of tumor heterotransplantation and the difficulties of accomplishing it are well-known. As Ehrlich [8] has already established, when a mouse carcinoma is subcutaneously implanted in rats, the tumor grows for a short time (up to 8 days), but then is resolved. In some studies [7, 10], a rather long preservation of the heterotransplanted Ehrlich's mouse tumor was obtained, but only in rats of the Hungarian strain. Tumor growth in a different type of host has been protracted by shortening the intervals between implantations of the heterogeneous carcinoma [1, 6], but even in this way, prolonged and steady development of heterotransplanted tumors could not be maintained for long. There are interesting works on the long preservation of tumors heterotransplanted from mice to newborn rats [9].

The majority of authors [3, 4, 6, 9, 10] mention that certain biological properties of mouse carcinoma are changed by heterotransplantation. However, there is as yet no definitive explanation of this question.

Our purpose was to obtain the prolonged development of a mouse ascitic cancer tumor heterotransplanted to rats and to study the immunobiological properties of the tumor during the implantations.

### EXPERIMENTAL METHODS

Three to five-day-old rats were intra-abdominally inoculated with a dose of 0.2 ml of Ehrlich's mouse adenocarcinoma (ascitic type). A considerable increase in the bulk of the animals was observed in the rats on the 3rd-6th day after implantation, and the rats died on the 6th-8th day. Material for subsequent implantations was obtained in the usual way under sterile conditions and bacteriologically inspected; at least 20 rats weighing from 6 to 9 g were used for each implantation (passage). When the increased volume of the rats' stomachs had reached considerable proportions, which usually occurred on the 3rd-6th day of the implantation, all the rats were injected with 1 ml of a physiological solution (0.85% NaCl) with penicillin (about 10,000 units); then, 2 ml or more of the ascitic fluid, thus diluted, was carefully pumped into a syringe. The ascitic exudate from the rats, even when diluted, was a rather dense suspension of pink or grey (depending on the erythrocyte content) tumorous cells. The material obtained was injected into the celiac cavity of normal rats in a dose of 0.2-0.4 ml.

The implantations of the mouse ascitic cancer in the 3-5-day old rats reached a frequency of 100%. In the last passages, the amount of tumorous cells per 1 ml of ascitic fluid fluctuated, although the amount was still rather considerable. It was 40 million cells in the 92nd passage and 66 million in the 108th passage, whereas, in ordinary mouse ascitic cancer, the amount of cancerous cells as a rule fluctuates between 99 and 120 million.

The cells were counted in a chamber with a Goryev's grid. The development of ascitic cancer in the

celiac cavity of the rats was also histologically proven. Smears of the ascitic exudate from the rats were fixed for one hour in a mixture of alcohol-ether and then stained with hematoxylin-eosin.

Five to eight white male mice weighing from 18-22 g and intra-abdominally inoculated with the tumor were used as the biological control for each passage.

We inoculated 2-week old or older rats and mice intra-abdominally with the passage cancer as an additional test to determine the virulency of the tumor in the passages. In these experiments, the implantation dose of ascitic cancer was 0.2-0.4 ml per 8 g of weight for the rats and 0.1-0.2 ml for the mice.

A total of 4,000 rats of various ages and 2,000 mice were experimented upon.

In this work, we shall present the general results of the observation from all 400 experiments.

## EXPERIMENTAL RESULTS

The first successful experiments transplanting Ehrlich's ascitic mouse cancer to the celiac cavity of rats were done in May-June 1954. At that time, we succeeded in transplanting the mouse tumor to rats for five passages with no decrease in the growth intensity of the tumor when it was reversely transplanted to mice. Further transplantations were impossible, however, due to the death of the experimental rats.

In April, 1955, we again transplanted Ehrlich's ascitic mouse cancer to rats intraperitoneally. This time, in view of the real danger of losing the strain, we conducted 2-3 parallel series of transplantations, using 15-30 animals, aged from 3-6 days, for each passage. More than 100 passages have now been made in the rats.

Twice, during 16 months of transplanting mouse tumors to rats, we were obliged to reimplant the tumor in the mice (after 30 and 73 passages) before we could conduct further passages to the rats.

These reverse passages to the mice were not reflected in the properties or behavior of the strain, as subsequent transplantations showed. The tumor grew intensively in the celiac cavity of the rats, which was indicated by the increase in the volume of the ascitic fluid and by the numerous mitotic forms in tumor cells.

We were very interested to find whether the immunobiological properties of mouse carcinoma changed after such prolonged heterogeneous transplantations of the tumor. The results obtained from our examination of the antigenic properties of mouse carcinoma cells after transplantation to rats will be presented in the next article. Certain changes in the biological properties were observed as follows.

The external aspect of the ascitic exudate changed gradually during the process of transplanting mouse cancer to 3-5-day-old rats; after 52-58 passages, a more fluid ascitic exudate, grey in color, in which white flakes occurred rather frequently (conglomerations of cancerous cells), began to replace the thicker, pink or cherry-colored exudate. In addition, when the rats were dissected, a thin, whitish film began to appear at about the same time on the organs of the celiac cavity (liver, spleen), which also consisted of tumorous cell conglomerations. However, in some of the rats, the ascitic exudate which developed was different, rather thick and grey without the flakes or films; this exudate was also used for further implantations. During this same period, we increased the inoculation dose from 0.2 to 0.4 ml and shortened the interval between implantations from 5-6 to 3-4 days. Tumorous cells of Ehrlich's mouse carcinoma were constantly found in the smears of the rat ascitic exudate.

When the ascitic cancer was transplanted back to the mice, we observed that 1-40 generations caused the amount of exudate to increase (to 9-10 or more ml) and that the mouse died, on the average, 10 to 14 days after the implantation. After 41-45 generations, the control mice, on the average, died sooner (on the 8th-12th day), but the accumulation of the ascitic cancer exudate was very slight (0.5-1.5 ml and less than 3 ml). The volume of cancerous exudate was also observed to decrease in further passages of the heterotransplanted tumor to mice; when the number of passages to rats (8, 20, 30, 44, 55 generations) increased, the amount of tumor implantations in mice (16, 11, 5, 4, 3 passages) decreased. In the last passages to the mice, no ascitic exudate was observed to develop in the mice, although the animals died.

Implantations of ascitic cancer to the celiac cavity of rats 2 weeks old or more were observed to first increase and subsequently to decrease the virulency of the tumor to more adult rats.

In contrast to the mice, the rats all survived the last passages, and the tumor was resolved.

Therefore, a new method of protracted mouse tumor heterotransplantation to rats has been developed

which permits more than 110 implantations of Ehrlich's ascitic carcinoma to be done within the celiac cavity of young rats (chiefly, of rats from 3-5 days old).

The heterotransplantate grew and developed like the original mouse carcinoma in both mice and week-old rats, retaining the basic morphology of the original Ehrlich's strain. However, protracted existence in heterogeneous surroundings caused some change in certain biological properties of the mouse ascitic carcinoma. For example, heterotransplantation gradually caused the tumor to lose one of the main features of mouse carcinoma, i.e., the ability to form a large amount of ascitic fluid with the implantation of tumorous cells in the celiac cavity of mice; the tumor became less virulent in relation to rats aged 2 weeks or more (after the 50th generation) and its rate of growth decreased in both rats and mice; the outward appearance of the ascitic cancer exudate in the young rats changed (after 52-58 passages). Therefore, the heterogeneous strain of the tumor cannot be considered identical to the original Ehrlich's ascitic mouse carcinoma.

#### SUMMARY

Over 100 intra-abdominal heterotransplantations of Ehrlich's ascitic adenocarcinoma to 3-5-day old rats were performed. Although the essential nature of the initial ascitic cancer was preserved for 16 months, some of its biological effects on mice and rats were altered, e.g., the mice did not form a large amount of ascitic fluid. In rats two weeks old and older, the growth rate of the heterogeneous implantations decreased from that of the original virulent mouse tumor.

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