ON CERTAIN BIOLOGICAL PROPERTIES OF MOUSE TUMORS SUBJECTED TO HETEROTRANSPLANTATION FOR PROLONGED PERIODS OF TIME*

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The question of preservation or change in the biological, cytological, and antigenic properties of tumors, associated with heterotransplantation, is a controversial one. A number of works [1, 12, 22, et al.] have emphasized the minimal changeability of tumors. Other investigators have concluded that during heterotransplantation tumors significantly alter their biological properties, morphology, and antigenic structure [7, 19, et al.].

In our previous work [2], we noted that a property of tumors such as sensitivity to chemotherapeutic preparations remains essentially unchanged during heterotransplantation. The subject of the experiments described in this work was the first generation of heterogenic tumors in animals. The results of the experiments justified the use of first generations of heterotransplanted tumors as subjects for chemotherapeutic investigation.

This work was devoted to studying a number of biological properties of mouse tumors subjected to heterotransplantation for prolonged periods of time.

EXPERIMENTAL METHOD

In the course of 130-140 days, the following mouse tumors were transplanted subcutaneously to rats treated with cortisone: carcinoma RSM, sarcomas 298 and 180 (Crocker). Transplantation to a new generation was carried out within 7, 8, and 9 days. Each of the strains was passed in rats 15-16 times.

In the experiments of this series we used 330 non-pedigreed rats, 3-5 wk of age. Characterization of the strains of mouse tumors is contained in the work of E. E. Pogosyants [8]. Cortisone was injected into the rats subcutaneously, twice a week, using a dose of 2.5 mg per animal.

In the therapeutic experiments, we employed embichin [16], sarcolysin [6], ftormezin [5]†, phenamet [11], thio-TEPA [14] and benzodet [13].† The results of these experiments were evaluated on the basis of a comparison of the changes in the mean tumor diameter in the control and treated animals, the mean weight of the tumors at the end of the experiment (calculation of the percent of tumor growth inhibition in the treated animals), and from a microscopic study of the transplants. There were at least 10 rats in each of the experimental and control groups; a total of 150 animals was used in this series of experiments. The results of the experiments were analyzed statistically, according to the method of Student. Control chemotherapeutic experiments were set up on non-pedigreed mice (40 animals), mice of the C57 line (30 animals), and mice of the C_3HA line (38 animals).

EXPERIMENTAL RESULTS

Crocker's Mouse Sarcoma. The tumor did not grow in rats that were not treated with cortisone. This property did not change, even after passage of 15 generations in rats that had received cortisone. Usually, the growth of sar-

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†Ftormezin = N-(p-fluorobenzoyl)-N', N'-diethylenephosphoric triamide (A-23 in Russian Drug Index of National Library of Medicine); benzodet = N-benzoyl-N', N"-diethylenephosphoric triamide (A-16). The identification of ftormezin is not completely certain [Publisher's note].

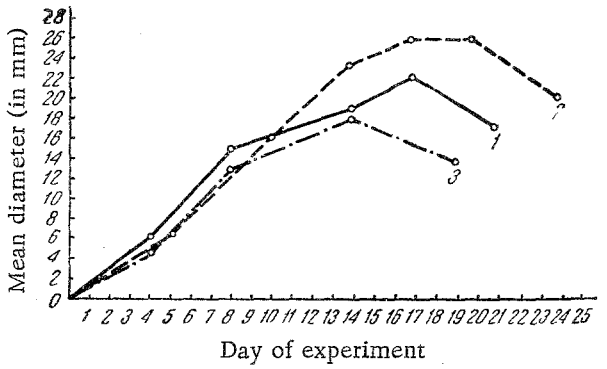

Fig. 1. Changes in the mean diameter of Crocker's sarcoma during growth in rats treated with cortisone. 1) 1st generation; 2) 10th generation; 3) 15th generation.

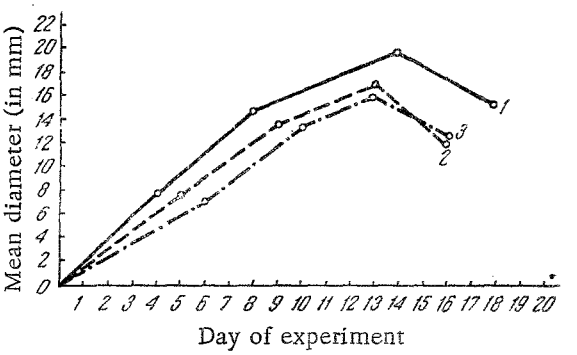

Fig. 2. Changes in the mean diameter of carcinoma RSM during growth in rats treated with cortisone. 1) 1st generation; 2) 10th generation; 3) 15th generation.

coma 180 in the rats continued up to the 14th day, and then, despite the injection of cortisone, the tumors gradually regressed. After the 10th passage to rats, the mean diameter of the tumors began to decrease starting the 17th day, and after the 15th passage, starting with the 20th day (Fig. 1).

Thus, prolonged passage apparently somewhat increased the viability of tumor cells under the conditions of heterotransplantation. After the Crocker's sarcoma had been transplanted over a course of 15 generations, to mice treated with cortisone, its transplant survival rate in non-pedigreed mice remained 100%.

We also studied the sensitivity of Crocker's sarcoma, repeatedly transplanted in rats, to chemotherapeutic preparations. Table 1 shows that as a result of heterotransplantation Crocker's sarcoma became somewhat more resistant to the preparations. However, as before, the tumor was highly sensitive to benzodet, and varied in its reaction to injection of different doses of sarcolysin. As before, the pattern of sensitivity to the preparations seen with Crocker's sarcoma growing in rats coincided with that observed for the sarcoma growing in mice.

Carcinoma RSM. The tumor was passed 16 times in rats treated with cortisone. Without the administration of this hormone, the tumors did not grow. The nature of the growth of carcinoma RSM in the 1st, 10th, and 15th generations in rats was similar. The mean diameter of the tumors began to decrease beginning with the 14th-15th day (Fig. 2).

Even after prolonged passage of carcinoma RSM in rats, the tumor grew in 100% of the cases when retransplanted back to mice. This is evidence of preservation of the basic biological properties of the strain.

We also studied the sensitivity of the tumor to chemopreparations. Table 2 shows that despite prolonged heterotransplantation, it remained essentially unchanged. As before, embichin and thio-TEPA did not affect the growth of carcinoma RSM, while ftormezin caused a high percent of growth inhibition in this strain.

Sarcoma 298. The tumor was passed 16 times in rats treated with cortisone. Regression of the tumor in the 1st generation began on the 14th day after transplantation. Decrease in the dimensions of the transplants of the 10th

TABLE 1. Growth Inhibition of Crocker's Sarcoma (in %)

Preparation	Dose	Growth			in mice (after 15 passages in rats)
		in mice	in rats		
			1st generation	15th generation	
Benzodet	3 mg/kg for rats, 6 mg/kg for mice	88	86	74	76
Sarcolysin	2 mg/kg for rats and mice	57	61	45	51
Sarcolysin	1.5 mg/kg for rats and mice	44	50	34	31

TABLE 2. Growth Inhibition of Carcinoma RSM (in %)

Preparation	Dose	Growth			
		in mice	in rats		in mice (after 15 passages in rats)
			1st generation	15th generation	
Thio-TEPA	2.5 mg/kg for rats, 4 mg/kg for mice	9	–10	8	20
Ftormezin	1.8 mg/kg for rats, 5 mg/kg for mice	98	82	87	88
Embichin	0.15 mg/kg for rats and mice	–14	16	–13	7

Note. The inhibition and stimulation of growth of carcinoma RSM caused by thio-TEPA and embichin were statistically insignificant.

and 15th generations was also observed beginning with the 14th day. With the increasing number of passages, the growth rate of the transplants increased somewhat during the first days after the transplantation (Fig. 3).

A special experiment was set up to study the linear specificity of sarcoma 298, repeatedly passed in rats. The starting tumor was strictly specific, growing only in mice of the C57 line. The tumor, retransplanted over a course of 16 generations in rats, did not grow in mice of the C_3H and C_3Hf lines, but did grow in 2 out of 5 inoculated mice of the Dba line, in one out of 4 mice of the ASn line, and in one out of 5 non-pedigreed mice. Thus, we noted a certain weakening of the linear specificity of the tumor. The therapeutic experiments showed that the sensitivity of sarcoma 298 to sarcolysin and phenamet decreased somewhat, as a result of prolonged passage in a foreign organism. Nonetheless, these preparations caused significant growth inhibition of the tumor (Table 3).

It is our opinion that the inconsistent results of the investigations on the biological, morphological and antigenic properties of heterotransplanted tumors may be treated as follows: transplantation to a foreign organism leads to less favorable conditions of nutrition, and the transplant is also subjected to the action of antibodies and the cellular elements of the connective tissue. One of the results of these actions could be the death of the heterogenic tumor, which is so often observed by experimenters. The new, "rigid", unfavorable conditions could lead to injurious effects on the heterotransplanted tumor. This injured tumor would not grow, or would grow more slowly, upon retransplantation back to the original host, which is sometimes [4] incorrectly taken as proof of changeability of the tumor's biological properties during heterotransplantation. The change in antigenic properties, described by a number of authors, can be explained by contamination with stromal elements of the recipient [10], or by adsorption of serum proteins of the recipient by the tumor tissue [3].

However, one cannot deny the possibility of real changes in the biological properties of tumors during heterotransplantation. It is interesting that the majority of authors that have described changes in the biological, cytological, and other properties of heterogenic tumors did not suppress the defense reactions of the recipient organism with roentgen rays or cortisone. As a rule, these authors transplanted heterogenic tumors to the lymph nodes, under the skin, or to the peritoneal cavity, increasing the transplant survival rate by frequent transplants or through transplantation to newborn animals [9, 15, et al.]. On the other hand, those investigators who created more favorable conditions for the growth of tumors in a foreign organism, by suppression of the defense mechanisms with cortisone or roentgen rays, and by transplantation to areas of the body with a small amount of connective tissue and a copious blood supply, very rarely observed changes in the properties of the tumors [17, 18, 20].

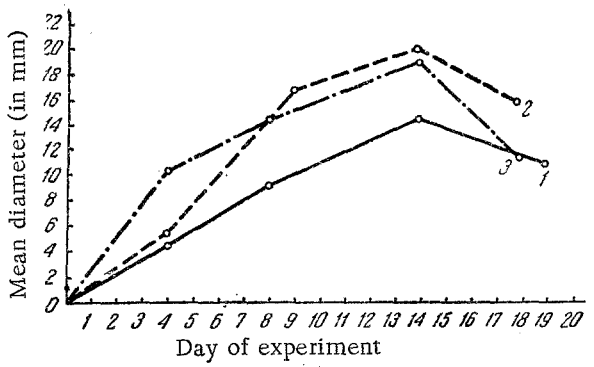

Fig. 3. Changes in the mean diameter of sarcoma 298 during growth in rats treated with cortisone. 1) 1st generation; 2) 10th generation; 3) 15th generation.

The concepts advanced above are well illustrated by

TABLE 3. Growth Inhibition of Sarcoma 298 (in %)

Preparation	Dose	Growth			
		in mice	in rats		in mice (after 15 passages in rats)
			1st generation	16th generation	
Sarcolysin	1.5 mg/kg for rats and mice	81	91	77	70
Phenamet	20 mg/kg for rats, 110 mg/kg for mice	80	92	63	67
Embichin	0.15 mg/kg for rats and mice	6	-12	-12	35

Note. The inhibition and stimulation of growth of sarcoma 298 caused by embichin were statistically insignificant.

the work of Stroud and co-workers [21]. In his experiments with intraperitoneal transplantation of the ascitic variant of one of the mouse tumors into rats, the morphology of the tumor cells was altered; their dimensions increased, and the shape of their nuclei was changed. However, if the intraperitoneal transplantations of this tumor were combined with simultaneous roentgen irradiation of the rats, or administration of cortisone, then the changes in morphology of the heterotransplants were not observed in a single case.

The experiments which we performed showed that when the mouse tumors RSM, 298, and 180 are repeatedly passed in rats (more than 100 days) treated with cortisone, they essentially retain their biological properties. The tumors grow when retransplanted back to mice, and do not grow in rats that have not received cortisone. The general pattern of sensitivity of the mouse tumors to chemotherapeutic preparations of the chlorethylamine and ethylenimine groups, subsequent to prolonged heterotransplantation in rats, remained the same. Minimal changes in the properties of the tumors were observed. These changes were manifested by a slight weakening in the linear specificity, intensification of the growth rate in rats with increased passages, and some reduction in the sensitivity of the tumors to chemotherapeutic preparations.

SUMMARY

Some biological characteristics of mouse tumor strains RSM, 298, and 180 transplanted to rats treated with cortisone were studied during 15-16 generations (over 100 days). Tumor biological characteristics were not significantly changed by heterotransplantation. Tumors grew after retransplantation on mice and did not grow on rats which were not treated with cortisone. The main character of sensitivity of mouse tumors to chemotherapeutic preparations from the groups of chlorethylamines and ethylenimine remained. Some insignificant alterations in the properties of tumors were also revealed. The author also noted some decrease of linear specificity, intensification of growth rate of heterotransplanted tumors during the series of passages and some diminution of sensitivity of tumors to chemotherapeutic preparations.

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