HETEROLOGOUS TUMOR TRANSPLANTS FROM MICE TO SPLENECTOMIZED RATS

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The question whether tumor growths can be obtained by heteroplastic grafting would appear to have been definitely answered. Woglom (15) gives a summary of the early failures to transfer human tumors to animals, and Andersen (1) has shown that the claims of Keysser and Heidenhain to have transplanted human tumors to mice are more than doubtful. More recent studies have dealt chiefly with tumor transplantation from one mammalian species to another or to avian species. While Ehrlich (6) demonstrated the ability of a malignant mouse tumor to grow for a very limited time in rats, the tumors receded early, would not thrive when transplanted from rat to rat, and revived only when transferred back to mice. Murphy (11) succeeded in growing rat and mouse tumors for a few generations in the membranes of developing chick embryos, but Stevenson (14) found that the tumor would not grow indefinitely even though transplanted to fresh embryos at frequent intervals.

Heteroplastic human grafts do not grow, however, in newly hatched chicks. Russell (13) thinks that this apparent resistance to growth is due to a failure of positive chemotaxis and to lack of development of the vascular stroma. Bullock (3) found that mouse tumors 37, 63, and 206 grew rapidly in new-born rats for ten to seventeen days, the tumor then gradually disappearing. Mouse sarcomata grew better in small rats than carcinomata.

Bullock and Rohdenburg (4) found that splenectomy did not prevent the recession of tumors nor favor the growth of inoculated primary tumors. They also showed that splenectomy did not favor either the inoculability or the growth of heteroplastic grafts (5). Keysser (8) has reported that he was able to transplant a human recurrent sarcoma of the testis into mice, and Keysser (9), as well as Hegner (7), reported the successful transplantation of human tumors into the vitreous humor of the rat's eye. A careful repetition of this work by Woglom (16) with a highly virulent mouse sarcoma showed that no growth could be obtained in the vitreous of the rat. For a further survey of the conflicting reports on this subject, the reader is referred to Woglom's critical review (17).

In a recent paper by Krebs and Busch (10) the statement is made that they were able to graft human tumors into mice and rats after the animals had been exposed to 475 r of x-rays. However, the growths were watched for a short time only and may have been survivals. The same may be said of other experiments not specifically mentioned here.
except, perhaps, in the case of Putnoky (12), who has described cultivation of the Ehrlich mouse carcinoma in rats for 28 generations.

Recently Brüda (2) reported the successful growth of a mouse carcinoma and sarcoma in splenectomized rats. These tumors, he said, developed readily in the rats if transplantation was done about two weeks after removal of the spleen. They did not regress and also grew when planted back into mice. Although experiments similar to Brüda's had failed in other hands, it seemed well to repeat his work, as it has been widely accepted by competent German and Italian investigators.

Splenectomized rats, normal control rats, and white mice were used. The rats were a homozygous strain raised in the Institute of Cancer Research and designated as August, and the mice were purchased from a dealer named Longacre. Both the young and adult animals were splenectomized and subcutaneous transplantation was done two or three weeks later, as suggested by Brüda.

Two mouse carcinomata, 63 and 11, and two mouse sarcomata, 180 and 37, were chosen for heteroplastic grafting. All the mice and one-half the number of splenectomized and control rats were inoculated by the usual trocar method, receiving 3.0 mg. of the tumor subcutaneously in the right side. The remainder of the rats received a larger fragment, about 5.0 mg., subsequently, in both axilla and groin. A total of 234 splenectomized rats, 147 normal control rats with spleens, and 64 control mice were used to test the viability of the tumors.

Mouse carcinoma 63 was inoculated in 12 mice, 24 young adult splenectomized rats, and 12 normal rat controls. Within ten days all the control mice showed tumors. The rats were under observation for eight weeks, and none of the 36 showed a tumor. At autopsy no gross or microscopic evidence of the tumor fragments remained.

Mouse carcinoma 11 also gave 100 per cent of takes in 6 mice one week after inoculation, but failed to grow in 24 splenectomized rats and 12 normal rats.

Mouse sarcoma 180 was inoculated in 12 mice, 26 splenectomized rats, and 12 normal rats. Control homotransplants grew in 100 per cent, one week after inoculation. Heterotransplants were consistently negative, though the 38 rats were under observation for ten weeks.

Mouse sarcoma 37 (Fig. 1) gave 100 per cent takes in 6 mice, after a week, and grew in the 12 splenectomized rats and in the 6 normal rats after eight days. In mice the tumor averaged 15 mm. in diameter. The heterotransplants measured about 6 mm. in diameter, appearing slightly larger in the splenectomized rats. In one of the latter (Box III, No. 2) an olive-sized tumor, 12 × 20 mm., grew in the left groin. The growths persisted for two weeks, after which period they began to retrogress and at the end of four weeks no trace remained. Autopsy revealed no evidence of growth, and microscopic examination showed no foreign tumor cells at the site of implantation.

Repetition of the above mentioned experiment with mouse sarcoma 37 yielded the following results: Six homotransplants gave 100 per cent
of takes after nine days. Heterotransplants in 12 splenectomized rats showed 30 per cent of takes, 3 mm. in diameter. In all the 6 young normal rats with spleens the tumor also grew within the same time. These tumors were soft and averaged 15 mm. They ulcerated early in some of the animals and receded rapidly in others. The ulcerated areas healed and the animals recovered. After four weeks the heterotransplants had completely disappeared, either through ulceration or absorption.

One of the growing heterotransplants of mouse sarcoma 37 was removed from a normal rat. The tumor was soft, spherical, light brown in color, and measured 18 mm. in diameter. It differed from the homo-

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Fig. 1. SECTION OF HOMOTRANSPLANT SHOWING AN EXTREMELY CELLULAR MOUSE SARCOMA SEVEN DAYS OLD (MOUSE INTO MOUSE)

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transplant in being less compact, more necrotic, and more vascular. The cells varied in size and shape from spindle to spherical or triangular, and stained more faintly and irregularly than the uniform spindle cells of the original sarcoma. The nuclei were often eccentric. Many cells showed multiple nuclei (Fig. 2).

Six Longacre mice and 6 young white rats, nine weeks old, were inoculated with fragments of the growing heterotransplant of tumor 37. After seven days minute growths about 1.2 mm. in diameter were noted in all the mice. In two weeks 3 of the 6 mice showed tumors 20 x 16 mm. in diameter. After six weeks no further growths were noticed, but the tumors did not recede in the mice. This tumor thus restored to its original habitat resembles the original sarcoma 37, grossly and microscopically (Fig. 3).
FIG. 2. SECTION OF TRANSPLANTED MOUSE SARCOMA 37 GROWING FOR ELEVEN DAYS IN A YOUNG NORMAL RAT, SHOWING AREAS OF NECROTIC CELLS
Karyorrhexis and pyknosis are present.

FIG. 3. MOUSE SARCOMA 37 TRANSPLANTED BACK INTO MOUSE AFTER GROWING NINE DAYS IN YOUNG NORMAL RAT
The heterotransplant, twenty-nine days old, is slower in growth and less cellular than the original mouse tumor. The cells are smaller and the nuclei less distinct. Compare with Fig. 1.
Of the 6 young rats, 2 died within a few days after transplantation, and in 4 bean-sized tumors developed after six days. At the end of three weeks the tumors had disappeared, and although the animals were kept under observation for six weeks, no recurrence was evident.

In the 3 mice with unsuccessful grafts autopsy revealed stationary organized transplants. In the rats no trace of tumor was seen on post-mortem examination.

Later a repetition of the experiments with sarcoma 37 (Fig. 1) was undertaken. Mice again yielded 100 per cent of takes with this tumor within ten days. Twelve young, normal, non-splenectomized rats gave 75 per cent of successful takes in the same time, but complete recession or absorption occurred by the sixteenth day in all the rats. Tumor 37 did not grow in 6 adult splenectomized rats. Reinoculation of young non-splenectomized rats in which the mouse tumor had disappeared, gave no second takes. At autopsy some of the animals had a yellowish discoloration of the subcutaneous tissue at the site of implantation, and in a few rats yellow pinpoint fragments persisted. A microscopic section of one stationary heterotransplant shows groups of mouse sarcoma cells lying about blood vessels. The cells are smaller than those in the mouse tumor, and the nuclei have few or no granules and no mitoses. Phagocytic giant cells are scattered through the section. The growth in the rat is more vascular than in the mouse. The discolored subcutaneous areas contain extravasated blood, necrotic cells, and an increased number of wandering phagocytes, with fibrosis.

On Oct. 10, 1928, a sarcoma 37 heterotransplant, nine days old, was removed from a young non-splenectomized rat and inoculated in 6 Longacre mice, 6 young rats four weeks old, and 6 adult splenectomized rats. This tumor originally taken from a mouse and growing in a young rat was soft, friable, 15 mm. in diameter, and covered by a thin transparent capsule enclosing a healthy periphery which surrounded a hemorrhagic center. This mouse-to-rat tumor is very cellular and vascular, contains no stroma, and shows many necrotic areas with disintegrating cancer cells. The nuclei of the cells of the original mouse sarcoma 37 are prominent, they take a more intense stain, and the granules and chromosomes are much more distinct than those of the heterotransplant.

After ten days' growth in the rat, transplants back into mice grew in only 50 per cent of the animals and reached a size $5 \times 6$ mm. From mouse to mouse tumor 37 grew in 100 per cent and in the same period of time reached twice the size of that derived from the rat. From rat to young normal rat and to splenectomized rat, transplants failed to grow. After one month 2 mice were still alive and carried soft olivesized tumors, which on section showed a glistening healthy margin and a soft reddish necrotic center. During its sojourn in the rat's body this mouse tumor is evidently devitalized, since a return to its native soil does not at first quicken its growth, for the latent period in the mouse is now longer, the growth in a given period of time is smaller, and the mouse lives longer.
Inasmuch as this tumor failed to grow in adult splenectomized rats, the next procedure was to inoculate young splenectomized rats with mouse sarcoma 37. Five mice, 5 young splenectomized rats six weeks old, 5 young normal control rats of the same age, and 5 adult splenectomized rats were used. The tumor grew in all the control mice, in 40 per cent of the young splenectomized rats, and in 60 per cent of the normal young rats. The 5 adult splenectomized rats gave negative results. After three weeks all the mice still had tumors, while in the rats, with one exception, the tumors had receded.

Homologous grafts of rat tumors, including sarcoma 340, fibrosarcoma 337, fibro-adenoma 308, and cystadenoma 342, were also transplanted in 58 splenectomized and 59 normal rat controls. There seemed to be no change in the growth or character of the tumors in rats without spleens, with the exception of a moderate retardation, attributed to severe intestinal infection to which splenectomized rats are prone. Otherwise there was no evidence that the lack of spleen influenced the taking or growth of this type of tumor.

It appears that more stress has been laid by investigators of the problem upon the resistance or immunity of the host than upon the proliferative capacity of the individual tumor cell. There can be no doubt that a reciprocal reaction must be established between the host or environment and the isolated fragment of living substance. From my experiments, which entirely confirm the work of Bullock and Rohdenburg (4), it is difficult to see how the spleen can be considered as responsible for inhibition of tumor growth, or that splenectomy encourages either homologous or heterologous transplants in their adjustment and growth. Certainly not the slightest confirmation of Brüda's work has been obtained.

**Conclusions**

1. Heterotransplants of mouse carcinoma 63 and 11 and of sarcoma 180 and 37 will not grow in adult splenectomized rats nor in adult normal rats.
2. Mouse sarcoma 37 will grow for a short time in young normal rats and young splenectomized rats, but recession and absorption of the tumor occur promptly, without visibly affecting the animals.
3. The growth energy of a tumor may be diminished during its stay in foreign soil, but is regained when the tumor is again transferred to its normal habitat.
4. The presence or absence of the spleen in a mouse or rat does not seem to hinder or accelerate the growth of sarcoma 37.
5. The age of the animal influences the growth of heterotransplants.

**Bibliography**

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