THE INFLUENCE OF MOUSE-RAT PARABIOSIS ON
THE GROWTH IN RATS OF A TRANSPLANTABLE MOUSE SARCOMA.*

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Plates XVI-XVIII.

In a former paper† it was shown that in a certain percentage of
cases a successful parabiotic union between rats and mice was
possible. The application of this procedure to tumor investigation
appeared promising in view of Ehrlich's² interesting work in zig-
zag transplantation. His results may be briefly stated: a virulent
mouse sarcoma when inoculated into rats grows rapidly for eight
to ten days and is then absorbed. If reinoculated into mice at the
height of growth the grafts are successful, and the cells con-
tinue to multiply with undiminished vigor. The resulting tumors
may again be used for rat inoculations. Transplantations direct
from rat to rat are, however, uniformly negative. Ehrlich ex-
plains this failure, as well as the limited growth in the first instance,
through the 'exhaustion of a specific food substance, "X-Stoff,"
brought over from the mouse.

Accepting Ehrlich's hypothesis, it seemed conceivable that the
deficient nutritive material might be supplied through a mouse-rat
parabiosis, since it has been shown that in animals thus united sub-
stances in the body fluids of one animal pass fairly readily to the
other.

Technique.—A free peritoneal anastomosis with wide skin
and muscle apposition was made between young rats, weighing
forty-five to fifty grams, and large "growing" or adult mice. Ad-

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Influence of Mouse-Rat Parabiosis.

Additional sutures through the skin of the necks with bands of adhesive plaster about the bodies and tails formed sufficient fixation. More extensive bandaging interfered with feeding and promoted soiling of the wound by excreta.

Primary healing took place in a small number of cases (plate XVI, Fig. 1, and plate XVII, Fig. 2), a relatively wide granulating zone without skin union in a larger number, and only an approximation of the granulating surfaces in others. Histological studies are necessary to determine the extent and character of the healing process.

In preliminary experiments proof of an interchange of fluids was made by injecting certain substances into one animal and subsequently demonstrating these in the urine and body tissues of the other. Potassium iodide was shown to be present in the urine of the second animal even when there was an incomplete union of the apposed surfaces, the free peritoneal anastomosis being apparently sufficient to carry over the injected chemical. Trypan blue injected subcutaneously was noticeable in the peritoneal fluid of the opposite animal before general diffusion was apparent.

Parabiosis of mice and rats gave a high mortality in mice, the majority not surviving ten days. Hernia of the rat's intestines into the mouse occurred very frequently and was probably an important factor in causing death. The limited duration of parabiosis was a serious obstacle in the present investigation. To overcome this partially, the tumor inoculations were made on the day of parabiosis or several days before. Rats from experiments 78 to 84 were separated on the eighth day in order to insure a longer observation period, and three rats, from experiments 74 to 76 and experiments 64 to 70, were separated after the death of the mice. The tumors in two of the surviving rats were excised on the sixteenth and seventeenth days and studied histologically. Details of the microscopic findings are given in a later paragraph (see p. 261).

The tumor used in the present study was an Ehrlich mouse sarcoma, which in mice gives from 70 to 100 per cent. of takes. It pursues a rapid course and rarely undergoes retrogression. In normal young rats it gives a growth in about 40 per cent. of the animals, a definite nodule ten days after inoculation being regarded
as positive. As a rule, the tumors in rats at this time are larger than those of corresponding age in mice. After ten days the size may remain stationary for a day or two followed by a rapid retrogression with complete disappearance by the fifteenth or twentieth day. Two rat tumors were inoculated into mice for parallel studies but only one positive growth (out of twenty) was noted, and this was microscopic in size.

The following tables show the percentage of "takes" in five experiments representing thirty parabioses and controls.

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The inoculations in the above table were made on the day of the parabioses.

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In experiments 34 to 39 and 78 to 84, the inoculation preceded parabiosis by one and five days respectively.

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<td>26</td>
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<td>16</td>
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Table III combines tables I and II.

Chart I shows the increase and decrease in the size of the tumors in the experimental rats 78 to 84 and in their controls.

In this experiment fourteen rats, weighing forty to fifty grams, were inoculated with mouse sarcoma. Five and six days later
seven were united with mice, weighing twenty to twenty-five grams. The seven remaining animals were kept for controls. After eight days the rats and mice were separated, for it was desirable to observe them for a longer period and experience had shown that, after the eighth day, the mortality was high. In spite of the shortness of the parabiosis it was conceivable that sufficient inter-change of fluid had taken place to influence the growth of the alien tumor. The tumors of three parabiotic rats were not absorbed until after the twenty-fifth day.

From table II it is seen that the percentage of takes in the parabiotic rats was 67 as contrasted with 33 in the controls. Since the inoculations preceded parabiosis by at least five days, it may be questioned whether this difference in percentage was not accidental. The larger size attained and the slow retrogression, however, can scarcely be explained other than through the favorable influence of

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**Chart I.** The figures 8, 12, 16, 20, 24, and 28, indicate the number of days after inoculation.
the union. Since the sarcoma does not give 100 per cent. of "takes" in mice of the size employed for parabiosis, some of the failures in parabiotic rats might be attributed to the insusceptibility of the associated mice. It did not seem desirable to test their susceptibility by simultaneous inoculation since another factor would thus have been introduced, one too, which according to our working hypothesis, might influence the results.

CONTROLS.

Before beginning the present series of experiments the growth of this tumor in normal rats was studied grossly and histologically. It was found that in the first ten days the appearances were practically the same as in the mouse tumors of the same age. After ten days, however, disintegration began in the central cells and rapidly involved the entire nodule. The blood vessels, however, remained patent for a time after the death of the cells. Absorption of the tumor was usually completed between the fifteenth and twentieth day.

A majority of the tumors in parabiotic rats was not studied histologically since it was desirable to determine the total duration of growth. Two tumors, however, excised on the sixteenth and seventeenth days, presented interesting pictures. Both tumors were removed from rats in which inoculation with tumor and the operation for parabiosis had been performed simultaneously and in both instances the rats and mice were separated on the eighth day. On the twelfth day the first tumor was 2.6 by .9 centimeters and remained stationary until excised on the sixteenth day. Sections showed that in the centre of the growth most of the tumor cells had disappeared, leaving the blood vessels—many with intact endothelium and filled with blood—standing out prominently. In the reticulum left by the disintegrated cells, normal and degenerating cells were scattered, and mitotic figures were frequently seen. Plate XVIII, Fig. 3, shows such a field.

In passing it may be suggested that this phenomenon can be explained by Ehrlich's hypothesis on the ground that the extensive necrosis of tumor cells, brought about by the limited supply of "X-Stoff," leaves the few surviving cells a proportionately larger share. This enables them to multiply actively until the same con-
Influence of Mouse-Rat Parabiosis.

dition occasions their death. In advancing this explanation it is assumed that through parabiosis there is circulating in the rat a certain amount of the specific nutritive substance.

The second tumor grew rapidly to the fourteenth day when it measured 1.6 by 1.2 centimeter. Before excision on the seventeenth day, the measurements were the same. Sections of this growth showed central necrosis, but less marked than in the first tumor. This softening evidently accounts for the lack of any increase in the size after the fourteenth day, since the presence of numerous dividing cells in the peripheral portions show that cell division had not ceased (plate XVIII, Fig. 4). Inoculations into mice were positive, confirming the microscopic evidence of vitality of the cells. The largest of the control tumors were excised simultaneously. Sections showed widespread granular necrosis, with no surviving cells.

CONCLUSION.

The results in these experiments seem to justify us in concluding that the growth of mouse tumors in rats is very definitely promoted by mouse-rat parabiosis, the percentage of "takes" being markedly increased, the rate of growth accelerated, and the duration of active growth extended to at least seventeen days.

These findings are in accord with Ehrlich's hypothesis, although, on account of the complexity of the factors involved in such experiments, other explanations may be advanced.

EXPLANATION OF PLATES.

PLATE XVI.

Fig. 1. Zone of union in a mouse-rat parabiosis of six days duration, showing perfect healing of all layers.

PLATE XVII.

Fig. 2. Mouse-rat parabiosis of eight days duration; x marks the point of skin union (approximate). The silk suture is visible.

PLATE XVIII.

Fig. 3. An area in the central portion of a sixteen day tumor in a rat of experiment 74 to 76, showing a patent blood vessel surrounded by normal and degenerating tumor cells, spaces left by dead cells, and three cells in stages of mitosis.

Fig. 4. Seventeen day tumor in a rat of experiments 57 to 63, showing mitotic figures.
Fig. 2.