PARABIOSIS AS A TEST FOR CIRCULATING ANTIBODIES IN CANCER.¹

FIRST PAPER.

BY PEYTON ROUS, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

The union of two animals, anatomical and physiological, which Sauerbruch and Heyde² have recently brought into prominence under the name of parabiosis, seems well adapted for a demonstration of the presence of circulating anti-bodies, if such there be, in rats and mice immune to transplantable tumors. The mass of evidence in the literature is against such anti-bodies, but the point remains unsettled. The recent cures of the infectious lymphosarcoma of dogs obtained by Crile and Beebe³ through replacing a large part of the blood of diseased animals with that from immune individuals have served to direct attention to it anew. And while the objection may be made that their work concerned itself with a neoplasm which many refuse to consider as a true "autonomous new growth," yet the question of circulating anti-bodies thus revived is too important to be let rest.

For the condition of parabiosis, two animals are joined by a large peritoneal opening and by union of the apposed skin and connective tissue. The connection brought about on healing is more than anatomical. Bacteria introduced into the blood of one animal can be isolated from that of the other; potassium iodide injected into one is excreted through the kidneys of both. Further, the life of each individual is directly dependent on that of its fellow. When one dies death of the other follows within a few hours. On such testimony for physiological union Sauerbruch and Heyde, Morpurgo,⁴

¹Received for publication September 1, 1909.
⁴B. Morpurgo, Münchener med. Woch., 1908, iv, 2446.
Jehn,\(^6\) and Forschbach\(^6\) have utilized the condition for experimental work on peritonitis, uraemia, pancreas diabetes and on sex determination. Their results bring out in a remarkable manner the closeness of the relationship in parabiosis.

In the idea that parabiosis can give important testimony on the presence of anti-bodies for cancer I have united tumor-bearing animals to others that have shown themselves strongly resistant to such growths, and have carefully noted the subsequent course of the tumors.\(^7\) Experiments are yet in progress, inclusive of tests with animals in which large tumors receded, but the early results having been so clear-cut as to warrant this note.

According to Sauerbruch and Heyde, it is necessary, in order that the union of the individuals shall endure, for them to be young, of the same sex, and above all, of the same litter. They worked with rabbits, dogs and cats, and their animals lived at best only three weeks. But Molpurgo, using young rats of the same litter, found that it was not necessary for them to be of the same sex. One couple lived three months. In my work the condition of like sex that he disregarded was alone complied with. The white rats joined were all adult, some old, none of the same litter, and those put together were of unequal size and age. Nevertheless union by first intention was prompt, and was found to be permanent in the life of the couple. Four such pairs (out of five) survived operation and figure in the present experiment. One pair lived thirty-four days, two others twenty-six and twenty-two days respectively, and the fourth was killed after two weeks. In considering these figures it should be remembered that one animal of each pair had a large growing tumor, and that an important physiological difference existed between the individuals joined, viz., that of behavior to a new growth. Manifestly those general

\(^8\) E. Ranzi and H. Ehrlich (*Zeit. f. Immunitätsforsch.*, 1909, iii, 38) have just published observations on the transmission of anti-bodies in parabiotic animals. Circulating anti-bodies are to be found, twenty-four hours after their introduction into the vessels of one animal, equally distributed through the blood of both.
tissue distinctions based on age, parentage and sex are much less marked in white rats than in some other species.

Strong animals bearing the Flexner-Jobling tumor were selected and united to others resistant to the same neoplasm. The Flexner-Jobling tumor is an adeno-carcinoma of quick growth, which yields 80 to 100 per cent. of takes on transplantation. It seemed especially suited for the test because of the small per cent. of cases, according to the most recent observations, in which the neoplasm, when once well started, retrogresses; and also because rats which do not develop this "virulent" tumor on repeated implantation have demonstrated their natural immunity as conclusively as the test by means of tumor-growth allows. The tumor-bearing individuals possessed midway on the right side, in the subcutaneous tissue, a mass of 1 to 3.4 centimeters diameter, in which growth was progressing steadily. Such neoplasms may possibly not be brought to complete disappearance through the slow and long-continued introduction of immune substances, but they should at least show changes in activity as evidenced by a delayed extension. Their size was therefore recorded with care every two or three days.

The resistant animals employed had failed on three implantations of tumor material to develop a growth. These implantations had been made at intervals of ten days to a month, and ten days after the last the animals were employed for the experiment. Other rats had received like implantations at the same time and these developed takes in the usual high percentage. The small bits of tumor were all placed, by means of a trocar, in the subcutaneous tissue of the left side. It was this left side, where local immunity was presumably highest, that was joined to the right side of the tumor animals, with, as has been said, union by first intention.

Operation was done according to the method outlined by Sauerbruch and Heyde. Each animal had been given beforehand an intraperitoneal injection of one-twelfth grain of morphine sulphate. The opening between the peritoneal cavities extended from the ribs to the groin, and the union of subcutaneous tissue and skin along the whole side from fore-leg to hind. Special pains were taken that the light yoke of adhesive plaster used to hold the animals together should not interfere with the tumor's blood-supply.
The individual protocols will not be given here. In all instances the resistant animal was the larger and stronger. This was intentional, the idea being to furnish the tumor-animal a good source for possible anti-bodies. After the animals of pair B 4,208 had completely united, methylene blue was injected subcutaneously into the immune and later was demonstrated in the urine from both. An emulsion of bone black was injected into the peritoneal cavity of the immunes, and later the omentum of three of the four tumor animals was found heavily charged with it. In the fourth case (A 4,170) an intestinal hernia had blocked off the opening. In all, during life, the intestine or the spleen (of the right-hand, or immune, animal) could be felt prolapsed through the tubular connection between the two rats. But the peritoneal route for the transfer of body fluids can hardly have the importance of that through the multitude of new capillaries and lymph-vessels in the zone of union.

**TABLE I.**

Those marked "A" were inoculated May 18, 1909, with material from the 28th generation, strain E, animal No. 4098.

Those marked "B" were inoculated May 19, 1909, with material from the 28th generation, strain E, animal No. 4110.

<table>
<thead>
<tr>
<th>Date, 1909.</th>
<th>June 11</th>
<th>June 14</th>
<th>June 17</th>
<th>June 20</th>
<th>June 23</th>
<th>June 26</th>
<th>June 29</th>
<th>July 2</th>
<th>July 5</th>
<th>July 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>B4,208 Parabiotic.</td>
<td>3.9*</td>
<td>3.7</td>
<td>6.4</td>
<td>6.5</td>
<td>5.9</td>
<td>7.5</td>
<td>Thin and ill, killed June 24.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4,201 Control.</td>
<td>3.5</td>
<td>5.5</td>
<td>6.0</td>
<td>6.2</td>
<td>8.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4,207 Control.</td>
<td>3.8</td>
<td>6.6</td>
<td>7.6</td>
<td>9.2</td>
<td>12.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4,178 Parabiotic.</td>
<td>1.2*</td>
<td>2.0</td>
<td>2.9</td>
<td>3.4</td>
<td>3.1</td>
<td>5.1</td>
<td>Died July 3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4,205 Control.</td>
<td>2.4</td>
<td>3.2</td>
<td>3.4</td>
<td>3.9</td>
<td>5.6</td>
<td>6.7</td>
<td>7.0</td>
<td>7.6</td>
<td>8.4</td>
<td>8.8</td>
</tr>
<tr>
<td>A4,170 Parabiotic.</td>
<td>2.2*</td>
<td>2.3</td>
<td>3.5</td>
<td>5.0</td>
<td>5.1</td>
<td>6.5</td>
<td>6.5</td>
<td>7.6</td>
<td>7.9</td>
<td>8.7</td>
</tr>
<tr>
<td>A4,174 Control.</td>
<td>1.4</td>
<td>1.7</td>
<td>2.2</td>
<td>1.3</td>
<td>2.4</td>
<td>3.2</td>
<td>3.6</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>A4,185 Control.</td>
<td>1.7*</td>
<td>1.8</td>
<td>1.8</td>
<td>2.5</td>
<td>3.1</td>
<td>4.4</td>
<td>4.7</td>
<td>6.5</td>
<td>7.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Tumor-growth in all the animals had been continuous to the time of the experiment.

* Operation this day.

The animals taken as controls were of the same lot, inoculation, and approximate age as the tumor-bearing ones in parabiosis; and their growths had run a like course, and were of much the same size as those in the experiment proper. They were not placed in parabiosis. In view of the tediousness of the procedure that was
deemed unnecessary until a first series of tests had brought out some differences in tumor-growth between parabiotic animals and controls. Such a difference has not been found.

The accompanying table brings out clearly the results. The figures representing tumor growth are a product of the multiplication of the length and breadth of each mass as measured in centimeters. Such a product does not represent the actual area of the disc-shaped tumors.

The table shows that growth of the tumors was in general unaffected by a parabiotic union of the host with a resistant animal. When it is recalled that the controls remained throughout in good health, whereas the parabiotic couples failed and died, the correspondence in progress of the tumors becomes the more noteworthy. Had retrogression appeared, a larger series of tests would have been necessary to prove it other than spontaneous, and thus independent of the parabiosis. But the freedom of the masses in the parabiotic animals in these experiments from even a general retardation of growth lends to the observations a distinct value.