# The Growth of Alien Strain Tumors in Parabiotic Mice*

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The method of parabiosis has been called into use by a number of workers in efforts to determine whether resistance to transplanted tumors is conditional on the presence of circulating antibodies in the host. In 1909 Rous (13) united tumor-bearing rats with those of another strain which were resistant to the growth of the tumor, but this procedure did not produce any observable effect on the growth of the tumors. Lambert (8), however, asserted that mouse sarcoma grew better and for a longer time in rats joined parabiotically with mice than in intact rats. According to Albrecht and Hecht (1), the parabiotic relation in itself led to a resistance against the transplanted tumors; mouse carcinoma grafted into either member of a pair of mice grew much more slowly than in controls. Similar results were reported in rats by Kross (7), who preferred to base an explanation on possible ill health of the animals. The illuminating researches of Morpurgo (11) tended to confirm the earlier conclusions of Rous. Morpurgo found that if susceptible and resistant rats were united and the tumor concerned placed in the peritoneal cavity common to the two animals, growth of the implanted cells occurred only in the tissues of the susceptible animal. Recently Furth, Barnes, and Brower (5) have used the parabiotic technic to investigate whether susceptibility or resistance to leukemia in mice is transferable. In varied series, involving the union of susceptible with resistant mice, no evidence was found that either of these hereditary qualities could be modified by parabiosis.

In all the investigations mentioned above the animals termed resistant had either acquired resistance prior to operation or possessed this condition as a hereditary trait. It is of additional interest to study the development of resistance in animals already in parabiotic connection, and in particular, the influence of regression of a tumor in one parabiont on the later inoculation of the same tumor in the opposite parabiont. With the development of pure inbred strains of mice and refined methods of inducing malignant growths, mouse tumors are now available that will grow temporarily in alien strains of mice but later regress, leaving the hosts 100 per cent immune from subsequent transplants of the same tumor (9). In the present experiments these have been utilized to produce immunity in parabiotic mice of alien strains, and to test for the spread of such resistance between members of a pair.

## MATERIALS

Mice of three inbred strains, A (Andervont), BA (Bagg albino), and C57 black were used, in each of which tumors were at hand for experimental work. These were sarcomas A274, BA1, C57-241, induced by subcutaneous inoculation of 1,2,5,6-dibenzanthracene and maintained by serial transplantation. M. R. Lewis (9) has described the growth behavior and immunity induced by these neoplasms on inoculation into alien strains of mice. Of the three, BA1 grows consistently in A or C57 mice to form a moderate sized tumor, which later invariably regresses. Subsequent transplants of BA1 in the same animals never show any growth. C57-241 nearly always grows to a fairly large size on inoculation into A or BA mice. Its growth energy is variable, and it may continue to grow progressively in the alien host or it may regress and disappear. In either event the mouse is immune from repeated inoculation of C57-241. The third sarcoma, A274, differs in that it usually grows progressively in both BA and C57 mice, and this process does not always confer an immunity on the host against later inoculations of the tumor. The differing growth characteristics of these three tumors will be of importance in interpreting the experimental results.

## METHODS

Parabiotic union of the mice was accomplished surgically under ether anesthesia. The method of Sauerbruch and Heyde (14), as modified by Bunster and Meyer (2), was followed throughout. In brief, this

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technic involves joining the two animals laterally by means of a skin suture from the level of the hind limbs to the back of the head. Internally, the abdominal wall is incised on the inner side of each animal and all four cut edges drawn together with a single continuous suture. In this way, a firm, extensive union of the two abdominal walls is obtained without, however, uniting the two peritoneal cavities (Fig. 1). With the use of pure inbred strains of mice, no apparent advantage was found in uniting mice from the same litter or of the same sex. In all pairs, however, both mice were normal animals of the same strain.

Tumor material was inoculated subcutaneously with a trocar. Aseptic precautions were observed in removing and injecting it, and only fresh, healthy portions

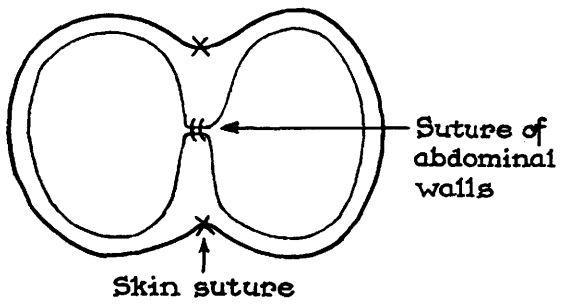


FIG. 1

were used. As controls, single mice of the same strain as the parabiotic pair were inoculated in all experiments.

The parabiotic procedure is apparently a drastic one and a physiologic incompatibility subsequently develops in many pairs, the causes for which are as yet poorly understood (3, 10). In addition, the animals are subject to infection to a greater degree than in ordinary operations, and may be unmatched temperamentally. Because of these factors a relatively high mortality was experienced in the present experiments, as in those reported by other workers. The results to be discussed here, however, are based only on pairs that survived the 2 month experimental period in good health; all other animals were discarded.

## RESULTS

Healing was variable, but usually fairly complete in 2 or 3 weeks. Typically, the skin of the two parabionts fused completely along the line of junction, leaving only a faint scar. In some pairs the anterior sutures tore free, but this condition could usually be controlled with a strip of adhesive tape around the inner forelegs of the animals. In harmonious parabiotic twins, of the type used for the experiments to be described, there was no outward sign of incompatibility over the experimental period and the nutrition of the parabionts was approximately normal. Tumor inoculation was not begun until at least 25 days after operation. By this time healing was practically complete, and an extensive connection between the two mice well established.

The series of inoculations of BA1 tumor into parabiotic twins yielded the most uniform results, and will be described first. In this group were included 11 C57 × C57 and 5 A × A pairs of mice. Each of these pairs received 2 tumor inoculations, spaced approximately 10 days apart. In the first injection, made 25 to 30 days after operation, a subcutaneous dose of tumor was placed in the outer flank of the left parabiont. In all animals this primary inoculation gave rise after a week or 10 days to a tumor measuring on the average about 7 by 7 by 10 mm. Within another 2 weeks these tumors had completely regressed and were no longer palpable. The second injection, made 10 days after the primary inoculation, consisted of a similar dose of tumor into the outer flank of the right parabiont. Single control mice of the same strain as the injected pair were also inoculated with the tumor. In contrast to the vigorous proliferation in controls, tumor inoculated into the right parabionts did not in any instance give rise to definite growth. About a week after the second injection, a slight lump, approximately 1 by 2 by 5 mm. was present at the site of inoculation. This mass disappeared within another week or two and probably represented reaction by the host for the most part, rather than true growth. Three pairs of mice were sacrificed one week after the second inoculation, and the tumor in the right parabiont was compared with autopsied normal controls that had been inoculated at the same time. Whereas the tumor fragments were well vascularized and actively enlarging in control mice, in the parabiotic animals there was no significant disturbance of the normal host vascular pattern near the tumor mass. The pieces of tumor were pale, avascular, and showed no apparent increase in size. The general picture of the tumor in such parabiotic mice coincided with that following injection of BA1 tumor into normal A or C57 mice that were immune from previous inoculation.

The failure of BA1 tumor to grow in the right parabionts could not be attributed to possible ill health of the animals. Several of these C57 × C57 pairs were further inoculated subsequently with A274 and vigorous growth ensued in all cases. Other C57 pairs were finally inoculated with the homologous tumor C57-241, which grew progressively at the same rate as in controls. Hence the failure of BA1 tumor to grow in the right hand member of the parabiotic pairs was apparently correlated with the previous inoculation of

BA1 tumor into the left hand member, and was not due to some general nonspecific effect.

A lesser number of pairs, 8 BA × BA and 2 A × A combinations, were given similar staggered injections of C57-241. The course of events in these animals, except for a few differences to be mentioned, followed the outline given above. In one pair the primary inoculation, for undetermined reasons, did not give rise to any apparent growth. All other primary injections were followed by vigorous proliferation of the implanted cells. In some animals the tumor fragments, inoculated into the left parabiont 25 days after operation, came to form a fairly large tumor, which eventually regressed and disappeared. Similar injections in other animals led to progressive tumor growth and the eventual death of the host. Regardless of whether the primary tumor in the left parabiont regressed or grew progressively, the inoculation of C57-241 tumor into the right parabiont, 10 days after the first injection, was not followed by any perceptible growth. A tiny mass, barely palpable and gradually receding, was the only evidence of inoculation. In one pair, however, the second injection resulted in a progressively growing tumor. It is significant that this was the same pair in which the primary inoculation had failed to take.

Only 3 parabiotic pairs, C57 × C57, were doubly injected with A274, and the results correspondingly lack statistical significance. They are nevertheless of considerable interest. The tumor in all 3 cases grew progressively as a primary inoculation in the left parabiont. After injection 10 days later into the right parabiont, progressive growth again occurred in 2 animals, with a negative result in the third. This finding correlates well with the known behavior of the tumor in control C57 mice, where a single inoculation does not always bring about an immunity from repeated inoculation.

The results of these experiments are summarized in Table I. While the number of animals in some categories is small, the results taken together are obviously significant. From these data it is clear that immunity develops in both parabionts after inoculation of one member of a pair with an appropriate tumor. The uninjected parabiont acquires the immune condition even though the tumor cells concerned were never present in the body.

## DISCUSSION

The degree of communication between members of a pair is a critical factor in all experiments involving the parabiotic technic. Early workers such as Friedberger and Nasetti (4) and Ranzi and Ehrlich (12) demonstrated that bacteria or bacterial antibodies placed in one parabiont were soon distributed between both members of a pair; more recently Hill (6) showed with parabiotic rats that dye injected into one animal reached equal concentrations in both partners within 6 hours. Until recently, however, it was uncertain whether blood vessels of the two parabionts were in actual anastomosis. This point has been clarified through the work of Furth, Barnes, and Brower (5) on parabiotic mice. Rat erythrocytes, injected intravenously into one mouse, could be detected by agglutination tests in serum from the opposite partner. Similarly, nucleated chicken erythrocytes injected intravenously into one animal were demonstrable later in histologic sections of organs from the other parabiont. Hence it may be assumed that a certain degree of direct continuity exists between the circulatory systems of parabiotic animals.

The results of the present experiments suggest that immunity from alien strain tumors depends on a distribution through the blood stream of products from the implanted cells. It is difficult on any other basis to explain the development of resistance in both para-

TABLE I: GROWTH OF ALIEN STRAIN TUMORS IN PARABIOTIC MICE

| Description of pair | Tumor used | Growth in left parabiont as primary tumor | Growth in right parabiont, inoculated 10 days later | Growth in control mice of same strain |
|---|---|---|---|---|
| C57 × C57 | BA1 | x x x x x x<br>x x x x x | 0 0 0 0 0 0<br>0 0 0 0 0 | x x x x x x<br>x x x x x x |
| A × A | BA1 | x x x x x | 0 0 0 0 0 | x x x x x x<br>x x x x x x |
| BA × BA | C57-241 | x x + +<br>+ + + 0 | 0 0 0 0<br>0 0 0 + | x x x +<br>+ + 0 0 |
| A × A | C57-241 | + x | 0 0 | + + + +<br>x 0 |
| C57 × C57 | A274 | + + + | 0 + + | + + |

Each symbol refers to an individual animal. + = Progressive growth of tumor; x = vigorous growth of tumor followed later by complete regression; 0 = no growth of inoculated tumor.

biotic animals, when only one is inoculated with a tumor. One possible explanation would assume that tumor cells in an alien environment give off characteristic biochemical factors that in the case of parabiotic animals circulate through both partners *via* the connected vascular systems. These factors, probably protein in nature, could call forth resistance simultaneously in both members of a pair. It is also possible that antibodies against the implanted cells are formed by the parabiont bearing the tumor, and later spread into the opposite animal, which would thus be passively immunized. At the present time this view may not be dismissed directly but is considered less probable, since the results of Rous, of Morpurgo, and of Furth, Barnes, and Brower, mentioned above, show that susceptible animals do not become resistant through parabiotic union with an immune animal.

## SUMMARY

1. Parabiotic mice have been used to study the development of immunity from alien strain mouse tumors and the spread of resistance between members of a pair. Mice of the same strain were united and inoculated with alien tumors known to induce an immunity in the hosts.

2. Primary injection of tumor into the left parabionts, 25 days after operation, resulted in a rapid proliferation of the implanted fragments. The eventual size of these tumors did not differ from that attained in single control mice of the same strain.

3. Inoculation of the same tumor 10 days later into the opposite parabionts was not followed by any perceptible growth of the implanted cells. Such fragments were not vascularized by the hosts, which appeared to be immune from the tumor.

4. It is suggested that inoculation of an immunizing tumor into one parabiont leads to simultaneous development of resistance in both animals. The mechanism of this process may involve chemical factors that are given off by the implanted tumor cells and circulate through the connected vascular systems.

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