Animals in which a transplanted tumor has regressed usually resist retransplantation of the same tumor; in at least some cases this immunity appears to be tumor-specific (1, 7, 10, 18). Resistance to retransplantation of tumors has been induced in rats and mice by surgical removal of an established graft (1), tourniquet strangulation of the graft (11), injection of nonliving tumor substances prior to grafting (15, 17, 20), or injection before grafting of either normal tissue or red blood cells foreign to the host (2, 12, 15). Whether circulating antibodies are associated with such resistance (immunity?) and whether antibodies may passively immunize a susceptible host are problems which have been analyzed to a degree as indicated below.

Serum from mice or rabbits actively immunized by injecting mouse leukemic cells inactivated the living antigenic cells in vitro so that their ability to transmit the disease was lost (8, 21). Immunity against leukemic cells arising within the strain has been produced in inbred mice by administering sublethal doses of cells prior to inoculation with massive doses. This immunity was passively transferred to susceptible hosts by implantation of spleen or liver from immune donors (12).

Parabiotic studies have on the one hand indicated transfer of immunity to normally susceptible animals (4, 9), whereas other reports appear to demonstrate the contrary (6, 14), to the extent that the immune condition was actually reported to have been converted to susceptibility (5). Bichel and Holm-Jensen have shown that such seemingly conflicting reports might stem from the genetic type of animals used. By means of labeling red blood corpuscles it was found that the crossed blood flow in homogenetic pairs was approximately 100 times that in pairs that were heterogenetic (9). Immunity was not transferred if the parabionts were heterogenetic, whereas homogenetic parabionts were passively immunized (4). It was concluded that large quantities of antibodies must be transferred to obtain passive immunity and that attempts at passive immunization by injection of serum and blood and by heterogenetic parabiosis have been unsuccessful because of insufficient antibody exchange.

MATERIALS AND METHODS

Induced regression of Gardner lymphosarcoma, immunity to retransplanted tumor tissue.—It was the purpose of the present study to determine whether a transplanted tumor which grows progressively would evoke immunity to retransplantation by its induced regression and whether such immunity could be transmitted to members of the same strain in parabiosis. In Bichel and Holm-Jensen's experiments (4) tumor immunity was passively transferred from animals in which spontaneous regression of the transplants had occurred.

When the Gardner lymphosarcoma (19) of strain C3H origin (also known as 6C3HED) is transplanted subcutaneously by trocar into CBA mice (obtained from Dr. L. C. Strong and inbred in this laboratory since 1941), a palpable tumor appears at the inoculation site within 8 days. This tumor is radiosensitive. At 10 days following transfer, when the tumor is 1 cm. in diameter, 600 r of x-rays directed to the tumor (remainder of animal lead-shielded) will induce regression within 4-9 days in 50 per cent of the cases.

Thirty-two mice in which tumor regression had been induced by x-radiation were subjected to a challenging reinoculation of the lymphosarcoma. Such transplants were made at various periods of time from 10-35 days following complete regression. Results are shown in Table 1.

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Once the tumor had regressed it did not reappear, and, of the 32 mice tested, 29 were immune to retransplantation of the same tumor. This immunity was demonstrable up to 105 days after tumor regression. Of twelve previously untreated CBA mice (Table 1) inoculated with the same tumor tissue, all succumbed to the disease within 12–48 days (average, 23 days). Although CBA mice are genetically distinct from the C3H strain of tumor origin, immunity to the Gardner lymphosarcoma may also develop in C3H mice. Stoerk and Emerson (19) reported that in riboflavin-deficient C3H mice the Gardner lymphosarcoma regressed, did not reappear, and the animals were subsequently resistant to retransplantation for at least 60 days.

Transfer of immunity by parabiosis.—In the present experiments immune CBA animals were placed in parabiosis with untreated CBA mice 15–45 days after a challenging inoculum into the immune mice. Litter-mates were not used, although CBA mice of the same weight, age, and sex were joined in parabiosis.

The method of parabiosis was that described by Sauerbruch and Heyde (16); it was an open coelomic method in which the peritoneum and abdominal muscles were united with one continuous suture. Another continuous suture united the skin of the two from the scapula to the crest of the ilium. Small strips of adhesive tape were used to bind together the inner forelegs, and similar tapes were applied to the tails to prevent strain on the sutures. Aseptic technic was employed. Skin healing was well established by the 5th postoperative day. At this time both parabionts were given subcutaneous inoculations of the Gardner lymphosarcoma. Only those animals which survived in parabiosis 15 or more days after transplantation are considered for analysis of data. There were fourteen such pairs whose survival ranged from 15 to 123 days after tumor transfer, the average being 34 days. Controls were twelve sets of nonimmune CBA parabionts which were inoculated with the same donor tumors as the test parabionts. These pairs averaged 30 days in parabiosis after transplantation, with a range of 20–36 days (Table 2).

Of the fourteen cases where actively immunized mice were in parabiosis with an untreated partner, in nine instances the latter resisted transplantation. In three sets only the untreated partner developed a tumor after inoculation, and in two pairs tumor growth occurred in both parabionts (Table 1). In eleven of the twelve control pairs, where both parabionts were untreated susceptible CBA mice, tumors developed in both members, and in one pair one of the animals did not develop a tumor (Table 2).

Immune mice in parabiosis with susceptible animals showed no local tumor upon reinoculation, whereas the susceptible member of the pair developed a barely palpable growth which subsequently regressed (nine of fourteen cases). It appears that the susceptible mice passively acquired sufficient resistance from the immune parabiont (by transfer of antibodies?) to check the growth of the tumor until their own defense mechanism destroyed it.

That the effect of x-rays in promoting tumor destruction was not the stimulus to immune reaction was demonstrated in the following manner. Twenty-one mice were simultaneously bilaterally

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**TABLE 1**

**GROWTH OF GARDNER LYMPHOSARCOMA IN MICE IN WHICH A PREVIOUS TRANSPLANT HAD REGRESSED FOLLOWING IRRADIATION AND IN UNTREATED MICE**

Regression was induced by 600 r of x-rays. Both groups of mice were of the same age and were inoculated with the same tumors.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. mice inoculated</th>
<th>Time after re-regression before challenging transplant (days)</th>
<th>No. immune mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice in which tumor had regressed</td>
<td>6</td>
<td>10–15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>18</td>
<td>6*</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>20–30</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>10–35</td>
<td>29</td>
</tr>
<tr>
<td>Previously untreated controls</td>
<td>12</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>* 105 days later these mice were still immune.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**TABLE 2**

**PASSIVE TRANSFER OF IMMUNITY BY PARABIOSIS**

Previously Untreated CBA Mice Were Joined to CBA Mice in Which a Gardner Lymphosarcoma Transplant Had Been Caused To Regress or, as Controls, to Untreated CBA Mice

**TEST**

<table>
<thead>
<tr>
<th>Immunized in parabiosis with susceptible mouse*</th>
<th>No. in which previously susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth in immune, no growth in susceptible parabiont: 9</td>
<td>No growth in immune, no growth in susceptible parabiont: 9</td>
</tr>
<tr>
<td>Growth in both immune and susceptible: 3</td>
<td>Growth in both immune and susceptible: 3</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Susceptible in parabiosis with susceptible mouse†</td>
<td>Susceptible in parabiosis with susceptible mouse†</td>
</tr>
<tr>
<td>No growth in either: 11</td>
<td>No growth in either: 11</td>
</tr>
<tr>
<td>Growth in only one: 1</td>
<td>Growth in only one: 1</td>
</tr>
</tbody>
</table>

* Survival time of 16–123 days—Average, 34 days.
† Survival time of 20–36 days—Average, 30 days.
inoculated with the Gardner lymphosarcoma. All 21 had large bilateral, subcutaneous tumors of similar size at death. A series of twenty mice were inoculated with the tumor on only the left side 10 days before a second inoculation was made on the right side. At autopsy only nine of the twenty had bilateral growths; the remaining eleven had enormous growths on the left but no tumor on the right side.

Apparently the Gardner lymphosarcoma provokes the development of resistance during its progressive growth in CBA mice, but the degree of resistance is ordinarily not sufficient to inhibit growth of the implant, whose presence caused the immune reaction. A second tumor inoculum is met with the already developed resistance, which, in 55 per cent of the cases, prevented progressive growth of the second transplant. X-radiation may merely cause the initial tumor to regress, allowing the animal to survive with its previously developed immunity. However, 90 per cent of the mice whose tumors had been caused to regress by x-radiation resisted retransplantation.

SUMMARY

A lymphosarcoma of strain C3H origin (Gardner lymphosarcoma, also known as 6C3HED) grew progressively when transplanted into CBA mice. When tumor regression was induced by x-radiation the mice were resistant to retransplantation of the same tumor. This resistance could be demonstrated up to 105 days after tumor regression and could be transmitted to previously susceptible animals of the same strain by parabiosis. Radiation effects per se may not have been responsible for producing the immune response, since 55 per cent of nonirradiated animals with rapidly growing tumors resisted a second tumor graft.

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Passive Immunization of Mice in Parabiosis Against a Transplanted Lymphosarcoma

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