In the last half century the use of x-rays, and other physical agents, in biological research has opened many fields of investigation. The basic work by Murphy and Taylor (9) has shown that x-rays have a definite effect on the artificially induced immunity of mice to transplantable cancerous tissues. This finding was adapted by Dempster et al. (3) to increase the survival of skin homografts. A high dosage of x-rays was employed by Main and Prehn (8), and by Trentin (11, 12), with the protective use of bone marrow. They found that homografts of skin from a foreign strain would grow in mice following the use of a high dosage of x-rays in conjunction with the injection of heterologous bone marrow, but not when isologous bone marrow was used. In experiments with human cancer, Toolan (18-20) found that the use of x-rays and/or cortisone enabled rats and hamsters to support the tumors. The tumors survived and proliferated for about 15 days, after which they showed signs of regression. By using a similar technic, Clemenzen (2) was able to pass a mouse tumor through x-radiated rats for 85 days. Fogh and Hok (5) also used x-rayed rats to grow cultured human cells. The entire field of conditioning the host animals is reviewed by Toolan (17).

The prolonged survival of skin grafts probably depends upon the fact that x-rays have an inhibitory effect on the antibody-forming mechanism of the mouse. Dixon (4) states: "The inhibitory effect of radiation on antibody response seems to be dependent upon radiation-induced changes prior to administration of antigen." Taliaferro (16) describes two effects of large doses of x-rays on antibody formation: "(1) the complete suppression of antibody formation which is associated with a very short radiosensitive stage at the beginning of the immune process and (2) a general retardation of the antibody process which is not necessarily incompatible with the formation of normal amounts of antibody but is associated with above normal peak titers in some animals. The radiosensitivity of the process is not limited to a short initial phase of immune process and may extend throughout the entire process." Gowen and Stadler (12) reported that the period of acute x-ray effects in the mouse was from 5 to 30 days after irradiation.

Gowen and Stadler (5, 12) also reported a series of experiments in which they studied the general effects of x-rays on mice. It was repeatedly found that, during the 25-day period of acute x-ray effects, a high number of deaths of mice could be expected for doses higher than 320 r. However mice...
surviving this critical period showed a varying degree of biological repair or regeneration—the amount of repair depending upon the x-ray dosage used. Results were also presented by these authors that whole-body irradiation had the greatest effect and that the resistance greatly decreased for 15 days after irradiation.

The effects of x-rays on a tumor of known genetic constitution have been reported by Reinhard et al. (10, 11) and by Warner and Reinhard (23). These workers state that x-ray doses as low as 25 r produce definite inheritable changes in a cancer, whereas in normal cells a much higher dose is required.

The experimental data reported in this paper are results obtained by combining these two approaches: (a) altering tumor characteristics by passage through a foreign host, and (b) altering the host's reactions by x-rays prior to challenge with the tumor.

**MATERIALS AND METHODS**

X-rays were administered at total doses of 350, 400, and 500 r, with doses of 175, 200, and 250 r on the 1st day, followed by an equal dose on the 2d day. Eight mice were irradiated at one time in a 150 mm. × 25 mm. petri dish with a perforated aluminum cover. X-rays were generated at 200 kilowatts, 30 ma., with the mice 30 centimeters from the tube. The x-rays were filtered by 0.5 mm. Cu and 1 mm. Al, and were administered at 226 r/min.¹

All mice used were raised in this laboratory and housed in wooden boxes with metal hoppers and kiln-dried sawdust as bedding. The food ration consisted of Mouse Blox and water fed ad libitum, plus a supplement of mixed grains and bread soaked in milk and cod liver oil. The boxes, metal hoppers, and bottles were changed at least once a week. Mice were kept in a room at 68–73° F. and 50–55 per cent humidity.

The two strains of mice used in this experiment were the pBr and F strains. The pBr strain was selected from the original NH strain. A mouse of the pBr strain developed a tumor of the forestomach which has been carried by subcutaneous transplantation for about 250 transfer generations (1).

The F strain, as reported by Strong (14), resulted when two unpedigreed mice were inbred for several generations. Mice of this strain develop lymphatic and myeloid spontaneous leukemias. This strain is completely unrelated to the pBr mouse and is genetically unable to support the progressive growth of the transplantable anaplastic carcinoma derived from a pBr mouse.

The pBr tumor used was designated 6N adapted, which signifies that the stomach tumor had been transplanted into mice of an ancestral strain, the N strain, for six generations, before being returned to mice of the pBr strain. The tumor was transplanted by the trocar method into the right flank.

**RESULTS**

The pBr tumor was passed through several generations in irradiated F mice. At first, alternate generations of pBr mice, the donor strain, were used to insure continuation of the tumor. This was done because of the small amount of tumor obtainable from irradiated F mice. After passage through irradiated F mice, the tumor was carried for a short time in pBr mice which received irradiation after transplants. Genetic tests were employed to test for any changes in the tumor.

Table 1 shows the results of transplants in F irradiated mice. Because many mice died before observation, they were classified separately. The per cent of takes is calculated for both the total and the corrected observed number of mice. A take is a transplant which can be observed in the mouse regardless of its eventual regression. When 294 F irradiated mice were inoculated with the tumor for 25 transplants generations, 172 grew the pBr tumor although for only a limited time, 14 failed to grow the tumor, and 108 died before observation. Death of the animals may be partly due to the fact that they had been irradiated. Chart 1 compares the persistence of the tumor in F irradiated and in pBr mice.

Table 2 gives the results of transplants in untreated pBr mice: 175 pBr have been used; of these, 155 were takes and 22 were rejects. A take

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¹ I am indebted to Mr. Melvin Reinhard of Roswell Park Memorial Institute for his supervision in the use of x-ray equipment.
in the pBr is the progressive growth of the tumor which eventually kills the mouse. In untreated F mice, 23 were takes and 90 rejects out of 113 mice (Table 3).

Table 4 shows the results in irradiated pBr mice. Of 22 mice, 30 were takes and two rejects.

Tables 5 and 6 show the results of genetic tests in F1 (pBr × F) and F2 (pBr × F) backcrossed to F. In all cases the tumor was first put in normal pBr mice to eliminate any transitory effects of x-radiation.

In the F2 generation, 260 mice were used, giving 78 takes and 182 rejects for a 30 per cent take.

The expected for normal pBr tumor, as shown by Strong (13) and Strong and Hardy (15), is a three-gene ratio with a 42.2 per cent take. Placing confidence limits around 30 per cent eliminates all except a four-gene ratio with a 31.6 per cent take.

In the F backcross, the same effect was demonstrated. The percentage changed from about 8 per

### Table 2

**RESULTS OF TRANSPLANTATION OF TUMOR FROM NON-IRRADIATED PBR, IRRADIATED PBR, AND IRRADIATED F MICE INTO NONIRRADIATED PBR MICE**

<table>
<thead>
<tr>
<th>No. transplant generations</th>
<th>Tumor origin</th>
<th>Total mice</th>
<th>Takes</th>
<th>Rejects</th>
<th>Per cent takes observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>pBr</td>
<td>44</td>
<td>43</td>
<td>1</td>
<td>97.7</td>
</tr>
<tr>
<td>2</td>
<td>pBr-Irr.</td>
<td>16</td>
<td>16</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>17</td>
<td>F-Irr.</td>
<td>110</td>
<td>94</td>
<td>16</td>
<td>81.7</td>
</tr>
<tr>
<td>Total: 26</td>
<td></td>
<td>175</td>
<td>153</td>
<td>22</td>
<td>87.4</td>
</tr>
</tbody>
</table>

* t, 2t, and 3t stand for test transplants of different tumor sublines.
DISCUSSION

After two transplant generations in irradiated F mice it was found necessary to use pBr intermediates to insure tumor life. Because the tumor grows in pBr mice, a small amount of tumor could be taken from F irradiated mice and increase its size in pBr before further transplant into F irradiated mice. The fact that 400 r irradiation caused acute death in the first transplant generations of F mice, plus the periodic shortage of F mice, also added to the need for pBr mice for the continued growth of the cancerous tissue.

After the first two transplant generations the irradiation dosage was changed from 400 r to 350 r (175 r on each of two days). This gave a slightly better survival of irradiated mice without showing any obvious effect upon the amount of tumor growth. The 500 r used in two generations were found to be lethally prohibitive. By the ninth transplant generation it was found no longer necessary to use pBr intermediates, and for several transplant generations the tumor was continued from F irradiated to F irradiated mice. However, it was seen that only small transitory takes could be produced on untreated F, and when genetic tests were begun transplantations into intermediate generations were resumed. This was mainly to insure that the results of the tests would be free of transitory changes caused by transplant into F irradiated mice.

In Table 1 the difference between the per cent of takes of the total and the per cent of takes of the observed can be explained by the fact that many of the mice died before observation could be made. These mice are tabulated separately, since, if all the mice were to live, the per cent of takes would be about the same as the 93.4 per cent take of the observed.

The persistence of the tumor in F irradiated and pBr mice, as shown in Chart 1, are roughly parallel for the first 20 days. However, after the biological repair begins in the F mice the tumor persistence begins to decrease, while that of the pBr mice remains generally constant. This correlation of the persistence of the cancer graft

TABLE 5
RESULTS OF TRANSPLANTATION OF DERIVED TUMOR FROM IRRADIATED F MICE INTO F (pBR X F)
The tumor was first passed through nonirradiated pBr mice.

<table>
<thead>
<tr>
<th>Transplant generation</th>
<th>Total no. in group</th>
<th>Takes</th>
<th>Rejects</th>
<th>Per cent take</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-St</td>
<td>14</td>
<td>5</td>
<td>9</td>
<td>34.7</td>
</tr>
<tr>
<td>18-St</td>
<td>39</td>
<td>15</td>
<td>24</td>
<td>38.5</td>
</tr>
<tr>
<td>21-St</td>
<td>24</td>
<td>25</td>
<td>29</td>
<td>46.3</td>
</tr>
<tr>
<td>21-St</td>
<td>11</td>
<td>4</td>
<td>7</td>
<td>36.4</td>
</tr>
<tr>
<td>22-St</td>
<td>20</td>
<td>6</td>
<td>14</td>
<td>30.0</td>
</tr>
<tr>
<td>24-St</td>
<td>19</td>
<td>4</td>
<td>15</td>
<td>21.1</td>
</tr>
<tr>
<td>26-St</td>
<td>18</td>
<td>5</td>
<td>15</td>
<td>16.7</td>
</tr>
<tr>
<td>27-St</td>
<td>45</td>
<td>7</td>
<td>38</td>
<td>15.6</td>
</tr>
<tr>
<td>34-St</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>50.0</td>
</tr>
<tr>
<td>35-St</td>
<td>38</td>
<td>5</td>
<td>27</td>
<td>16.0</td>
</tr>
<tr>
<td>Total:</td>
<td>260</td>
<td>78</td>
<td>182</td>
<td>Av.: 30.0</td>
</tr>
</tbody>
</table>

* See footnote to Table 3.

TABLE 6
RESULTS OF TRANSPLANTATION OF DERIVED TUMOR FROM IRRADIATED F MICE INTO F1 (pBR X F) BACKCROSSED TO F
The tumor was first passed through nonirradiated pBr mice.

<table>
<thead>
<tr>
<th>Transplant generation</th>
<th>Total no. in group</th>
<th>Takes</th>
<th>Rejects</th>
<th>Per cent take</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-St</td>
<td>17</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>24-St</td>
<td>37</td>
<td>4</td>
<td>33</td>
<td>10.8</td>
</tr>
<tr>
<td>26-St</td>
<td>19</td>
<td>0</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>34-St</td>
<td>46</td>
<td>0</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>35-St</td>
<td>61</td>
<td>1</td>
<td>60</td>
<td>1.6</td>
</tr>
<tr>
<td>Total:</td>
<td>190</td>
<td>5</td>
<td>175</td>
<td>Av.: 2.8</td>
</tr>
</tbody>
</table>

* See footnote to Table 3.
an extent, attributed to the poor quality of tumor that it was necessary to use. At times, tumor from the irradiated F mouse was very necrotic. The take of 30.6 per cent in untreated F mice, where none is normally observed, may be significant even though the tumors are small and transitory. However, this may also be a transitory effect of passage through x-rayed animals.

The final analysis of the results lies in tests of its genetic characters. Strong (13) and Strong and Hardy (15) reported that the pBr tumor needed the presence of three genes in the F2 (pBr X F) before it would grow. However, 30 per cent of the F3 (pBr X F) indicate that four genes are necessary for progressive growth of the pBr tumor after passage through irradiated F mice. All three of the above series of F2 mice were done in the same laboratory at about the same time. In the F1 (pBr X F) backcrossed to F, the same change is observed, although the gene requirement for tumor growth does not correspond completely with the requirement in the F2. It appears that there is a deficiency of susceptible mice in the backcross animals, which as yet is unexplained.

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A Change in a Transplantable Anaplastic Carcinoma by Passage through X-radiated F Mice

Warren Zahler


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