Mechanism of Tumor Immunity as Investigated by Means of the Intraocular Inoculation of the Brown-Pearce Carcinoma

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The mechanism of the immunity which can be induced in rabbits against the highly malignant Brown-Pearce carcinoma remains obscure.

Following the regression of intraocular implants of tumor tissue, Besredka and his collaborators (4-5) were unable to demonstrate the existence of humoral antibodies against the tumor. These observations were confirmed and extended by Gross (9) and by Cheever and Janeway (6). Furthermore, Favorite and Cheever (7) did not observe any deleterious effect on tumor cells growing in roller tube tissue cultures when sera and tissue extracts from immune rabbits were added. The pathological studies so far reported (1, 6) have failed to elucidate the problem.

Greene's (8) success in transferring homologous and heterologous tumors into the anterior chamber of the rabbit's eye suggested that this technic might offer certain advantages for the analysis of the immune mechanism, especially as Besredka and Bardach (2) had failed to obtain takes in resistant animals following intraocular implantation. Although the original purpose of the present investigation was to determine whether the tumor cells actually succumbed in the aqueous humor of the immune animal or merely remained inactive but viable, the recently reported failure of Saphir and his collaborators (12) to confirm the results of the French workers led us to reinvestigate the entire problem.

MATERIALS AND METHODS

A hybrid lot of brown-gray and white rabbits were used. The routine preparation and propagation of the tumor have been described (6). The methods of immunization were essentially those described by Pearce and Brown (11) and further developed by Besredka and his collaborators (3). The technic of intraocular inoculation closely followed that described by Krichesky and his collaborators (10). Each batch of tumor material used for challenging inoculations (i.e. to determine the presence or absence of immunity against the carcinoma) was tested for viability by the inoculation of a portion into the anterior chamber of the eye of one or more normal animals; in all cases typical takes resulted.

Primary inoculations.—To test the validity of the experimental procedure, 29 rabbits were inoculated intraocularly with tumor fragments. Seven of these animals received intratesticular inoculations also at the same time. The results are presented in Table I.

In the cases of successful grafts, growth was usually obvious at the end of a week, and the subsequent proliferation of the tumor was sufficiently rapid to fill the entire orbit within a month. The animals were sacrificed at this time. No metastases were found at autopsy. Only once was regression of an apparently well established growth observed: one rabbit receiving implants simultaneously in both eyes showed bilateral proliferation for the first 10 days followed by spontaneous regression of both tumors.

These preliminary experiments confirmed previous observations that unilateral or bilateral intraocular inoculations of the Brown-Pearce carcinoma in normal animals gave rise to progressive growths in at least 85 per cent of the cases, that regression was rare, and finally that gross metastasis did not take place within a month. Simultaneous intraocular and intratesticular inoculations gave rise to progressive growths at both sites.

Secondary eye inoculations.—Sixteen animals received inoculations of the tumor material in one eye.

### Table I: Primary Inoculations of Normal Animals

<table>
<thead>
<tr>
<th>Number of rabbits inoculated</th>
<th>A. One eye inoculated</th>
<th>B. Both eyes inoculated simultaneously</th>
<th>C. One eye and one testicle inoculated simultaneously</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Takes</td>
<td>Failures to take</td>
<td>Takes in both eyes</td>
</tr>
<tr>
<td>14</td>
<td>12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* John Ware Memorial Fellow.
At varying periods thereafter fresh tumor material was introduced into the other eye and the fate of this 2nd graft observed.

Tumors growing in one eye for as long as 3 weeks were usually ineffective in preventing the growth of carcinomatous material subsequently implanted in the other eye. Takes resulted in all but 4 out of 17 animals tested. There was no evidence that the state of the first tumor was of importance in affecting the fate of the 2nd graft in 76 per cent of the animals tested.

Rabbits were then immunized by several of the methods referred to above (6) and at varying intervals thereafter each was given an intraocular inoculation of viable tumor material. In addition to these methods, several animals from which growing tumors were removed by operative procedures and a group with proliferating tumors in various sites were tested for susceptibility by intraocular tumor implants. The results are summarized in Table III.

The presence of a growing tumor in the testicle or skin prevented the growth of a tumor in the eye in all but 1 of 5 animals so treated. The spontaneous regression or the surgical removal of previously implanted tumors appeared to render 97 per cent of the animals refractory to subsequent intraocular inoculations of the carcinoma. In 4 instances (rabbits No. 51, 95, 64, 339) unsuccessful attempts were made to break down this immunity by vascularizing the cornea to provide a rich capillary bed, previous to the implantation of the tumor in the anterior chamber of the eye.

From these results one may conclude that all methods of immunization used appeared to be equally effective.

Removal of tumor from eye of immunized animals for reimplantation.—It was noticed in the previous experiments that intraocular implants, while failing to grow and becoming pale and slightly shrunken in the anterior chambers of the eyes of immune rabbits, did not become necrotic or disintegrate for long periods of time. Accordingly it became of interest to determine whether these fragments were merely lying dormant or whether some agent in the aqueous humor (antibody?) had caused the cells to lose their property of malignant proliferation. Rabbits immunized by the regression of testicular or intracutaneous tumors were inoculated in one eye with carcinomatous material as a test for immunity. An equal number of normal animals were inoculated simultaneously and typical takes were observed in all this latter group, but none among the immune rabbits although they were carefully observed for 6 weeks. The animals were then subjected to a 2nd intraocular inoculation with fresh tumor material in the opposite eye. Again no takes were obtained, although portions of the same malignant tissue introduced simultaneously into the eyes of normal rabbits gave rise to typical proliferative lesions in every instance.

After remaining for varying periods of time (5 to 14 days) in the anterior chambers of the eyes of the immune rabbits, these fragments of tumor material were removed and introduced into the eyes of normal rabbits. At the time of transfer the fragments appeared quiescent; they were pale and slightly shrunken but of relatively firm consistency with no evidence of either necrosis or inflammatory reaction. These fragments (which were somewhat larger than those employed in the previous experiments) gave rise to typical

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Primary inoculation</th>
<th>Time between primary and secondary inoculation, days</th>
<th>State of implant in 1st eye at time of inoculation of 2nd eye</th>
<th>2nd inoculation</th>
<th>Time between and inoculation and autopsy, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eye</td>
<td>Result</td>
<td></td>
<td>Eye</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Left</td>
<td>Take 4</td>
<td>Definite take with increase in size</td>
<td>Right Take 23</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>&quot;</td>
<td>5</td>
<td>No activity</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>&quot;</td>
<td>5</td>
<td>Definite take with increase in size</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>&quot;</td>
<td>6</td>
<td>No activity</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>&quot;</td>
<td>6</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>369</td>
<td>&quot;</td>
<td>6</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>Right</td>
<td>No take 7</td>
<td>&quot;</td>
<td>Left No take 38</td>
<td></td>
</tr>
<tr>
<td>156</td>
<td>&quot;</td>
<td>8</td>
<td>Definite take with considerable growth</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>79</td>
<td>Left</td>
<td>Take 12</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>&quot;</td>
<td>12</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>&quot;</td>
<td>13</td>
<td>Anterior chamber half filled</td>
<td>No take 35</td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>&quot;</td>
<td>13</td>
<td>Definite take, fragment tripled in size</td>
<td>Right Take 15</td>
<td></td>
</tr>
<tr>
<td>409</td>
<td>Right</td>
<td>15</td>
<td>Anterior chamber 2 filled</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>Left</td>
<td>21</td>
<td>Anterior chamber filled</td>
<td>Left 20</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>&quot;</td>
<td>21</td>
<td>&quot;</td>
<td>Right 14</td>
<td></td>
</tr>
<tr>
<td>75*</td>
<td>&quot;</td>
<td>21</td>
<td>Anterior chamber 1 filled</td>
<td>No take 14</td>
<td></td>
</tr>
<tr>
<td>319</td>
<td>Right</td>
<td>120</td>
<td>Anterior chamber filled</td>
<td>Left Take 14</td>
<td></td>
</tr>
</tbody>
</table>

* Received a bilateral testicular inoculation at same time. Bilateral tumors resulted.

Table II: Secondary Eye Inoculations

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The susceptibility of these rabbits to the tumor was

The procedures employed in these experiments will be apparent from the data included in Table IV. The results are summarized in Table V.

**Table III: Intraocular Inoculations in Immunized Animals**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Route and method of immunization</th>
<th>Interval between immunizing and test inoculations, days</th>
<th>Test inoculation</th>
<th>Interval from test inoculation to autopsy, days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GROUP I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Regression of testicular tumor</td>
<td>90</td>
<td>Left No take</td>
<td>90</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>40</td>
<td>Right &quot;</td>
<td>48</td>
</tr>
<tr>
<td>95</td>
<td></td>
<td>109</td>
<td>Left &quot;</td>
<td>90</td>
</tr>
<tr>
<td>385</td>
<td></td>
<td>50</td>
<td>&quot;</td>
<td>60</td>
</tr>
<tr>
<td><strong>GROUP II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Regression of intraperitoneal tumor</td>
<td>54</td>
<td>Left No take</td>
<td>56</td>
</tr>
<tr>
<td><strong>GROUP III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Regression of testicular and intraperitoneal tumors</td>
<td>91</td>
<td>Left No take</td>
<td>60</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td>58</td>
<td>Right &quot;</td>
<td>41</td>
</tr>
<tr>
<td>41B</td>
<td></td>
<td>49</td>
<td>Left Take followed by regression</td>
<td>51</td>
</tr>
<tr>
<td>39B</td>
<td></td>
<td>99</td>
<td>Right &quot;</td>
<td>41</td>
</tr>
<tr>
<td>98</td>
<td></td>
<td>145</td>
<td>Left &quot;</td>
<td>54</td>
</tr>
<tr>
<td><strong>GROUP IV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>339</td>
<td>Regression of intracutaneous tumors</td>
<td>57</td>
<td>Right No take</td>
<td>90</td>
</tr>
<tr>
<td>402</td>
<td></td>
<td>49</td>
<td>Left Early take, subsequent regression</td>
<td>60</td>
</tr>
<tr>
<td>348B</td>
<td></td>
<td>14</td>
<td>Left No take</td>
<td>37</td>
</tr>
<tr>
<td><strong>GROUP V</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Excision of testicular tumor 14 days previous to test inoculation</td>
<td>154</td>
<td>Left &quot;</td>
<td>32</td>
</tr>
<tr>
<td>346</td>
<td>Excision of testicular tumor day of test inoculation</td>
<td>14</td>
<td>Left &quot;</td>
<td>32</td>
</tr>
<tr>
<td>344</td>
<td>Testicular tumor</td>
<td>14</td>
<td>Left &quot;</td>
<td>32</td>
</tr>
<tr>
<td>348</td>
<td>&quot; &quot;</td>
<td>14</td>
<td>&quot;</td>
<td>32</td>
</tr>
<tr>
<td>281</td>
<td>&quot; &quot;</td>
<td>7</td>
<td>&quot;</td>
<td>25</td>
</tr>
<tr>
<td>282</td>
<td>&quot; &quot;</td>
<td>7</td>
<td>&quot;</td>
<td>25</td>
</tr>
<tr>
<td>206</td>
<td>5 intracutaneous tumors</td>
<td>14</td>
<td>Take</td>
<td>26</td>
</tr>
</tbody>
</table>

**Table IV: Reimplantation of Tumor Fragments from Eyes of Immunized Animals**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Date of immunization, 1941</th>
<th>Method of immunization</th>
<th>Date of test of immunity, 1941</th>
<th>Test inoculation</th>
<th>Number of days in eye of immune animal</th>
<th>Transplant to normal animal</th>
<th>Test of susceptibility of normal rabbit Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>385</td>
<td>Jan. 23</td>
<td>Regression of testicular tumor</td>
<td>Mar. 13</td>
<td>Left No take</td>
<td>Right</td>
<td>14</td>
<td>No take</td>
</tr>
<tr>
<td>348B</td>
<td>Aug. 8</td>
<td>Regression of intracutaneous tumors</td>
<td>Aug. 18</td>
<td>Right &quot;</td>
<td>Left, 2 fragments</td>
<td>292</td>
<td>Take</td>
</tr>
<tr>
<td>350</td>
<td>Nov. 15</td>
<td>&quot;</td>
<td>Nov. 29</td>
<td>Left &quot;</td>
<td>Right, 2 fragments</td>
<td>14</td>
<td>No take</td>
</tr>
</tbody>
</table>

proved by the subsequent inoculation of fresh malignant tissue into the anterior chamber of the other eye. In each case a typical take resulted.

From 3 of the animals with reimplanted fragments of tumor which had resided in the eye of an immune animal for 14 days, fragments were taken for micro-
scop examination 4 months following the reimplanta-
tion. The fragments at this time were pale and slightly
shrunken but not necrotic. Study of the stained sec-
tions revealed that the tumor cells were replaced by
a mass of collagenous material containing a few capil-
laries and occasionally an isolated tumor cell or a
small group of such cells which showed no signs of
proliferation. This experiment suggested a possible
relationship between the increasing length of time a
tumor fragment remained in the eye of an immune
animal and its decreasing chance of proliferating when
transferred into a normal rabbit. That the actual
transfer of tumor material from one eye to another
did not affect its chances of proliferation in the 2nd
animal was shown by observations that fragments
growing in normal rabbits as long as 10 and 14 days,
and up to 3 months in one case, always continued to
proliferate when transferred to other normal animals.

TABLE V: SUMMARY OF REIMPLANTATION OF TUMOR FRAGMENTS

<table>
<thead>
<tr>
<th>Number of days tumor fragments remained in eyes of immunized rabbits</th>
<th>Number of fragments inoculated into eyes of normal rabbits</th>
<th>Number of takes</th>
<th>Number of failures to take</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>11</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Besredka and Bardach (2) failed to engraft the
Brown-Pearce carcinoma in the eyes of rabbits previ-
ously immunized against the tumor. Our experiments
bear out their observations, and are not in accord
with those of Saphir and his coworkers (12). These
investigators suggest that the French workers did not
extend their observations for a sufficient period, but
this explanation of the discrepancy does not seem
applicable to the present experiments since the great
majority of our animals were not killed until at least
6 weeks or more had elapsed after the test inoculations.

A growing testicular tumor immunized against
subsequent intraocular inoculations, but a proliferating
carcinoma in one eye failed to render the animal re-
fractory to subsequent inoculations of tumor material
into the other eye. Intraocular tumors appeared to be
exceptions to the general rule that proliferating
growths immunize the animal against subsequent
inoculations of the same material. The reason for
this is not clear, but the failure of Greene (8) and
of the present authors to discover metastases from
anterior chamber tumors may have some relation to
this lack of immunity since the failure of tumor cells
to escape from the eye may prevent the inauguration
of a general systemic resistance which is dependent
upon some hypothetical immunizing agent in such
material.

By what mechanism is the proliferation of tumor
grafts in the anterior chamber of the eyes of immu-

nized rabbits prevented? The implanted fragments
remained intact for several weeks, showing no signs
of necrosis. Ultimately they became pale and some-
what shrunken but we never observed any evidence
of an inflammatory reaction in either iris or cornea;
in fact these structures in the immune animal appeared
indifferent to the presence of the malignant cells.

There was no indication of a foreign body reaction
such as has been described surrounding regressing
tumors implanted elsewhere (13). The microscopic
examination of 3 of these tumor fragments, which
on removal from the immune host were reimplanted
in a normal animal, likewise showed no evidence of
necrosis or inflammatory reaction though the tumor
cells were largely replaced by connective tissue in-
cluding large amounts of collagen.

The failure of the host to develop an angiofibrous
supporting stroma for the tumor is a possible explana-
tion for the inability of the latter to take. Working
with the rabbit carcinoma H31 Greene (8), however,
have shown that in the eyes of guinea pigs the tumor
may lie latent for as long as 120 days before pro-
liferating. During this period there is no reaction
on the part of the iris and no obvious blood supply to
the tumor. He also noted that fragments remaining
inert for over 500 days showed typical and rapid
proliferation when removed from the guinea pig and
transplanted into its native host, the rabbit.

Fragments of the Brown-Pearce carcinoma remain-
ing but 5 days in the anterior chamber of the eyes
of immune animals showed a 50 per cent mortality,
and only 2 fragments out of 11 proliferated upon
reimplantation into normal rabbits' eyes after a sojourn
of 2 weeks in the anterior chambers of the eyes of
immune animals. Although these findings are ad-
imittedly inconclusive they suggest the existence of an
agent in the aqueous humor of the eyes of immune
animals which acts slowly but nonetheless with dele-
terious effect upon specific tumor grafts. Its poten-
cy is probably not great since 2 fragments were able to
proliferate upon transfer into normal animals after 2
weeks' contact with it in the anterior chamber of the
eyes of immune animals. However, these tumor
fragments were of considerable size so that malignant
cells in the center of the mass were protected by
adjacent cells from any agent diffusing into the piece
of tumor tissue.

**SUMMARY**

1. The implantation of fragments of the Brown-
Pearce carcinoma into the anterior chamber of the eye
of normal animals was followed by successful takes in most instances, regardless of whether the inoculation was unilateral or bilateral.

2. Secondary intraocular inoculation in animals immunized by any one of several standard methods was unsuccessful in 18 of 20 cases.

3. Secondary intraocular inoculations as long as 4 months after the primary implantation of tumor into the other eye were generally successful, regardless of the state of activity of the first growth at this time.

4. Of 11 tumor fragments remaining as long as 14 days in the eyes of immune animals only 2 proved viable and capable of delayed but sustained proliferation when implanted into the eyes of normal animals.

5. This evidence, though far from unequivocal, suggests the existence of an agent in the aqueous humor of immune rabbits' eyes capable of destroying or rendering nonmalignant the cells of the Brown-Pearce carcinoma.

REFERENCES


Mechanism of Tumor Immunity as Investigated by Means of the Intraocular Inoculation of the Brown-Pearce Carcinoma

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