THE ENDURING PARTNERSHIP OF A NEOPLASTIC VIRUS AND CARCINOMA CELLS*

CONTINUED INCREASE OF VIRUS IN THE V2 CARCINOMA DURING PROPAGATION IN VIRUS-IMMUNE HOSTS

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The rabbit papilloma virus (Shope) gives rise under natural conditions to horny cutaneous growths in wild cottontails (1), and to papillomas of similar sort when inoculated into domestic rabbits. The growths of both species may become malignant after a while through alterations in the virus-infected cells (2); but the virus has never been recovered from the cancers, nor can it be got ordinarily from many of the papillomas induced with it in domestic rabbits. Yet it persists in masked or altered form in the cancers as well as in the papillomas, as serological tests have proved: an antibody directed specifically against the virus appears in the blood of rabbits carrying the growths, and its titer rises as the tumors enlarge (3).

One of the cancers originating in a virus-induced papilloma and transplanted successfully—the V2 carcinoma—has now been carried through 21 tumor generations in the course of more than three years. It grows rapidly in a considerable proportion of hosts and often metastasizes. Tests have been made for the specific antiviral antibody in the blood of rabbits of every tumor generation,—more than a hundred animals in all. This has never been found in the blood of normal control animals, nor in noteworthy titer in rabbits implanted with the tumor but negative; but it has regularly appeared in the blood of rabbits in which the V2 carcinoma has grown progressively, attaining a titer as high as that in animals which have long carried large papillomas, or even higher. The strength of the antibody has been as great in recent tumor generations as in the early ones (3).

The persistence of virus in the transplanted V2 carcinoma does not seem remarkable when the fact is recalled that wholly extraneous, parasitic viruses—vaccinia, virus III, yellow fever, infectious ectromelia, to name but a few—can ride along as passengers in other transplanted tumors (4), as can also spirochetes, pleuropneumonia-like microorganisms, and various other bacteria (5).

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But the fact is now becoming manifest that passenger viruses may disappear spontaneously from the tumors they ride upon, while some have been got rid of by transplanting the growth to hosts immune to the virus. Rivers and Pearce observed long ago that virus III could vanish from the Brown-Pearce tumor as casually as it had appeared, and Andrewes recently noted that a rabbit sarcoma—the RSI—became free of passenger virus III when transplanted through a rabbit previously immunized against it (6). He also found that the Brown-Pearce carcinoma could be rid of virus III by propagation in two successive virus III-immune rabbits, and from vaccine virus by passage through a single animal immune to it. Identical results were got with the same materials by Pearce (7).

These findings bring up the possibility that the V2 carcinoma can be freed of associated virus by growing it in a succession of hosts immunized against this. The work here reported was done to learn whether such is the case. The general plan was to propagate the V2 carcinoma in five successive groups of animals all hyperimmunized beforehand against the papilloma virus, and then to determine whether, upon return to normal hosts, the growth would elicit antiviral antibodies in the same high titer as before.

Methods

To hyperimmunize the rabbits to which the tumor was to be transferred, four “pancake” papillomas were produced on the flanks of the animals by rubbing a potent virus suspension into areas of skin about 6 × 8 cm. which had been freshly scarified with sandpaper. Confluent papillomatosis usually resulted after an incubation period of about 10 days, and the growths rapidly enlarged into characteristic horny masses, these often rising 2 cm. or more above the skin level. Growth of this sort usually elicits antiviral antibody in considerable titer, and this can be greatly increased by repeated injections of large quantities of virus intraperitoneally, as previous work has shown (8). Accordingly, 10 days to 3 weeks after the scarifications, 10 cc. of a 1:20 Berkefeld V filtrate of wild rabbit papillomas which were known to contain virus in quantity was injected intraperitoneally into each animal. The intraperitoneal injections were repeated 7 days later, and after a further interval of 7 to 10 days the rabbits were bled from an ear vein and the sera tested. At this time they were implanted with the tumor.

Much use was made of the complement fixation test to titrate the antiviral antibody. Previous work had demonstrated its specificity and reliability as a gauge of immunity to the papilloma virus (9). The test was carried out as previously described (9), using 2 units of complement (titrated immediately beforehand) and 2 hours at room temperature for fixation. Antigen from two sources was used—the natural papillomas of W. R. (wild cottontail rabbit) 1-28, and the pooled natural papillomas of five cottontails, P. Both materials yielded much virus. A 1:120 saline extract of the glycerolated papillomas from both sources had been found in previous experiments to provide an optimal quantity of antigen (virus), and hence this dilution was uniformly used. Mention need hardly be made here of the fact that the complement
fixation reaction provides a comparative, not an absolute, measure of the titer of antiviral antibody. Sometimes the results with a single serum varied by as much as a twofold dilution in different tests; but the variation was never greater. A serum can be considered very potent if, after dilution to 1:24 or more, it contains enough antibody to fix completely 2 units of complement in mixture with an optimal dose of antigen. Such a serum is capable of neutralizing many thousand infectious doses of virus, and an animal yielding it is usually resistant to infection with the virus, as previous studies have shown (8). In the present work the antibody titer has been expressed in terms of the highest dilution of serum that gave complete or almost complete fixation in the standard test. Dilutions beyond 1:128 were not tested, for when fixation is complete at this dilution the quantity of antibody is great, and hyperimmunization may be deemed maximal.

The neutralization test for antiviral antibody was also used. It was applied as previously described (14, 9).

The virus suspensions for primary inoculation and subsequent hyperimmunization were made by grinding the glycerolated natural papillomas of cottontails in a mortar with sand and suspending the ground paste in physiological saline, usually 1:10 to 1:20 (10 per cent and 5 per cent extracts). The suspension was then spun clear, and sometimes passed through a Berkefeld V filter in addition. To spread the conditions, five different virus materials were used, all highly pathogenic. Always a different material was injected intraperitoneally from that with which the rabbit had been cutaneously inoculated.

Normal adult gray brown rabbits (agouti hybrids) were used throughout, males and females indiscriminately. All weighed 2.5 kilos or more.

Transplantation of the tumor was effected by a method somewhat different from that used in the previous work, in order to provide conditions under which the antiviral antibody might have better opportunity to act. A fine suspension of carcinoma tissue was used instead of hashed pieces, and the tissue was kept moist and finally suspended in Tyrode to which immune serum had been added. The tumors were procured with aseptic precautions, and the "healthiest" portions carefully selected from nodules taken from at least two different situations in each animal. By means of a small pestle, the tumor tissue was pressed through a 40-mesh monel metal sieve, with the addition of Tyrode-immune serum (usually 10 parts of Tyrode to one of the animal's own serum), the result being a turbid, finely particulate suspension. 1 cc. portions of the suspension were then implanted into the leg muscles of the new hosts at six situations,—both forelegs and the anterior and posterior muscles of both thighs. The fastest growing tumors were sometimes selected for transplantation and again slower-growing ones were utilized to spread the conditions.

Successive Transplantations of the V2 Carcinoma in Virus-Immune Rabbits

The course of the transplantations is summarized in Chart 1.

The Starting Tumor.—The history of the V2 carcinoma has already been given in detail from its inception to the 13th serial transplantation, when the present experiments were begun (3). To procure material for them a 12th generation tumor was implanted into the muscles of the upper forelegs and anterior and posterior thighs—
Passage of the Vs Carcinoma through Immunized Rabbits

Chart 1. A summary of the transplantations. The black circles denote individual rabbits in which tumors grew progressively; half-white ones, animals in which regression occurred; white ones, rabbits in which no palpable nodules developed. The animals remain anonymous except for those providing material for transfer and the ones bled for serum in the final generation—see Table III. D. R. = domestic rabbit; K = killed; † = died from tumor. For further explanation see text.
the usual situations—in D. R. (domestic rabbit) 12-37, amongst others. Palpable
nodules were present in three of the situations when the animal was examined on the
17th day, and in two more later on. By the 42nd day the five nodules range from 1.2
to 3.2 cm. in size. All enlarged greatly in the ensuing 8 weeks, at length reaching
diameters of 7.5 to 10.0 cm., the animal during this time becoming thin and weak.
The antiviral antibody titer of its blood as determined by complement fixation was
found to be 1:128 on the 107th day, when it was killed and the tumors procured for
transplantation. They were huge cysts of the sort already described (3), filled with
thick, glairy, mucoid fluid in which floated yellowish gouts of necrotic material, and
with walls 1 to 3 mm. thick, composed of pale-pink, close-texture tumor tissue. The
healthiest portions of the neoplastic tissue were pressed through the monel metal sieve
and suspended in a mixture of Tyrode and the animal's own serum (proportion 20:1).

First Transfer to Hyperimmunized Rabbits, 14th Generation.—The tumor suspen-
sion of D. R. 12-37, described above, was implanted into the six muscle situations of
six hyperimmunized rabbits. At the time all bore four large confluent papillomatous
masses, each about 6 X 8 cm. across and raised 0.4 to 0.8 cm., as result of inoculation
30 days before with W. R. (wild rabbit) 1-72 virus, 1:20. Intraperitoneal injections
of 10 cc. of virus filtrate W. R. 1-68, 1:20, had been made on the 14th and 21st days
and the serum antibody titers now ranged from 1:32 to 1:128. Progressively enlarg-
ing carcinomas developed in three of the implanted animals, early regression took
place in one, and no palpable tumors developed in the other two (see chart).

Further Transfers in Hyperimmunized Animals.—By
the 40th day after implanta-
tion the six growths of D. R. 1-77 (14th generation—hyperimmunized) had become
cysts of characteristic sort, varying from 3.5 to 7.5 cm. in diameter. The rabbit was
bled and the complement fixation titer found to be 1:48. It was killed and a sus-
pension made by sieving the healthy portions of its tumors into Tyrode plus the
animal's own serum (10:1). The suspension was then implanted into the leg muscles
of twelve hyperimmunized rabbits (15th generation) all of which carried large con-
fluent papillomas resulting from the inoculation of W. R. 1-28 virus, 1:10, 24 days
before. The animals had been injected intraperitoneally with 10 cc. of virus filtrate
W. R. 16-96, 1:10, on the 12th day, and with 10 cc. of virus filtrate W. R. 1-68, 1:10,
on the 18th day, and they now had serum antibody titers ranging from 1:48 to 1:128.
The implantations resulted in progressively enlarging tumors in four rabbits, regress-
ing ones in five, and no palpable growths in three.

The details of the remaining transfers into hyperimmunized animals need not be
given, for they do not differ significantly from those just furnished.

The Eventual Transfer to Normal Animals.—Tumors grew progressively in seven
of the eight implanted rabbits of the 18th generation—the fifth hyperimmunized
group. The growths of three (D. R. 14-21, 14-22, and 14-23) were implanted in
normal rabbits, as indicated in the chart, each material into a different group of eight
animals. For the final transfers sieved suspensions were made as usual, but plain
Tyrode was used instead of Tyrode plus host serum, since it was deemed advisable to
avoid the passive transfer of antibody as possibly affecting the outcome of tests of the
blood of the new hosts. As the chart shows, the implantations from D. R. 14-21
grew progressively in half of the eight normal rabbits, regressing in the others; the
D. R. 14-22 tumors gave progressively enlarging growths in two instances, regress-
ones in four, and no palpable nodules in two; while the D. R. 14–23 material provided progressively enlarging tumors in five rabbits, regressing growths in two, the eighth individual remaining negative. Details of the serum tests will be given further on.

### General Findings

When viewed in the large, the results of the transfers, as summarized in Chart 1, leave no doubt that the V2 carcinoma can be readily propagated in animals hyperimmunized against the papilloma virus. It was maintained in this way in five successive tumor generations during nearly 8 months in all. Implantation of the growth into animals having very high titers of antiviral antibody often resulted in tumors that appeared promptly and grew progressively, frequently killing the hosts; but the outcome of the implantations varied much from one generation to the next and from individual to individual in the same generation. The question arises, therefore, whether the tumor grew as well in virus-immune animals as in comparable normal ones. Chart 1 goes far towards answering the question. The cancer flourished in many of the hyperimmunized hosts, its course comparing favorably with that in the tumor generations before hyperimmunization was undertaken (3), and when it was eventually returned to normal animals the number of “takes” and of progressively enlarging and regressing growths, though differing somewhat from one group of hosts to another, was on the whole no different from that obtaining in the last generation of hyperimmunized rabbits. To safeguard the tumor it had been propagated in normal animals as well as in the hyperimmunized ones throughout the period of experimentation. Hence the material for an additional comparison is available (Table I).

The passages in normal rabbits were made during the same months as those through the hyperimmunized but were not done simultaneously, and the materials used, though of the same tumor generations, came of necessity from different groups of animals. Furthermore, hashed tissue was implanted into the new normal hosts, and sieved suspensions into the hyperimmunized; and the animals of both groups were market bought, which is to say that none was pure bred. Even so, it becomes evident from Table I that the outcome of implantations of the V2 carcinoma, though varying somewhat as in the case of other propagated neoplasms, was not significantly different in the hyperimmunized and the normal rabbits. “Takes” developed in two-thirds or more of the implanted rabbits in all of the groups (Table I). Progressively enlarging tumors developed in from 28 to 87 per cent of the hyperimmunized animals and in from 17 to 54 per cent of the normals; while regression took place in from 13 to 56 per cent of the hyperimmunized rabbits and in from 33 to 83 per cent of the controls.

From the findings just given it is manifest that the V2 carcinoma grew as well in the hyperimmunized rabbits as in the normals; and this fact makes it seem altogether unlikely that the antibody titer of the hyperimmune rabbits at the time of implantation would have any influence on the outcome. That it had none can readily be seen from an inspection of Chart 1. In the 14th gen-
eration, for example, tumors enlarged progressively in three animals having antibody titers when implanted of 1:64, 1:64, and 1:32, respectively; no palpable nodules appeared in a rabbit with antibody titer of 1:64; and regression of the growths took place in two animals with titers of 1:32 and 1:128, respectively. So too in the 15th and the succeeding generations: the tumors grew, or failed to develop, or regressed irrespective of the antibody titer at time of implantation.

### TABLE I

**Outcome of Implantations of the V2 Carcinoma in Rabbits Hyperimmunized against the Papilloma Virus and in Normals**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Number of rabbits implanted</th>
<th>Number developing palpable nodules</th>
<th>Number in which tumors grew progressively</th>
<th>Number in which tumors regressed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyperimmunized</td>
<td>Normal</td>
<td>Hyperimmunized</td>
<td>Normal</td>
</tr>
<tr>
<td>14th</td>
<td>6</td>
<td>11</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>15th</td>
<td>12</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>16th</td>
<td>7</td>
<td>12</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>17th</td>
<td>8</td>
<td>12</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>18th</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Hybrid agouti rabbits procured from various dealers were used throughout. The transplantations in corresponding hyperimmunized and normal generations were made during the same months but not simultaneously.

No distinction is made in the table between the animals in which regression was complete and the occasional ones in which it was partial and transitory, i.e., individuals in which all of the tumors dwindled for a time and some disappeared but one or more later enlarged.

Attention may be called in passing to the fact that the antibody titers of the hyperimmunized rabbits that provided tumors for transplantation were lower when the animals were killed than at time of the implantations, 40 to 52 days before (Chart 1). The finding was not unexpected. For the high titers resulting from the injections of large quantities of virus intraperitoneally are not maintained, as previous experience had shown, and the antibody response was presumably at or near its height at the time the implantations were made. In many unimplanted rabbits immunized in other experiments the antibody titers fell during the 6 to 8 weeks following the intraperitoneal injections to less than one-half the maximum. Manifestly the virus present in the large papillomas growing on the bellies of the rabbits of the present experiments and in the V2 carcinomas proliferating in their leg muscles failed to keep the antibody
titer at its highest level. Such findings will not seem strange to students of the infectious diseases. Two possible reasons for them may be mentioned in the present case. The papillomas of domestic rabbits provide in general comparatively little antigenic stimulus, eliciting much lower titers of antiviral antibody than do growths of comparable size and duration in cottontails (9); and the V2 carcinoma, which eventually elicits very high titers of the antiviral antibody, does so only after the tumors have become large and cystic and have persisted for many weeks, as observations made recently with the collaboration of Dr. William F. Friedewald attest.

It is of interest to compare the course of the implanted V2 carcinomas with that of the cutaneous papillomas present on the same animal, for the reason that their relation to the host is very different. The cells of the V2 carcinoma come from another animal—the one in which this tumor first arose—whereas those of the papilloma are the animal's own, now infected with virus. Regression of the V2 carcinoma, like that of other transplanted cancers, would appear to be consequent upon an induced general resistance, directed against the foreign cells (10); whereas regression of the papilloma involves a resistance which is directed against such of the animal's own cells as have been rendered neoplastic by the virus (11). In neither case does the antiviral antibody have any influence on the fate of the tumor: growths of both sorts often dwindle away in rabbits having little or no antiviral antibody in their blood, and they frequently grow progressively in other animals having high serum antibody titers. Can it be that regression of the V2 carcinoma and that of the papilloma depend upon the same mechanism?

A summary is given in Table II of the course of the papillomas and carcinomas in the 41 hyperimmunized rabbits of Chart 1. The papillomas grew progressively in all of the rabbits except two, whereas in six rabbits no carcinomas appeared and in fourteen others they regressed after having attained a diameter of 1 cm. or more by the 16th day after implantation. In twelve of them the nodules had regressed completely by the 40th day or shortly thereafter; in the other two regression was partial and transitory, with dwindling of the nodules between the 16th and 30th days, complete disappearance of several of them, and later enlargement of one or two.

The V2 carcinoma grew progressively in the one rabbit in which the papillomas regressed entirely. This animal (D. R. 12–83, 15th generation—hyperimmunized) developed only scattered discrete papillomas as result of the inunction of the highly pathogenic W. R. 1–28 virus, 1:10, and these attained a height of 1 to 2 mm. on the 25th day; but had all regressed completely by the 38th day. The V2 carcinomas, implanted on the 25th day after virus inunction, grew vigorously and progressively from the start at every situation, becoming 1.5 to 2.4 cm. in diameter by the 38th day, that is to say, during the period when the surface papillomas were regressing. They continued to enlarge, formed huge cysts 4.0 to 6.0 cm. across, and brought about the animal's death on the 88th day (63rd day after the implantations).

From the data just given (Table II) it becomes evident that virus-induced papillomas of autochthonous origin and V2 carcinomas resulting from transplantation may grow or regress independently of one another in the same host.
The findings do not allow a definite conclusion as to whether regression of the two types of growths is brought about by similar mechanisms, but they provide a sound basis for doubting that the mechanism is identical in the two cases. Local conditions and other factors as well have an important influence upon the course of virus-induced papillomas (11), and similar influences may be responsible for the highly various outcome of implantations with the V2 carcinoma.

The passage of the V2 carcinoma through the hyperimmunized animals did not result in any perceivable alteration in the neoplasm. Autopsies were done as routine on all animals dying from the tumor, and representative blocks were taken for microscopic study. No differences could be made out between the growths in the hyperimmunized rabbits and those in the normals.

TABLE II

<table>
<thead>
<tr>
<th>Fate of Autochthonous Virus-Induced Papillomas and Transplanted V2 Carcinomas in the Hyperimmunized Rabbits of Chart 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillomas and carcinomas grew progressively................</td>
</tr>
<tr>
<td>Papillomas grew progressively—carcinomas failed to “take”..</td>
</tr>
<tr>
<td>Papillomas grew progressively—carcinomas regressed...........</td>
</tr>
<tr>
<td>Papillomas dwindled (partial regression) and carcinomas regressed completely.</td>
</tr>
<tr>
<td>Papillomas regressed completely and carcinomas grew progressively...............</td>
</tr>
<tr>
<td>Total ........................................................................</td>
</tr>
</tbody>
</table>

* Partial regression in two.

Tests for Presence of Virus

To determine whether the virus had persisted in the V2 carcinoma during its transfer serially through hyperimmunized animals, the growths of three rabbits of the 18th generation (hyperimmunized) were transplanted into as many groups of normals (Chart 1). Later on tests were made for the antiviral antibody in the blood of the rabbits in which the tumors had grown progressively.

Table III summarizes the results of the serum tests. It will be seen that five of the rabbits with carcinomas derived from D. R. 14-23 all had huge growths when bled on the 61st day following implantation. Their sera contained much antiviral antibody, as manifested both by their capacity to react with the virus in the complement fixation test and by their ability to neutralize a potent suspension of it.1 The tumors grew progressively in only two of the rabbits implanted with the growths of D. R. 14-22 and in only four of those implanted with the growths of D. R. 14-21. The antiviral antibody was present in quantity in the sera of all these, as the table shows. The sera

1 The fact is conspicuous that the sera failed to neutralize the virus completely, though many had high tilters of antiviral antibody as determined by complement fixation. The phenomenon has already been discussed in relation to the limitations of the neutralization test as a gauge of antibody titer (12).
### TABLE III

*Tests for Antiviral Antibody in the Blood of Rabbits Carrying V2 Carcinomas*

19th Tumor Generation: Growth Implanted in Normal Animals after 5 Serial Transfers in Virus-Immune Rabbits

<table>
<thead>
<tr>
<th>Source of serum</th>
<th>Implant with</th>
<th>Diameter of growths (six situations)</th>
<th>Complement fixation tests†</th>
<th>Neutralization tests</th>
<th>Papillomas due to mixtures of serum and virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal rabbits</td>
<td></td>
<td>Serum dilution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>implanted</td>
<td>cm.</td>
<td>1:2</td>
<td>1:4</td>
<td>1:8</td>
</tr>
<tr>
<td>From immune rabbit</td>
<td>No.</td>
<td>days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. R. 14:23</td>
<td>15-32</td>
<td>61</td>
<td>8 - 12 - 14 - 8 - 12</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>15-33</td>
<td>&quot;</td>
<td>6 - 8 - 10 - 14 - 6 - 8</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>15-35</td>
<td>&quot;</td>
<td>5 - 7 - 9 - 14 - 8 - 4</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-36</td>
<td>&quot;</td>
<td>8 - 8 - 11 - 9 - 10</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-37</td>
<td>&quot;</td>
<td>10 - 9 - 12 - 14 - 9 - 8</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>D. R. 14:22</td>
<td>15-42</td>
<td>57</td>
<td>5 - 8 - 8 - 8 - 4 - 5</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-43</td>
<td>&quot;</td>
<td>6 - 8 - 10 - 10 - 4 - 10</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-44</td>
<td>&quot;</td>
<td>N - N - N - 3.5 - N</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D. R. 14:21</td>
<td>15-48</td>
<td>55</td>
<td>10 - 7 - 10 - 10 - 6 - 6</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-50</td>
<td>&quot;</td>
<td>5 - 4 - 7 - 8 - 4 - 2</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-51</td>
<td>&quot;</td>
<td>8 - 7 - 10 - 10 - 9 - 7</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-54</td>
<td>&quot;</td>
<td>4 - 5 - 6 - 8 - 8 - 6</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>15-55</td>
<td>&quot;</td>
<td>Regressed—neg.</td>
<td>±</td>
<td>0</td>
</tr>
<tr>
<td>Normal controls—implanted</td>
<td>15-56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15-57</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15-58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† 2 units of complement in all tubes.  
Antigen, P, 1:120.  
None of the sera was anticomplementary when tested concurrently in double volume, nor was the antigen.  
‡ Equal parts serum and 1 per cent virus filtrate P, incubated 2 hours at 37°C.
of the three normal controls exhibited no capacity to fix complement and were devoid of ability to neutralize the virus.

Special attention should be called to the findings with the sera of D. R. 15-44 and 15-55, rabbits implanted respectively with the growths of D. R. 14-22 and 14-21. In D. R. 15-44, nodules 1.0 to 2.5 cm. in diameter had appeared at all six situations by the 17th day following implantation. They measured from 1.8 to 4.0 cm. on the 29th day, but during the ensuing 2 weeks all abruptly vanished except the largest, which dwindled to a softish nodule 1.2 cm. across. Later on this gradually enlarged and it had become a thin-walled cyst 3.5 cm. across at the time of bleeding. The serum of this rabbit had no detectable capacity to fix complement, but it possessed the power to neutralize partially a 1 per cent virus filtrate (Table III). The growths of D. R. 15-55 were indolent. Palpable nodules 0.4 and 1.2 cm. across were present in only two of the six implanted situations on the 15th day. Five nodules measuring 0.4 to 2.0 cm. had appeared by the 27th day, but these dwindled during the next 2 weeks and all had disappeared when the animal was bled on the 55th day. The animal's serum had only the slightest complement-fixing capacity, though it neutralized a considerable proportion of the test virus as the table shows. The results contrast sharply with those got with the sera of rabbits in which the tumors had grown big. They conform to a general rule, namely, that rabbits in which the V2 carcinoma fails to grow, and those in which it regresses before it has got large, fail as a rule to develop the antiviral antibody in quantity (3).

The telltale antibody in the blood of all of the rabbits in which the cancer grew (Table III) makes plain the fact that the virus was still associated with the V2 carcinoma despite the propagation of the tumor during a period of nearly 8 months in hosts hyperimmunized against it. The fact that the antibody appeared in as high titer as previously when the growth was returned to normal animals indicates that the tumor tissue contained the usual amount of antigenic material, namely, masked or altered virus. The conclusion would seem warranted that the latter had increased to the usual extent as the V2 carcinoma grew in the hyperimmune hosts.

COMMENT

It is generally recognized now that viruses are protected from the action of specific antiviral antibodies so long as they remain associated with living susceptible cells. No exception to the rule has been discerned: even the necro-

2 It is of theoretical interest to speculate upon the mechanism whereby living cells protect viruses from the action of circulating antiviral antibodies. Perhaps simplest is the assumption that viruses live within cells and have no contact with antibodies, these being presumably kept away by the semipermeable protoplasm. But it is conceivable that specific antiviral antibodies might still be ineffective even if they reached the vicinity of the virus. This might be the case, for example, if, in the living cell, there were a special association between the cell constituents and that part of the virus to which the antibody would become attached in the absence of such an association.
tizing viruses are protected until they kill their cell hosts and thus expose themselves to the action of the antibodies they have elicited (13). The principle finds exquisite illustration in the case of the papilloma virus, which, inducing neoplastic proliferation, continues to exert its effects and to increase in amount in association with cells that are nourished by blood which would promptly render the virus inactive in the lack of cell protection (14).

The same state of affairs exists in the V2 carcinoma, and under conditions even more extreme; for the virus continues to increase even when the growth is propagated in hosts previously immunized against it, as the foregoing experiments have shown. The outcome is notably different, however, in the case of certain of the extraneous viruses that sometimes ride along as passengers in transplanted cancers. For, as already mentioned, virus III and vaccine virus—the only ones tested thus far in this relation—are eliminated if the tumors they ride upon are propagated in hosts previously immunized against them (6, 7). It is conceivable that the passenger viruses disappear because not sufficiently protected, but this is not necessarily so. Both of the aforementioned viruses can survive in tumors after the hosts become immune to them (16),— findings which show clearly that tumor cells are capable of protecting some passenger viruses, at least against ordinary amounts of antibody.

The disabilities inherent in the characters and effects of passenger viruses will sufficiently account for their disappearance from tumors propagated in virus-immune hosts. Many of them sooner or later kill the cells susceptible to them; and under ordinary circumstances they rely upon passage to new cells for survival and increase. None is a neoplastic virus, going along with cells that proliferate continually as result of its action. Their disappearance from tumors propagated in virus-immune hosts could be explained by supposing that they infect relatively few of the tumor cells at any one time, that they seriously interfere with cellular proliferation, and that they maintain their association with the tumors chiefly by virtue of their ability to pass from dead or dying cells to living susceptible ones, instead of going along as the cells divide. For under such circumstances the neutralizing antibody already present in the new host would inactivate any virus liberated from the infected cells and preclude the infection of susceptible ones, and the uninfected cells would sooner or later outgrow those hampered by the parasite.

This does not mean that all passenger viruses would necessarily be eliminated by similar means. Certain extrinsic viruses can adapt themselves notably well to tumor cells, as witness the persistence of fowl pest and lymphogranuloma viruses in tumors apparently unaffected by them (17). But it is one thing to go along with a tumor and another to be its cause or to modify its neoplastic character. None of the passenger viruses thus far studied has either of these effects though some induce the formation of inclusion bodies in tumor cells and many cause cellular necrosis and thus deter tumor growth (4). In sum, how-
ever, the passenger viruses are all mere riders. The papilloma virus on the other hand is not only responsible for the causation and continued proliferation of tumors, but it has proved capable of rendering malignant many benign tar tumors of the rabbit and of hastening the growth and modifying the character of others, both malignant and benign, when brought into contact with them experimentally (15). In previous papers facts have been presented which attest to the continued neoplastic activities of the virus in partnership with the V2 carcinoma cells (3, 15).

SUMMARY

The V2 carcinoma—a transplanted rabbit cancer derived originally from a virus-induced papilloma and carrying in masked or altered form the virus primarily responsible for it—was propagated in five successive groups of animals all previously hyperimmunized against the papilloma virus. The cancer grew as well in the hyperimmunized hosts as in normal animals implanted during the same months; and serological tests, made when the tumor was eventually returned to ordinary hosts, proved that the virus was still associated with the carcinoma cells: it had increased to the usual extent as the tumor grew in the hyperimmune animals.

The continued increase of the neoplastic virus during propagation of the V2 carcinoma in hyperimmunized hosts contrasts sharply with the elimination of certain extraneous passenger viruses when the tumors they ride upon are grown in hosts previously immunized against them. The facts as a whole would seem to warrant a distinction between the enduring partnership of a neoplastic virus and carcinoma cells on the one hand and the casual association of passenger viruses with tumor cells on the other.

BIBLIOGRAPHY

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