An Increase in Host Specificity of a Transplantable Spindle-Cell Tumor in Mice*

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A tumor of the forestomach of a mouse of the pBr inbred strain which had been treated with methylcholanthrene has been used in these experiments. Bagshaw and Strong (1) reported the origin of this tumor and previous transplantation experiments with it. It may be referred to as the “pBr spindle-cell tumor,” since it arose in the pBr (pink-eyed brown nonagouti) inbred strain of this laboratory and because it is typically made up of spindle-shaped cells with large vesicular nuclei. The individual cells vary greatly in size in sections and interlace with one another at various angles. The histopathology of this tumor has been discussed (1), and for reasons presented there the descriptive term spindle-cell tumor is used.

The tumor has been transplanted subcutaneously in mice of the pBr strain at 5-8 weeks of age, with very uniform results. All the present transplants were made by the author, routinely. The tumor tissue is relatively soft and may fragment while being passed through the trocar. Beginning with the 36th transplant generation (T36), weights of the tumor were taken at 14 days. By this time the tumor typically forms an elongated hollow mass, and the center is generally filled with blood and tissue debris. Many of the tumors present a segmented or beaded appearance, possibly from fragmentation of the tumor. Weights do not include fluid or tissue debris; in pBr mice the tumors at 14 days average between 0.8 and 1.0 gm., quite consistently. No regressions of tumors have appeared in mice of the pBr strain.

Also beginning at the T36 generation, transplantations were made into mice of the N strain. Surprisingly, the tumor grew, though much more slowly than in pBr mice. Weights of these tumors in hybrid mice were not determined, as the mice were allowed to live until killed by the tumors. The present analysis is based on the percentage susceptibility in the various generations rather than the growth rate of the tumors.

Additional information has also been gained in tests employing hybrids between mice of the N and pBr strains. The tumors, both the original and the N-adapted one, had grown in all mice of the N strain at the time the crosses were made between mice of the N and the pBr strains (see Table 1). The data are summarized in Table 1. Weights of the T45—T59 (original series) were employed in these tests, as well as N-adapted tumors between the thirteenth and seventeenth transfer generations (following return to pBr hosts). Tumors of the T59—T80 (fourth generation) were employed in the last five generations of the N-adapted tumors. Weights of these tumors in hybrid mice were not determined, as the mice were allowed to live until killed by the tumors.

It is notable that the tumor grew progressively and rapidly in the F1 mice—100 per cent. Summarization of all transplant results with pure pa-

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rental types shows 1.4 per cent negatives in pBr
(the original series gave these exclusively). The N
series gave negatives in the early series T1-T3, but
no negatives between T10 and T36 or at the time the
genetic crosses were made.

In the F1 generation, the original tumor grew
very well, in general. There was a total of about 5
per cent negatives (see above); there were only 3.3
per cent negatives at 14 days, and the others re-
gressed in the next 2 weeks. However, the N-
adapted tumor showed an unexpected number of
negatives—at the 14th day 27 per cent of the
mice had no tumors, and, of the positives, in 2 or
more weeks many more regressed, so that the final
percentage of negatives is 45 per cent.

DISCUSSION

The poorer growth of the tumor in the N series
must be attributed to genetic differences between
N and pBr. These differences are not limited to
color genes, in all probability. In the F2 from the
cross, various new combinations presumably oc-
cur, some of which are possibly unfavorable for
growth of the tumor.

CHART 1.—Data on the average (for either eight or sixteen
mice) size of the original spindle-cell neoplasm at 14 days of
growth in (a) mice of the N-strain (dash line), (b) mice of the
pBr strain (solid circle, and solid line), and (c) the N-adapted
altered tumor in mice of the pBr strain (solid line). The trans-
plant generations for mice of the N strain are given on the base
line and average weight of tumors along the vertical line.
Transfer generations for pBr mice with the original tumor
begin with T9 (i.e., the 25th generation is really T9).

TABLE 1

The Numbers of Mice Positive and Negative to the Growth of the Original Spindle-
Cell Neoplasm and the Altered N-Adapted Tumor Derived from It

<table>
<thead>
<tr>
<th></th>
<th>pBr</th>
<th>N</th>
<th>F1*</th>
<th>No. mice</th>
<th>At 14 days</th>
<th>Final Classification (90+ days)</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original tumor</td>
<td>489+: 7—</td>
<td>275+: 41—</td>
<td>78+: 0—</td>
<td>182</td>
<td>176+: 6—</td>
<td>163+: 10—</td>
<td>94.8±1.6</td>
</tr>
<tr>
<td>T1—T3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1r—T3n</td>
<td>65+: 41—</td>
<td>109+: 0—</td>
<td>206</td>
<td>151+: 55—</td>
<td>115+: 93—</td>
<td>94.9±3.46</td>
<td></td>
</tr>
<tr>
<td>Expected ratios (1 gene)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“N—adapted”</td>
<td>272+: 0—</td>
<td>189+: 0—</td>
<td>109+: 0—</td>
<td>206</td>
<td>151+: 55—</td>
<td>115+: 93—</td>
<td>54.9±3.46</td>
</tr>
<tr>
<td>Expected ratios (2 genes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>761+: 7—</td>
<td>564+: 41—</td>
<td>187+: 0—</td>
<td>388</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* From a cross between pBr and N mice.
† + means progressive growth of tumor; — means no growth or only a temporary nodule which completely regressed.
But such facts cannot account for the wide difference of growth success of the original versus the N-adapted tumor in the F1 mice. It seems more plausible to assume a change induced in the tumor's growth capacities, perhaps in its genetic constitution, as a result of its sojourn in N mice. The difference in the two F2 ratios, upon which the following discussion is based, as measured by χ², is 56.39, a highly significant difference. That the change took place in the N-adapted tumor and not in the original pBr tumor is indicated by the fact that the pBr tumor continues to grow in about 95 per cent of all F1 mice (unpublished data).

Alterations in the transplantability potentials of tumors in animals are one of the oldest observations in cancer research (90). It was not until 1926, however, that some of these changes were placed on a factorial or genetic basis (18, 19). Since that time, sudden permanent changes in the transplantation of tumors have been verified by several investigators, particularly Bittner (3) and Cloudman (4). These changes in transplantability occurred sporadically but progressively during the continuous transplantation of the tumor over a period of years. The fluctuations were, however, somewhat more than due to chance alone, since the changes, as far as discoverable, were always in the same direction. The tumor progressively acquired the ability to grow in more and more kinds of mice.

Since 1926, many ways have been found to permit a highly specific tumor to grow in more and more animals. These changes have, in part, conditioned the host to tolerate a transplantable tumor, and, in part, probably brought about changes in the tumor cells themselves. It is not always clear, however, whether these changes are of a permanent or only of a temporary nature.

Another problem bearing upon the biological nature of these adapted tumors has also not been convincingly settled. The advocates of the somatic mutation hypothesis for the origin of tumors would like to look upon these altered transplantability potentials as being brought about by some such a process as somatic mutation. However, it should be kept clearly in mind that many tumors, perhaps all, are made up of a mixed population of cells. This is indicated (a) by the great pleomorphism of some tumors, (b) by the variety of tumors that can be extracted with very little effort from one tumor mass or single cell by tissue culture (10), or (c) by transplantation into other animals with only the criterion of a different growth rate in a given group.

There are at least eight ways by which tumor-host relationships can be altered, thus permitting tumors derived from one strain of experimental animal to grow progressively when transplanted into individuals of other strains or even species. These are, as follows:

1. X-raying mice before the transplantation of the tumor (Murphy [14]).
2. Mixing a tumor with cotton or wool fiber before transplantation (Jones [11]).
3. Growing a tumor in the anterior chamber of the eye or in the brain (Green [6, 7], Duran-Reynals).
4. Growing a tumor in an F1 mouse (Barrett and Deringer [8], verified by Hauschka [8, 9]). This observation is based partly on the fact that susceptibility to transplanted tumors is dominant in crossing tests.
5. The aging of tumors through numerous transplant generations permits successive changes to take place; that is, adapted changes take place spontaneously (Strong [18, 19]).
6. The treatment of the host by estrogenic hormones before the injection of the tumor (Gardner [5]).
7. The treatment of the host by tumor or normal tissue extracts before the inoculation of the tumor (Snell et al. [15, 16] and Kaliss and Snell [12]).
8. The growth of tumors in the newborn (Strong [17], Little [18], Gross).

There appear to be different mechanisms involved in these different adaptive procedures. Some changes (X-ray) appear to be altering the host mechanism, whereas other procedures (3, 4, and 5) appear to be bringing about changes in the tumor cells or in tumor cell relationships. (For an excellent review see Hauschka [9].)

The present data demonstrate that the process of "adaptation" may reverse the process of decrease in specificity of transplanted tumors that takes place spontaneously. A dependent tumor which will grow in an F1 mouse when either one of two alternative genes or factors are present in the genetic constitution of the host (indicated by a 15+ : 1— ratio in the F2) has now become dependent for its progressive growth on the simultaneous presence of two genes (9+ : 7— ratio in F2). What relation, if any, exists between the two alternative genes in the growth of the original tumor and the two dependent genes for the survival of the N-adapted or derived tumor has not, as yet, been determined.

It is also not clear whether this increased dependence of the derived tumor has occurred spontaneously or has been brought about through adaptation. One can only select between these two alternative explanations by repeating the adaptation of the pBr or other strain tumors into mice of the N or other strains repeatedly.

Selection between the two ideas whether (a)
somatic mutation is brought about by selective adaptation or (b) growing a mixed population of cells in a foreign host permits the process of selective survival of the best adapted cells to take place, cannot be determined with the present data.

There appears, however, to be one fundamental difference between the original observation, reported in 1926 and interpreted in terms of "somatic mutation," and the present data. The original work on transplantation demonstrated that sudden and very significant deviations occurred in the growth capacity of the tumor and that these sudden alterations were permanent from the point of origin. In the present data, no sudden or dramatic change took place in the growth capacity of the N-adapted transplants. Certainly the cyclical changes which occurred in the growth capacity of the pBr tumor growing in mice of the N strain is more likely the resultant of some selective process. Again, the stimulated growth capacity of the N-adapted tumor, when placed in pBr mice, was gradually acquired over several generations (T1 - T1) and gradually lost. The stimulated growth capacity of the spindle-cell neoplasm was not permanent but merely of a temporary nature.

However, the two alternative points raised by the present investigation which would be of interest to geneticists or cancer biologists cannot be decided. These are as follows: (a) It is not clear whether the change in the transplantability of the spindle-cell neoplasm was of a spontaneous nature or brought about through the process of adaptation. (b) The alternative view of "somatic mutation" or a selection of potentially different cancer cells also cannot be decided, but the evidence tends more toward selection.

The change in transplantation, whatever it is or by whatever mechanism or means it is brought about, is probably of a factorial nature.

**SUMMARY**

Mice (1,948) have been inoculated subcutaneously with a spindle-cell neoplasm which arose originally in the forestomach of a mouse of the pBr descent. The mice used belonged to the pBr inbred strains and to the F1 and F2 generations of a cross between the pBr and N strains. Nine generations of growth in mice of the N strain the tumor (N-adapted) was returned to the originan pBr mice for thirteen generations and then into a new group of F1's and F2's. The original tumor from the pBr strain gave a 15+ : 1− ratio in a group of F1 mice. This would indicate that progressive growth is dependent upon either one of two alternative genes. The N-adapted tumor gave a 9+ : 7− ratio expected when two genes are simultaneously needed for progressive growth of the tumor. From a dependent tumor the transplant has become more dependent through some change of a genic or factorial nature. The phenomena of adaptation and changes in the transplantation potentials of tumors are discussed.

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