Effect of Normal Tissue Inocula on Homologous Tumor Transplants

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Although transplantation of normal or neoplastic tissue separately from one animal to another has been extensively employed in studies of tumor or non-neoplastic growth, the interaction between these tissues, following simultaneous transplantation of tumor and non-neoplastic tissue to the same host site, has not been sufficiently investigated. Browning (1) has employed this technic to investigate the conditions for autonomy of heterologous tumor fragments. The technic, however, permits more general application to the problem of interaction between growing tumor and contiguous transplanted normal embryonic or adult tissue.

In the experiments reported here, the interactions of two synchronously transplanted tissues have been investigated, using minced cell suspensions rather than tissue fragments. In addition, only homologous transplants have been made, and the suspensions of embryonic cells or cell clumps consisted of whole minced embryos rather than fragments of particular organs.

MATERIALS AND METHODS

Transplant generations of a spontaneous adenocarcinoma of mammary origin were used as the source of neoplastic tissue in these investigations. Embryonic tissue was obtained from 15—18-day-old embryos. Carworth Farms CFW strain mice were employed throughout. The suspensions of embryo cells were prepared by mincing whole mouse embryos and adding to this an equal volume of sterile physiological saline and then repeatedly forcing the resultant suspension through a 1-cc. tuberculin syringe; the suspensions of adult tissues were prepared by mincing a mixture of lung, liver, spleen, kidney, heart, ribs, muscle, and skin in an equal volume of saline and forcing this mixture through the syringe; the tumor suspension was prepared by similar technic. A given quantity of the cell suspension, or combination of cell suspensions, was injected subcutaneously by tuberculin syringe into 2-4-month-old male and female mice.

The amount of growth of the inoculum within a specified period of time (usually 10 days) was determined by sacrificing the animals, weighing the excised mass on a torsion balance, and comparing this weight with the weight of the original inoculum.

Specimens were taken at selected times from representative growths and prepared by standard procedure for microscopic examination.

RESULTS

Results of injection of suspension of embryonic cells alone.—The subcutaneous injection of a cell suspension prepared from whole 15—18-day-old mouse embryos into an adult host of the same strain resulted in the organization of a discrete mass. This mass did not increase in weight beyond the original value, and, in fact, showed a decided progressive decrease with time. Table 1 shows data obtained by direct measurements of the weight of the tissue mass removed from the sacrificed host 10, 14, and 42 days after inoculation and indicates that, as time after inoculation increased, the amount of tissue persisting decreased. At the end of 10 days, the average tissue mass persisting was only 39 per cent of the total original inoculum; at the end of 14 days, only 20 per cent of the original inoculum persisted; while at 42 days, the per cent of the original inoculum had decreased to 7. In no case did either these
or serial embryonic transplants kill the host.

Histological examination of the 10-day-old inoculum showed a preponderance of cartilage, loose connective tissue, and epithelium (Fig. 1). Microscopic examination of tissue masses which had remained in the host for a longer period of time showed cartilage to be the last remaining element.

**Growth of tumor inocula.**—The amount of growth after 10 days of tumor cell inocula is shown by the data presented in Table 2. These data show that the average ratio of final to original weights was 5.4, as compared to 0.32 for embryo alone.

**Injection of mixed suspensions of tumor (Tu) and embryo (Eb) cells.**—The subcutaneous injection of a cell suspension consisting of 10 mg. of Tu cells and 90 mg. of Eb cells into an adult host of the same strain resulted in the formation of a discrete mass grossly resembling a vascularized tumor. At the end of 10 days, the weight of this mass was 2–7 times that of the original inoculum. Evidence derived from three sources establishes that the increase in mass of this complex may be attributed primarily to the small tumor component. First, microscopic examination of the tissue from the 10-day Tu-Eb complex showed the tissue to consist primarily of Tu cells in active mitosis, with only scattered Eb elements, principally cartilage and loose connective tissue (Fig. 2).

Second, as indicated by the data in Table 3, animals bearing such Tu-Eb complexes died in approximately the same length of time after inoculation as animals injected with Tu cells alone. Microscopic examination of the primary lesions at death revealed a marked preponderance of tumor cells with even less representation by Eb elements than noted from animals sacrificed at the end of 10 days.

A third source of evidence is based on the results of transplantation of the 10-day complex into a new host. Examination of data presented in Table 3 shows that, within 11 days after injection of such an inoculum, death resulted from the presence of a large tumor. As already pointed out, transplantation of a 10-day growth of Eb cells alone did not prove lethal. Microscopic examination of the second serial Tu-Eb transplant in most cases revealed only tumor cells. In those instances where Eb elements persisted they comprised an exceedingly small fraction of the total mass.

On the basis of this evidence, it appears justifiable to consider the tumor component responsible for the increase in size of the Tu-Eb inoculum. Accordingly, the increase in mass of the Tu-Eb complex for a given time interval may be expressed in the following way:

\[
W_f - (KW_{E_b})
\]

\[
W_{T_o}
\]

In this expression, \(W_f\) represents the final weight of the Tu-Eb complex for the selected time interval, in this case, 10 days; \(W_{E_b}\) is the weight of the original embryo inoculum; \(W_{T_o}\) is the weight of the original tumor inoculum; and \(K\) is derived from growth data of embryo cells alone. Thus, for these series of 10-day experiments, \(K\) has the value 0.32, and \(0.32W_{E_b}\) represents the weight of the embryonic component after 10 days' growth of the complex.

Average values for the weight of a Tu-Eb com-

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**TABLE 2**

<table>
<thead>
<tr>
<th>TUMOR-EMBRYO</th>
<th>TUMOR-ADULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10 mg.)</td>
<td>(10+290 mg.)</td>
</tr>
<tr>
<td>Av. wt.</td>
<td>Range</td>
</tr>
<tr>
<td>54</td>
<td>15–60</td>
</tr>
<tr>
<td>1,438</td>
<td>350–3,000</td>
</tr>
<tr>
<td>605</td>
<td>210–2,025</td>
</tr>
<tr>
<td>22</td>
<td>2–73</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>SIZE OF TRANSPLANT AT DEATH OF ANIMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplant (days)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Tumor (10 mg.)</td>
</tr>
<tr>
<td>(10+290 mg.)</td>
</tr>
<tr>
<td>27 1,500</td>
</tr>
<tr>
<td>28 2,500</td>
</tr>
<tr>
<td>29 3,500</td>
</tr>
<tr>
<td>30 5,544</td>
</tr>
</tbody>
</table>

Note: These inocula consist of 10 mg. of tumor tissue and 90 mg. of normal tissue.

† In the case of tumor-embryo transplants, growth increment is calculated by:

\[
\frac{W_f - (0.32W_{E_b})}{W_{T_o}}
\]

In this expression, \(W_f\) represents the final weight of the Tu-Eb complex for the selected time interval, in this case, 10 days; \(W_{E_b}\) is the weight of the original embryo inoculum; \(W_{T_o}\) is the weight of the original tumor inoculum; and \(K\) is derived from growth data of embryo cells alone. Thus, for these series of 10-day experiments, \(K\) has the value 0.32, and \(0.32W_{E_b}\) represents the weight of the embryonic component after 10 days' growth of the complex.
plex after 10 days growth are presented in Table 2.

The amount of growth of the tumor component, calculated on the basis of the relationship presented above showed an average ratio of final weight to original tumor weight of 51.4. This ratio indicates, for this period, a rate of growth of tumor cells in the presence of Eb that was 6–10 times that of tumor cells alone.

Injection of mixed suspensions of tumor (Tu) and adult (Ad) cells.—The subcutaneous injection of cell suspension consisting of 10 mg. of Tu and 290 mg. of cells from selected adult organs caused an enhanced growth of the tumor component of the complex in some respects similar to that evoked in a tumor-embryo transplant. Thus, at the time of death of the animal, the size of the Tu-Ad mass usually equalled that of Tu or Tu-Eb.

It may be noted, however, that the time for initiation of the enhancement by Ad was longer than that required for enhancement by Eb. Thus, within the initial 10-day period, no discernible growth of the Tu-Ad complex occurs.

DISCUSSION

Subcutaneous injection of a suspension containing either normal embryonic, tumor, a mixture of tumor and embryonic, or a mixture of tumor and adult cells into a homologous adult host results, under the conditions of these experiments, in the formation of a discrete cell mass at the site of inoculation. By the end of the initial 10-day period after inoculation, however, only the cell masses resulting from inoculation of tumor cells alone or tumor cells with embryonic cells have shown any real growth, as judged by the criterion of weight increase. The Tu-Eb complexes, initially containing only 10 mg. of tumor cells, may, within 10 days, reach the same mass as do inocula consisting of 300 mg. of Tu alone. The increase of the tumor component of the Tu-Eb complex greatly exceeds the increase in size of the mass produced when Tu cells alone are present.

In view of other investigations concerning the growth-promoting ability of normal and neoplastic tissue extracts (2–4), it is possible that growth enhancement of the tumor component of the Tu-Eb complex may be attributed to the elaboration of some growth-stimulating substance. Furthermore, it may be noted that, while mixtures of Tu-Ad cells eventually bring about the same end effects as the Tu-Eb mixtures, there is not the same enhancement in the initial phases of growth. This indicates that the early enhancement of the Tu-Eb complex cannot be attributed to dilution of the Tu cells by other tissue components, with such dilution thereby producing the enhanced condition due to multicentric growth.

SUMMARY

Inoculation of suspensions of embryonic cells into homologous host mice results in the formation of a discrete cell mass which, within 10 days after inoculation, is only one-third of the original weight and shows a further decrease with time.

Inocula consisting of tumor and embryo cells also form a discrete cell mass at the site of inoculation, and this mass continues to increase in weight until it proves lethal. Interaction between contiguous surviving transplanted tumor and normal cells is indicated by the fact that this tumor component of the complex shows a growth rate 6–10 times that of tumor alone within the first 10-day period.

The tumor component of the complex is the one primarily stimulated to proliferate.

ACKNOWLEDGMENTS

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REFERENCES

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Effect of Normal Tissue Inocula on Homologous Tumor Transplants

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