Transplantation of Heterologous Tumors by the Intravenous Inoculation of the Chick Embryo*†‡

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This report is concerned with the transplantation of cells of certain mammalian neoplasms into the living chick embryo by injection of these cells into the allantoic vein. Although the technic of allantoic vein injection has been reported previously, it has not been used heretofore as a technic for implanting tumor cells from the same species or from another.

It is well known that tissues transplanted from one species to another fail to grow except under certain conditions. Such heterologous transplants have been successful in the anterior chamber of the eye and on the chorio-allantois or in the yolk sac of the chick embryo. In specific instances other methods have had limited success.

The fact that many types of tissue have been grown for short periods of time on the chick chorio-allantois may be related to the absence of antibody formation. The absence of complement from the serum of the chick embryo was noted as early as 1907 (1). In 1929, Grasset (2) observed that the chick embryo lacked the ability to produce antibody. Polk, Buddingh, and Goodpasture (3) in 1938 showed that complement for sensitized sheep cells was not present in the serum, extraembryonic fluids, or tissues of the chick embryo before hatching. Just at hatching or immediately afterward it was suddenly present and gradually increased to a maximum in the adult fowl. Murphy (4) in 1914 observed that there was no reaction upon the part of the chick embryo to foreign tissue growing on the chorio-allantois until the eighteenth day of incubation.

The failure of growth of heterologous tissue, either normal or neoplastic, has been thought to be based on immunity reaction and species specificity. Therefore, it seemed reasonable that successful transfer of heterologous tumor tissue might be effected in the chick embryo if only for a limited period of time. The presence of growth-promoting substances in the rapidly growing chick embryo should make it an ideal medium for growth of heterologous tissue. Furthermore, it seemed that the environment of the tumor tissue within the embryo itself would approximate more closely the environment of such tissue as seen in patients than would tumor growth upon the chorio-allantois or within the yolk sac.

Previous experience of Lee, Stavitsky, and Lee (5) and Lee and Stavitsky (6) with intravenous inoculation of chick embryos with suspensions of Mycobacterium tuberculosis suggested that this method might be useful in establishing heterologous tumor growth within the embryo. The use of this technic also offered an opportunity to determine what tissues were most likely to be the site of metastasis following blood stream dissemination.

Sterile tumor tissue was obtained from patients at operation, from tumor-bearing rats and mice, and from tissue culture. The tumor tissue was collected under aseptic precautions. It was then forced through a 70-mesh Monel wire screen, and a sterile suspension of tumor cells in physiological saline was prepared. Lewis (7) has determined that such suspensions contain viable cells.

Chick embryos incubated for 11 days were selected and the eggs candled. A portion of the egg shell over the air sac was removed and the shell membrane carefully stripped off, exposing the chorio-allantois. An inoculum of 0.05 cc. of the tumor-cell suspension was then injected intravenously. The eggs were sealed with Scotch tape, which formed a window through which one could observe the developing chick embryo. The surviving embryos were sacrificed on the twentieth day of incubation, or 9 days after inoculation.

In all, 278 embryos were injected intravenously with 0.05 cc. of sterile tumor-cell suspension in a series of 17 experiments. Following the intravenous injection of physiological saline alone in 11-day-old
embryos, 70 per cent survival is expected. Survival following the intravenous inoculation of chick embryos with sterile tumor-cell suspensions averaged about 50 per cent. This survival has been improved recently by the addition of small amounts of penicillin to the tumor-cell suspension, which is allowed to stand at room temperature for 1 hour prior to inoculation into the embryos.

By this technic the C57 strain mouse sarcoma (Table 1) was successfully grown. In 81.7 per cent of surviving embryos injected with this tumor-cell suspension no growth was demonstrated, while 18.3 per cent of surviving embryos showed evidence of tumor growth. In several cases the growth was small, but definite nests of tumor cells could be found histologically in sections of the liver or brain or both (Figs. 1—3). No evidence of tumor metastasis was found in the kidney or spleen. In one instance, although the chick itself appeared to be normal in size and development, diffuse nodulation of the liver was observed grossly. On histologic examination, about one-fourth of the liver tissue appeared to be replaced by tumor tissue, and the brain was diffusely invaded by nests of tumor cells. These appeared to be similar to the parent tumor cells. Mitoses were present as was frequent blood vessel invasion. Occasionally a bile canaliculus appeared to be invaded by tumor cells.

The C3H strain mouse mammary carcinoma failed to grow.

Tumor transfer from human beings (Table 2) was accomplished in four instances. Nine and six-tenths per cent of the surviving embryos injected with such tumor suspensions showed microscopic takes in sections of the liver but not in other embryonic tissues. These takes were all small. The tumors from patients which showed evidence of growth within the embryo were two neuroblastomas, a cerebral hemangioblastoma, and a cerebral metastasis secondary to a bronchogenic carcinoma. The lapse of time between removal of the tumor from the patient and inoculation of the tumor-cell suspension into the chick embryo may be responsible for the few takes and small growth of human tumor tissue within the embryo.

Following the intravenous injection of normal cells from the embryonic chick liver or brain, perivascular cellular infiltration and occasional focal necroses were seen histologically in the livers of the surviving embryos, but there were no changes suggestive of tumor formation.

### TABLE 1

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of embryos surviving after inoculation</th>
<th>No. of takes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 strain mouse sarcoma</td>
<td>60</td>
<td>11 (18.3%)</td>
</tr>
<tr>
<td>C3H strain mouse carcinoma</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>11 (14.1%)</td>
</tr>
</tbody>
</table>

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Following the intravenous injection of normal cells from the embryonic chick liver or brain, perivascular cellular infiltration and occasional focal necroses were seen histologically in the livers of the surviving embryos, but there were no changes suggestive of tumor formation.

### TABLE 2

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of embryos surviving after inoculation</th>
<th>No. of takes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td>6</td>
<td>9 (15.0%)</td>
</tr>
<tr>
<td>Chondrosarcoma (cadaver)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Metastatic adenocarcinoma*</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>7</td>
<td>4 (28.6%)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>7</td>
<td>4 (28.6%)</td>
</tr>
<tr>
<td>Neurofibroma (cerebral)</td>
<td>7</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Hemangioblastoma (cerebral)</td>
<td>2</td>
<td>3 (18.2%)</td>
</tr>
<tr>
<td>Metastatic carcinoma† (cerebral)</td>
<td>3</td>
<td>15 (91.2%)</td>
</tr>
<tr>
<td>Metastatic adenocarcinoma‡</td>
<td>10</td>
<td>1 (6.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>4 (9.6%)</td>
</tr>
</tbody>
</table>

* Probably from colon.
† Probably from lung.
‡ Probably from cecum.

In the entire series of experiments there was evidence of tumor growth in the brain or liver of 12.4 per cent of surviving embryos injected intravenously with tumor-cell suspensions. The neoplastic cells maintained their histologic character in the embryo and closely resembled the parent tumors. This morphologic evidence suggests the probable identity of the transplants with the parent tumors. Experiments are now under way to effect serial transfer of heterologous tumor transplants. In the case of transplants of animal tumors we are attempting to return such transplants to a member of the original host species. Attempts are...
Fig. 1.—Metastatic nodule in the liver of a 20-day-old chick embryo following intravenous inoculation with the C57 strain mouse sarcoma. Mag. X330.

Fig. 2.—Diffuse involvement of the brain of a 20-day-old chick embryo injected intravenously with the C57 strain mouse sarcoma. Mag. X660.

Fig. 3.—Higher magnification of a section of the brain of a 20-day-old chick embryo injected intravenously with the C57 strain mouse sarcoma. Mag. X160.
also being made to determine what happens to the tumor transplants after the chick is allowed to hatch.

At this time it can be stated that it is possible to grow heterologous tumors by the intravenous inoculation of the chick embryo with sterile tumor-cell suspensions, although we have not been able as yet to propagate tumors by this method consistently.

REFERENCES

7. Lewis, M. R. Personal communication.
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