Pattern of Tumor Cell Spread in Tissues and Organs as a Lethal Factor in Tumor-bearing Animals*

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The mechanism of the lethal effect of a tumor on the host has been only briefly noted in clinical studies (3, 11, 12, 15-17, 19, 22, 23) and has not been widely studied in experimental animals (1, 2, 4, 10, 14, 20). The complexity of this problem has been emphasized by a clinician, as follows: "The cause of death in cancer is a problem which interests many physicians. Why does the patient die? . . . We see a patient with a small local carcinoma which has not produced metastases or evidence of extensive destruction, and yet for unexplained reasons the patient dies. On the other hand a patient may live for an extremely long time with large tumors and even extensive distant metastases" (15). Investigators using the biochemical approach (92, 4, 10, 14) to this problem tend to incriminate the systemic effect of tumors, suggesting that "the effects elicited by tumors may be produced either by a liberation into the blood stream of material elaborated by the tumor or by abstraction by the tumor of some components in the blood stream essential for the maintenance and proper function of the distant tissue" (10). On the other hand, pathologists retort that "many cachexia producing tumors are neither bulky nor degenerated." (92).

We have attempted to elicit some information about the lethal factor by recording the survival span in series of mice given inoculations of the same doses of the same specimens of tumor cell suspension by various routes and at various body sites. The results were correlated with macroscopic and microscopic autopsy findings. The data and an attempt at their interpretation are presented below.

MATERIAL AND METHODS

Tumor and mouse strains.—Sarcoma 87, Sarcoma 180, and Harding-Passey melanoma were carried in CFW mice; carcinomas E 0771, B1, BW1888, and leukemia C-1498 in C57-6 mice; spindle-cell Carcinoma 1509a in CAF1 mice; leukemia P1884 in DBA-2 mice; and AKR leukemia in AK4 mice.*

Methods of inoculation.—The technic of cell counts for inoculation of requisite numbers of tumor cells has been described elsewhere (6). Material for inoculation was obtained from peritoneal growth; free tumor cells, mashed implants, or both were used. Injections were given (a) intrapleurally, through the right thoracic wall at the intersection of the posterior axillary line and the lowest intercostal space, i.e., slightly above the diaphragm; (b) intraperitoneally, into the lower left quadrant of the abdomen; (c) subcutaneously, into the flank, the back, and the legs by usual technics, and into the scalp by introducing the needle through the right external ear into the nose tip. Doses of 10 to 50 million cells were generally used; in a few cases 100 and 200 million cells were transplanted. Nonviable 5-87 and 5-180 cells were obtained by heating cell-containing exudate at 45° C. for 80 minutes or by freezing at —80°.

Mortality records.—Survival of mice in each series was checked daily. Dead and sacrificed animals were examined for the number of implants, their size, their weight, their topographic position, and for the presence of tumor growth and of pathological changes in their organs and tissues (sections).*

RESULTS

In the first group of experiments the survival spans of mice after intracavitary and subcutaneous inoculations with the same doses of tumor cells were compared. For each type of inoculation two different routes were used, i.e., intrapleural or intraperitoneal for intracavitary growth, and into the flank (loose, widely extending tissue) or into the scalp (tissue compressed between the skin and the bone) for subcutaneous growth. The results are recorded in Table 1.

It appears (Table 1) that in leukemic mice the results of inoculation by various routes were only slightly different (no difference for AK4), since the implantation of tumor cells at the site of inoculation was relatively scanty and overshadowed by their early blood-borne invasion and extensive infiltration of hematopoietic organs (7).

1 CFW and AKR mice were supplied by Carworth Laboratories, New City, N.Y., and C57-6 and CAF1 mice by Jackson Memorial Laboratories, Bar Harbor, Maine.

2 Mr. Wendell Gillett, medical photographer, Vanderbilt University Medical School, prepared the photographs.

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For sarcomas and carcinomas, death from intracavitary inoculation occurred in most animals earlier than that following inoculation by subcutaneous routes. The shortest average and minimal survivals were observed in the intrapleural series. At autopsy nodular implants 1–8 mm. in diameter were found at the site of inoculation and in the mediastinal pleura. The average total weight of macroscopic implants was, in this series, below 1.0 gm. In the lungs distinct thickening of interalveolar septa and of interstitial tissue, hemorrhages, and perivasular infiltration were observed consistently (Fig. 1) and were often associated with free tumor cell invasion (Fig. 2) followed by organized microscopic tumor growth in the lung tissue. Elsewhere we have described the growth of free tumor cells in the peritoneal exudate, their implantation into the pleura (9), and their detection in the lung by assay grafts (8).

There was a wide range of survival variations in each group of mice with slowly growing melanomas, and therefore averages were not conclusive. However, the minimum survival span was lowest in intracavitary-inoculated mice, and, moreover, in some of them tumor cell invasion (Fig. 7) and advanced degeneration of organs (Fig. 8) were observed.

The use of large numbers of S-37 or S-180 cells (100 to 200 million) by intraperitoneal route shortened the survival span to a minimum of 8 days (average, 4 days) but never below this. At autopsy the amount of the exudate and the average number of free tumor cells/cmm were not greater than in sarcoma-bearing mice (Table 1), but the perivasular infiltration of the mesentery by tumor cells was more massive. Fat tissue tightly packed with S-180 cells eventually became partly necrotic and separated from the healthy tissue, appearing as large, waxy, jelly-like lumps ("jelly tumors," not to be confused with necrotic organized tumors) (Fig. 9).

Average and minimum survivals were slightly, but consistently, longer for intraperitoneal sarcomas than for intrapleural—more distinctly so for Carcinoma 15091a. The characteristic features of intraperitoneal growth were: (a) formation of exudate (abundant in sarcomas, scarce in carcinomas) with floating and proliferating free tumor cells; (b) presence of implants, 1–10 mm. in diameter (sometimes welded together in a cluster) at the site of inoculation—formerly (6) described as autoinoculation—and in the visceral peritoneum (total weight of implant 0.5–2 gm., in a few instances 3 gm.); (c) infiltration of the peritoneal fat tissue (Fig. 3) by free tumor cells, sometimes growing into an organized tumor, around the vessels or close to abdominal organs; (d) tumor cell spread into the liver (Fig. 4), spleen (Fig. 5), pancreas (Fig. 6), and other visceral organs, associated consistently with perivasular lymphocytic infiltration and, in the liver, also with infiltration of sinusoids by free tumor cells and lymphocytes (Fig. 4).

### Table 1

**Survival of Mice Inoculated with Tumor Cells by Different Routes**

<table>
<thead>
<tr>
<th>Tumor strain</th>
<th>Intrapleural (days)</th>
<th>Intraperitoneal (days)</th>
<th>flank (days)</th>
<th>scalp (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia AK4</td>
<td>6(4–6)</td>
<td>6(5–7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia P1854</td>
<td>7(6–9)</td>
<td>9(7–12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia C1498</td>
<td>7(6–10)</td>
<td>9(7–12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma 37</td>
<td>6(4–9)</td>
<td>8(7–5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>6(4–5)</td>
<td>8(7–5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma 15091a</td>
<td>8(5–11)</td>
<td>17(14–22)</td>
<td>24(16–56)</td>
<td>22(13–52)</td>
</tr>
<tr>
<td>Carcinoma E 0771</td>
<td>12(11–16)</td>
<td>15(11–17)</td>
<td>21(16–50)</td>
<td>18(14–53)</td>
</tr>
<tr>
<td>Melanoma Harding-Passey</td>
<td>6(5–8)</td>
<td>7(4–10)</td>
<td>9(6–7)</td>
<td>7(6–9)</td>
</tr>
</tbody>
</table>

Thirty mice were used in each group (three series of ten mice). Average survival period (days) and variation extremes are indicated. Data on leukemias were obtained in experiments described elsewhere (7).
range of variation were higher than those in mice with tumors of the extremities. In the case of carcinomas, only mice with tumors of the back survived much longer than other groups. No significant difference in survival times was found between mice with tumors of the foreleg and those with hindleg tumors. However, in each experiment the first dead mice were found in the group with tumors of the hind leg.

The greater age of tumors on the back was reflected by larger size and weight (up to 9 gm., average, 6 gm.) of these tumors as compared with other subcutaneous tumors (up to 7 gm., average, 4 gm.). Tumors on the back infiltrated only superficial spine muscles and grew mainly in the loose subcutaneous connective tissue above these muscles. Tumors on the flank spread downward to the pelvis, overgrowing connective tissue and muscles between the skin and the peritoneum and occasionally penetrating into the peritoneal cavity with formation of ascitic fluid and free tumor cell growth. Tumors of the extremities spread upward, obliterating muscles and large vessels (Fig. 11). Foreleg tumors extended into axilla and breast muscles but never invaded the pleural cavity macroscopically. Hindleg growth spread on lower abdominal and pelvic muscles, probably compressing some of the pelvic organs.

Requisite numbers of tumor cells were inoculated subcutaneously either in a single dose injected at one site of the flank or as ten fractions of the same dose injected at ten sites on both flanks. The spread and the amount (weight) of tumor growth and the survival span of the host were recorded for mice of each group, and the results are summarized in Table 3.

It appears (Table 3) that tumor cell proliferation spreading from several growth centers resulted in larger amounts of malignant tissue and earlier death of the host than tumor growth from a single site.

<table>
<thead>
<tr>
<th>TUMOR STRAIN</th>
<th>SITE OF SUBCUTANEOUS INOCULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flank</td>
</tr>
<tr>
<td>Sarcoma 37</td>
<td>14(11–20)</td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>16(10–21)</td>
</tr>
<tr>
<td>Carcinoma E0771</td>
<td>18(15–27)</td>
</tr>
<tr>
<td>Carcinoma 15091a</td>
<td>19(11–26)</td>
</tr>
</tbody>
</table>

TABLE 2
SURVIVAL OF MICE GIVEN INOCULATIONS OF TUMOR CELLS SUBCUTANEOUSLY AT DIFFERENT SITES

Thirty mice were used in each group (three series of ten mice). Average survival period (days) and variation extremes are indicated. Data for leukemic tumors (AK4, P1574, and C1498) were not included, since they did not reveal any significant difference in results for different groups.

TABLE 3
SURVIVAL AND AMOUNT OF TUMOR GROWTH (WEIGHT) IN MICE INOCULATED SUBCUTANEOUSLY WITH TUMOR CELLS AT ONE OR SEVERAL SITES

Each animal received the same number of tumor cells either as a single dose at one site or as fractional doses at ten different sites.

<table>
<thead>
<tr>
<th>TUMOR STRAIN</th>
<th>METHOD OF INOCULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single injection of full dose</td>
</tr>
<tr>
<td></td>
<td>Survival (days)</td>
</tr>
<tr>
<td>Sarcoma 37</td>
<td>14(7–19)</td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>16(8–17)</td>
</tr>
<tr>
<td>Carcinoma E0771</td>
<td>18(18–19)</td>
</tr>
<tr>
<td>Carcinoma 15091a</td>
<td>22(18–34)</td>
</tr>
<tr>
<td>Carcinoma B-1</td>
<td>20(sacrificed)</td>
</tr>
<tr>
<td>Carcinoma BW1898</td>
<td>20(sacrificed)</td>
</tr>
</tbody>
</table>

Averages of 20 mice and variation extremes are indicated. Data for leukemias showed no consistent differences in the results by the two methods.

DISCUSSION
Our data on the mortality of mice given inoculations by various routes and at various topographical sites illustrate the condition of early
death in spite of the small amount of tumor tissue growth and of the late death of animals with large organized tumors. Leukemic tumors showed the most rapid effect with minimum primary tissue growth; independently of the route of inoculation their cells followed the same route of massive invasion, i.e., the blood stream, and induced death by infiltration of organs (13, 18).

Disruption of lymph and blood circulation in the lung by thickening of interalveolar septa and of interstitial tissue was apparently responsible for the rapid death of animals with small tumor implants in the pleura.

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Table 4

Red Blood Cell Counts (Millions) in Mice with Unicentric and Pluricentric Subcutaneous (Flank) Tumors

<table>
<thead>
<tr>
<th>Tumor strain</th>
<th>Age (days)</th>
<th>Unicentric growth</th>
<th>Pluricentric growth</th>
<th>Noninoculated controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma E 0771</td>
<td>17</td>
<td>4.975 (4.325-5.975)</td>
<td>4.145 (4.325-5.975)</td>
<td>9.550 (7.270-12.180)</td>
</tr>
</tbody>
</table>

Averages of ten mice and variation extremes.

The short survival of the series with intraperitoneal implants could be correlated with the invasion of the mesentery, the abdominal organs, and the fat tissue surrounding these organs by free tumor cells from the peritoneal exudate. Tumor cell invasion could be demonstrated in intraperitoneally inoculated mice by organ assay grafts in our previous experiments (8) occasionally in any abdominal organ but in the liver of nearly all animals. Longer survival (in melanoma-bearing mice) allowed the development of early damage into severe degeneration of liver parenchyma. Occasionally, tumor hemorrhages. The role of blood-borne metastases as an eventual lethal factor in mice will be discussed in a special study.

No damage was produced by injection of dead cells structurally intact (killed by heating at 45°C) or disintegrated (frozen and thawed). Moreover, a minimum period of 3 days was necessary to kill the animals even by inoculation of the largest doses of viable cells. These findings suggest that the lethal effect of tumor cells is associated with their ability to grow and to infiltrate the tissues.

It appears from the above data that the survival
FIG. 7.—Liver, 40 days after intraperitoneal inoculation with melanoma (Harding-Passey) cells; invasion of liver tissue by scattered tumor cells. ×200.

FIG. 8.—Liver, 60 days after intraperitoneal inoculation with melanoma; hyaline necrosis. ×150.

FIG. 9.—Fat tissue, 5 days after intraperitoneal inoculation of 50 million S-180 cells; “jelly tumor,” tissue infiltration with free tumor cells. ×150.

FIG. 10.—Muscle of the flank, 7 days after subcutaneous inoculation with S-180 cells; tumor cell lines advancing from the solid tumor, sometimes in a single line, surround areas of normal tissue. ×150.

FIG. 11.—Muscle of the hind leg, 12 days after subcutaneous inoculation with S-180 cells; tumor growth replacing the muscle and extending to the bone. ×150.

FIG. 12.—Brain, 12 days after inoculation of S-180 cells into the scalp; large hemorrhage into the cerebral tissue located not far from a dense infiltration of the arachnoid tissue by tumor cells which infiltrate in this area into the brain tissue and which are found in a few capillary lumens. ×100.

Hematoxylin-eosin stain used throughout.
span of tumor-bearing mice could be correlated with the pattern of spread of tumor cells from the primary growth. These experimental data in mice are in agreement with conceptions of some pathologists (15, 22) and clinicians (3, 15, 16) about the mechanism of death in cancer patients.

Experiments presented in Table 8 show that pluricentric tumor growth induced more abundant tissue proliferation and earlier death than unicentric tumors. The large amount of tumor growth and short survival span were not associated with a systemic effect of hematopoietic organs. The occurrence and the importance of pluricentric tumor growth were frequently discussed in human pathology (16, 19, 21, 28) and in the research on experimental carcinogenesis (20). Our data suggest that the rapid growth and the early lethal effect of some malignant tumors may be explained by their multicentric origin.

SUMMARY AND CONCLUSIONS

1. Requisite cell numbers of various tumors were inoculated into mice by different routes.
2. Death occurred earliest (average and variation extremes) in the series injected intrapleurally and was attributed to cell infiltration and interstitial tissue thickening in the lung.
3. The short survival span of mice given inoculations intraperitoneally of sarcomas and carcinomas was correlated with the massive infiltration of peritoneal tissue and of abdominal organs by free tumor cells.
4. The earliest death in the series injected subcutaneously was recorded in mice with tumors on the leg and the latest in those with tumors on the back (the amount of proliferated tumor tissue could not be correlated with mortality), which observation suggested as an important lethal factor the compression or obliteration of blood vessels and lymph spaces by spreading tumor growth followed by hemorrhages and atrophic changes. Cells from tumors on the scalp invaded brain tissue and vessels inducing lethal hemorrhages.
5. Unicentric tumor growth, initiated at a single site by a single dose of tumor cells, induced less proliferation and spread of malignant tissue and killed later than pluricentric tumor growth, initiated with ten fractions of the same dose at ten sites.
6. It was concluded that the lethal effect of tumor growth in mice can be interpreted by tracing the pattern of its spread in tissues and organs.

ACKNOWLEDGMENTS

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REFERENCES

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