Factors Affecting the Number of Tumor Metastases
Experiments with a Transplantable Mouse Tumor*

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It is a matter of common knowledge that in human cancers the number of metastases bears no relation to the size of the primary tumor. Since the factors responsible for this lack of correlation are not understood, it seemed of interest to inquire into their identity. However, with human tumors it is difficult—or even impossible—to investigate this problem quantitatively, because in man the variety of neoplasms is great, the environmental conditions are dissimilar, and the hosts are of widely different constitution. To obviate these unmanageable variables, it is highly desirable to use a standardized transplantable tumor in hosts that have been inbred for many generations and that are kept under uniform environmental conditions. The present experiments, using Sarcoma 241 in C57 mice, were designed to meet these requirements. Their purpose was to ascertain whether the number of metastases is related to the size and duration of growth of a primary tumor.

Since the process of metastasis is primarily dependent upon embolism of tumor cells, it was decided to determine first the quantitative relationship between the number of viable cells artificially introduced into the circulation and the number of metastatic tumors so produced. If it could be shown that a strongly positive correlation exists, then variations in the number of "spontaneous" metastases probably could be attributed to factors in the primary tumor that affect the number of emboli released into the circulation.

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RELATIONSHIP OF THE NUMBER OF EMBOLIC CELLS TO THE NUMBER OF RESULTANT TUMORS

MATERIALS AND METHODS

A suspension of tumor cells was prepared by forcing mouse Sarcoma 241 (1) through the mesh of a stainless steel screen, and by adding a few cc. of a mixture of equal parts of serum and physiological salt solution. This suspension was allowed to stand for 10 minutes to permit settling of clumped cells. The supernatant suspension used in the experiment consisted almost exclusively of single cells.

The number of cells per cubic centimeter of this suspension was determined with a counting chamber. Because many of the cells were damaged by the sieving procedure, it was necessary to ascertain the percentage of living cells. This was done by using trypan blue as an indicator of viability.

A dilute solution of trypan blue was made in a mixture of salt solution and serum. Cells were mixed with this solution and observed in a counting chamber. Under these conditions, viable cells are unstained and refractile, whereas the nuclei of dead cells are stained blue and the cells are not refractile. By this method it was found that only about 6 per cent of the suspended tumor cells in this particular suspension were viable.

Serial dilutions of the original suspension were made, using the saline-serum mixture as the diluent. These suspensions were then injected into the tail veins of C57 mice, using 0.3 cc. for each injection. Eighteen days later the mice were sacrificed, and the tumors in the lungs were counted with the aid of a dissecting microscope.

RESULTS

The results are shown in Table 1. There was direct proportionality between the number of cells injected and the number of resultant pulmonary tumors. It is also apparent that relatively
large numbers of embolic cells were needed to produce a tumor, indicating that most of the cells failed to survive the process of embolism and lodgement in their new site, the lungs.²

### TABLE 1

#### RELATIONSHIP OF NUMBER OF VIABLE EMBOLIC TUMOR CELLS TO THE NUMBER OF RESULTANT PULMONARY TUMORS

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>Number of viable cells injected</th>
<th>Average number of pulmonary tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>104,400</td>
<td>11.5</td>
</tr>
<tr>
<td>10</td>
<td>19,800</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>5,400</td>
<td>1.7</td>
</tr>
<tr>
<td>10</td>
<td>900</td>
<td>0.3</td>
</tr>
</tbody>
</table>

#### RELATIONSHIP OF THE SIZE AND DURATION OF GROWTH OF THE PRIMARY TUMOR TO THE NUMBER OF METASTASES

Having established the fact that the number of metastases is directly proportional to the number of viable embolic cells, the question may now be asked: Do the size of the primary tumor and the duration of its growth determine the number of emboli released?

### MATERIAL AND METHODS

The material selected was mouse Sarcoma 241 implanted in C57 mice. In this highly inbred strain, 100 per cent of "takes" occur and "spontaneous" pulmonary metastases appear almost invariably.

Sixty-two mice were inoculated subcutaneously in the flank with fragments of tumor approximately 3 mm. in diameter and of as uniform a size as possible. Mice were sacrificed at intervals, the lungs were removed, and the subpleural metastases were counted with the aid of a dissecting microscope. As a check on these counts, a pulmonary lobe from each of four mice was sectioned serially, and metastases were counted with the microscope. In this way, a few additional subpleural tumors of microscopic size and a still smaller number of more deeply located tumors were picked up, but the counts on serial sections were proportional to counts made by the method described above—which thus appeared satisfactory for the present purpose. The ultimate sizes attained by the primary tumors were determined by measuring the volume of fluid displaced by them when immersed in isotonic salt solution.

### RESULTS

#### Effect of the duration of growth.—The period of growth of the primary tumor on the number of pulmonary metastases is shown in Table 2. There is a consistent increase in the number of secondary tumors with time, beginning on the eleventh day and reaching a peak on the 21st. Thus, duration of growth of the primary tumor was found to be an important factor in affecting the number of metastases.

#### Effect on the number of metastases of the original size of the tumor implant.—Experiments were designed to answer the question: Is the number of pulmonary metastases proportional to the size of the tumor implant? Twenty mice were inoculated in one flank with large tumor fragments measuring about 5 mm., and twenty mice (of which eighteen survived) with small fragments measuring about 2 mm. All animals were sacrificed on the seventeenth day. When the pulmonary metastases were counted, a very wide range in their numbers was found—from 0 to 105. The mean number of metastases resulting from large fragments was 21.7, with a standard error of ±5.41. In mice inoculated with small fragments, the mean with its standard error was 9.4 ± 1.89. The ratio of the difference (12.3) to its standard error was 2.185, which corresponds to a probability of between 0.03 and 0.04. In other words, a difference of this (or greater) magnitude would occur by chance only 3 or 4 times in 100, and therefore is regarded as significant. In this experiment, therefore, greater numbers of metastases resulted from implantation of large tumor fragments than from small ones.

#### Relation of the number of metastases to the final size of the primary tumor.—The data given above were based on the original size of the fragments of tissue at the time of implantation. Now, the relationship of the number of metastases to the final size of the resulting tumors, as determined at the conclusion of the experiment 17 days later, will be considered.

The volumes obtained were arranged in numerical order and divided into two groups of nineteen.

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² Such a mortality of embolic cells is consistent with the conclusions of Schmidt (4), Iwasaki (3), and Warren and Gates (5). See also Willis (6).
each, one group containing the larger values (ranging from 4.5 to 1.8 cc.), the other the smaller values (ranging from 1.75 to 0.30 cc.). Comparison was then made between the number of metastases corresponding to each group. In those animals having the larger primary tumors, the mean number of metastases was 19.8, with a standard error of ±5.74; the group with smaller tumors had 11.8 ± 2.25 metastases. However, because of the wide scatter in numbers of metastases, the difference between the two groups cannot be regarded as significant (P = .19). Likewise, when final volumes of primary tumors are correlated with numbers of metastases, the value of the correlation coefficient is 0.18, and P = > 0.1, a value considerably short of significance. It is, therefore, concluded that in this experiment the number of pulmonary metastases was not definitely related to the final size attained by the primary tumor.

DISCUSSION

As demonstrated in these experiments, the likelihood of metastasis from a cancer depends on the number of emboli released from the primary tumor. It depends also on the ability of emboli to establish themselves and grow where they lodge, as discussed by others (3—6) and in recent (2) and forthcoming papers from this laboratory. In the present investigation, attention centers on factors in the primary tumor regulating the number of emboli given off. Among the most important of such factors may be reckoned the ability of cancer cells to invade vessels and there to release emboli into the circulation. If these properties can be held relatively constant by using highly standardized transplantable tumors in inbred hosts, such simple physical factors as the size of the primary tumor and the duration of its growth would be expected to affect the number of metastases to a demonstrable extent. It is, therefore, surprising to find that even under these standardized conditions there is little correlation between the size of the primary tumor and the number of metastases it produces. It may be assumed, therefore, that the wide variation in numbers of “spontaneous” metastases must depend on factors existing in the primary tumor, as yet unidentified, that affect the number of emboli given off.

SUMMARY AND CONCLUSIONS

Experiments were designed to determine the relationship between the number of embolic viable tumor cells and the number of resultant tumors. Serial dilutions of a suspension of viable cells from mouse Sarcoma 241 were injected into the tail veins of C57 mice. A direct proportionality was found between the number of cells injected and the number of tumors resulting in the lungs. It was also shown that very few tumors were formed in relation to the number of tumor cells injected, indicating a high mortality of the cells.

Further experiments were designed to determine the relationship of size and duration of growth of the primary tumor to the number of emboli released into the circulation. Using C57 mice with transplantable Sarcoma 241 it was found that:

1. The longer a primary tumor existed, the greater the number of emboli released, as judged by the number of metastases appearing in the lungs.
2. Mice inoculated with large tumor fragments had a greater number of metastatic tumors than did mice inoculated with smaller fragments.
3. There was not a significant correlation between the number of pulmonary metastases and the final size attained by the primary tumors.

It is concluded from these experiments:

1. That the number of metastases is directly proportional to the number of viable tumor cell emboli released into the circulation.
2. That although the number of emboli released may be affected by such simple factors as the initial size of the tumor inoculum and duration of growth of the primary tumors, it depends to an even greater extent upon factors within the primary tumor as yet unrecognized.

REFERENCES

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