The mechanisms by which tumors metastasize have been of interest to the oncologist for some time, and several papers (1, 3, 4) have appeared relating to factors which modify metastases and the mode of cell transport. Little information, however, is available concerning the chemical nature of the cells which break free of the primary tumor mass and give rise to the secondary tumor growth. It is generally assumed that there are no chemical differences between the primary and the secondary tumor growths, although little proof has been offered. This paper is a preliminary attempt at the delineation of possible differences between the primary and the secondary growths of 2 different tumors, the Morris hepatoma No. 5123 and a transplantable rhabdomyosarcoma.

**MATERIALS AND METHODS**

All animals were bred and maintained in air-conditioned animal rooms and fed Purina laboratory chow and water *ad libitum*.

The Morris hepatoma No. 5523 was originally obtained from a hepatoma induced in Buffalo rats by feeding acetylaminofluorene (7). Our line was obtained from Dr. Harold Morris in 1961 and has been maintained since then by transplantation to male or female Buffalo rats.

The rhabdomyosarcoma to be described was originally obtained in 1950 from Dr. Elizabeth U. Green (5) in its 70th transplant generation. The tumor was induced by the intramuscular injection of 0.5 mg of 20-methylcholanthrene in filtered lard. The tumor appeared 71 days after the administration of the carcinogen and was transplanted 24 days thereafter. The recipient strain was C3H but was later changed to (C3H X DBA)F1 hybrids. In our laboratories the tumor has been carried in C3H mice of both sexes by s.c. trocar implantation.

Animals which were to receive transplants of either tumor were shaved on the abdomen and thigh and the area disinfected with 70% alcohol. The rhabdomyosarcoma to be implanted was excised, cut into small pieces, washed in isotonic saline containing 0.5% streptomycin and 0.25% penicillin and transplanted s.c. into the axilla by trocar. The Morris hepatoma No. 5123 was excised, washed in isotonic saline and transplanted into muscle of the hind thigh of the recipient animal by trocar. The tumor was transplanted in this manner every 2–2½ weeks for the rhabdomyosarcoma and 5–6 weeks for the Morris No. 5123. All measurements reported were made in 2 dimensions with vernier calipers and expressed as sq cm. Transplanted tumors were isolated from the animal following decapitation and the Morris hepatoma was perfused through the femoral artery with cold isotonic saline. The tumor mass was then excised, minced with scissors and homogenized in a VirTis “45” homogenizer (cells and nuclei are broken by this procedure). After a preliminary low speed centrifugation to remove large particulates, the supernatant was centrifuged at 105,000 X g for 60 min in a Spinco preparative ultracentrifuge. The metastatic lesions of the lung were recovered from host animals which were kept 2–3 times beyond the normal transplantation date. After the rats were killed, the lungs were perfused through the right ventricle, the minute tumors were dissected free of surrounding lung and treated in an identical manner as were the transplant tumors.

The supernatants were then chromatographed on DEAE-cellulose using a gradient elution system described by Moore and Angeletti (6) and the protein content of each fraction was determined at 280 m\(\mu\) in a Beckman DU spectrophotometer. The individual samples were then pooled to give 15–20 fractions which were concentrated to a volume of 100 \(\mu\)l. Aliquots (20 \(\mu\)l) of these concentrated samples were subjected to gel electrophoresis by the method of Smithies (9) using the discontinuous buffer system described by Poulak (8). Photographs were taken of the gels after staining and washing and a sketch of each
TABLE 1

AVERAGE SIZE OF RHABDOMYOSARCOMA IN DIFFERENT AGE GROUPS OF C3H MALE MICE

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ANIMALS PER GROUP</th>
<th>AVERAGE AGE (MO)</th>
<th>DAYS AFTER TRANSPLANTATION*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>2.75</td>
<td>0.1+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.04-0.18)²</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>9</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.04-0.16)²</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>16</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.06-0.16)²</td>
</tr>
</tbody>
</table>

* All measurements expressed as sq cm.
² Mean tumor size.
³ Variation in tumor size given in parentheses.
² Mean without tumors (not included in calculation of average size).

was made to show location of the various protein bands. Also, since both the primary tumor and its lung metastases were chromatographed in an identical manner, gels were run with concentrated fractions from primary and secondary tumors which appeared at the same point in the elution sequence. In order to obtain a better comparison, these similar fractions of primary and secondary tumors were electrophoresed in adjacent slots in the gel. The small size of the metastatic tumors (0.5–1.5 mm in diameter) necessitated the combination of many nodules to give sufficient protein for study.

RESULTS

Growth properties of the rhabdomyosarcoma.—In order to be able to define more accurately the process of metastasis, the temporal relationships between tumor growth and appearance of lung lesions in C3H mice of different ages were determined.

Table 1 shows the average size of the rhabdomyosarcoma at various times after transplantation to C3H mice of both sexes and 3 different ages. Considering the large individual variation in the size of the tumor at the times indicated, there seems to be little, if any, difference in the growth of this tumor in mice of widely divergent ages of either sex.

The average survival time of the animals with tumors, deduced from the subsequent experiments, was 40 days with a variation of 22–53 days. The reported growth rate of the tumor was calculated for the 280th transplant generation and represents an increase over that observed for the same tumor at earlier periods.

In order to determine the over-all incidence of metastatic lesions in the lung, 100 C3H mice of both sexes, 3–4 months old, received tumor implants which were allowed to grow until the recipient mice appeared to be dying. The mice were then sacrificed and the lungs examined meticulously for gross lesions. All lesions, positive or suspected, as well as portions of the lung itself, were taken for microscopic examination. The tabulation of the results obtained for all mice studied is shown in Chart 1. Other than the few animals that died early and were without observable metastases, the incidence of secondary pulmonary tumors reached 100% at approximately 25 days; practically all mice examined thereafter had obvious multiple lesions of the lung. It should be noted that those animals that died tumor free were included in this tabulation, even though it was difficult in many cases to determine from gross examination whether lesions were present. Of the 94 mice with lung lesions, 89 had more than 1 lesion. In order to determine more accurately the time at which the lung metastases appear, 34 C3H mice of both sexes, 9 months old, received transplants of rhabdomyosarcoma as previously described (all transplants were derived from the same tumor). Groups of mice were then killed 10, 17, 21, 25, 28, and 32 days after transplantation and the percentage of animals with lung nodules calculated. The results are included in Chart 1. It was observed that the incidence of lung metastases increased with time, reaching a maximum about 28 days after transplantation. The number of nodules in the lung also increased, so that essentially all the mice had multiple nodules after 25 days.

Morphologic examination of tumors.—Microscopically, the rhabdomyosarcoma at the 70th transplant generation consisted of characteristic spindle-shaped cells with large nuclei, interspersed with large but varying numbers of...
FIG. 1.—Rhabdomyosarcoma, 282nd transplant generation. H & E, x 220.

FIG. 2.—Metastatic lesion of the rhabdomyosarcoma in the lung. H & E, x 220.

FIG. 3.—Cross-striations in a rhabdomyoblast of the transplanted rhabdomyosarcoma. Phosphotungstic acid—hematoxylin, X 950.

FIG. 4.—Transplanted Morris hepatoma No. 5123. X 25.

FIG. 5.—Lung metastasis of Morris hepatoma No. 5123. X 25.

Chart 2.—A representative drawing of the protein bands of 2 starch gel electropherograms of the soluble proteins of a rhabdomyosarcoma (unprimed numbers) and the soluble proteins of the lung metastases of the rhabdomyosarcoma (primed numbers). Each number represents a single slot in the starch block, filled with a pooled and concentrated aliquot of the eluate of a DEAE ion exchange chromatogram. A line extending vertically through 2 or more horizontal bands indicates an essential identity of the protein in the 2 tumor samples.

Giant cells. In the strongly eosinophilic cytoplasm of these giant cells, longitudinal striations were occasionally observed. An increase in the number of mitoses was noted during the period of time that the tumor was maintained in these laboratories.

The growing tumor was highly invasive, so much so that even 8 days after transplantation it was observed infiltrating the surrounding muscle. Fig. 1 represents the 282nd transplant generation of the rhabdomyosarcoma and displays the same structure as was apparent on the 70th transplantation.

The metastases to the lungs (Fig. 2) and lymph nodes very closely resembled the primary tumor in all aspects, with an equally large number of giant cells.

Cross striations were rarely observed on slides stained with phosphotungstic acid-hematoxylin (Fig. 3), but never on slides stained with hematoxylin and eosin.

Microscopic examination of the Morris hepatoma No. 5123 (Fig. 4) indicated that the primary tumor was composed of chords of parenchymal cells which had lost the characteristic organization existing in liver. The lung
metastases (Fig. 5) were noted to resemble the primary tumor in all respects. Many microscopic foci of cells which arose from the implantation of a few cells of the transplanted tumor were evident in the lung specimens.  

_Gel electrophoresis of the 2 tumors._—The results of at least 2 electrophoretic analyses for 2 different samples of each primary tumor, and 2 different collections of lung metastases, are shown in Chart 2 (rhabdomyosarcoma) and Chart 3 (Morris hepatoma No. 5123). As can readily be seen, there was no obvious difference between the protein profile of either primary tumor and its lung metastasis, even when the method of analysis allows rather high resolution. Previous data (2, 8) indicate that there are rather large alterations in protein pattern and enzymes content of primary tumors when compared to their tissue of origin. The process of metastasis formation, however, from the data presented, indicates that it is related simply to the growth rate of the primary tumor and host factors other than age, rather than to some intrinsic property of the tumor cell itself. Since the individual nodules probably arise from very few cells (possibly even one), the collected nodules represent a clonal selection of the primary tumor. As such, then, the data reflect the comparison of the progeny of a few (10–500) selected tumor cells which have established themselves in the host lung.  

It thus seems that the formation of metastases is related to factors which influence cell transport through the lymph and blood and the surface properties of the cells themselves, rather than a selection of some cells of the primary growth which have special properties.

**REFERENCES**


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The Protein Profiles of Primary Tumors and Their Lung Metastases

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