The Malignant Melanoma of Hamsters

I. Pathologic Characteristics of a Transplanted Melanotic and Amelanotic Tumor*

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SUMMARY

The pathologic characteristics of the amelanotic and melanotic variants of the transplanted melanoma of hamsters (Fortner) are described. Outstanding differences between the two variants are the more rapid growth and invasiveness of the nonpigmented strain. Similarities between the Cloudman S91 tumor and the melanotic melanoma are pointed out.

In 1957 Fortner (1) reported on the occurrence of an assortment of spontaneous tumors in the golden Syrian hamster (*Mesocricetus auratus*), a laboratory animal introduced into the United States in 1938 by the Public Health Service laboratory at Carville, Louisiana. In a subsequent paper he described the characteristics of the primary malignant melanomas which he found in this animal (2). A total of ten such tumors were observed in a group of 523 hamsters. Fortner noted that: "Their gross and microscopic appearances, histogenesis and biologic behavior are counterparts of the human melanomas" (9). At least five of the ten malignant melanomas were successfully transplanted to other animals, and only rarely did the tumor fail to take on subsequent transplantation. It was also noted that many of the transplanted tumors which were originally pigmented soon developed amelanotic areas. The nonpigmented tumors contained dopa-positive cells, and on histologic examination appeared more anaplastic than did the pigmented neoplasms. An increased growth rate and capacity to metastasize also accompanied the loss of pigment production by the tumor cells.

In February, 1958, Dr. Fortner was kind enough to provide us with an opportunity to establish two transplantable melanomas in this laboratory. A melanotic (#1) and an amelanotic (#3) tumor have since been maintained here in an inbred colony of hamsters. The following study was designed to extend Fortner's observations on the pathologic characteristics of the transplanted hamster melanoma. It is part of a larger investigation of the *in vivo* and *in vitro* growth and behavior of this tumor as a means of gaining insight into the biologic properties of the melanoma.

MATERIALS AND METHODS

The tumors studied were all originally transplanted into the subcutaneous or muscular tissues of the right femoral area by implantation of tumor fragments of melanoma measuring about 5 mm. in greatest diameter. This was found to produce a tumor which corresponded to one resulting from the injection of 0.5–1.0 ml. of a 50 per cent (w/v) tumor suspension. Anywhere from 100,000 to 400,000 cells obtained by trypsinization of tumor tissue, as for the preparation of an inoculum for tissue culture (7), also produced tumors of similar characteristics. Of the three methods used to transplant the tumor, the first of the above-mentioned was found most reliable. Under these circumstances we could confirm Fortner's observa-
tion that only rarely did the tumor fail to take. With a 50 per cent tumor suspension, about an 85 per cent take of the amelanotic tumor was observed, and even lower per cent takes were found with the melanotic tumor. Less satisfactory results were obtained when trypsinized cell suspensions were used. In selecting fragments of tumor for implantation, an attempt was made to obtain the most viable-appearing and characteristic pieces of tissue available—i.e., the most pigmented and nonpigmented areas were used for the melanotic and amelanotic tumors, respectively. Under these conditions, there was little tendency for the melanotic tumor to become less pigmented. The

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Melanotic</th>
<th>Amelanotic</th>
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<tbody>
<tr>
<td>Survival (days):</td>
<td>32-118</td>
<td>22-88</td>
</tr>
<tr>
<td>Range</td>
<td>66.3</td>
<td>38.7</td>
</tr>
<tr>
<td>Mean</td>
<td>±7.4</td>
<td>±4.2</td>
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<tr>
<td>Standard error</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamsters dying with metastases (%)</td>
<td>89</td>
<td>95</td>
</tr>
<tr>
<td>Sites of metastases (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>11</td>
<td>95</td>
</tr>
<tr>
<td>Liver</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>Kidney</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>Heart</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Lung</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>Subcutaneous tissues</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

GROSS PATHOLOGY

Survival following implantation of tumor.—The hamster’s life span following implantation of the melanotic and amelanotic tumors differed (Table 1). Those that received the nonpigmented tumor lived for 38.7 ± 4.2 days (mean ± standard error), whereas those with the pigmented variant lived for 66.3 ± 9.3 days.

Implanted tumor.—The tumor which grew at the site of implantation was usually discrete, ellipsoidal to spherical in shape, and often attained an extremely large size, i.e., one-quarter to one-third the size of its host. At post-mortem examination, its surface was usually ulcerated and infected. Most of the hamsters demonstrated extreme cachexia as the tumor attained a large size (Fig. 1).

The central portion of both types of tumors was usually grossly necrotic. Necrosis was more prominent in the melanotic tumors; most of the central portion of this tumor was occupied by black, mostly liquefied material which contained a moderate amount of blood. Only a rim of viable tissue was present at the periphery. Smaller tumors were less necrotic. Blood vessels were readily seen coursing along the surface of the tumor. However, arteriographic studies revealed that both tumors were sparsely vascularized in the more central portions (Fig. 2).

The amelanotic tumor was usually not as well demarcated from the surrounding tissue as was the melanotic tumor. It had a firmer consistency than the melanotic tumors. Gross evidence of extensive direct extension was not generally seen at the site of the implanted tumor.

Metastases.—At death, 95 per cent of the hamsters with the amelanotic melanoma had metastases. Of the animals with the pigmented tumor, 89 per cent had metastases. The anatomic distribution of the metastases found in a small

1 Dr. L. Wattenberg kindly performed the dopa-oxidase reactions which are reported in this paper.
group of animals studied separately is indicated in Table 1. Not all the sites of metastases which occurred are indicated in this table because of the small sample size used for this particular phase of the investigation (21 hamsters with the amelanotic tumor and eighteen hamsters with the melanotic tumor). Nonetheless, a striking difference was noted in the metastatic pattern of the two tumors, and it is this point which we seek to demonstrate here. All the animals with amelanotic tumor metastases had lymph node involvement (Fig. 3). In contrast to this 95 per cent involvement is the 11 per cent lymph node involvement observed in the animals with melanotic metastases. A further contrast in the distribution of metastases is seen when the percentages of animals with metastases to the lungs are compared. No animals with amelanotic tumor had metastases to the lung (except for occasional instances of direct extension from lymph nodes), whereas 72 per cent of the hamsters with melanotic tumor did show definite metastases (Fig. 4). Cardiac metastases were occasionally seen in both types of melanoma, but metastasis to the brain, spleen, or osseous tissues was rare.

Metastases from the amelanotic tumor were of the same general appearance as the primary tumor. The distribution of the lymph node metastases in a group of 29 animals is shown in Table 2. Of the periaortic nodes, the group located at the level of the renal arteries was most commonly enlarged (Fig. 3). Next in frequency was the group of nodes present in the retro-sternal area. The thymus was included under this designation. This organ was frequently replaced with tumor. Mesenteric nodes containing tumor were usually accompanied by spread of tumor to the gastrointestinal tract. The hepatic metastases found were either diffusely distributed throughout this organ, to produce a finely speckled appearance to the cut surface of the liver, or present in the form of larger tumor nodules. When spread to the liver occurred, hepatomegaly was common.

Metastases from the melanotic tumor never attained the large size that the amelanotic metastases did, nor were they ever as large as the implanted tumor. They were usually confined to the thorax and appeared less pigmented than the implanted tumor. In addition to pulmonary metastases, deposits of melanotic tumor were found on the diaphragm and parietal and mediastinal pleura.

**Histopathology**

The histologic appearance of the transplanted tumors and their metastases was similar in most respects to that described in the primary and metastatic tumors by Fortner and Allen. The melanotic tumor and the amelanotic variant were recognizable as arising from the same origin and showed more similarities than differences.

In both types of growth, the tumor was generally formed of solid sheets and masses of cells which were more polygonal than fusiform. There was little tendency to form fascicles or whorls, and the characteristic alveolar clusters of human melanomas were almost completely lacking. There was little fibrous stroma, and the tumors were not remarkably vascular. A perivascular arrangement was seen only in otherwise extensively necrotic areas. There was often extensive degeneration and necrosis, making observations of pattern difficult.

**TABLE 2**

<table>
<thead>
<tr>
<th>Regional lymph nodes</th>
<th>Per cent of animals showing involvement</th>
</tr>
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<tr>
<td>Periaortic</td>
<td>97</td>
</tr>
<tr>
<td>Pelvic</td>
<td>90</td>
</tr>
<tr>
<td>Axillary:</td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>84</td>
</tr>
<tr>
<td>Contralateral</td>
<td>57</td>
</tr>
<tr>
<td>Mediastinal</td>
<td>76</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>64</td>
</tr>
<tr>
<td>Cervical:</td>
<td></td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>41</td>
</tr>
<tr>
<td>Contralateral</td>
<td>28</td>
</tr>
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</table>

*29 animals received 0.8 ml. of a 50 per cent tumor suspension subcutaneously in the right inguinal area and were autopsied when they died with tumor metastases.

The melanotic tumor was histologically the more uniform in cellular appearance (Fig. 5). It was formed of polygonal melanocytes with scattered, more irregular or elongated melanophages. The nuclei were somewhat oval but rarely spindle-shaped. Variation in nuclear size was only moderate, the largest cells generally measuring only up to twice the diameter of the smallest. Nucleoli were easily seen but were generally small and basophilic and occasionally multiple. The chromatin had a finely stippled appearance with distinct nuclear membranes.

The amount of pigment varied from one area to another but was present in all the tumors classified as melanotic on gross examination. In general, pigmentation was not as extensive in the secondary growths as has been reported in the spon-
taneous tumors. It was not usually necessary to
depigmentize the sections to examine the cyto-
logical detail. In the melanocytes, the pigment
could generally be seen as a fine, diffuse granula-
tion of the cytoplasm. In the melanophages, the
pigment granules generally formed coarser aggre-
gates, filling the cell (Fig. 6). Pigment was particu-
larly evident around areas of necrosis and in the
vicinity of fibrous trabeculae and vessels, where
phagocytes were more numerous.

Other occasional sites of pigment deposition
were the regional lymph nodes and the glomeruli
(Fig. 7). Pigment was not seen within the renal
tubules, nor were the changes characterized as
"melanuric nephrosis" observed (6). The numbers
of mitotic figures seen in the tumors were variable.
In some areas of cellular uniformity there might
be none to one per high-power field. Elsewhere,
particularly at margins of tumor nodules, or
where there was more irregularity, there might
be four to six or even more mitoses per high-power
field. Giant forms or multinucleate cells were not
encountered in the melanotic tumors, even in those
areas having high mitotic activity.

In contrast, the amelanotic variant produced
tumors with greater cellular pleomorphism. De-
genenerative changes were more frequent, and the
tumor architecture less well preserved. The ar-
range ment was again in broad sheets of cells with-
out specific pattern. The cells were nearly always
polygonal in shape without any significant tend-
ency to develop spindle forms. While the average
cell size did not seem to differ remarkably from
that of the melanotic tumor, nuclear and cell size
were generally variable. There were scattered gi-
gantic forms with bizarre nuclei. The largest
nuclei were several times the diameter of the
smallest. Large, often eosinophilic, nucleoli, some-
times occupying half the diameter of the nucleus
were common. Nucleoli were sometimes multiple.
Multiple nuclei or irregular masses of chromatin
were observed. The chromatin pattern, however,
was generally finely stippled or reticular. These
large cells did not appear to be the same as those
which were observed in tissue culture by Rosen-
berg et al. (7) but did resemble those described by
Symeonides (9) in amelanotic melanomas of
humans (Fig. 8).

The mitotic activity was fairly equal from area
to area, and mitoses might average 1 to 3 per high-
power field. They were found without difficulty in
all areas. No areas of spindle-celled, sarcomatous
appearance were found as described by Greene (3),
and no pigmentation occurred. The dopa reaction
was negative.

A reticulum framework was found which varied
with cell size and density. It generally surrounded
individual cells or small groups of two to ten cells
in the amelanotic tumors. The reticulum seemed
somewhat less uniform in the melanotic tumor.
Larger groups of cells and irregular nests or
fascicles were marked off by thin strands of fibers,
often paralleling vessels. This contributed to a
fasciculated appearance which was not consistently
observed and which was not apparent in hema-
 toxylene and cosin preparations (Fig. 9).

Of particular interest was the apparent differ-
ence in manner of growth of the melanotic and
amelanotic tumors. The melanotic tumor gener-
ally formed a discrete nodule within an organ or
tissue and appeared to grow by expansion, main-
taining a distinct solid boundary with the adjacent
normal tissue (Fig. 10). The amelanotic variant,
on the other hand, frequently grew as infiltrat-
ing columns or strands of cells within the invaded
tissue, preserving the architectural framework, at
least in the early stages. This was particularly evi-
dent in liver (Fig. 11), kidney (Fig. 12), heart
muscle, and lymph nodes. Whereas the infiltrating
mode of growth might resemble that of a ma-
lignant lymphoblastoma, the infiltration of the
sinuses of lymph nodes did not.

DISCUSSION

Aside from the more rapid growth and greater
metastasizing capacity of the amelanotic mela-
noma, there is also a difference in the metastatic
pattern of these tumors. The amelanotic tumor

Fig. 1.—Hamster with amelanotic melanoma. The tumor
was injected into the right inguinal area 70 days prior to the
time this photograph was obtained. Note the large ulcerating,
partially infected growth which has replaced the entire lower
extremity.

Fig. 2.—Arteriogram of a hamster that has had both the
melanotic (left) and amelanotic (right) tumors implanted at
the same time. Note the larger size of the amelanotic tumor and
the sparse arterial network within the tumor, particularly at its
center.

Fig. 3.—Same animal as in Figure 1. The tumor has been
cross-sectioned. Note the necrotic and infected areas at the
distal end of the extremity. Below this portion of the tumor is
the shriveled remnant of the foot. Periaortic nodes can be seen
situated between the kidneys (upper arrow). A large pelvic
node is present over the fundus of the bicornuate uterus (lower
arrow). These nodes are completely replaced with tumor tissue.

Fig. 4.—Metastatic nodules of melanotic melanoma in the
lung. These subpleural deposits are most characteristic for this
tumor.
was impossible to evaluate specific differences in cytotoxic responses as described before or between biopsies, in relation to lesions of the same type. This group did not include any primary and metastatic tumors from the same patient. There was no significant difference in sensitivity or resistance to any agent based on the therapy within 7 months prior to biopsy. All specimens were excised from patients treated with one or several of the drugs included administration of carcinostatic agents and response to any test in vitro.
Fig. 5.—Spherical melanocytes from the amelanotic cultures. Note similarity to pigmented melanocytes. May-Grunwald-Giesma, ×1500.

Fig. 6.—Spindle-shaped fibrocytes with pigment aggregated around nucleus. Giesma, ×1500.

Fig. 7.—Wandering macrophage containing much ingested pigment. Giesma, ×800.

Fig. 8.—Living unstained giant cell with an ingested melanocyte. The melanocyte is beginning to disintegrate, ×800.
It was impossible to evaluate specific differences in cytotoxic responses as described given before or between biopsies, in relation to lesions of the same type. This group did not include any primary and metastatic tumors from the same patient. There was no significant difference in sensitivity or resistance to any agent based on prior therapy, except in a few cases which responded in a similar manner to thioTEPA and phenylalanine mustard. All agents tested produced direct objective cytological changes. All five agents under investigation produced changes that were qualitatively similar and apparently: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard.

To investigate the relation between the response of primary versus metastatic tissues, the responses of 154 specimens excised from patients treated with one or several of the drugs were compared with the responses of 311 specimens from patients with no previous treatment and subsequent response for all normal, malignant, and benign neoplastic tissues tested. Normal treated tissue cultures never displayed any primary and metastatic tumors from the same patient. There was no detectable correlation between the response and drug concentration. These data may be useful in clinical procedures involving the use of agents administered at the tissue site as, for example, in perfusions. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard.

There was no detectable correlation between the prior growth rate and response to any test agent other than that expected due to chance. All agents were investigated by COBB et al.--Chemotherapy Studies on Human Tissue Cultures and response to any test agent individually, except in a few cases which responded in a similar manner to thioTEPA and phenylalanine mustard. All agents tested produced direct objective cytological changes. All five agents under investigation produced changes that were qualitatively similar and apparently: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard.

The effect of prior therapy seems to be more closely related to success or failure of growth in vivo than to what was observed in vitro, according to the observations of Cobb and Walker (5) on a series of melanomas. The relation of prior therapy to cellular changes in a wide variety of human benign and malignant tumors and normal tissues was investigated. Prior treatment of primary and metastatic tumors from the same patient was investigated. An analysis of the experimental data failed to reveal over-all sensitivity or resistance as a result of prior therapy. There was no relation between tissue culture and in vivo responses of these tumors, despite their different rates of growth in vivo. Some cultures of tumor tissues exposed to identical drug concentrations. These data may be useful in clinical procedures involving the use of agents administered at the tissue site as, for example, in perfusions.
Fig. 9.—Giant cell with an ingested melanocyte. Note the clear area surrounding the melanocyte and the second binucleate cell beside it. May-Grunwald-Giemsa, X600.

Fig. 10.—Giant cell with many ingested heavily pigmented melanocytes. Smaller pigment granules are seen in the periphery of the giant cell cytoplasm. Note the nucleus at the lower right corner of the giant cell. Giemsa, X800.

Fig. 11.—Unstained fixed preparations of two giant cells. Many melanocytes have been ingested, and many small clumps of ingested cytoplasm and pigment are to be seen, X400.

Figs. 12, 13.—A giant cell which has partially engulfed a spherical melanocyte. Pseudopodial extensions have nearly surrounded the melanocyte. Note the large size of the nuclei of these giant cells. May-Grunwald-Giemsa, X1000.
was impossible to evaluate specific

fore, differences in cytotoxic responses as described
given before or between biopsies, in relation to

prior growth rate

There was no detectable correlation between the

tical manner, regardless of the tissue type. There-

constant responses owing to the small sampling of

patients with identical drug therapy.

flections of either differences in growth rates of

type in the group evaluated.

clude any primary and metastatic tumors from the

responded, in the main, as individual tissue

specimens. Cells were either sensitive or resistant
to each agent individually, except in a few cases
which responded in a similar manner to thioTEPA
agents caused mitotic inhibition and cytolysis.

and phenylalanine mustard. All agents tested pro-
duced direct objective cytological changes. All five
compounds in decreasing order of effective-

was investigated. Prior treatment

therapy to cellular

as a result of prior therapy. All specimens were excised from

treatment and subsequent response for all normal,
solid tissues were handled and cultured in an iden-

in vitro

of primary versus metastatic tissues, the responses

cultures prior to drug addition or methods of cul-

in vitro. An analysis of the

of 154 specimens excised from patients

therapy were compared with the

responses of 30 randomly selected culture series of metastatic

therapy within 7 months prior to biopsy. The re-

response

in sensitivity or resistance to any agent based on

of primary and metastatic tumors from the same

patients treated with one or several of the drugs

of 30 culture series of primary melanoma and car-

therapy, despite their different rates of growth

Studies based on a large group of malignant neo-

f) the resistance of breast carcinomas to chlor-

c) the sensitivity of fibrosarcomas to direct ex-
d) the sensitivity of certain carcinoma types to
b) the sensitivity of lymphomas to thioTEPA;
e) the resistance of lymphosarcomas to metho-
a) the sensitivity of lymphosarcomas, Hodg-

Of interest was the absence of a relation between

apparent:

The five agents under investigation produced

Walker (5) on a series of melanomas.

DISCUSSION

prior therapy, whereas tumor types in general responded indi-

in vitro.

study of drug effects based on the

the severe cytotoxic changes that were observed in

some cultures of tumor tissues exposed to identical

ministered at the tissue site as, for example, in

spent the most time in vitro.

in vivo.

a list of human tumor types with respect to drug

therapy, drug concentration, and drug agent.

in vivo.

the antibiotics, actinomycin D, chlorambucil, and thio-

in vitro.

in vitro.

in vitro. CDp was able to induce changes similar to

methotrexate, actinomycin D, and thioTEPA; and

Walker's, and lymphomas of undetermined type to di-

the relation between migration and growth in tissue culture and

in vitro. AFP expressed a certain degree of differ-

in vivo. AFP did not differ from normal breast tissue in its

cancerous tissue.
seems to have a greater affinity for the lymphatic tissues, which may result from a predominantly lymphogenous mode of spread. On the other hand, the less active pigmented tumor may metastasize by a hematogenous route, explaining its more frequent occurrence in the lungs. This difference in the melanotic and amelanotic tumor may be related to the more invasive character of the growth of the amelanotic tumor.

The question arises whether or not the amelanotic tumor should be considered a true melanoma. The cells of the nonpigmented melanoma are dopa-negative, and its behavior is that of an undifferentiated neoplasm. It would appear, however, from morphologic and tissue culture studies, that the amelanotic tumor is similar to its pigmented counterpart and that they are variants of the same tumor. The origin of this amelanotic tumor, as described by Fortner, also supports the concept that this is a true melanoma. Characteristic junctional changes were present at the site of the tumor. Nonetheless, we believe that the pigmented tumor should be used in experiments designed to investigate the biologic characteristics of the hamster melanoma.

A comparison of the pathologic characteristics of this tumor, with its counterpart in mice, reveals that the transplanted hamster melanotic melanoma is similar to the Cloudman S91 melanoma which has been intensively studied in recent years (4, 5, 8). Most striking is the affinity of the metastases for pulmonary tissue.

When compared with the pathologic characteristics of the human melanoma, it can be seen that the hamster tumor is more similar than the mouse melanoma. Nonetheless, significant differences exist. The unpredictable metastatic pattern of the melanoma of man is in sharp contrast to the uniform behavior of these transplanted hamster melanomas, thus compromising the use of this tumor as an experimental tool in the investigation of human melanomas.

REFERENCES

The Malignant Melanoma of Hamsters I. Pathologic Characteristics of a Transplanted Melanotic and Amelanotic Tumor

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