A Spontaneous Melanoma in the Hamster with a Propensity for Amelanotic Alteration and Sarcomatous Transformation during Transplantation*

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Relatively few spontaneous tumors have been reported in the hamster despite the wide use of this animal in experimental laboratories. The apparent low incidence may relate to the age groups observed rather than to fact, for the stock provided by dealers consists almost entirely of young animals, and, in laboratory colonies maintained for supply purposes, the breeders are generally discarded at the end of their characteristically short period of reproductive activity. Older hamsters have not been studied, and the possibility that the incidence in this group may be significantly higher is suggested by the discovery of a number of spontaneous growths in our colony among normal, untreated animals of more than 2 years of age. One of the more interesting of the tumors was a malignant melanoma, and the object of the present paper is to report its occurrence and to describe its behavior on transplantation.

MATERIALS AND METHODS

The hamsters utilized were of the Syrian golden variety, the mice of DBA strain, and the guinea pigs of mongrel stock.

The technics were entirely those of transplantation. The subcutaneous space, eye, and brain were used as transplantation sites, and the methods employed have been described in detail (4, 5). All transfers were effected by a trocar with fragments of tissue measuring 0.5–1 mm. in diameter.

RESULTS

The tumor occurred in the pigmented spot of the right flank of a male hamster approximately 2½ years old. It was first discovered 2 months before death when it appeared as a black, rounded mass ½ cm. in diameter. The animal had not been thoroughly examined during the previous 6 months, and there are no data to indicate the age of the tumor at the date of discovery. It had undoubtedly been present for a considerable length of time for, when first noted, both axillary and inguinal nodes were involved. Biopsies and transplantation experiments were performed at the time of discovery and 6 weeks later. The animal was found in extremis 2 weeks after the last biopsy and was killed for further study.

The histological structure of tumor tissue obtained at biopsy and autopsy was identical. Melanoma cells were arranged in an alveolar pattern, and there was an abundance of pigment (Fig. 1). At autopsy, black, metastatic nodules were found widespread throughout the viscera. Some of these were minute, but others were of such size as to suggest that they had been present for a considerable period of time before death.

The homologous subcutaneous transfer of tissue obtained from both biopsy and autopsy specimens resulted in 100 per cent of takes (Fig. 2). All the transplanted tumors grew progressively and all the recipient animals died within 5 months of diffuse metastasis (Fig. 3). Transplants of the second biopsy specimen were selected for further passage, and the tumor has been maintained by serial transfer through six generations over a 2-year period. During this time, approximately 400 hamsters have been used for transfer, with continuance of the 100 per cent incidence of takes and death from metastases. Morphologically, there have been two dominant growth patterns. The alveolar structure observed in the primary tumor persisted through the second generation but is only occasionally seen at present. The replacing arrangement is one of solid, cellular masses without definable structure (Fig. 4). Necrosis is common in older tumors and results in an apparent perivascular pattern of living cells. The change in histological structure has not been associated with a significant increase in growth rate.

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A variation in pigment content, from pitch black to gray, was noted in one animal of the third passage, and transfer of this tumor resulted in growths varying in color from the original black to pale white. Continued transplantation of the gray variant produced a similar spectrum of colors, but the white variant gave rise exclusively to white tumors. Histologically, the gray and white tumors differ from their black parent only with respect to pigment content, and detailed microscopic study of the white form shows it to be completely amelanotic (Fig. 6). This tumor has been carried by continued transfer for six serial generations, and in none of the growths borne by several hundred animals has there been any trace of melanotic pigmentation. Its behavior on transplantation is identical with that of the original melanoma.

A further variation in transplants of the melanotic tumor of greater morphological consequence is a sarcomatous transformation resulting eventually in a complete replacement of neoplastic elements (Figs. 7-13). This occurs in two forms. The more common appears to arise in the stroma, and, in early stages, tumors so involved are characteristically black throughout their greater bulk but contain scattered nodules of white tissue. Microscopically, such nodules are made up of sarcomatous connective tissue, and sections of adjacent melanoma show similar tissue largely replacing its stroma. Further, random sections taken from distant black, melanomatous regions sometimes show a less extensive but identical stromal transformation. Later in the course of the tumor, the fibrosarcoma overgrows the melanoma, and the resulting growth consists solely of white fibrosarcomatous elements.

Transformations of this type have been observed to occur in melanomas borne by eight different animals. Each of the animals had been treated, but in each instance the type of treatment was different and varied from accidental hyperthermia to the transplantation of a second tumor to another bodily region. The only common factor in the various treatments was a resultant slowing of growth rate, but this occurred as well in other animals without affecting the morphology of the transplanted melanoma.

The second form of sarcomatous transformation differed in that no histological alterations were found in the melanomas of treated animals, but transplants of the melanomas to normal untreated hamsters resulted in growths made up exclusively of fibrosarcoma from the very beginning. The treatment in these cases consisted of the intramuscular injection of an emulsion of melanoma tissue stored in deep freeze for 6 months. The injected tumor did not grow, and a month later living melanoma tissue was transplanted to a different bodily region. Takes occurred as a result of the latter transfer, but the rate of growth was significantly slower than that of controls. Metastases were found at death, but both secondary and primary tumors showed the alveolar pattern of melanoma cells typical of the original growth, and there was no indication of a fibrosarcomatous transformation. Yet transfer of tumor fragments to normal hamsters resulted in growths of fibrosarcoma without melanomatous elements. It should be noted that fibrosarcomatous transformation did not follow the transfer of tumor from all the treated animals. In most instances, the transplanted tumors were typical, unaltered melanomas, and the described mutation was unpredictable and comparatively uncommon. In the group in question, four out of fifteen treated animals bore melanomas which yielded fibrosarcomas on transfer.

A type of sarcomatous transformation of different character from that described above has been observed in a single hamster. In this instance, the morphology of the transplanted melanoma was unaltered, and the sarcomatous change was present only in the metastases. The tumor concerned was a second-generation transplant of the amelanotic variant of the original melanoma, and the tissue used for transfer had been stored overnight in the refrigerator prior to use. The transplants grew rapidly and at the end of a 2 months' period had attained a mass of 2½ cm. diameter. At this time, a 1-cm. tumor was palpated in the muscle of the contralateral thigh, and the animal was killed. At autopsy, metastatic tumor was also found in the pelvic nodes and in the liver. Histologically, the transplanted growth was characteristic of the other transplants of the same tumor, and no aberrant cells were found on detailed examination. However, the metastases were made up of cells entirely different from those of the primary tumor and contained none of the elements of the primary tumor. The cells were small, rounded, with clear or vacuolated cytoplasm, and resembled those of a liposarcoma both in structure and arrangement; but special stains failed to reveal the presence of fat (Fig. 14).

The primary transplanted tumor concerned in this variation was transferred to other hamsters but, in all cases, its behavior was characteristic of the amelanotic melanoma, and the metastases were identical with the parent growth. The sarcomatous metastases were also transplanted and
have been carried through nine serial passages in an 8-month period (Figs. 15 and 16). With the exception of a more rapid growth rate, the transplant behave like other derivative tumors of the melanoma, growing progressively in all cases and metastasizing diffusely throughout the body.

All the variants of the melanoma described above maintain their morphological character on transfer, and in no instance to date has reversion to parental cell type occurred. All the tumors are transplantable heterologously but, like the original melanoma, display a highly selective preference for certain species. The melanoma itself and all its derivatives, with the exception of the clear-cell sarcoma, survive transfer to the brain and subcutaneous space of DBA mice; but only the latter growth has been successfully transferred to the guinea pig, and none of the tumors survives transplantation to the rabbit.

Various possibilities have been investigated in an effort to bring about sarcomatous transformation experimentally. These have all been based on the assumption that the site of alteration was the stroma of the tumor, and in no instances have the attempts been successful. The chance that the melanotic pigment evolved by the parenchyma might be the inducing agent was investigated in several ways. Pigment, freed from living tumor cells, was injected subcutaneously into adult hamsters; and, when the animals were killed a year later, large masses of melanin-filled macrophages were found, but there was no sarcomatous reaction in the surrounding connective tissue. In further experiments of this nature, the high susceptibility of embryonic tissue to carcinogens was utilized (3), but transplants of embryonic hamster tissue mixed with pigment grew and persisted in an entirely normal manner. The possibility that some other constituent of parenchymal cells either normal or viral in nature might be concerned was investigated in a similar manner, with fresh filtrates, but again no confirmatory results were obtained. Finally, in an attempt to determine whether or not the tumor stroma was more susceptible to carcinogens than other connective tissues of the host, methylcholanthrene was injected into both tumor and normal subcutaneous regions of a number of melanoma-bearing hamsters. At the time of death, several months later, no changes were found in the stroma of the tumor, but small nodules of sarcoma were present at other inoculation sites.

DISCUSSION

Malignant melanomas in man are distinguished by vagaries in behavior which set them apart from more conventional tumors and warrant a characterization as black sheep of the neoplastic family. The hamster tumor reported in the present paper is not an exception to this generalization; but, in its case, the instability relates more to morphological appearance, and, in actuality, its different morphological derivatives show an unusual degree of similarity in behavior.

The loss of the capacity to produce pigment is not an uncommon development in human melanomas and has been observed during the course of transplantation of a mouse melanoma (1). However, the proclivity of the hamster tumor to undergo sarcomatous transformation is apparently unique and has not been noted in its human counterpart or in the transplantable mouse melanomas. It should be emphasized that the sarcomatous transformation observed does not represent a growth of fusiform melanoma cells in fasciculate arrangement but rather an independent neoplasm involving separate histological elements and constituting a fibrosarcoma.

Carcinosarcomas have been described and studied in mouse and rat breast (2, 6) and mouse lung (7), with the general conclusion that the sarcomatous element represents a neoplastic transformation of the stroma of a primary carcinoma. Available evidence indicates a similar origin in the melanomas in question, although, in some instances, no morphological evidence of a stroma change could be found in melanotic tumors that on transfer gave rise to growths made up solely of fibrosarcoma. In these instances, the specimens used for transfer were grossly identical with those fixed for histological section, but the possibility of a microscopic difference cannot be excluded.

A point of particular interest concerns the
Fig. 3.—Second-generation transplant in the subcutaneous space of DBA mouse. Mag. X400.

Fig. 6.—Fourth-generation transplant of amelanotic variant. Mag. X400.

Fig. 7.—Sarcomatous transformation in melanotic tumor. Note broad band of sarcomatous connective tissue separating masses of melanoma. Mag. X200.

Fig. 8.—Diffuse sarcomatous transformation with encroachment of growth on pre-existing melanoma. Mag. X400.
FIG. 9.—Separation of solid melanoma into islands by sarcomatous growth. Mag. X400.

FIG. 10.—Distribution of reticulum fibers in tumor shown in Figure 9. Note intercellular distribution in sarcomatous tissue and absence in residual melanoma. Mag. X400.

FIG. 11.—More extensive sarcomatous transformation of tumor, with almost complete obliteration of melanoma. Mag. X400.

FIG. 12.—Transplant of sarcomatous element showing growth of fibrosarcoma without melanoma. Mag. X260.
Fig. 13.—Transplant of fibrosarcoma from hamster to the brain of a DBA mouse. Mag. X260.

Fig. 14.—Metastatic tumor in liver derived from subcutaneously transplanted melanoma. Mag. X260.

Fig. 15.—Transplant of metastatic tumor (Fig. 14) to subcutaneous space of hamster. Mag. X260.

Fig. 16.—Transplant of metastatic tumor (Fig. 14) to anterior chamber of guinea pig’s eye. Mag. X260.
nature and mechanism of the neoplastic transformation if, in the present cases, the sarcoma is a stromal derivative. It is generally believed that tumor stroma consists of normal connective tissue acting merely as a scaffolding for parenchymatous cells. Further, there is much evidence to indicate that, except in highly inbred strains, transfer of a tumor results in death of the stromal component with subsequent replacement by growth of connective tissue from the new host. In such cases, sarcomatous transformation must be accomplished with unusual rapidity, since the full developmental course of the sarcoma from initiation to the attainment of metastasizability transpires during the period of residence of the transplanted melanoma in one hamster—and this period has been as brief as 3½ months. It should be noted that in our experiments a standard carcinogen, methylcholanthrene, induced no changes in melanoma stroma in a comparable length of time.

The occurrence of sarcomatous transformation in scattered foci throughout affected melanomas suggests a tumor-wide distribution of the causative factor. Pertinent experiments failed to show that the characteristic pigment of the tumor was of influence in this respect, and investigations are being continued in an attempt to determine the possible role of other substances of melanoma derivation.

SUMMARY

The occurrence of a spontaneous melanoma in the hamster and its behavior on transplantation have been described. Significant features observed during the course of study were the occurrence of amelanotic derivatives and a proclivity for sarcomatous transformation.

REFERENCES

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