Spontaneous Transformation from Carcinomatous to Sarcomatous-like Growth*

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This report describes a spontaneous morphological change which occurred in a mouse mammary adenocarcinoma during the course of its propagation in hosts of origin. A number of investigators have previously described similar phenomena. Ehrlich and Apolant (1905) were the first to note a sudden appearance of sarcomatous tissue between the alveoli of a mouse mammary tumor, and they reported that on three separate occasions sarcomatous tissue was observed to replace the carcinomatous tissue with the resulting formation of pure spindle-cell tumors. In one instance the carcinomatous growth became sarcomatous in the ninth generation; in a second, the change took place during the third and fourth generations; and in a third instance the transformation from carcinomatous to sarcomatous growth was completed during one or two generations (1, 4).

Following Ehrlich and Apolant, other investigators reported similar observations. A comprehensive review of the literature on the subject up to 1945 can be found in Dr. Thelma B. Dunn's publication on the morphology and histogenesis of mouse mammary tumors (3) and in the paper by Stewart et al. in which is described the development of sarcomatous growth during serial transplantation of mouse pulmonary epithelial tumors (13). Taylor et al. noted a sarcomatous change occurring in a mouse mammary tumor during cultivation in egg embryos in vitro (15, 16). Sarcomatous transformation in mammary carcinomas which arose in one mouse of each of the R III, A, and C3H strains was reported by Dmochowski in 1950 (2).

The tumor herein described has been carried through serial transplants in mice of the DBA inbred line, from which it originated about 3 years ago. It has proved to be very useful for experimental purposes, and it might be of interest and useful to other investigators in the cancer field.

MATERIALS AND METHODS

The tumor arose in an 11-month-old female mouse of an inbred DBA line. This line was inbred in our laboratory from a single litter, consisting of two males and four females, obtained in 1949 through the courtesy of Dr. E. U. Green of the Institute for Cancer Research in Philadelphia.1 The tumor was seated on the right side above the inguinal region, and it was dissected under aseptic conditions on January 7, 1950. Grossly, the tumor appeared encapsulated, rather firm to the touch, and moderately vascular. Portions from the edges of the tumor were cut with a sharp scalpel into particles about 2 mm. in diameter. These were aseptically implanted by means of a trocar and cannula into five male DBA mice, each mouse receiving one tumor fragment placed subcutaneously between the groin and subaxillary region on the right side. The implanted grafts developed into palpable tumors of measurable size in all five mice within 23 days. The tumors grew steadily.

Several cross-sections were removed from the original spontaneous tumor and fixed in Zenker's acetic acid fluid. After routine paraffin embedding, histological sections about 6 μ thick were stained with Bulliards hematoxylin and eosin. Microscopic analysis revealed acinar structures of rather uniform size in nearly the entire tumor section. These were separated by a delicate stroma of fibrous connective tissue. The histological pattern was that of a typical, well differentiated mammary adenocarcinoma (Figs. 1, 2).

One of the five mice was sacrificed and its

1 The litter was received on May 2, 1949, with the following information: "They were born January 25, 1949, in our cage no. 56. The stock has been bred brother by sister in our colony since December, 1944. We obtained the original parents from the main Institute colony. The record says: from Jackson Memorial Laboratory, 7/5/33, inbred strain 'D' described by W. S. Murray, 1934, Am. J. Cancer, 20:575."
tumor removed aseptically. Cross-sections of this second-generation tumor were fixed for histological studies, and several fragments dissected from the edges of the tumor were implanted into five other DBA male mice, in which tumors of measurable size developed within 18–24 days. Successive transplantations of this tumor into other DBA mice were performed regularly, and fragments of the tumor were fixed for histological analysis. Up to the sixth generation, the daughter tumors retained the histologic appearance of the original spontaneous tumor, maintaining a uniform distribution of acinar structures. After latent periods varying from 12–18 days, the daughter tumors grew steadily and uniformly in their DBA hosts.

Microscopic analysis of sections of the sixth-generation tumor revealed nests and sheets of closely packed epithelial cells among the regular acinous forms. In the seventh generation, an abrupt change was noted in the histologic appearance of the tumor as well as in the rate of growth. The mice which had been implanted with particles dissected from the tumor of the seventh generation developed palpable tumors of measurable size within 7–9 days instead of 12–18 days. Microscopic analysis of sections from the tumor of the seventh generation showed it to consist mainly of whorls and bundles of large spindle-shaped cells, among which mitotic figures were frequently noted. Nests of epithelial cells were still present here and there. The mixed structure of the seventh generation tumor can be seen in Figure 3. Sections of tumors from subsequent generations consisted entirely of large spindle cells, among which mitotic figures were frequently present. Thus, the spindle-cell tissue replaced the epithelial tissue of which the original tumor consisted. The histologic change which took place in this tumor apparently occurred almost within a single generation.

The change in the vigor of growth of this tumor is evident when a comparison is made of the mitotic activity of the tumors of the first six generations, before the histologic change took place, with that of tumors of subsequent generations. Using Chalkley’s method, as adapted by the author (8), the volume ratio of dividing to resting cells was obtained by counting 700 intact resting cells. The volume ratio of mitotic to resting cells, determined at random intervals from the 25th to the 108th generation, ranged from 1:40 to 1:50, instead of 1:116, which was the average of the six generations before the histologic change took place (see Table 1).

The tumor, in its 188th generation at the time of this writing, has attained a uniform rate of growth; its latent period is 4–6 days, and histologically it consists mainly of large spindle-shaped cells, among which mitotic figures are frequent. Other characteristic features of this tumor are its tendency to grow in the form of interlacing bundles of varying thickness and its rapid invasiveness by direct extension into the surrounding normal tissue when grown either subcutaneously or intraperitoneally.

Attempts were made to elucidate the histogenesis of this transformed tumor by using the following classical staining technics: Van Giesen’s stain for collagen; Mallory’s phosphotungstic-acid-hematoxylin for fibroglia fibrils, and Foot’s modification of Bielschowsky’s silver stain for reticulum. No collagen formation could be visualized by the use of Van Giesen’s stain in sections taken at random from tumors of the tenth up to the 60th generation. It is difficult to draw conclusions from this negative observation, since it is known that in vigorously growing fibrosarcomas very little or no collagen is noted (14). The staining reaction for fibroglia was not conclusive. The silver-stained sections of these tumors showed that the reticulin fibers formed a network which intimately surrounded groups of large spindle cells or individual cells (Fig. 5).

Dr. Thelma B. Dunn of the National Cancer Institute kindly reviewed the microscopic sections of the primary tumor and of tumors of successive generations up to the 60th. According to Dr. Dunn’s diagnosis, the primary tumor (Fig. 1) represents a mammary adenocarcinoma type A. The sections of the tumor at its sixth generation of transfer represent a mammary adenocarcinoma type B. Sections of tumors from subsequent generations consisted of large spindle-shaped cells only. Figure 4 illustrates a typical example.

This tumor has been used for radiation experiments. Observations regarding its cellular response will be mentioned here, and further details regarding its radiosensitivity will be described in a separate paper. Sections of tumors which had

<table>
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<th>Generation</th>
<th>Ratio of dividing to resting cells</th>
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<tr>
<td>1–6th</td>
<td>1:116 (Av.)</td>
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<tr>
<td>6–16th</td>
<td>1:70–1:87 (Range)</td>
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<tr>
<td>17th</td>
<td>1:58</td>
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<tr>
<td>24th</td>
<td>1:33</td>
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<tr>
<td>25–108th</td>
<td>1:40–1:50 (Range)</td>
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been exposed in situ to x-radiation showed bizarre nuclear changes (Fig. 6).

DISCUSSION

That the primary tumor herein described was an epithelial tumor of an acinous form is evident from Figures 1, 2, and that the pattern of this tumor changed to a spindle-shaped cell type is also evident from Figure 4. The transition from the epithelial to the spindle-cell pattern took place within the seventh daughter tumor, since the tumor of the sixth generation still showed a pure epithelial pattern, whereas the seventh-generation tumor consisted mainly of whorls and bundles of large spindle-shaped cells and several nests of epithelial cells. Thus, the change occurred during one or two generations. This observation coincides with one of those described by Ehrlich and Apolant (1, 4) and with an observation of Haaland (10), who also noticed the first appearance of bundles of large spindle cells in a seventh-generation mouse mammary carcinoma.

Various theories have been advanced to explain the possible mechanism involved in the development of spindle-cell tumors following serial transplantation of mammary epithelial tumors. The earlier investigators, Ehrlich, Apolant, and Haaland, were of the opinion that the normal connective tissue cells were biologically altered under the influence of the malignant epithelial cells with resulting sarcomatous growth. Ludford and Barlow (11, 12) brought forth conclusive evidence from their studies in tissue culture that the mammary carcinomatous tissue had a stimulating influence upon the normal fibroblasts, causing them to undergo a progressive sarcomatous change. Taylor (15, 16), using a mammary tumor bearing the milk agent or virus, explained the malignant change in the normal stroma as a consequence of virus infection. Taylor's interpretation may be subject to question, since transitions from carcinomatous to sarcomatous tumors have been noted in mice, both with and without the milk agent. In connection with Taylor's hypothesis, it should be mentioned that two mammary adenocarcinomas which arose in two females of two different inbred lines of mice, both bearing the milk agent, have been carried through serial transplants in mice of their respective inbred lines for more than 3 years in this laboratory; the daughter tumors have retained the original acinous structure of their respective spontaneous mother tumors. There exists another school of thought in interpreting the histogenesis of the spindle-cell tumors arising from epithelial tumors: Ewing (5) suggested, but did not conclude, that the spindle cells might be altered epithelial cells.

The question whether the spindle cells of the tumor herein described are altered epithelial cells from the original tumor, or whether they are derived from the connective tissue cells under the influence of the malignant epithelial cells, cannot be answered with any certainty. It is difficult to speculate on the mechanisms involved in this case, since the histologic change occurred abruptly in vivo. That a fundamental biologic change accompanied the histologic one is evident from the increase in the vigor of growth. The bizarre nuclear changes occurring following irradiation in this transformed tumor are similar to those noted by the author in mouse Sarcoma 180 following x-radiation (6). According to the records of the Crocker Institute of Columbia University, Sarcoma 180 also originated from a mouse mammary adenocarcinoma. No such bizarre nuclear changes were noted by the writer in an anaplastic, rapidly growing mouse mammary adenocarcinoma designated dbkB, following irradiation in vitro (9) or in vivo (7).

The histologic pattern of this new transformed tumor, its vigor of growth, and the bizarre nuclei appearing following irradiation all suggest sarcomatous growth. Experiments on the physiological characteristics of this tumor are in progress.

SUMMARY

A spontaneous mouse mammary adenocarcinoma type A which arose in a female mouse of an inbred DBA line retained its original acinous structure during five generations of transfer into DBA mice of this line. The histological pattern of the sixth-generation tumor was that of a mammary adenocarcinoma type B, whereas that of the seventh generation represented a mixed type of tumor, with whorls and bundles of spindle cells and occasional acinar forms. All subsequent generations of this tumor consisted entirely of large spindle-shaped cells with vigorous mitotic activity. The tumor in its present state has proved very useful for experimental purposes.

ACKNOWLEDGMENTS

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REFERENCES

2. DWOROWSKI, L. Sarcomatous Transformation in One Tumor of Each High Cancer Strain Mice B III; A; CS.


**FIG. 1.— Primary tumor. Low magnification to show general structure. Note the uniformly distributed acini and the delicate stroma. Adenocarcinoma type A. H & E. X100.**

**FIG. 2.—Second generation. Note acinar forms in which the cells having round nuclei dominate. H & E. X300.**

**FIG. 3.—Seventh generation. Note change in histological structure. The first appearance of large spindle-like cells, with deeply stained elongated nuclei. Few nests of cells which appear epithelial with round nuclei. H & E. X300.**

**FIG. 4.—Sixtieth generation. The growth consists of large spindle-shaped cells. Note the elongated nuclei having pointed edges, the deeply stained prominent nucleoli, the three mitotic figures. H & E. X350.**

**FIG. 5.—Eighth generation. Stained with Foot’s silver method. Note the reticulin fibers surrounding single cells and groups of cells.**

**FIG. 6.—Microscopic view of the 96th-generation daughter tumor 168 hours after irradiation in situ with 2,500 roentgens. Note the multinucleated cells, cells with large eccentrically located nuclei, cells with variously shaped nuclei, the deeply stained eosinophilic cytoplasm. H & E. X400.**
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