Sarcomatous Transformation of the Stroma of Mammary Carcinomas That Stimulated Fibroblastic Growth in Vitro*

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INTRODUCTION

Sarcomatous transformation of the stroma of transplantable mouse carcinomas was first described by Ehrlich and Apolant (5). The following year a case was reported by Loeb (13). Subsequently the phenomenon was observed to occur twice in a strain of mammary carcinoma maintained in these laboratories, and a preliminary note on the subject was published by Bashford, Murray, and Haaland (4). Later a detailed histological investigation was carried out by Haaland, whose paper (8) effectively dealt with criticisms that had been raised as to the reality of sarcomatous transformations. "All evidence," he wrote, "seems to speak for a gradual process by which apparently normal connective tissue cells evolve into sarcomatous elements, endowed with altered biological qualities." Since this was written other cases have been reported from time to time, but sarcomatous change has been regarded in the past as a "rare occurrence." Thus in a general review of the tumors studied in these laboratories up to 1911, Bashford (2) commented, "the carcinomata which induce sarcomatous transformation of their stroma are of rare occurrence." This conclusion was based on observations upon more than 650 primary tumors and 85 strains of propagated tumors of various histological types. Sarcomatous transformation was observed in only four strains. "In two the change supervened during transplantation. In the other two the change took place in the primarily affected animal."

Of the various suggestions advanced as to the cause of sarcomatous change it is of interest to recall that Apolant and Ehrlich (1) attributed it to "a stimulating influence proceeding from the carcinomatous cells, which in a certain phase of their development determines the sarcomatous transformation of the connective tissue scaffolding of the tumor." Now, 38 years after this was written, we have been able to adduce evidence that mammary carcinomas that had "a stimulating influence" upon fibroblasts in tissue cultures have undergone sarcomatous change. In our previous paper (15) we demonstrated stimulation of fibroblastic growth in vitro by carcinomas of high mammary cancer strain mice. The present communication is concerned with the sarcomatous changes occurring in those tumors that were maintained by transplantation. Contrary to the experience of the earlier investigators, who worked with hybrid mice of unknown genetic constitution, our experiments indicate that sarcomatous change is a common occurrence in mice of high mammary cancer strains.

It should be pointed out that the evidence for the occurrence of sarcomatous transformation has not been universally accepted as conclusive, based as it is on the histological interpretation of transitional stages. An alternative explanation negates the idea of neoplastic changes in the stroma, and attributes what appear to be sarcoma cells to morphologically altered epithelial cells (spindle cell metaplasia). "Such transformations are relatively common in the early or advanced stages of many human tumors, and especially in recurrences after operation, and this fact establishes a probability that a similar change in mouse tumors has a similar significance." So wrote Ewing (6), who concluded: "Since such an interpretation is at least admissible, it may be urged that further evidence is required before the sarcomatous transformation of mouse carcinoma can be accepted as proved."

Our purpose in this paper is to furnish further evi-
The irregular graph is the result of partial digestion of the plasma medium. When the plasma is liquefied these mammary carcinoma cells will usually spread out on the surface of the coverglass in the liquefied areas. The very different type of growth of a tumor of the sixth generation approximately 6 months after the first transplantation is illustrated in Fig. 2. The culture medium was the same in both cases, but Fig. 2 represents a 5 day old culture. It is composed of spindle-shaped cells becoming progressively more separated as they migrate peripherally. Another very significant difference between the 2 cultures is the large number of cells of the monocyte-macrophage type seen in Fig. 2. It is, in fact, a typical sarcoma culture, and sections of the tumor from which this culture was prepared show it to be a spindle cell sarcoma.

Part of another 3 day old culture prepared from an eighth generation transplant of a different mammary carcinoma of a Strong A mouse is represented in Fig. 3. Here again is seen the typical sheet-like growth characteristic of carcinomas in vitro. The irregular form of the growth, with small isolated islands of cells, is the result of explanting numerous small fragments of tumor, some of which coalesced as they grew. This culture again contains very few fibroblasts or cells of the monocyte-macrophage type. It will be observed that after 8 transplantations, the eighth being approximately 7 months after the primary tumor was first transplanted, this tumor was still growing in vitro as a typical carcinoma.

A sarcomatous growth from a tumor of the 19th generation transplant is shown in Fig. 4. This is comprised of numerous separate large spindle-shaped to polymorphous cells, again with very considerable numbers of cells of the monocyte-macrophage type. These extend out into the medium far beyond the outermost of the sarcoma cells.

This particular tumor was unique amongst our 6 cases in that large atypical cells were first observed in cultures from a tumor of the 11th generation, approximately 9 months after the first transplantation. Since these cells exhibited the same cytological characters as have persisted throughout subsequent generations and now comprise the greater part of the tumor, we consider their appearance as indicative of the sarcomatous transformation. But they did not rapidly overgrow and replace the carcinoma cells in vitro, which is what occurred with the other 5 tumors. Instead, they grew along with the carcinoma cells, constituting a carcinosarcoma. When small fragments of a tumor were explanted, 3 types of cultures resulted; (a) sheets of carcinoma cells, (b) growths of large spindle cells with innumerable monocytes and macrophages, and (c) mixtures of these two. Sometimes there was a sheet growth on one side of a culture and spindle cell
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Fig. 1.—Four day old culture of a mammary carcinoma of a Strong A mouse (primary tumor).

Fig. 2.—Five day old culture of the sarcoma that originated as a result of sarcomatous transformation of the stroma during transplantation of the mammary carcinoma shown in Fig. 1, at the same magnification.
FIG. 3.—Three day old culture of another mammary carcinoma of a Strong A mouse (eighth generation transplant).

FIG. 4.—Three day old culture of the sarcoma resulting from sarcomatous transformation of the stroma of the mammary carcinoma illustrated in Fig. 3, at the same magnification.
growth on the other side. Most frequently there was a considerable outgrowth of sarcoma cells and mono-
cytes and macrophages, and a residual compact growth of carcinoma cells within the explant. In the culture
illustrated in Fig. 4 there are considerable numbers of carcinoma cells in the explant. Though there are
well defined cytological differences between the carci-
noma cells and the sarcoma cells, in addition to their
shape and mode of growth, it is not intended to enter
here into a discussion of the detailed cytology of these
tumors; this will be reported in a later paper.

Sections of this tumor during its carcinosarcomatous
phase exhibit large compact groups of carcinoma cells,
in an extensive stroma consisting of hyperchromatic
cells varying notably in size, with a considerable ad-
mixture of cells of the monocyte-macrophage type.
Sections of the 20th generation still show areas of
carcinoma and sarcoma, but suggest that the carci-
nomatous element will eventually be eliminated.

With the other 5 tumors the sarcomatous change
was completed very rapidly; as far as we are able to
determine, within a single generation, though our
methods for distinguishing between fibroblasts, pre-
sarcomatous fibroblasts, and sarcoma cells lack the
precision necessary for an unequivocal decision. Cul-
tures prepared from tumors, the sections of which
exhibited the presarcomatous changes of a more abun-
dant and cellular stroma, were characterized by a
considerable growth of fibroblasts with macrophages,
usually extending outwards beyond the sheet growth
of carcinoma cells. The fibroblasts that first appeared
differed considerably from the sarcoma cells that grew
ultimately. Although it is not always possible to dis-
inguish with certainty whether any one cell is an
abnormal fibroblast or a definite sarcoma cell, yet, as
Lewis (12) has pointed out, sarcoma cells in general
are characterized by an "increase in size of cell and
nucleus, increase in density of cytoplasm, increase in
number and decrease in size of the mitochondria,
increase in the amount of nucleolar material, increase
in thickness of the nuclear membrane and the granu-
lar condition of the nucleoplasm."

Were our evidence for sarcomatous transformation of
these mammary carcinomas limited to differences in
cellular morphology and in their growth patterns in vitro it would amount to little more than an amplifi-
cation of the histopathological data. But we are able
to add to it the demonstration of a difference in the
biological properties of the cells that exhibit the dif-
ferent morphological features. In a previous paper
(15), we adduced evidence that cultures of mam-
mary carcinomas from high cancer strain mice stimu-
late the growth of fibroblasts, while sarcomas induced
by carcinogenic hydrocarbons inhibit fibroblastic
growth. We have employed the same technic to deter-
mine the influence on fibroblastic growth of these
tumors when they are growing as typical carcinomas
(Figs. 1 and 3) and as sarcomas (Figs. 2 and 4).

Mouse fibroblasts were grown between two explants
of carcinoma and later, when the tumor exhibited the
morphological indications of complete sarcomatous
change, between two explants of this tumor. Growth
of fibroblasts in the presence of the 2 tumors was com-
pared with the growth of fibroblasts in the presence
of other cultures of the same fibroblasts. The technic
of cultivation and the method of measuring fibroblas-
tic growth was the same as previously described. The
carcinomas exhibited fibroblastic growth stimulation
of the same order as described in our former paper.
The tumors derived from their stromas, which were
diagnosed histologically as sarcomas, inhibited fibro-
blastic growth to varying degrees, as had the sarcomas
induced by carcinogenic hydrocarbons, with which our
previous experiments were conducted. Figs. 5 and 6
demonstrate the difference in the growth of fibroblasts
in the presence of a transplanted RIII carcinoma
(Fig. 5) and of the spindle cell sarcoma to which it
gave origin (Fig. 6). Both were photographed at the
same magnification. Fig. 5 represents the best growth
of fibroblasts obtained in this particular experiment
after 7 days. Since fibroblastic growth in untreated
cultures invariably ceases sooner in the presence of
sarcoma than in the presence of carcinoma, the culture
shown in Fig. 6 of the series, was fixed after 5 days,
as it had begun to exhibit early indications of cellular
degeneration. The 2 cultures are thus not strictly
comparable, but in spite of the discrepancy in age of
these 2 fibroblast cultures it is obvious that they have
been subjected to different types of action, which have
been determined by the different biological proper-
ties of the cells responsible.

DISCUSSION

As the foregoing observations indicate, tumors that
from histological evidence are considered to have origi-
nated from fibroblasts of the stroma of mammary
carcinomas are indeed true sarcomas. Their sarcoma-
tous nature is indicated in cultures by their growth
pattern and general cellular morphology, which re-
sembles that of fibroblasts rather than of epithelial
cells; by their high content of cells of the monocyte-
macrophage type; and by their property of inhibiting
the growth of fibroblasts. That the first 6 tumors with
which we commenced this work should all have under-
gone sarcomatous transformation, apart from others
now showing presarcomatous changes, implies that
such transformations are a common occurrence in high
cancer strains, at least in the Strong A and RIII
strains. The selection of these tumors for transplanta-
tion in the first instance was purely arbitrary. They
Fig. 5.—Culture of embryonic mouse fibroblasts grown between two explants of a mammary carcinoma from an RIII mouse (third generation transplant). Seven day old culture.

Fig. 6.—Culture of embryonic mouse fibroblasts grown between 2 explants of the sarcoma that originated by sarcomatous transformation of the stroma of the RII tumor that stimulated the growth of fibroblasts illustrated in Fig. 5. Five day old culture, same magnification as Fig. 5.
were taken at random from our stocks of inbred mice as being of sufficient size to yield abundant tissue for transplantation into a large number of mice. The method of transplantation varied from time to time. When large numbers of mice were to be inoculated, tumors were minced to a pulp with scissors and injected with a syringe, but when transplantation was limited to a few mice, fragments of tissue, about 2 mm. in diameter, were inoculated with a trochar. We have no evidence that either method of transplantation specifically influenced the induction of the sarcomatous change.

In seeking an explanation for the frequency of the sarcomatous change certain factors demand special consideration. Particularly significant is the fact that all our mammary carcinomas that underwent this change had been found to be powerful stimulants of fibroblastic growth in vitro, as previously reported. This can mean only that something was liberated by the growing carcinoma cells that excited the fibroblasts to increased growth. We have no definite proof that a similar stimulation was operative in vivo, but its occurrence was indicated by the more abundant and cellular character of the stroma that preceded the sarcomatous changes. As was pointed out in our previous paper the carcinomas that stimulate fibroblastic growth most conspicuously are the mammary carcinomas of high cancer strain mice, and these are the tumors that have exhibited sarcomatous transformation of the stroma. In their etiology the "mammary tumor inciter" of Bittner is of fundamental importance, but we have as yet no evidence whether or not it is concerned in the sarcomatous transformation. Experiments directed towards elucidation of this aspect of the problem are still in progress.

That the special genetic constitution of the high mammary cancer strains may be an important factor seems highly probable, but here again our evidence is as yet equivocal and will be left for discussion in a later communication. Attention should be directed, however, to the difference between our transplantation experiments with inbred strains of mice and the work of the earlier investigators, who used mice of mixed genetic constitution. Thirty-nine years ago Bashford, Murray, and Cramer (3) published the first account of the "source of the constituent elements of new growths obtained by artificial propagation." They confirmed the earlier observations of Jensen (9), that the new tumor parenchyma is derived solely from that introduced, and demonstrated that the "stroma and vascular structures are merely a reaction on the part of the successive hosts, whereby the parenchyma is nourished and supported by an artificial circulation renewed from time to time." They described the stroma of carcinoma grafts as beginning to degenerate 24 hours after inoculation, as being "extremely degenerated in all its elements" after 3 days, and as reaching "the last stage of degeneration" after 4 days. In his study of sarcomatous transformation Haaland (8) was led to consider the possibility of fibroblasts of the stroma surviving transplantation, and their "survival after repeated subtransplantation into successive hosts" was suggested as a contributing factor to the induction of malignancy. He contributed corroborative evidence about which he wrote: "In examining early stages of tumours in this presarcomatous stage with abundant and cellular stroma, we found in single cases strong evidence of connective-tissue elements being transplantable, before any sarcomatous change shows itself histologically. We have seen the difficulties in the way of deciding when this transplantability of individual stroma elements has appeared for the first time, and the possibility remains that the transplantation of individual stroma elements may go further back than can be proved by our methods." It might be expected that when a tumor is transplanted into mice of the same genetic constitution, the likelihood of stroma elements surviving would be much enhanced, since the cells of the transplanted tumor stroma are then homozygous with those of the new hosts. We have investigated this possibility and, without entering into the details of our findings here, it is pertinent to point out that our evidence indicates that stromal cells from even the first generation transplant of a mammary carcinoma appear to survive when transplanted into a new host. Stromal cells at the periphery of such a graft do not undergo the early degenerative changes that Bashford, Murray, and Cramer described, but it is not possible to be absolutely certain that they survive to proliferate indefinitely, owing to the difficulty of distinguishing between these cells and others that invade the graft in bringing about the new vascularization.

Of the various factors, then, that might be responsible for the frequency of sarcomatous transformation during the transplantation of mammary carcinomas of high cancer strain mice, our present evidence emphasizes particularly the significance of the stimulation of fibroblastic growth by the carcinoma cells, and the greater survival of stromal cells when transplanted because they are homozygous with the cells of their new host.

SUMMARY
1. Sarcomatous transformation of the stroma is a common occurrence during the transplantation of mammary carcinomas of high cancer strain mice.
2. The histological evidence of sarcomatous change is confirmed by study of the growth characteristics of tumors in vitro before and after they have undergone transformation.
3. In tissue cultures mammary carcinomas exhibit the typical epithelial growth pattern, with few cells of the monocyte-macrophage type, and stimulate fibroblastic growth (Figs. 1, 3, and 5).

4. The sarcomatous nature of the transformed tumors is indicated by their growth pattern and general cellular morphology, resembling fibroblasts; by their high content of cells of the monocyte-macrophage type; and by their inhibiting fibroblastic growth (Figs. 2, 4, and 6).

5. Of the factors responsible for the frequency of sarcomatous change in the high mammary cancer strains, special significance is attributed to: (a) the considerable stimulation of fibroblastic growth by the carcinoma cells; and (b) stromal cells surviving transplantation because the cells of the graft are homozygous with those of the new host.

REFERENCES


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