Differential Invasion of Embryonic Chick Tissues by Mouse Sarcomas 180 and 37

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INTRODUCTION

The method of tumor transplantation to the extra-embryonic membranes of the chick embryo was inaugurated by Murphy and Rous (8, 9), who successfully transplanted the Rous chicken sarcoma to the chorioallantoic membrane. Shortly thereafter, Murphy (6, 7) made the first transplantation of mammalian tumor (Jensen rat sarcoma) to the extra-embryonic membranes of the chick. Since that time, the technic has been elaborated, and it is now widely used for the propagation and maintenance of tumors (11).

The compatibility of mouse tumors and chick embryos suggested to Bueker (2) the possibility of the implantation of mouse sarcomas into the embryo proper, in connection with certain neuroembryological problems. Bueker implanted small pieces of mouse Sarcoma 180 into the flank of 3-day embryos and found that the implanted tumor grew rapidly. It was invaded by sensory nerves of the chick, resulting in hyperplasia of the corresponding ganglia. The experiment was continued in this laboratory by Levi-Montalcini and Hamburger (5), who confirmed and extended Bueker’s findings. These authors also implanted mouse carcinomas dbrB and C3HBA and found that neither would grow intra-embryonically, although Coman (3) has successfully cultured mouse carcinomas on the extra-embryonic membranes of the chick.

Levi-Montalcini and Hamburger reported some observations on the growth of the sarcomas and their infiltration of tissues of the host embryo. A distinct affinity of the neoplastic tissue for certain organs, particularly the mesonephros, was evident; other structures, such as the metanephros, skeletal elements, and nerve tissues were not invaded, although they were equally accessible to the tumor. This phenomenon of selective affinity and destruction seemed to deserve a more detailed investigation. In this connection, the study of the growth pattern of the tumor became a point of interest. The present investigation is concerned with the period from tumor implantation, at 2½ days of incubation, to the ninth day, by which time the tumor has become well established at its permanent site.

MATERIALS AND METHODS

The original stock of mouse Sarcomas 180 and 37 (hereafter referred to as S180 and S37) was obtained from the Jackson Memorial Laboratory at Bar Harbor, Maine; it was carried in closely inbred white mice. It was maintained in our laboratory in a colony of white mice of unknown genetic background.

Much of the material used in the present study was loaned by Drs. Levi-Montalcini and Hamburger; this was supplemented by a number of similar experiments by the author.

The operative technic was described by Hamburger (4) and Levi-Montalcini and Hamburger (5). Tumors which had grown from 4 to 14 days in the mouse and were readily palpable were removed to sterile saline. Pieces of about 1 c. mm. were selected from a healthy, non-necrotic portion of the tumor. The transplant was placed in a small slit in the right body wall, lateral to the somites and at the base of the wing- or hind-limb bud (Chart 1). It was exposed at the surface and extended into the body cavity in most cases, being held in place by the tension of the walls of the wound. Implantation was always made at 2½ days; the age of the cases as recorded in these experiments refers to age in days of incubation of the chick host.

Embryos were fixed at regular intervals from 12 hours to 7 days after operation. They were fixed with Bouin’s fixative, stained in hema- toxylin (B. Wenger’s modification of the Heidenhain technic [12]), and sectioned at 10 μ. Some were counterstained lightly with Eosin B.

RESULTS

Some General Characteristics of the Tumor

The two sarcomas used in this study appeared to be histologically identical. During the period under consideration (i.e., through the ninth day of...
incubation), they invaded the same organs in essentially the same way. Some differences in degree of invasiveness, which may show a trend toward greater activity by SS7, will be discussed later. Except where otherwise noted, the following descriptions refer equally to both sarcomas, representative cases being selected without prejudice.

The tumor mass had characteristic properties which made its identification certain in all instances. The tumor cells were larger than the host cells. The tumor nuclei, and to a lesser extent the cytoplasm, stained more deeply in hematoxylin than did most normal chick tissues. A higher percentage of mitotic figures was seen in the tumor tissue than in most tissues of the chick embryo, and many of them were atypical. The tumor mass often included necrotic areas and nests or strands of host cells. Ingrowth of nerves into the tumor, which began at 6\textfrac{1}{2} days, was the subject of a detailed investigation by Levi-Montalcini and Hamburger (5). Blood vessels within the tumor were distinctly recognizable by 5\textfrac{1}{2} days.

It was not always easy to identify individual neoplastic cells which had migrated out from the main tumor mass. The large size of the tumor cells, their previously mentioned differential staining capacity, and large, granular nuclei, which were often polymorphic, were sometimes found to be useful characteristics for their identification.

**GROWTH PATTERN OF THE TUMOR**

For a few days after implantation in the chick embryo, the tumor tissue underwent considerable necrosis. During and following this necrotic period, surviving tumor cells divided rapidly, infiltrated host tissues, and established centers of growth in certain nearby organs. It is convenient to divide the period under consideration into a regressive phase (3-5\textfrac{1}{2} days), characterized by the necrosis, and a later phase of active growth (from 5 days on). The two phases are not sharply separated but overlap considerably in 4- to 6-day cases. It should be emphasized that active growth of the tumor did not cease at 9 days, but continued as long as the
chick remained alive, i.e., until near the end of the incubation period in some cases. The following description of the growth pattern of the tumor is derived from our study of a closely staged series of cases (Table 1).

Whereas the tumor at first extended outside the body wall, the incision wound usually closed over within the first day after operation. The tumor remained centered in the somatopleure during the regressive phase; its relatively few viable cells migrated out for short distances within the mesenchyme of the body wall.

The necrotic area always lay in the center of the tumor mass, surrounded by a fringe of healthy, dividing cells (Figs. 1, 2). It had two components: (a) a hyaline substance in which no cell boundaries could be distinguished; and (b) small basophilic granules, usually clustered in the center of the hyaline area. These had the appearance of droplets of chromatin, possibly the remnants of cell degeneration, or possibly pyknotic nuclei.

The cells at the periphery of the tumor were very irregular in shape; they often showed blunt pseudopodia or pointed cytoplasmic extensions. They were found at increasing distances from the tumor center in 3- to 5-day cases. These characteristics suggested that the marginal cells possessed a high degree of amoeboid motility and reached new sites by active migration.

The tumor mass as a whole had a loose texture in early stages. All its cells were interspersed with host cells. As the necrotic region decreased in size and finally disappeared (at 6 days in almost all cases), more densely arranged tumor cells filled its position in the center of the tumor mass. These cells lost their irregular outlines and became spherical or spindle-shaped, probably due to dense packing.

At 5 days of incubation, the expanding tumor had kept pace with the increase in the width of the body wall and with the considerable distension of the coelomic cavity of the chick (Chart 2). As a rule, the tumor was still centered in the body wall. It was farther from the epidermis than in earlier stages, although in several cases a strand of cells extended to the incision scar. The medial side of the tumor now formed a broad bulge into the coelom (Fig. 1), and occasionally locally filled the right side of the body cavity. Through this extension, contact was established with various visceral organs, especially the developing mesonephros.

In this way, a bridge was formed from the body wall across the coelom (Fig. 2). Many migratory tumor cells reached the mesonephros in this way; here they infiltrated the interstitial spaces so that soon all mesonephric tubules, at the level of the tumor, were surrounded by tumor tissue. Other structures, like the liver and gut wall, were superficially invaded.

From approximately 6 days on, the bulk of the tumor shifted inward to the right mesonephros and coelom, where it became established; it proliferated extensively and progressively invaded adjacent host tissues (Chart 2). One had the impression that once a considerable mass of tumor cells had become lodged in the mesonephros, the tumor became stationary at this site. In most cases in which the tumor had shifted to a more medial position, a narrow strand of cells still extended across the body wall toward the point of implantation.

In older cases the tumor mass was often extremely large and very dense throughout. However, the cells at its margin remained scattered and apparently migratory.

With a single exception, all cases of S180 were smaller and somewhat less invasive than those of S37. It is also interesting to note that necrosis ended sooner in the latter series (see Table 2, column 9). However, it should be noted that in the period covered by this investigation (i.e., through 9 days of incubation) these differences were not great, and showed, at most, only a trend. Our data showed no significant differences in the number and type of structures invaded by the two tumors, the difference indicated being one of degree of invasiveness only. Levi-Montalcini and Hamburger (5) reported that in older cases (beyond 9 days) S37 is much more invasive than is S180, especially with respect to musculature and connective tissue. This behavior of S37 does not become apparent until later stages, beyond the period covered by this investigation.

**DIFFERENTIAL INVASION**

A systematic survey was made of all structures within the range of the growing tumor, using a series of arbitrary estimates of the degree of invasion or refractoriness (see legend of Table 2). It will be noted that the degree of invasion of the mesonephros, as well as the total number of structures invaded, increased steadily during the phase of ac-

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**TABLE 1**

**SUMMARY OF CASES**

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<th>Age (days of incubation)</th>
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1 Levi-Montalcini, personal communication.
CHART 2.—Various stages in the growth pattern of the tumor in the chick embryo. The tumor area is represented by diagonal hatching. Abbreviations: A, dorsal aorta; C, coelom; G, gut; L, liver; M, mesonephros; N, notochord; S, tumor; T, meta-

nephros; an arrow indicates the position of the Müllerian duct.
# TABLE 2

## DEGREE OF INVASION OF HOST STRUCTURES BY THE TUMOR

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<th>Mucous gland</th>
<th>Yolk sac</th>
<th>Adrenal gland</th>
<th>Liver</th>
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+ to ++++:+ invasion by a few tumor cells to extensive invasion and destruction.
0 to 0000: a few tumor cells in the region of, or surrounding the structure, structure completely imbedded in the tumor mass but not invaded or destroyed by it.
Each rating was made at the level of maximum invasion and destruction, or maximum refractoriness, respectively.
* Denotes transplants into wing level.
of invasion, the metanephros begins its excretory function, and the mesonephros is degenerating (10). Some of our cases showed severe destruction (Fig. 6). On the other hand, in a few cases the kidney was capable of taking on any extra excretory activity required.

When invasion began at about 5 days, tumor cells first migrated into the interstitial spaces, until only the tubules and a few glomeruli remained imbedded in the dense tumor mass (Fig. 5). Subsequently, the tubules disappeared, and in later stages some sections showed no remnants of the right mesonephros (Fig. 6). It is interesting to observe that the Wolffian duct often remained intact even after most of the tubules had been destroyed (Fig. 6). On the other hand, in a few cases the first invading cells entered the mesonephros near the Wolffian duct, and the latter was occluded early. In case S180-33, as a result of complete occlusion of the duct by the tumor, the Wolffian duct was greatly distended anteriorly and exceeded in size the entire left (control) mesonephros. When invasion of that structure was evident, tissuepenetrating these structures. In older embryos with large tumors, the tumor cells sometimes penetrated and destroyed these structures locally.

As is shown in Table 2, the mesonephros seemed to be equally invaded, whether the tumor was implanted in the hind-limb or wing-level. At 6 days, invasion of that structure was evident, tissue which was to form mesonephros extended from the wing- to hind-limb level and was easily accessible to tumors implanted at either site. However, some structures in the following group (e.g., liver, lung) were more accessible to wing-level implants and were invaded in most cases only by tumors implanted at the more anterior site.

**Other structures adjacent to the tumor.**—The tumor in its expansion encountered many other structures which it might have invaded. Apparently, none of these were as favorable for tumor growth as was the mesonephros. One group of organs (liver, lung, gut wall, and coelomic walls) became superficially invaded, but the tumor did not establish itself in them. A second group (gonad, adrenal, Müllerian duct, muscle) were temporarily refractory, but they were often eventually invaded and destroyed. A third group of structures (nerves, ganglia, blood vessel walls, cartilage, metanephros) were often adjacent to the tumor or imbedded in its mass, but seemed to be highly refractory; they were almost never invaded by tumor cells in the period up to 10 days. We shall use this tentative grouping in the following description.

**First group.**—Between 5 and 6 days the liver was close to the expanding tumor and exposed to invasion. Of 40 embryos of 5 days or older, 13 showed superficial liver invasion (Fig. 4). Since the liver was more accessible to tumors implanted at the wing level, 10 of the 13 cases were from wing-level implants. It is significant that older cases showed no deeper penetration of the liver by the tumor than did the youngest. Although the tumor probably destroyed and replaced a small amount of liver tissue, histological evidence of this process was not seen.

The edge of the splanchnopleure of the gut wall was similarly invaded superficially in a few cases. Again, there was no progressive increase in the amount of neoplastic tissue at this site. Altogether, only four cases displayed a slight superficial invasion of the lung. Again, neither expansion nor progressive tumor growth followed the initial invasion. Only few tumor cells were found in the loose mesenchymous tissue between bronchiole buds.

The body wall was placed in this group, because it never became a major center for tumor growth. However, in all cases a number of tumor cells remained in the body wall, which was the site of original implantation, and occasionally tumor cells crept along the somatopleure (Fig. 4).

**Second group.**—Gonad, adrenal, Wolffian duct, Müllerian duct, and muscle were invaded slowly by the tumor. Marginal tumor cells sometimes migrated between dense cords of gonad or adrenal cortical cells, or around the wall of the Wolffian duct or Müllerian duct (Fig. 5), without at first penetrating these structures. In older embryos with large tumors, the tumor cells sometimes penetrated and destroyed these structures locally.
The Müllerian duct retained temporary refractoriness longer than the other structures. In some cases it remained the only intact structure within the dense center of the tumor after the right mesonephros had been entirely destroyed (Fig. 5) locally. However, in eleven advanced cases even the Müllerian duct had been destroyed at the level of the tumor.

The tumor never became well established in any of the structures in either of the above groups. Rapid proliferation evidently did not follow as it did when cells infiltrated the mesonephros.

The secondary centers of tumor growth to which we refer above did not conform strictly to the commonly adopted definition of metastases; they did not result from the transport of cells to distant sites, but were always contiguous with the primary tumor. In older cases of this series (beyond 9 days), true metastases have been observed by Levi-Montalcini.2

Third group.—These structures were characterized by complete refractoriness to the tumor. It has already been noted that nerves, ganglia, cartilage, blood vessels, and metanephros often became surrounded by, or imbedded in, the expanding tumor mass (Figs. 4–6). These structures were almost always exposed to the tumor but remained entirely free from invasion up to the tenth day.

DISCUSSION

Mechanics of growth and expansion.—The initial necrosis which invariably followed implantation undoubtedly involved some degenerating mouse tissues included with the implant. Since it occurred in the center of the tumor rather than at the fringes, it probably did not represent a reaction between tumor tissue and the chick host. It is suggested that the bulk of the necrotic substance involved originally healthy tumor tissue which became necrotic before the tumor mass received adequate vascularization and before it shifted to a more favorable location than was afforded by the body wall.

Whereas the tumor mass was at first centered in the body wall where it was implanted, it was later found to be centered in the right mesonephros and coelom. Several possibilities suggest themselves as to the mechanism of this shifting, such as a gradual inward shift of the bulk of the tumor and an infiltration by migratory cells. After the closure of the incision wound, the median surface of the expanding tumor mass bulged into the coelom (Fig. 1), where it encountered little or no resistance. The inward shift of the lateral surface of the tumor, away from the body wall (Chart 2, Fig. 1), may have been merely passive—i.e., it may have been due to rapid increase in thickness of the host somatopleure, lateral to the tumor.

A process involving infiltration of viscera by migratory cells was suggested by the loose texture of the tumor and the amoeboid shape of many of its cells. Often isolated groups and strands of tumor cells were observed at considerable distance from the center of the tumor mass. It is likely that, when such strands of cells reached the mesonephros, they established there a vigorous local center of proliferation, which rapidly outgrew the original center. Usually a narrow cone of cells extending toward the body wall indicated the original site of the tumor (Chart 2).

Infiltration by migratory cells can easily account for all sites of tumor invasion in our material. However, it is possible that still another mechanism of spreading occurred, especially in older cases. Isolated clusters of cells were found floating freely in the body cavity whenever the tumor mass had penetrated through the somatopleure (Fig. 8). Some of these cells may have been transported in the coelomic fluid to nearby organs, where they became lodged. On the other hand, we never observed tumor cells inside of blood vessels, and this mechanism of transport seems to be ruled out for young embryos. However, it should be remembered that our observations extended only through the ninth day of incubation.

Our observations are in accord with the evidence of Coman (3) that tumor cells are less cohesive and show a higher degree of motility than their normal counterparts. Material like that used in our investigations would be favorable for a more detailed study, in vivo, of these properties, since closely timed series can be obtained, and intraembryonic tumor transplants in the chick would be readily accessible to experimentation.

Selective destruction of host structures.—The problem of selective invasion by the tumor, resulting in the preferential destruction of some host tissues, while others seem to be refractory, is sharply pointed up in this study. The fact stands out that the mesonephros alone became the center of tumor growth, whereas other organs, although they may also have been invaded, did not support further expansive growth of the tumor equally well.

The following explanations of this situation suggest themselves: (a) the early onset of function of the mesonephros may have increased its susceptibility to invasion; (b) the tumor may have established itself in the mesonephros because of its high degree of vascularization; (c) density or mechanical resistance may have "protected" other organs; (d) the topographic position of the mesonephros, in
All photomicrographs are of cases stained with hematoxylin.

Abbreviations: A, dorsal aorta; C, coelom; G, gut; L, liver; M, mesonephros; N, notochord; S, tumor; T, metanephros; an arrow indicates the position of the Müllerian duct.

Fig. 1.—S180-32 (5 days). Tumor with a large necrotic area in its center. The viable cells are scattered in the body wall (left); the tumor mass bulges into the coelom (right). ×65.

Fig. 2.—S180-59 (5 days). Tumor with necrotic area. A bridge spans the coelom, from body wall (left) to mesonephros (M), and a few tumor cells have invaded the interstitial spaces of the mesonephros. The adjacent liver (L) is not invaded. ×80.

Fig. 3.—S180-71 (5 days). A large tumor lies in the ventrolateral body wall. At its inner margin cells have become detached, and float freely in the coelom (C). The gut is not invaded. A few tumor cells have reached the mesonephros (M). ×70.

Fig. 4.—S180-5 (7 days). Tumor cells have infiltrated the outer wall of the coelom (left). Center of tumor (S) in the right mesonephros which has been almost completely destroyed at this level. Müllerian duct (arrow) and dorsal aorta (A) remain refractory, while the liver (L) is superficially invaded. ×30.

Fig. 5.—S37-3 (7 days). The right mesonephros has been entirely replaced by the tumor at this level, and a few peripheral cells have migrated to the left mesonephros (M). Dorsal aorta and Müllerian duct (arrow) are intact. Right gonad and adrenal are absent. ×25.

Fig. 6.—S180-125 (8 days). Cartilages of the pelvic girdle, nerves (upper left), and a sympathetic ganglion are partially imbedded in the tumor, but not invaded. The Wolffian duct (just left of “S” label) is imbedded in the tumor but remains intact. ×25.
the neighborhood of the advancing tumor, may have increased its chance of becoming a target for attack. Yet the mesonephros, invariably the most severely invaded, holds each of these properties in common with other structures, which were equally accessible to the tumor but remained partly or completely refractory. Whatever biochemical constituents of the mesonephros may have combined with the mechanical factors suggested above to favor it as a site of tumor establishment are yet to be demonstrated.

SUMMARY
Small portions of mouse Sarcomas 37 and 180 were implanted in the somatopleure of 2½-day chick embryos. The growth pattern of the tumor in the host is described. Two phases were distinguished: an initial regressive phase, which is followed by a phase of active growth. During the latter period the tumor was highly invasive of various chick tissues. The mesonephros assumed a unique position in that it was preferentially attacked and partially destroyed; it became invariably the growth center of the tumor. The metanephros, blood vessel walls, cartilage, nerves, and ganglia were completely refractory. Some other structures fell into an intermediate category, being only temporarily refractory or slightly invaded. The reasons for this selectiveness by the tumor are not yet evident, although a number of factors which may have contributed to the susceptibility of the mesonephros to tumor invasion are discussed.

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Differential Invasion of Embryonic Chick Tissues by Mouse Sarcomas 180 and 37

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