In Vitro Experiments on the Effects of Mouse Sarcomas 180 and 37 on the Spinal and Sympathetic Ganglia of the Chick Embryo

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INTRODUCTION

Previous experiments have given evidence that mouse Sarcomas 37 and 180 produce an agent which promotes the growth of spinal ganglia (1) and of sympathetic ganglia (12) in the chick embryo. This effect was first observed in experiments in which small pieces of tumor were implanted in the body wall of 3-day embryos, where they grew vigorously. Later, the same effects were obtained when the tumors were transplanted extra-embryonically to the allantoic membrane of 4-day embryos (10, 11, 13). The latter result was considered as conclusive evidence that we are dealing with a diffusible agent.

The response of the nervous system was found to be selective and complex. Only the sensory and para- and prevertebral sympathetic ganglia showed a reaction, whereas all centers in the spinal cord were refractory. The hyperplasia of the ganglia was due partly to an increase in cell number and partly to a cellular hypertrophy. The differentiation of nerve fibers was accelerated, and their number was increased to an extraordinary degree. The supernumerary fibers emerging from the hyperplastic ganglia flooded the adjacent viscer: meso- and metanephros, gonads, spleen, adrenal, thyroid, parathyroid glands, and also the tumor, in cases of intra-embryonic transplantations. Normally, these organs receive only a very scant nerve supply or none at all, in corresponding stages of development.

These results confronted us with two major problems: (a) the chemical nature of the agent and (b) its mode of action. Concerning the latter problem, the previous experiments gave no definite clue whether the agent acts directly on the ganglia or indirectly, by producing complex metabolic changes in the embryo. In this connection, it should be mentioned that the embryos carrying a tumor showed signs of toxic effects (edema, perfusion of the liver by bile, stunted growth) which were eventually fatal to the embryo.

It seemed that the tissue culture method might offer a new approach to the analysis of both problems. This method has several advantages: it permits the direct exposure of the ganglia to the tumor, thus excluding possible influences of the organism; furthermore, extracts of tumors can be easily tested, and other tumors and normal tissues can be screened for possible nerve growth-stimulating effects. Since the behavior of the spinal ganglia of the chick embryo in vitro, under normal and experimental conditions, has been studied in great detail (6, 8, 9, 17, 18), one can build on a solid foundation.

MATERIALS AND METHODS

The experiments consisted of the combination of spinal ganglia with fragments of mouse Sarcomas 180, 37, 1, adenocarcinoma dbrB and neuroblastoma C1800. Fragments of embryonic chicken or mouse heart were used as controls. In a limited number of experiments, paravertebral sympathetic ganglia or pieces of spinal cord were exposed to these tumors and to control tissues. Usually, several fragments of the same tumor or of control tissue were placed at a distance of 1-5 mm. from the ganglion; this has been found to be the optimal distance. In no instance were the explants placed in direct contact with each other, but usually the spreading of the tumor resulted in a contact at about 48 hours. In one series, the distance between the ganglion and the tumors was varied, in order to study the range of diffusibility of the tumor agent.

Table 1 gives a summary of all data. It should be pointed out that the number of experiments performed was considerably larger, since, in most instances, several groups of explants were placed

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in the same hanging drop, at a considerable distance from each other.

The spinal ganglia were in most instances lumbo-sacral ganglia of 6- to 7-day embryos. In a few instances, ganglia from 9- and 10-day embryos were used. They were dissected in physiological salt solution under a binocular microscope and explanted in toto, that is, surrounded by their capsule. The isolation of sympathetic ganglia is much more laborious than that of spinal ganglia; hence only a limited number of experiments of this type was done. The sympathetic ganglia were obtained from older embryos ranging from 8 to 15 days, since the isolation of younger ganglia is not practicable. Long segments of the paravertebral chain were dissected out and cut into fragments, including one ganglion. Spinal cord fragments were obtained from 6-day embryos. The lumbo-sacral level was isolated and cut into small square fragments corresponding approximately to one segment. Each fragment was split apart in the median plane, and the lateral halves were explanted separately.

All tumors were obtained from the Jackson Memorial Laboratory at Bar Harbor. In the earlier series, the tumor explants were taken directly from the mouse, but they had a strong inhibitory effect on the ganglia. These unsatisfactory results suggested that an adaptation to the chick embryo might be necessary. For this purpose, the tumors were implanted in the body wall near the hind-limb bud of 2- to 3-day embryos (for technic see [12]) and allowed to grow there for 4-8 days. They were then used for explantation or transferred to other chick embryos for one or more additional passages. Tumors with at least one passage in the chick embryo proved to be optimally effective. Only the "healthy" peripheral parts of the tumors were selected; the central necrotic and hemorrhagic parts were discarded. The pieces were approximately 1 c. mm. in size.

As controls, the following tissues were used: heart tissue of 7-day (or in a few instances 8- to 10-day) chick embryos, heart tissue of mouse embryos or fetuses, or of newly-born mice.

The standard culture medium was chicken plasma and chick heart organ culture. The plasma was obtained by the standard technic of bleeding a rooster through the carotid artery. In all experiments, the extract was diluted 1:3 with Earle's solution. The plasma was ordinarily used undiluted; in a few experiments the plasma was diluted 1:1 with Earle's salt solution.

The hanging-drop technic was used throughout; Maximow depression slides with two cover glasses were employed in most instances. Most experiments were discontinued after 48 hours. A limited number of cultures which were carried beyond this period were washed every second day with Earle's solution, and nutrient (serum and extract) was added. In all instances, a set of tumor experiments and a set of control experiments were done on the same day, using the same medium, the same technic, and ganglia from the same embryo.

TABLE 1

<table>
<thead>
<tr>
<th>Combination of</th>
<th>Spinal ganglion</th>
<th>Sympathetic ganglion</th>
<th>Spinal cord</th>
<th>Chick embryo</th>
<th>Chick organ</th>
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<tr>
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<td>92</td>
<td>6</td>
<td>12</td>
<td>21</td>
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<tr>
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<tr>
<td>Miscellaneous chick organs</td>
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<td>0</td>
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<tr>
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<td>108</td>
<td>24</td>
<td>3</td>
<td>7</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>688</td>
<td>108</td>
<td>49</td>
<td>51</td>
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</table>

The growth of neural tissue in vitro

Ganglia of 6- to 7-day embryos were chosen for our experiments. In these stages, the ganglia are still in a phase of active proliferation, and some of the neuroblasts have not yet sent out nerve fibers (4). In our previous experiments of in vivo transplantsations of sarcomas, the spinal ganglia had shown the first responses to the tumor agent on the 7th or 8th day of incubation; hence ganglia of 6-7 days were expected to be in the best condition for reaction in vitro.

When ganglia of the lumbo-sacral level are cultivated in vitro in the absence of other tissues, they show almost no fiber outgrowth during the first 16 hours. The migration of spindle-shaped cells begins at about 10-15 hours. These spindle cells, whose active migration has been observed by all previous workers, represent a heterogeneous population of Schwann cells, satellite cells, and mesenchyme cells of the capsule. At 24 hours, the migration of the spindle cells is well advanced, and a small number of nerve fibers has grown out (Fig. 3). The fibers are distributed irregularly and take a wavy course. Between 24 and 48 hours, the number of nerve fibers increases, but they are still rather sparse (Fig. 7). They have a tendency to fasciculate and to associate with rows and columns of spindle cells. Those fibers which do not join with others follow tortuous routes; occasionally they grow tangentially or in circular paths around the ganglion (8). In accordance with the observations of Levi and Meyer (8), we find that nerve cells do not migrate out of the ganglion, but that the spreading of its surface and the decrease of its density come about by a migration of the spindle cells. Nerve fiber and spindle cell outgrowth continues during the 3d day. After this time, a considerable number of centrally located neurons begin to degenerate. They are disposed of

1 Since there is no difference between single ganglia and ganglia combined with embryonic chick heart tissue, with respect to nerve fiber growth and spindle cell migration (see p. 83), only the latter experiments were chosen for illustrations.
by macrophages which appear in increasing numbers in older cultures. Our observations on normal ganglia are in agreement with the basic studies of Levi and Meyer (8).

**Experiments with Sarcoma 180**

Excellent growth of this tumor was obtained after it had undergone one or more passages in the chick embryo. The migration of cells gets well under way during the first 16 hours. At 24 hours, a uniform margin of typical tumor cells surrounds the explant; the cells are large in size and spindle-shaped; they usually adhere to one another, forming long strands. The growth zone appears to be a homogeneous population of sarcoma cells, not contaminated with small-sized fibroblasts of chick origin (Fig. 14, a).

Between 24 and 48 hours, the area of migrating cells is further expanded; the neoplastic cells are facing the tumor, the fibers become gradually less contaminated with small-sized fibroblasts of chick macrophages in the explant and in the growth zone. This occurs even if the cultures are washed and nutritive material is added every other day. The growth conditions could probably be improved by transfer, but in our present investigation, we were particularly interested in the maintenance of the tumor without resection and transfer.

**Combination of spinal ganglia with Sarcoma 180.**

—One or more small pieces of tumor were implanted at distances of 1–2 mm. from a lumbo-sacral spinal ganglion of a 6- to 7-day embryo. The results obtained in a large number of experiments (Table 1) were entirely consistent and uniform. The nerve fiber outgrowth was definitely precocious in the presence of the sarcoma. At 16 hours, few, if any, fibers are present in control ganglia (see above), whereas in the combination experiments with sarcoma, a rather large number of nerve fibers has grown out at that stage; they are limited to the side facing the tumor. Shortly thereafter, fibers begin to sprout from the entire surface of the ganglion. In contrast to control cultures, no spindle cells have migrated out during this period. At 24 hours, the ganglion represents a remarkable picture (Fig. 1). It is surrounded by a “halo” of nerve fibers. They show maximal density and a very straight course on the side facing the tumor. The tips of the fibers branch profusely and form a brushlike border. This phenomenon has never been observed in normal cultures. On the sides not facing the tumor, the fibers become gradually less dense and longer; the direction of their outgrowth is less straight, and some take more winding routes. However, they never wander in all directions nor do they form bundles, as is characteristic of the control cultures. In all well-growing cultures in which the tumor is rather close to the ganglion, the halo of nerve fibers has a very characteristic contour. Toward the tumor, the border of the fiber tips is sharply demarcated and often ellipsoid in outline. One gets the impression that the fibers face an invisible barrier which none of them trespasses. A close inspection shows that the borderline of the fibers is particularly sharp at the interface between cover glass and plasma clot, whereas the fiber length in deep layers of the same culture is somewhat more uneven. The line of demarcation of the fiber tips becomes less sharp with increasing distance from the tumor, the fibers become gradually longer and more wavy and, at the same time, more variable in length. The migration of the spindle cells is greatly reduced, as compared to the control cultures. They are completely blocked on the side facing the tumor, but a few do grow out on the opposite side. This feature becomes more distinct during the following period.

No significant changes were observed between 24 and 48 hours, but all features described above are accentuated. In many instances, the fibers on the side toward the tumor form a very dense, felt-like matting (Fig. 8). When the tumor is close to the ganglion, neoplastic cells reach the nerve fibers and mingle with them. Very few or no spindle cells are found on the side toward the tumor, but they migrate out in increasing numbers on the other side, where they may occasionally form bundles or ribbons combined with nerve fibers. In cultures older than 48 hours, the results were less consistent than in earlier stages, and a systematic study of this material has not yet been made. In general, we have noticed the disappearance of the sharp contour of the fibers on the side facing the tumor; instead, they show a tendency to form weblike networks superimposed on the tumor cells (Fig. 6). The migration of spindle cells is no longer blocked. A strange phenomenon was observed in older cultures which needs further study: The cellular degeneration which is characteristic of control ganglia of 3–6 days does not seem to occur in the presence of the sarcoma, even though the sarcoma itself shows regressive changes.

**Combination of Sarcoma 180 with sympathetic ganglia.**—These experiments were done in only a limited number of cases and not continued beyond the 2d day. The growth of sympathetic ganglia of 8- to 18-day chick embryos in tissue culture has been briefly described by Levi and Delorenzi (7). Control ganglia show at 24 hours a rather uniform
fibers are mingled with the spindle cells; they do not form bundles, and they grow in a wavy course. On the following days, the number of nerve fibers increases moderately.

The combination with Sarcoma 180 results in a very precocious and exuberant outgrowth of nerve fibers, closely resembling the pattern found in spinal ganglia (Fig. 14). Again, the fibers facing the tumor are shorter than those on the opposite side, and their contour is sharply delimited. The orientation of the fibers is always radial and very straight, and their density is greater toward the tumor. The fibers are much finer than the sensory fibers, and the halo is formed of a very dense, regular matting.

Combination of Sarcoma 180 with chick embryo heart.—The striking inhibitory effect of Sarcoma 180 on the spindle cells of spinal ganglia raised the question of whether fibroblasts of an entirely different origin might also be affected. Fragments of heart from chick embryos of 7-9 days were used. In most instances, no effect of the sarcoma on the growth pattern of heart fibroblasts was observed, even when the sarcoma was actively growing and close to the heart fragment. In a few instances, the migration of heart fibroblasts seemed to be somewhat impaired. The number of experiments is not sufficiently large to establish this point definitely; however, the effect is not at all comparable to that observed in ganglia.

Experiments with Sarcoma 37

Growth of Sarcoma 37 in vitro.—Consistently good results were obtained with this tumor when it had been grown in the chick embryo for one or more passages. During the first 24 hours, its growth is even more vigorous than that of Sarcoma 180. In distinction to the latter, its cells, which are spherical rather than spindle-shaped, migrate individually and have no tendency to form rows or bands. As a result, the area of expansion of the culture, which increases considerably between 24 and 48 hours, is not as compact as in cultures of Sarcoma 180. After 48 hours, the cultures show signs of deterioration, and an increasingly large number of macrophages is found among the neoplastic cells.

Combination of Sarcoma 37 with spinal ganglia.—The effects on the fiber outgrowth and the spindle cells of spinal ganglia are identical with those observed with Sarcoma 180 (Figs. 4, 10, 12).

The density of nerve fiber outgrowth and the demarcation line of the fibers facing the tumor are, if anything, more pronounced than in the case of Sarcoma 180, and the same is true for the inhibitory effect on the spindle cells.

In several series, the distance between the tumor fragments and the ganglion was varied. Maximal effects were obtained when the distance ranged from 1 to 2 mm. At a distance of 3 mm., the effect was somewhat delayed; the fibers were less dense, and their contour toward the tumor was not so sharp as at close distances. However, the general pattern of nerve growth and spindle-cell inhibition was similar to that obtained at shorter distances (Figs. 18, 15). At a distance of 5 mm., a faint effect is still noticeable. On the side toward the tumor, the fibers are distinctly more numerous and longer than on the other sides; however, they are wavy and do not take a straight course.

Combination of Sarcoma 37 with sympathetic ganglia.—This experiment gave exactly the same results as combinations with Sarcoma 180 and therefore requires no separate description.

Explants of spinal cord, alone and combined with Sarcoma 37.—The explants were taken from 6-day embryos. In contrast to cultures of spinal ganglia, no spindle-shaped cells migrate out of the spinal cord (see also [8]). After 24 hours, one finds a limited outgrowth of epithelial sheets which are probably derived from ependymal cells. They are always restricted to some parts of the explant. The nerve fiber outgrowth differs also from that found in sensory ganglia. The fibers are not distributed regularly over the entire surface of the explant, but they are limited to one or a few sites from which they emerge in long strands. Fibers which bridge a localized area of liquefaction show a very straight course, due to passive stretching.

The combination of spinal cord with Sarcoma 37 did not result in an increase of nerve fibers, nor in a change in the general growth pattern. Occasionally, strands of fibers were directed toward the tumor, but the same behavior of fibers was observed in control combinations of spinal cord with embryonic chick heart, and additional fiber groups were always found emerging from other parts of the explant. Altogether, we consider the spinal cord as completely refractory to the tumor. A small number of experiments with Sarcomas 180 and 1 gave the same results.

Experiments with Sarcoma 1

This tumor grows in vitro even more actively than do Sarcomas 180 and 37. However, there are some differences in the morphology of the cells and in their distribution pattern. The cells are
spindle-shaped and much smaller than those of the other two sarcomas. They migrate individually in a radial direction and distribute themselves very evenly, covering a large area in a short period (Fig. 17). During the 2d day, one finds a considerable number of round cells in the growth zone; to judge from their size and cytological characters, they are transformed neoplastic cells. After 48 hours, the explant is almost invariably surrounded by a liquefied area.

**Combination with spinal ganglia.**—In all cases, the fiber outgrowth is enhanced, as compared to control cultures, and the density of the fibers is increased on the side facing the tumor. However, the results are not consistent. In the majority of cases, the effects were considerably milder than with other sarcomas. The fibers do not show the growth pattern characteristic of the combinations with Sarcomas 37 and 180. They are much less dense, and they do not grow out in a regular straight radial direction nor do they show a sharp line of demarcation in front of the tumor. During the 2d day, the fibers in the area facing the tumor collect in bundles which penetrate into the growth zone of the tumor. The migration of spindle-shaped cells is apparently not affected. In a smaller number of cases, the effects are stronger and more similar to the effects of the other two sarcomas. A typical halo of nerve fibers is present, and, at the same time, the migration of spindle cells is inhibited on the side facing the tumor. Nevertheless, the fiber density never reaches the same degree as in the other tumors. It is perhaps significant that, in the latter group, the plasma had not been diluted and the medium was therefore more dense; no liquefaction occurred in these cases.

**Experiments with Adenocarcinoma dmbB**

Typical epithelial growth of this tumor was obtained by placing the tumor fragment on the surface of the medium which had been allowed to begin clotting. Since the tumors had gone through one or several passages in the chick embryo before being used for explantation, they contained chick fibroblasts in their stroma, and these cells migrated out along with the epithelial sheets. As has been observed by others, the epithelial growth of adenocarcinomas in vitro is delayed in comparison with the growth of sarcomas.

**Combination with spinal ganglia.**—In all experiments, several fragments of the tumor were placed near one side of the ganglion. The carcinoma did not stimulate the outgrowth of nerve fibers beyond the normal range of controls, and spindle cells migrated out actively in all directions (Fig. 11). In some instances, they were even more numerous than in control cultures. A striking stimulation of the outgrowth of mouse fibroblasts by carcinomas, in vitro, has been described by Ludford and Barlow (15).

**Experiments with Neuroblastoma C1800**

This tumor is not compact, as are the others, and is rather difficult to grow in tissue culture. However, typical epithelial sheets surrounding the explants were obtained in some cases (Fig. 5, N). In addition, a large number of spindle-shaped cells, probably of chick origin, and macrophages were observed in the growth zone.

**Combination with spinal ganglia.**—In all cases, a number of fragments of different sizes were placed around the ganglion. The results were different from those obtained with other tumors. As a rule, both the fiber outgrowth and the migration of spindle cells were impaired. In a few cases, nerve fibers did grow out, but they were rarely as numerous as in the controls (Fig. 5).

**Control Experiments with Embryonic Chicken Heart**

The outcome of a large number of combination experiments of spinal ganglia with heart fragments of 7- to 9-day embryos was consistently negative. The presence of the chick tissue did not change the rate, density, and growth pattern of the nerves. The migration of spindle cells from the ganglia was normal in all instances (Figs. 3, 7).

**Control Experiments with Heart Tissue of Embryonic, Fetal, or New-Born Mice**

This tissue grows fairly well in vitro (in a medium of chicken plasma and extract), but its rate of growth is much lower than that of mouse sarcoma cells or of chicken heart fibroblasts.

**Combination with spinal ganglia.**—Toward the end of the first day, the number of nerve fibers on the side facing the mouse tissue is consistently higher than in isolated control ganglia and in ganglia facing embryonic chicken heart. However, the features which are characteristic of combination cultures with sarcomas are entirely missing (compare Fig. 1 to Fig. 2), and the general appearance resembles that of control ganglia. During the 2d day, the preferential growth of nerve fibers toward the mouse tissue is accentuated, and the over-all density of fibers is greater than in controls, but the difference in the growth pattern, between combinations with sarcoma and combinations with mouse heart, is as distinct as it was before (compare Fig. 9 to Figs. 8, 10).

**Discussion**

**Parallelism of tumor effects in vivo and in vitro.**

—The tissue culture experiments had been under-
taken with the expectation that it might be possible to duplicate in vitro some of the remarkable effects on ganglia which had been observed in vivo. The results obtained in the two sets of experiments show striking similarities, but also differences in some essential points. In both instances, the nerve fiber outgrowth is far beyond the normal range, and the fibers begin to grow out at earlier stages than they do normally. In both experiments, the agent acts at a distance, and the tumor does not require contact with the ganglion or with nerve fibers to exert its influence. Furthermore, the specificity of the target, which was one of the essential features of the sarcoma effect in vivo, is also characteristic of the in vitro experiments; only spinal and sympathetic ganglia show a response, whereas the cells of the spinal cord are refractory. The parallelism between the two phenomena extends to the quantitative aspects of the response. The two sarcomas, 180 and 87, show very strong effects both in vivo and in vitro. A third sarcoma, 1, which was tested by Bueker and Hilderman (2) in vivo, was found to have only a mild effect on adjacent ganglia and none on remote ganglia. In vitro, it was likewise much less effective than the other two sarcomas.

The question arises whether we are dealing with a general tumor effect or with a specific sarcoma effect. The results of both in vivo and in vitro experiments were clearly in favor of the second alternative. Two epithelial mouse tumors were found to be entirely negative. Neuroblastoma C1800 grew intra-embryonically to a considerable size, but it was not invaded by nerve fibers and did not call forth a hyperplasia of ganglia (9). In vitro, this tumor grew in an epithelial fashion, though its growth was not very extensive. It did not stimulate nerve growth, but, on the contrary, had an inhibitory effect on both nerve fibers and spindle cells. Mammary adenocarcinoma dbrB, which was first grown successfully in the yolk sac (16), attained in coelomic transplantations a very conspicuous size, exceeding even Sarcomas 180 and 87 (unpublished observations of Levi-Montalcini). Nevertheless, it had no effect on ganglia, and it was not invaded by nerve fibers. This tumor grew well in vitro; it formed epithelial sheets mixed with stroma cells, but it was again entirely ineffective as far as nerve fiber stimulation is concerned.

At present, it seems that the agent which promotes nerve fiber growth is restricted to some sarcomas. The parallelism between the tumor effects in vivo and in vitro is very striking and suggests strongly that we are dealing in both instances with the same agent.5

On the other hand, we wish to emphasize some important differences in the mode of response of the ganglia in vivo and in vitro. In tissue culture, we have found an outburst of fiber growth which begins as early as 12 hours after explantation, reaches a peak between 24 and 48 hours, and regresses during the following days. In intra- and extra-embryonic transplants, the first responses were observed about 3½—4 days after implantation. Following these initial responses, the effects increased steadily until the death of the embryo. It seems that these differences can be accounted for by differences in the experimental set-up. The embryonic transplantations are done when the embryos are 2—4 days old, at which time the ganglia are in early stages of differentiation. The small tumor fragment undergoes an initial regression (5) and does not reach an appreciable size until the embryo is 6—7 days old. After reaching this stage, the tumor enlarges progressively and increases its activity proportionally. On the other hand, the explanted tumor fragment does not undergo regression. On the contrary, cell migration begins a few hours after transplantation, and mitotic figures are present at that time. These features attest to the strong vitality of the tumor explant from the first hours on. The tumor fragment is placed closely adjacent to a ganglion which is apparently in an optimal stage for reaction; therefore, all conditions are given for an immediate response. The rapid decline of fiber growth after 8 days is paralleled by a concomitant regression of the tumor because of the depletion of the medium which was not renewed.

Two other sarcoma effects on ganglia had been observed in embryonic transplantation: an increase in mitotic activity and a cellular hypertrophy of neuroblasts. These two aspects were not studied in tissue culture.

Among the many problems raised—but not resolved—by our previous transplantation experiments was that concerning the immediate target of the tumor agent. Two alternative hypotheses were advanced (11, 18): Either the agent acts directly on the potential neuroblasts or indirectly by breaking down the normal resistance of viscera against hyperneurotization, thus inviting an abnormal in-flow of pathfinders into the viscera. According to this second hypothesis, the hyperplasia of the ganglia would be the end result of a complex chain of reactions beginning with a change at the periphery. The nerve fibers would mediate the effect from the periphery to the centers.

5 In our previous extra-embryonic transplantation experiments (18) only the responses of the sympathetic ganglia were described in detail. However, responses of the spinal ganglia were clearly manifest both in these experiments (unpublished observations) and in our previous intra-embryonic transplantation experiments (12).

Cancer Research
If one admits a basic identity of the tumor effects in vivo and in vitro, the present experiments can be taken as a strong argument in favor of the first hypothesis cited above. In our experiments in vitro, we have observed a precocious outburst of fiber formation almost simultaneously in all parts of the ganglion. The fibers grow straight radially, they do not converge toward the tumor, and, what is most important, they do not establish contact with the tumor until after the peak of the reaction has passed.

The growth pattern of nerve fibers in relation to the culture medium and to other conditions.—All arguments presented so far support the assumption that the main features observed in vitro, namely, the conspicuous increase in nerve fiber density and the precociousness of fiber outgrowth, are due to a diffusible agent released by sarcomas. However, in tissue culture experiments, the physical condition of the culture medium cannot be ignored, and the question arises whether some of the strikingly regular features of the growth pattern may be due to this factor. This problem is particularly pertinent in experiments with nerve fibers. Weiss (17) has shown that in many instances where the directional outgrowth of nerve fibers, in vitro and in vivo, had been attributed to a chemical action at a distance (“chemotropism,” “neurotropism”), the “guidance” of nerve fibers was actually achieved by the structural organization of the microscopic or micellar constituents of the ground substance; the fibers do not follow diffusion gradients, but specific pathways or track systems established in the substrate on which they grow (“contact guidance”). The density of fiber outgrowth can also be determined in this way, by the channeling of nutrient supply along preferential lines laid down in the matrix (17, p. 487).

Can any of the sarcoma effects on ganglia be attributed to a structural organization called forth by the tumor in the culture medium? In this connection, it should be pointed out that in our experiments the fiber outgrowth is not directed toward the tumor, but straight radially in all directions (Figs. 10, 13, 14). However, the greater density of the fibers on the side facing the tumor (Figs. 1, 4, 8) could be interpreted in this way. This feature suggests the presence of invisible barriers between nerve fibers and sarcoma. If two ganglia are explanted adjacent to each other, a large tract of nerve fibers and spindle cells eventually connects the two, whereas fiber outgrowth in other directions remains sparse. It is assumed that the two ganglia call forth a dehydration and thus create lines of tension in the micellar components of the medium. These lines would be mutually reinforced in the area between the ganglia and become a preferential pathway for cells and fibers. A similar explanation may hold in our case; however, the gradual decrease of fiber density with increasing distance from the tumor speaks more in favor of a diffusion gradient set up by the tumor.

The very straight, radial course of the nerve fibers in the presence of sarcomas is in contrast to the somewhat wavy and bent course which is characteristic of fibers emerging from isolated ganglia (compare Fig. 7 to Figs. 8, 10, 14). It is conceivable that, under the impact of the very actively growing sarcoma, the matrix surrounding the ganglion undergoes a particularly regular micellar orientation in a radial direction which would be reflected in the straight and almost geometric pattern of nerve growth. A liquefaction of the medium which is sometimes responsible for a straight fiber course plays no role in our experiments.

Physical factors could also be responsible for the sharp and very regular line of termination of the fibers in front of the tumor (Figs. 10, 12, 14). This feature suggests the presence of an invisible barrier in the culture medium. No evidence for an interface in the plasma clot along this line was observed, but the possibility remains that such an interface is created by the apposition of sarcoma and ganglion.

The seemingly paradoxical phenomenon that the nerve fibers are shorter on the side facing the tumor than on the other sides (Figs. 10, 13, 14) could be ascribed to the same factor or to a threshold of tolerance for the sarcoma agent. An analysis of the role of the culture medium in the nerve growth pattern is in progress.

An inhibitory effect of sarcoma on spindle cells.— Sarcomas 180 and 37 had an inhibitory effect on the migration of spindle cells. The inhibition was always complete on the side facing the tumor (Figs. 10, 12, 14). It decreased with the distance from the tumor, and, in cases of mild tumor effects on the nerves, it was barely noticeable on the side opposite to the tumor. A close correlation between the stimulatory effects on nerve fibers and the blocking effect on spindle cell migration was consistent feature of all experiments with these sarcomas.

Temperature experiments with spinal ganglia showed a similar relationship (8). When tissue cultures of spinal ganglia of 11-day embryos were reared at low temperatures (39° C.), the spindle-cell migration was inhibited, but the branching of nerve fibers was enhanced.

Does a causal relation exist between these two phenomena? And, if so, which is the primary response? Our present material does not permit us to give a conclusive answer to these questions, but a few pertinent observations may be mentioned. It
was shown above (p. 51) that the nerve fiber outgrowth in sarcoma experiments is very precocious; in fact, it begins a few hours earlier than the spindle-cell migration in control cultures. This indicates that the response of the nerve cells is independent of the spindle cells. Furthermore, a correlation between spindle-cell inhibition and nerve fiber stimulation does not exist in combination experiments with neuroblastoma. This tumor inhibits both nerve fibers and spindle cells. The relation between these two phenomena deserves further study, both in vivo and in vitro, since it touches upon the basic problem of the interaction of tumors with host tissues.

Ludford (14) and Ludford and Barlow (15) have also observed slight inhibitory effects of mouse sarcoma on mouse fibroblasts in vitro. The same authors found a very strong stimulatory effect of carcinomas on the outgrowth of mouse fibroblasts. Our own carcinoma experiments gave only a slight and not consistent effect of this type.

The effects of normal mouse tissues.—Whereas the combination of chick tissue (from embryonic heart) with spinal ganglia gave entirely negative results, the combination of mouse tissue (from embryonic or fetal heart) with chick ganglia resulted in a consistent increase in the number of nerve fibers on the side facing the mouse tissue (Fig. 2). However, as was pointed out above (p. 53), the growth pattern of the nerves did not show any of the characteristics of the sarcoma effects, but was merely an accentuation of the normal pattern (compare Fig. 9 to Fig. 10). This result suggests that the sarcoma agent may be present in low concentration in normal mouse tissue. However, mouse adenocarcinoma and neuroblastoma were negative, and the question of the distribution of the agent in mouse tissue requires further studies. In this connection, some data of Ludford and Barlow (15) are of interest. These authors reported that kidney tissue of mouse embryos has a moderate stimulating effect on mouse fibroblasts which is comparable to that of some mouse carcinomas, but much milder in degree.

SUMMARY
Small fragments of mouse Sarcomas 180 and 87 were placed at a distance of 1–2 mm. from spinal or sympathetic ganglia of a chick embryo in a hanging-drop tissue culture. Under this condition the ganglion produces precociously, within 24 hours, an excessive number of nerve fibers which grow very straight radially in all directions, forming a dense "halo" around the ganglion. Their density decreases, and their length increases, with increasing distance from the sarcoma. In addition, the migration of spindle cells from the ganglia is inhibited by the sarcomas. Mouse Sarcoma 1 has a similar, but milder, effect. Mouse adenocarcinoma dbBR and mouse neuroblastoma C1800 do not stimulate nerve growth. Control experiments with heart tissue from chick embryos were entirely negative, but heart tissue of fetal mice was found to have a mild stimulating effect. However, in the latter instance, the growth pattern is very different from that found in the presence of sarcomas and very similar to that found in normal, isolated ganglia. Sarcomas have no effect on spinal cord fibers.

It is concluded that the mouse sarcomas tested produce a diffusible agent which strongly promotes the nerve fiber outgrowth of ganglia. The results obtained in vitro are compared to previous results obtained by intra-embryonic transplantation of the same sarcomas, and the conclusion is reached that the in vitro and the in vivo effects on the spinal and sympathetic ganglia are due to the same agent.

Magnification of all figures X65.
Fig. 1.—Lumbar ganglion of 7-day embryo, combined with Sarcoma 180 (S). 24 hrs.
Fig. 2.—Lumbar ganglion of 7-day embryo, combined with heart of mouse fetus (to the left of ganglion). 24 hrs. Note fiber outgrowth to the left.
Fig. 3.—Lumbar ganglion of 7-day embryo, combined with heart of chick embryo (C). 24 hrs.
Fig. 4.—Lumbar ganglion of 7-day embryo, combined with 2 fragments of Sarcoma 87. 24 hrs.
Fig. 5.—Lumbar ganglion of 7-day embryo (G), combined with 2 fragments of neuroblastoma C1800 (N). 24 hrs.
Fig. 6.—Lumbar ganglion of 6-day embryo, combined with Sarcoma 180 (S). 72 hrs.
Magnification of all figures ×65.

Fig. 7.—Lumbar ganglion of 6-day embryo, combined with heart of chick embryo (to the left). 48 hrs.

Fig. 8.—Lumbar ganglion of 7-day embryo, combined with Sarcoma 180 (to the left). 48 hrs.

Fig. 9.—Lumbar ganglion of 7-day embryo, combined with heart of mouse fetus (to the right). 48 hrs.

Fig. 10.—Lumbar ganglion of 7-day embryo, combined with Sarcoma 37 (S). 48 hrs.

Fig. 11.—Thoracic ganglion of 7-day embryo, combined with adenocarcinoma dbrB (A). 30 hrs.

Fig. 12.—Lumbar ganglion of 7-day embryo, combined with Sarcoma 37. 48 hrs.
Magnification of all figures X 65.

FIG. 13.—Lumbar ganglion of 7-day embryo, combined with Sarcoma 37 (to the left). Actual distance between ganglion and sarcoma = 1 mm. (compare with Fig. 15). 48 hrs.

FIG. 14.—Paravertebral sympathetic ganglion of 13-day embryo, combined with Sarcoma 180 (S). 24 hrs.

FIG. 15.—Lumbar ganglion of 7-day embryo, combined with Sarcoma 37 (located at some distance from the left lower corner). Actual distance between ganglion and sarcoma = 34 mm. 48 hrs.

FIG. 16.—Paravertebral sympathetic ganglion of 13-day embryo, combined with Sarcoma 37 (S). 44 hrs.

FIG. 17.—Lumbar ganglion of 7-day embryo, combined with Sarcoma 1 (S). 44 hrs.

FIG. 18.—Paravertebral sympathetic ganglion of 13-day embryo, combined with heart of chick embryo (H). 36 hrs.
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REFERENCES


In Vitro Experiments on the Effects of Mouse Sarcomas 180 and 37 on the Spinal and Sympathetic Ganglia of the Chick Embryo

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