SCHWANN CELL VERSUS FIBROBLAST AS THE ORIGIN OF THE SPECIFIC NERVE SHEATH TUMOR *

Observations Upon Normal Nerve Sheaths and Neurilemomas In Vitro

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Introduction

Since the description by Verocay [1] in 1908 of the specific nerve sheath tumor which he named neurinoma, two opposing views of its cellular origin have prevailed. One, represented by the work of Nageotte,[2,3,4] and of Masson,[5] is based on the study of the morphology and physiology of normal nerve sheaths under various experimental conditions. By comparing the histology of these tissues with that of the tumors, these workers feel that they have proved that the tumor originates from the sheath of Schwann. They therefore give it the name schwannoma, or peripheral glioma.

The opposing opinion, represented by the work of Mallory [6] and of Penfield,[7] emphasizes mainly the study of the extracellular components of these tumors, and rests on the assumption that collagen and reticulin can be formed only by cells of mesodermal derivation. Since the tumors contain considerable collagen and reticulin, this line of reasoning induces the conclusion that the tumors are derived from the endoneural or perineural components of the nerve sheath, and the term perineural fibroblastoma is adopted. Both schools accept Harrison's [8] demonstration in 1924 of the origin of Schwann cells from the neural crest.

Since neither side has been able to convince the other by histological methods, and since in some instances the same evidence is interpreted differently by exponents of opposing theories, an attempt has been made in this laboratory to solve the problem by

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another method. We have employed the method of tissue culture in the hope of obtaining critical evidence which the purely histological approach has failed to reveal.

The procedure has been: (1) to cultivate normal and experimental nerve sheaths *in vitro* in an indifferent medium, studying the morphology and the physiology of the outgrowing cells, thus assembling criteria by which the Schwann cell may be distinguished from its accompanying fibroblasts under conditions of uncontrolled or unorganized growth; and (2) to obtain spontaneous tumors of the nerve sheath and cultivate them under similar conditions, comparing the outgrowth with the norms established in Group 1.

Our primary aim has been to establish a concept of the general morphology and physiology of the cell types in question, when freed from normal relations and from the control exerted by the organism. Specific staining, though often an aid in diagnosis, is not wholly to be relied upon in tissue culture, nor in the study of tumors. The tumors with which we are concerned, however, are slow growing and of a considerable degree of organization; differentiation is high and cellular aberrancy at a minimum in certain areas, a fortunate circumstance for the elucidation of the problem.

The appearance and behavior of the various cell types are described and recorded as far as possible by microphotographs. By means of these the reader may compare the tumor cultures with cultures of normal and of experimental nerves of man and the rat. When dealing with a normal nerve in culture it is possible to identify the Schwann cells in the explant, as they assist at wallerian degeneration, and to observe them as they emerge in alignment from tufts of nerve fibers. Since they have a characteristic form and definite peculiarities in staining, it is possible also to recognize them when they are free and isolated in an outgrowth composed of all the constituents of the nerve sheath. It should be borne in mind that in these preparations one sees always the whole cell — never a cell dismembered by sectioning.

During the past year we have obtained and cultivated two sets of multiple tumors from two different individuals, and we now submit evidence that these are of schwannian derivation. We shall refer to these tumors as neurilemomas, the term proposed by Stout [9] in 1935 for the specific nerve sheath tumor.

Preliminary Experiments

Cultivation of Normal and Experimental Nerve Sheaths In Vitro

To prevent this paper from exceeding a convenient length, we shall give here only a brief description of the procedure and the material by means of which we have studied the morphology of the sheath cells *in vitro*, relying on microphotographs of the material to convey the essential evidence. The treatment and the culture mediums have been uniform, as far as practicable in dealing with material from different species. The Maximow hanging-drop method was used.

In the course of the investigation we have cultivated and studied *in vitro*:

(*1*) *Fetal Spinal Nerves from Man and the Rat:* The human specimens (5 months fetuses) were not particularly instructive because of the relatively undifferentiated character of the sheath cells, or because of the exuberance of the fibroblastic growth which tended to obscure other cells.

The rat material came from 20 day rat fetuses in which the spinal nerves were still unmyelinated. In many of these the sheath cells migrated and grew out freely. They were frequently discrete, though more often syncytial, with fine anastomosing filaments. With Mallory's phosphotungstic acid hematoxylin they stained deeply with a purplish cast quite distinct from the accompanying fibroblasts, which were pinkish in addition to being of a wholly different form (Figs. 1 and 2). These cultures digested fibrin with inconvenient rapidity, especially in the areas richest in Schwann cells. As a result the explant was often lost in the course of washings. The outgrowth then filled the empty space.

(*2*) *A Normal Splanchnic Nerve from a 16 Year Old Girl:* In this the growth was slow and sparse, but definitely schwannian. Syncytia and cells with long filiform branches emerged from the bundles of nerve fibers (Fig. 3). Fibroblasts were scarce or absent from this outgrowth. (The normal adult fibroblast, especially from man, grows out very slowly, as a general rule.)

(*3*) *Sciatic Nerves from Young Rats (2½ Months Old) in Simultaneous Wallerian Degeneration and Nerve Sheath Outgrowth:* It is not necessary to give a complete description of wal-

lerian degeneration here. Suffice it to say that Nageotte's [2] exact and comprehensive description of this process as it takes place *in vivo* (1922) is substantiated by our observations *in vitro*. Nageotte also placed segments of nerves in balanced salt solutions and observed the first steps in the myelin degeneration, but since the cells of Schwann did not survive in this medium the process was arrested there. In our culture medium, composed of a balanced and buffered saline solution, homogeneous plasma and tissue juice, the Schwann cells survive and become very active. As Nageotte states, they bud from the parent stalk, but within 48 hours they begin also to wander out as single units into the medium. There they may divide and the daughter cells remain attached in syncytial form. The sheath cells from the fibers of Remak are precocious; these closely resemble the embryonic form of Schwann cell referred to above. The cells from the myelinated fibers, whose cytoplasm is more abundant, nevertheless emerge later through interstices in the gelatinized Plenk-Laidlaw sheath.

Wallerian degeneration proceeds rapidly in our cultures and regeneration takes place simultaneously, after a lag of about 2 days. There is a high degree of resolution and considerable proliferation within 6 days (Figs. 4 and 5). At this time the collagenous sheaths of the large medullated fibers have the gelatinized reticulated appearance described by Masson [5] in 1932, with the longitudinal fibers staining with aniline blue (Fig. 5).

Since under normal conditions there are relatively few phagocytes present in a nerve, the transplantation of a nerve segment into culture medium allows it to pursue its degenerative course practically free from systemic interference. Under these circumstances, as has been shown, resolution of the myelin inclusions is accomplished none the less, presumably by the activity of the Schwann cells and probably by their subsequent absorption of the liquid products of this digestion. They are, as Nageotte states, slightly, but only slightly, phagocytic. These cultures have a strongly liquefactive effect on the fibrin clot in which they grow. This digestive activity suggests an explanation for the infiltrative tendency of proliferating schwannomas resulting from nerve section. The sheath cells from the Remak fibers play an important part in the migratory and proliferative activity which goes on in the cultures (Fig. 6), as do the endoneural cells. However, the

latter take no part in the resolution of the dead neurite or of its myelin sheath.

While it is impossible to exclude the possibility of amitotic division in the schwannian ribbons, it is apparent that under these conditions the usual form is mitotic (Fig. 7). Although the result of a division often suggests that the nucleus has divided longitudinally, we have seen only one longitudinal division actually take place. All the others we have observed have been transverse, with the exception of some suggestive fixed cells.

Limitations of the method have prevented us from carrying the study of regeneration *in vitro* beyond its early stages. When the nerve segment is implanted it may be teased a little with glass needles, and in successful preparations the course of degeneration may be observed. Any severe trauma destroys the Schwann cells. Even with washings, the preparations cannot be kept more than 10 days, as a rule, in their original situation. On transferal to a fresh medium the explant contracts and fibroblasts gain the upper hand, so that the culture becomes worthless for our purposes.

(4) *Cultivation of Schwannomas Formed In Vivo:* This material * was obtained by extirpating 1 cm. of the sciatic nerve in adult rats and by explanting portions of the proximal and the distal stumps after 7 weeks.

Here, as in the preceding section, we shall not give a detailed description of material already handled in a masterly manner by Nageotte and by Masson. The growth we used for explantation represented approximately Stages 2 and 3 in Masson's schema of the transformation of a degenerated fiber into an aneuritic bundle of regeneration.

The outgrowth of the schwannian elements was characterized by syncytial cords, single (Fig. 9), two abreast (Fig. 10), or parallel strands very close together with frequent forking but not much parallel anastomosis (Fig. 8). There were no sheets of schwannian growth such as appear in the later stages of the regeneration, and there were many clusters of discrete cells drawn out lengthwise with many single cells actively wandering. One type of cell in the outgrowth closely resembled the embryonic sheath cells or the outgrowth from the Remak fibers (referred to under heading No. 3), with somewhat hypertrophied nuclei and

* With the aid of Dr. T. P. Eberhard.

exceedingly fine, long drawn out cytoplasmic filaments (Fig. 8). The derivation of these cells, whether from myelinated or unmyelinated fiber, can only be presumed since there is no way of distinguishing them in the explant.

In all the Schwann cells both nucleus and cytoplasm were hyperchromophilic (Mallory's phosphotungstic acid hematoxylin stain). In the type of sheath cells which is assumed because of its more voluminous cytoplasm to have originated from the medullated nerve fibers, the nuclei were frequently lobated; they were somewhat hypertrophied and correspondingly endowed with chromatin, sometimes containing three nucleoli instead of the customary one or two. The cytoplasm of these cells is not always smooth and hyaline (Fig. 9). The fibroblasts had little or no share in this hypertrophic and hyperchromophilic condition, and their behavior was quite commonplace. In one tumor in which adipose tissue was present there was a considerable growth of capillary endothelium, which served for purposes of comparison.

On the subject of division it is difficult to be explicit. The appearance and disposition of the nuclei in the schwannian bands make it tempting to infer that division is frequently amitotic and longitudinal (Masson [5]). But on only one occasion have we seen a nucleus actually complete an amitotic division. We have seen a number of mitoses, particularly at the ends of ribbons. On the other hand, the number of mitoses seen among the Schwann cells is small — much less than the number among the fibroblasts although the fibroblasts are less numerous and less actively proliferating in the culture. On this ground, and on the ground that an amitotic division is difficult to detect *in vitro* (there being many nuclear constrictions proceeding from other causes, such as locomotion), one might be justified in the inference that amitosis is a frequent mode of division among the schwannian nuclei.

Among the sheath cells of the Remak fibers densely chromatic bodies, approximately spherical, are often seen which bring to mind the homogeneous bodies described in this location by Nageotte [2] in 1922. However, close observation of favorable specimens shows them to be nuclei in mitotic division. In these cultures the liquefaction of the clot was rapid and widespread, difficult to control from the standpoint of both growth and suitable histological treatment.

In none of our cultures have we observed collagen produced anew in the outgrowth by any type of cell, schwannian or mesoblastic. This is not to be wondered at since the period of usefulness for observation in our cultures is short. According to Nageotte, it requires 2 weeks, in a regenerating neuroma of the rabbit, for the primitive membrane of Schwann to reach the point where it stains red with Van Gieson's solution, and a longer time for the longitudinal collagen fibers to appear. In cultures of lymph nodes from an adult rabbit, however, McKinney [10] in 1929 found collagen fibers appearing during the 6th day. We have not so far been able to apply Laidlaw's reticulum stain successfully to cultures, probably because of their unequal thickness and because of the presence of successive layers of plasma.

In order to compare various types of syncytia, we have cultivated (*5*) *ossifying ribs of fetal rats*, in which we studied the abundant capillary outgrowths; and (*6*) *fetal skeletal muscle*, which forms long, solid multinucleated ribbons and bands.

It is evident from our material that Schwann cells are capable of separating themselves from the group and wandering off singly during the more differentiated stages of existence of the spinal nerves, as well as in the early developmental stages, as described by Harrison [8] in 1924, and by Speidel [11] in 1932. In our cultures it is more common for proliferating cells to remain in contact with one another after nuclear divisions have taken place. We have not attempted microdissection. However, many observations of living and fixed cells give the impression that they remain in organic continuity, as Nageotte's work in 1922 [2] and that of De Renyi [12] in 1929 indicate (Figs. 2, 3, 7, 8 and 9).

Résumé of Characteristics Distinguishing the Cells of Schwann from the Mesoblastic Elements of the Nerve Sheath Cultivated In Vitro

It is not our intention to state that throughout these studies we can unerringly classify any and every cell in our cultures. In tissue culture, perhaps more often than elsewhere, there are borderline cases in which certain characteristics appear to overlap. However, we do state that for the majority of the cells with which we are dealing the distinction is clear beyond any reasonable

doubt, since the Schwann cells retain a considerable degree of differentiation *in vitro*.

Outgrowing Schwann cells characteristically align themselves in formations wherein the individual cells adhere to one another at one or more points, giving the appearance of syncytia. These consist of ribbons in which the filiform cells lie tandem, or in parallel ribbons or bands with anastomosing branches. Single cells frequently dissociate themselves from the group and wander off slowly to another locality, in much the same way Speidel [11] describes in his studies of sheath cells in the developing tadpole's tail. The discrete cells are usually quite distinct from fibroblasts in form and staining. They are frequently dichotomous (Nageotte and Guyon [3]), especially at the anterior end. The syncytial structures are absolutely distinct from any syncytial arrangement adopted by fibroblasts; and though the hypertrophied ribbons from the medullated fibers may occasionally be confused by an inexperienced observer with capillary outgrowths, the texture and relative width of the cytoplasm, and the number of nucleoli, will usually dispel any doubts. The single or the syncytial structures formed by the sheath cells from the Remak fibers cannot possibly be mistaken for formations of mesoblastic cells.

Let us consider the individual Schwann cell *in vitro*: this cell is long, thin and compact, as compared with its companion fibroblasts. It has a generally bipolar form, often filiform, while the fibroblast is amorphously branching. We should mention here that the endoperineural fibroblast, like other races of fibroblasts, has a perceptible individuality. We shall not treat endothelium separately from the endoperineural fibroblast except where capillary formations are in question.

The Schwann cell has an oval nucleus which, especially in the cells from the fibers of Remak, is wider than the empty protoplasmic ribbon which contains it, thereby causing this to bulge in a characteristic manner; the diameter of the fibroblast nucleus, on the other hand, is always considerably less than the diameter of the cell. The Schwann cell nucleus in the rat and in man is poor in chromatin, and has usually one or two prominent nucleoli — only rarely three or four. Fibroblasts and endothelial cells in the same species have a variable number from two to seven, with four being the most common number. By this criterion alone a group

of Schwann cells can often be distinguished from a group of fibroblasts.

After fixatives such as Bouin's and Zenker's, the cytoplasm of the Schwann cell is smooth in texture as compared with the granular or fibrillar fibroblast; in the embryonic material and in the Remak sheath especially it has a hyaline appearance. With Masson's trichrome stain, also with Mallory's phosphotungstic acid hematoxylin, the Schwann cell shows certain clear and definite peculiarities in coloring. Its cytoplasm takes a deep clear red in the former, and a bluish purple in the latter stain. Nageotte likewise reports a distinctive picture for the Schwann cell by the use of hemalum after treatment with alcohol and nitric acid. In his figures of Schwann cells of the unmyelinated neurites of the cornea, and of the fibers of Remak, Nageotte gives illustrations that can be duplicated to the minutest essential detail in our cultures of nerves and in the cultures of the tumors under discussion.

The Spontaneous Tumors

Case 1: The patient was a male neurologist (S.P. 69049) aged 68 years, with tumors in the left posterior tibial nerve in the popliteal space, in the right ulnar nerve near the head of the ulna, and in a branch of the anterior crural nerve beneath the rectus femoris muscle in the left thigh. These tumors had been present for several years and had recently occasioned tingling in the left foot and right hand. All of them lay within the sheaths of the respective nerves with the nerve fibers spread out over their surfaces. They were encapsulated and were removed by pushing the nerve fibers aside and enucleating them. They were all essentially the same in gross appearance, without any gross cyst formation. The cut surfaces were a mottled yellowish gray. The largest tumor came from the posterior tibial nerve and measured 25 by 20 by 22 mm. The others were only half as large. There was no evidence of von Recklinghausen's disease.

All three tumors show both A and B type tissue (Antoni [13]) with marked organoid differentiation, especially in Tumor 2 from the ulnar nerve, and in Tumor 3 from the anterior crural nerve. In Tumor 1 there are blood vessels with thick collagen sheaths, but in Tumors 2 and 3 the blood vessel walls are little if any thickened. Sections impregnated by Cajal's method show neurites

scattered at intervals throughout all three tumors. They are delicate and non-myelinated, and course at haphazard throughout both the A and the B tissues. All three tumors were considered characteristic neurilemomas.

CASE 2: The patient was a negress (S.P. 69535) 28 years old, with three tumors. The largest tumor measured 12 by 7.5 by 3.5 cm. and lay within the sheath of the right sciatic nerve in the midthigh, where its presence had been noticed for 6 years. The other tumors were of shorter duration. One tumor 4 cm. in diameter lay within the sheath of the right tibial nerve in the popliteal space, and the other was attached to a branch of the peroneal nerve just posterior to the right medial malleolus. The tumor attached to the tibial nerve caused some pain which radiated down to the great toe and the heel. The others were symptomless. There was no evidence of von Recklinghausen's disease. All of these tumors were excised by enucleation, sparing the nerves as much as possible. Following the operation there were some disturbances of sensation.

The tumor from the sciatic nerve was encapsulated and largely cystic, with spaces filled with gelatinous and sometimes blood-tinged fluid. The other tumors were less cystic but were encapsulated and made up of both soft and firm, yellowish gray semitranslucent tissue.

All of the tumors are composed of both A and B types of tissue without much organoid differentiation but with collagen and reticulum fibers in both types and long tapering cells sometimes arranged in tandem formation with two or more nuclei. Cajal impregnations show delicate, non-myelinated neurites coursing in all directions in both the A and B type tissues in the two smaller tumors, but none is found in the largest tumor from the sciatic nerve. All of the tumors have blood vessels surrounded by thick collagen sheaths. They were all accepted as neurilemomas.

THE TUMOR CELLS IN VITRO

When the tumors are being cut up for examination, and especially after the explants have been placed on coverslips, it is usually possible to distinguish between the A type and the B type explants. The A type has a whitish, shiny, almost translucent appearance, while the B type is blotchy, opaque, and often yellow-

ish. The fasciculated form of the A type often may be discerned at the edges of the explant.

Type A Tissue: All the distinguishing characteristics of the Schwann cell *in vitro*, evident in mixed cultures from normal peripheral nerves and from experimental schwannomas, may readily be seen in the outgrowth from the A type tumor tissue, and none of the distinguishing marks of the fibroblast are found (Figs. 11, 13). The tissue has a pronounced liquefactive effect (very difficult to control) upon the plasma clot in which it is grown. It has a tendency to adhere to surfaces, healing over cut edges, and in many instances it grows out in the form of sheets or cords (Fig. 14). These latter probably emerge from the palisaded nodules. The A type of tissue does not seem to undergo degeneration or dedifferentiation *in vitro*. At the end of 12 weeks cultivation it still grows out in the original form even though parts of the explant may have accumulated fatty degeneration granules. Sometimes sheets of interlacing cells are formed. These are always inconveniently liquefactive.

In the outgrowth from normal sciatic nerves undergoing wallerian degeneration *in vitro* there are present a great many sheath cells from the fibers of Remak. These can be identified by appearance and behavior while still situated in the nerve bundle, or when emerging from it, or at some distance away in the outgrowth (Figs. 4 and 6). Their hyaline, threadlike cytoplasm is distinctive, as is also their small size. Their anastomosing branches which rejoin the parent stalk (Fig. 6) are instantly to be recognized in Nageotte's figures of such cells,[2] which have been deprived of their nerve fibers. They have differentiated very little beyond the embryonic type of Schwann cell (Figs. 1 and 2).

The outgrowth from the A type areas of the tumors is composed very largely of such cells, slightly hypertrophied and of slightly more than normal variation in size. They are the most stable type of cell in the tumors. The picture once seen is unmistakable (Figs. 11–13), and appears in culture after culture. This is the morphological norm in cultures of the A type areas, overshadowing the sheets and cords, which probably emerge from palisaded nodules (Fig. 14).

It is not surprising that this should be so when one considers the large numbers of Remak's fibers which are carried by the

peripheral nerves. Ranson *et al.*[14] have made careful counts of the myelinated and unmyelinated nerve fibers in a number of the spinal nerves in man, and find widely varying ratios between them but always a substantial number of Remak's fibers. For instance, the superficial branch of the ulnar nerve gives 2.12 unmyelinated to 1 myelinated nerve fiber; the deep branch 0.69 unmyelinated to 1 myelinated; in the lateral cutaneous nerve of the thigh, 3.85 unmyelinated to 1 myelinated. It will be seen, therefore, that although the bulk of the peripheral nerve appears to be taken up by the myelinated fibers, this is a false impression due to the relatively large size of the individual fibers. It is therefore not difficult to conceive, on grounds of opportunity, that the sheath cells of unmyelinated fibers might afford an origin for the A type of tumor tissue.

The Palisaded Nodules: A second type of growth is sometimes obtained from the hyaline explants, probably from the palisaded nodules which they contain. This is more solid than the usual A type of outgrowth, and resembles the sheets and cords formed by epithelium when it is growing through a clot, as these cells are (Fig. 14). After 12 weeks cultivation, in which it produced only this type of outgrowth, one of these cultures began to produce large numbers of cells characteristic of the B type of explant, which is described below.

Type B Tissue: Cultures of the B type, or reticulated tumor areas, produce cells that at first appear to be quite different from those of the A type. They liquefy their medium so rapidly that in the vicinity of the explant, where they are present in greatest concentration, they are often found in the retracted form induced by lack of solid support, and bear a superficial resemblance to mesothelial wandering cells (Fig. 17). They do not, however, stain brilliantly with neutral red intravitally, as these latter cells do; a few granules in them stain a pale orange, and only occasionally are there bright red inclusions. Furthermore, the nucleus, when fixed and stained, is entirely unlike that of the macrophage or lymphocyte (Fig. 16).

Where the clot is dense enough for them to exist in the extended state the B cells are elongated, knobby and branching, and much more irregular in form than the A type. Because the nucleus tends to be less vesicular, it is thinner and more elongate, and the

ratio of cytoplasmic to nuclear diameter is greater than in the cells of the A type; hence the nucleus does not bulge conspicuously from the band. The average number of nucleoli is less than two. The nucleus is often lobated or constricted, suggesting amitotic division, or, where the cytoplasm is constricted in the same area, suggesting locomotion by constriction bands as described by Speidel. The cytoplasm is not as hyaline as in the A type but tends to be opaque. These cells may form syncytial bands in tandem (Fig. 15), but often are united into an amorphous appearing structure reminiscent of the reticulated B tissue from which they emerge (Fig. 16).

If a small strand of cotton or a fine rod of spun glass is introduced into the medium in which these cells are wandering, they tend to accumulate on it, losing the opacity which characterizes them in the actively wandering state and often sending out tendrils along and around the rod of support (Fig. 18). Sometimes a pocket of liquefied plasma can be seen between the cell and the glass rod.

These cells were the first to make their appearance in the cultures. At the end of 12 weeks cultivation they were still present in scarcely modified form. They show a generic similarity to the cells of the A type but maintain also these constant differences. One is obliged to call them Schwann cells. They are not fibroblasts, endothelium or macrophages.

Although the B type areas of the explants gradually accumulate fatty granules and vacuoles *in vitro*, the B type of cell for a long time continues to migrate from them, and once free in the medium is very active. Of the two cell types it has the shorter lag period and the greater capacity for digesting fibrin. It does not die out and there is no clear evidence that under tissue culture conditions it metamorphoses into the A type, unless it be sometimes into sheets or cords. In Figure 17, which shows clumps of B type cells in an extreme liquefactive state, such that the medium is wholly fluid, a few cells may be seen in a more normal form, extended against the coverslip which affords them support. The whole picture of the B type cell is not primarily that of a degenerating cell but of a cell that lacks osmotic equilibrium; of one that is somewhat plasmolyzed as the result of excessive secretion of digestive ferments.

The stellate, retiform shape of the B type cells in the plasma clot is similar to that of the normal Schwann cell on its medullated fiber with its extensions into and through the myelin. *In vitro* these cells form syncytial structures with prolongations anastomosing in a network; essentially a reproduction of the Büngner bundles described by Nageotte and by Masson in experimental schwannomas. Only the directive forces for compressing them or of pulling them out lengthwise are (of necessity) lacking *in vitro*. The Büngner bundles appear to be derived from the sheaths of the medullated nerves.

The solitary cells or tandem arrangements in the tumor cultures are similar in appearance to cells seen on or emerging from medullated fibers during wallerian degeneration *in vitro* (Figs. 3 and 5). They are not incompatible with Nageotte's illustrations of wallerian degeneration *in vivo*, although this stage precedes the tumor formation in the stump. They are similar to Masson's figures of young schwannian bands invading the connective tissue in experimental schwannomas. In our cultures of experimental schwannomas this type appears also (Fig. 9).

Endothelial Elements: Endothelial elements in the cultures are even less conspicuous than in the sections of the tumors. There were at the beginning of cultivation a number of true wandering cells of lymphocytic nature, as in practically all tumors, but these were quickly lost. There was no capillary formation *in vitro*.

Cell Division: In the matter of cell division in these tumor cultures it is difficult to be explicit. In the whole course of our observations we have seen but two mitoses, both in outgrowths from the palisaded areas. Although it is true that the rate of outgrowth in the A type is slow, still it seems incredible that more mitoses should not have come to light if mitosis were the sole method of division. In the B type particularly, whose outgrowth or outwandering is not slow, no mitoses have been seen, but many nuclear constrictions have been present. In both types (A and B) unequal nuclear divisions are common. It seems unlikely that in the course of 12 weeks cultivation and many passages there should be no cell division; one is therefore impelled to regard amitosis as the probable method.

Inclusion of Normal Tissue with Tumor: The third tumor from Case 2 had infiltrated a considerable portion of the endoneurium

of a branch of the right peroneal nerve. Consequently when it was removed it was necessary to take a good deal of the nerve with it. In several cultures of this tumor we had areas of normal nerve, and from these normal Schwann cells and characteristic endoneural fibroblasts grew out.

Discussion

It should be emphasized that in our cultures of these two sets of neurilemomas no typical fibroblasts have appeared in the outgrowth, and (except in two cultures from a tumor which contained strands of normal nerve) nothing even remotely similar to the endoperineural fibroblasts which are common in our cultures of normal nerves in wallerian degeneration has been observed. Yet the tumors are quite well supplied with collagen in the B type areas, and reticulin or collagen in the A type areas.

It is evident that the neoplastic elements in this material are represented by the two types of Schwann cells emerging from the A and B type areas of the tumors, and by the epithelioid growths emerging from the palisaded nodules.

That Schwann cells can and do form collagen and reticulin seems a reasonable inference from our observations, since these tumors of peripheral nerves containing neurites and solid homogeneous bands of tumor cells are well supplied with fibers that stain with aniline blue and with silver. Characteristic tumor cells, which appear to be schwannian, invariably grow out from the explants, but fibroblasts do not. Careful and repeated examination of the tumor sections has not disclosed the presence of a stroma, unless the sharply circumscribed collars around blood vessels could be given that name. Consequently the conclusion seems strongly indicated that the collagen and reticulin which characterize the A and B type areas are formed under the influence of the A and B type tissues — which our evidence classifies as schwannian.

The presence of two rather distinct and stable cell types in the outgrowth from the tumors is especially interesting in the light of the classical morphology of the neurilemoma — *i.e.* its tendency to be divided into A and B type areas, so-called. Observations on the physiology of the two cell types *in vitro* do much to explain the differences in morphology between the A and the B type areas,

which are regarded, we believe rightly, to have the same cellular origin.

The B type of tissue was thought by Antoni to be a degenerative phase of the A type. Yet, cultivated *in vitro,* these cells do not die; on the contrary they are highly active metabolically and they persist, constantly migrating out from the explant, through many passages. The life of these cultures was never terminated spontaneously during the 3 months through which they were kept growing. It may be of significance that in our two sets of three multiple tumors the largest tumors have contained the largest proportion of B type tissue. *In vitro* this type maintains its individuality, thus presenting us with the problem of a tumor in which there appear to coexist at least two types of Schwann cells.

We have speculated inconclusively whether this situation could be the result of the presence of two types of Schwann cells in the nerve of origin. Certainly two types of nerve fiber, each with its investiture, are there. Speidel's observations indicate that while there is no specificity of Schwann cells in the early phases of fiber development, and while a cell from a non-myelinated fiber may migrate at any time to a myelinated fiber, the relation of cell to myelinated fiber, once established, is quite a stable one. Possibly, therefore, the subsequent differentiation of the Schwann cell is not altogether reversible and we may be dealing with two types of sheath cells which have a tendency to remain distinct. In cultures of normal nerves we have often found it possible to distinguish between the two types. There is a striking resemblance between the A type tumor outgrowth and the outgrowth from the Remak fibers; and except in its extremely liquefactive phases the B type tumor outgrowth bears considerable resemblance to that from the medullated fibers. If our hypothesis should be verified, we should have to believe that the palisaded nodules arise from the sheath cells of medullated nerves, since their outgrowth has been seen *in vitro* to metamorphose into the B type. It may be of interest in this connection that the Wagner-Meissner corpuscles are found at terminal branches of myelinated nerve fibers.

In 1916 Ingebrigtsen,[15] studying the phenomena of nerve degeneration, cultivated sections of sciatic nerves from rabbits, which had been allowed to degenerate *in vivo* for 5 days or more. In the schwannian outgrowth which he obtained he made morpho-

logical distinctions between the outgrowths from nerves in the earlier stages of degeneration, which tended toward a "syncytial network," and straight outgrowths composed of parallel rows of cells similar to the bands of von Büngner, from the later stages of degeneration. His figures suggest that in his cultures it would be possible to distinguish the cells from the medullated fibers from those of the fibers of Remak, and that the cells from the medullated fibers form the bulk of the later outgrowth. His figures also bear a generic resemblance to our two types of tumor cells.

An alternative hypothesis to that of double schwannian origin just outlined, would regard the differences observed among the normal and experimental Schwann cells as non-specific; *i.e.* as relating merely to the cells' original dimensions or degree of hypertrophy. The different tumor areas and types of tumor outgrowth would then represent degrees of tumorous modification of the original Schwann cell, with the A type as the least modified, and the cystic B type as the most modified. There is evidence to support this theory also in both the tumor sections and the cultures of normal and tumor cells. But we are not able at this time to offer conclusive observations on either side. It is difficult to fit the palisaded nodule satisfactorily into either picture.

The progressive degeneration, jellification and eventual liquefaction of the collagen and reticulin in the B type areas of tumors is not of itself remarkable if the behavior of the B type cells *in vitro* be taken into consideration. It will be remembered that they have a marked proclivity to digest the fibrin clot into which they have grown or migrated, and thus to surround themselves by liquid areas from which their protoplasm is retracted, a striking equivalent of microcystic degeneration. When one considers the intimate nutritive relationship which is normally maintained between the nerve fiber and the Schwann cell, and the latter's influence on both the formation of myelin (Speidel [11]), and on its resorption in wallerian degeneration (Nageotte [2]), one may conclude that the normal Schwann cell of the medullated nerve is a particularly active producer of digestive catalysts. After injury to the nerve, or sectioning of it, the Schwann cells not only become hypertrophied and proliferate, but often infiltrate their surroundings as well. A tumor cell, aberrant in respect to its reproductive function, and deprived of its normal relation with the nerve fiber,

might be expected to suffer an exaggeration of its normal digestive function as a result of its general disequilibration.

Hyperactivity of the liquefactive function, as in newly emerged B type cells from cystic areas, works against the morphological expression of the cells' differentiation, even *in vitro*, by the liquefaction of the fibrin adjacent to the cell and the coincident destruction of the solid support for finely branching processes (which characterize the normal Schwann cell *in situ*). These, therefore, are retracted and appear as the knobby branches typical of this type of tumor cell *in vitro*. Experimentally this condition may be overcome to some extent by providing the cells with an exceedingly thick clot which they cannot easily liquefy. In the outgrowth of B type cells from sclerosed portions of tumors, the morphological differentiation appears unhindered since it is not digestion but collagen formation which is the aberrant function here. These cells are often solitary and are provided with numerous finely branching processes. It is this condition which predominates in cultures of tumors generally classified as neurofibromas.

By the train of reasoning outlined above it is possible to bring into harmony the phenomena of sclerosis and of Antoni's B type of tissue, or myxoid metamorphosis, as being due to the unbridled exercise of different normal proclivities by disequilibrated sheath cells. The choice and the timing of the aberrancy among these respective functions might well be dependent on local or systemic conditions varying within the tumor and from tumor to tumor. Where the cell engages in either sclerosis or liquefaction in great excess, it is inevitable that it should destroy itself, as uncontrollably liquefactive cells do *in vitro*.

Summary

We have offered evidence, obtained by means of tissue culture, that the specific nerve sheath tumor or neurilemoma is of schwannian origin. This evidence is based on morphological and physiological similarities between the schwannian outgrowth from normal and experimental nerves and the outgrowth from spontaneous tumors. It is inferred that Schwann cells can and do condition the formation of collagen without the intervention of fibroblasts, since these tumors, whose outgrowth is wholly schwannian, contain considerable collagen.

By the study of the tumor cells' behavior *in vitro* we are able to offer an explanation of the so-called cystic degeneration in these tumors and to harmonize it with the sclerosis seen in neurofibromas.

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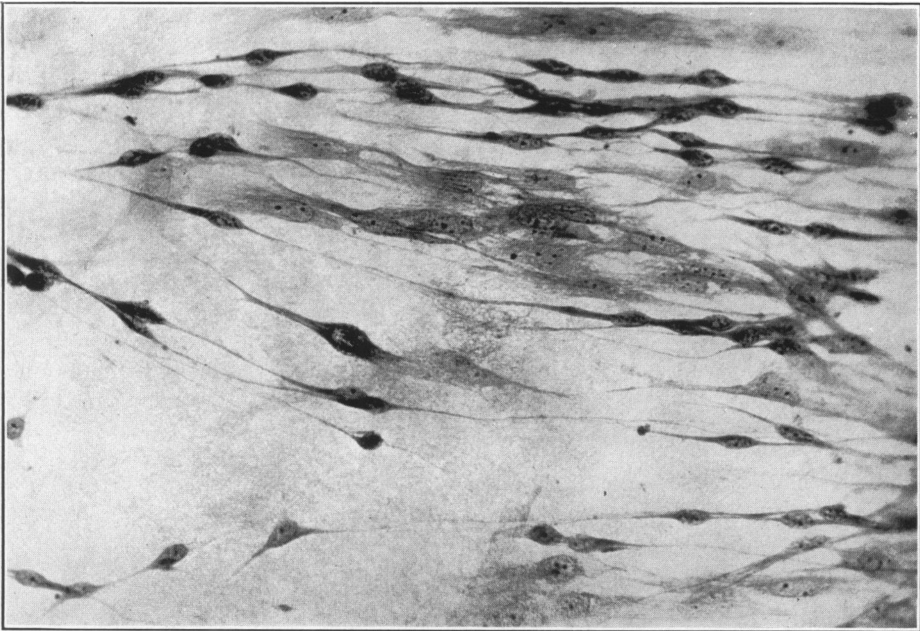
DESCRIPTION OF PLATES

Plate 10

Fig. 1. Schwann syncytium from the spinal nerve of a 20 day rat fetus. One fibroblast is seen at the right. Tissue culture 1 week *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.

Fig. 2. Schwann syncytia and fibroblasts from the same culture as in Figure 1.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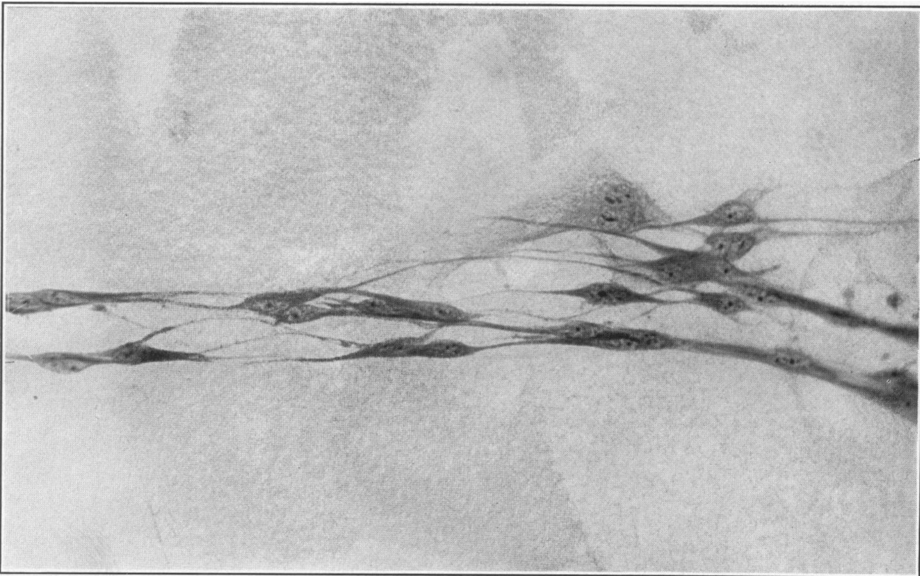
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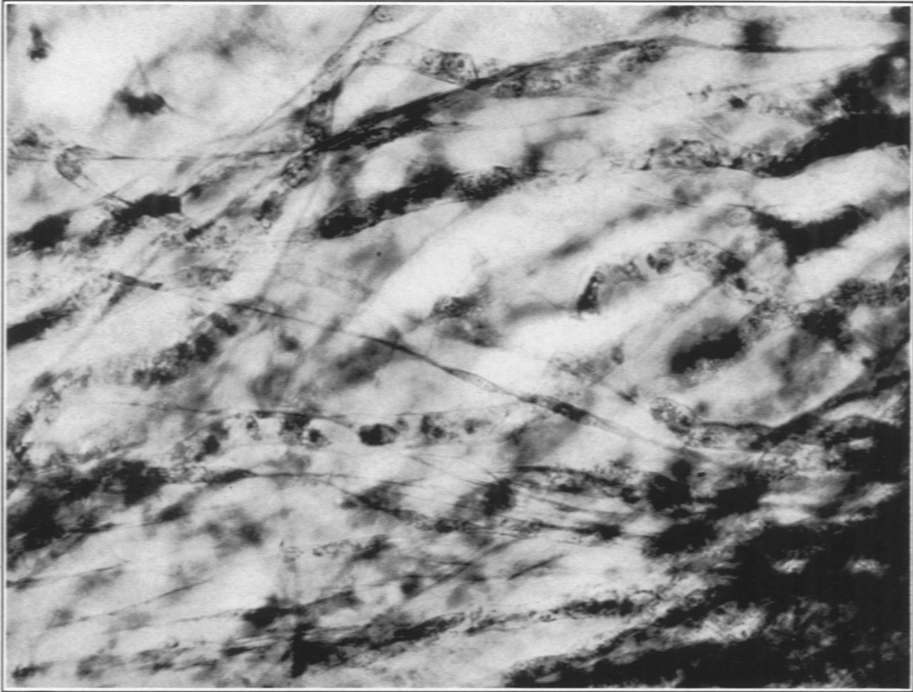
Plate 11

Fig. 3. Schwann cells from the splanchnic nerve of a 16 year old girl. Tissue culture 10 days *in vitro*. Toluidine blue stain.

Fig. 4. Wallerian degeneration *in vitro*. Note the Remak fibers. Sciatic nerve from a 2½ months old rat. Tissue culture 8 days *in vitro*. Phosphotungstic acid hematoxylin stain.



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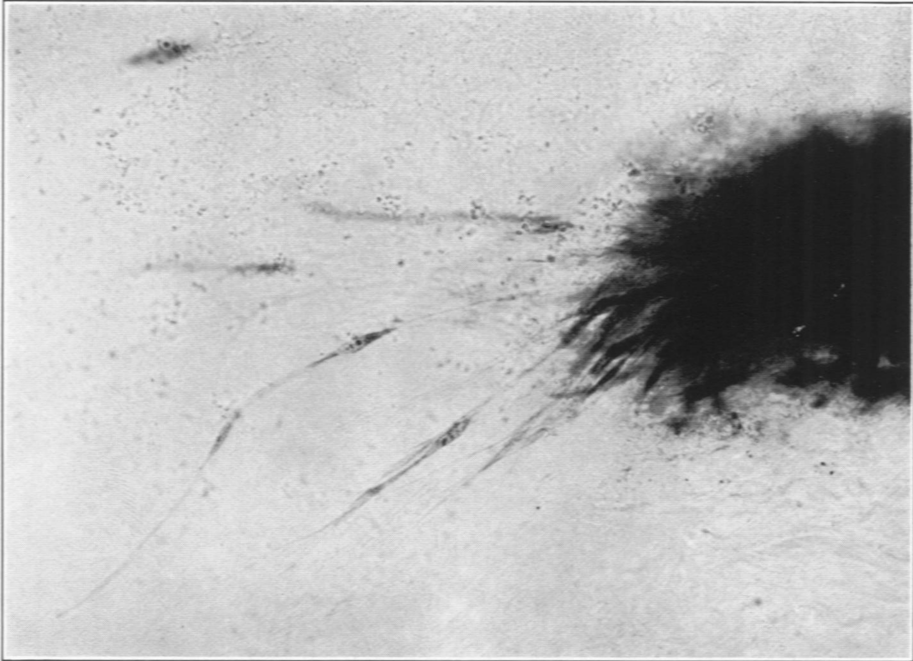

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PLATE 12

FIG. 5. Wallerian degeneration *in vitro*. Schwann cells of medullated fibers. Sciatic nerve from a 2½ months old rat. Tissue culture 6 days *in vitro*. Zenker's fixation, Masson's fuchsin-ponceau, aniline blue stain.

FIG. 6. Cells from Remak's fibers; endoperineural fibroblasts in the background. Same culture as shown in Figure 5.

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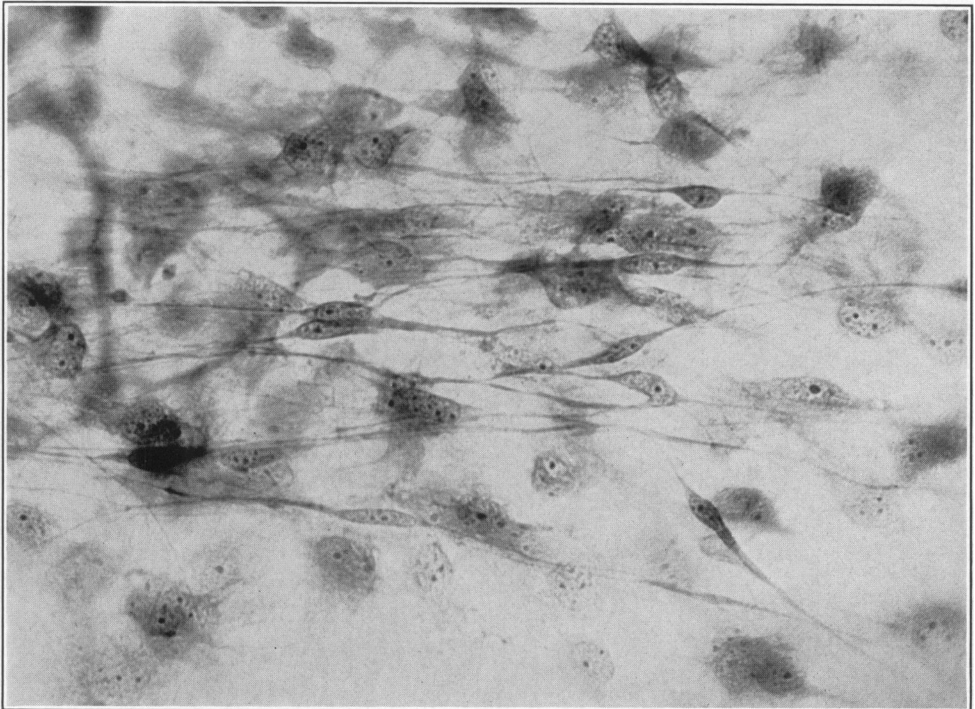

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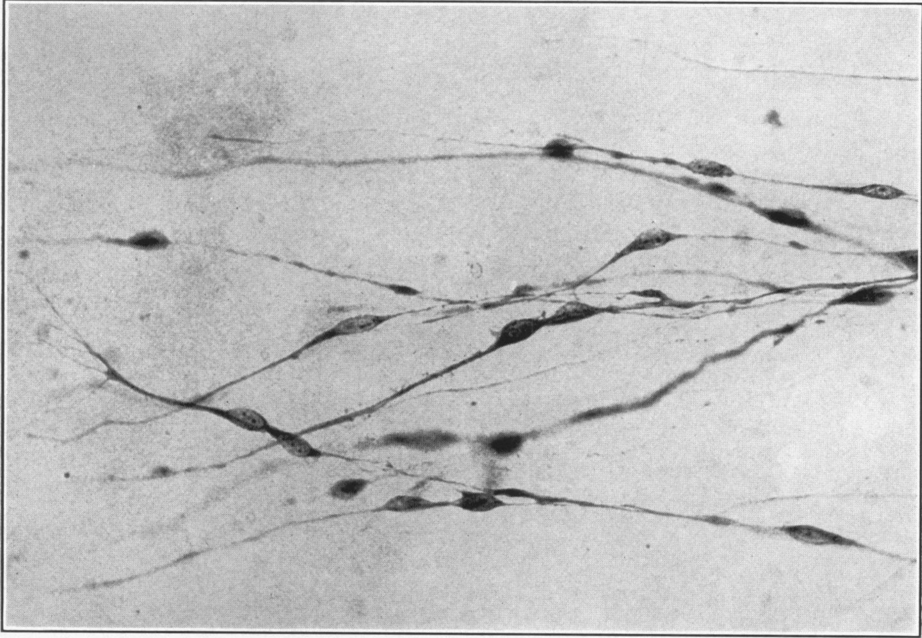
Plate 13

Fig. 7. Early stage in palisading. Outgrowth from a sciatic nerve from a rat. Tissue culture 9 days *in vitro*. Delafield's hematoxylin stain.

Fig. 8. Schwann syncytia from a proximal amputation neuroma (7 weeks) of a sciatic nerve from a rat. Tissue culture 5 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.



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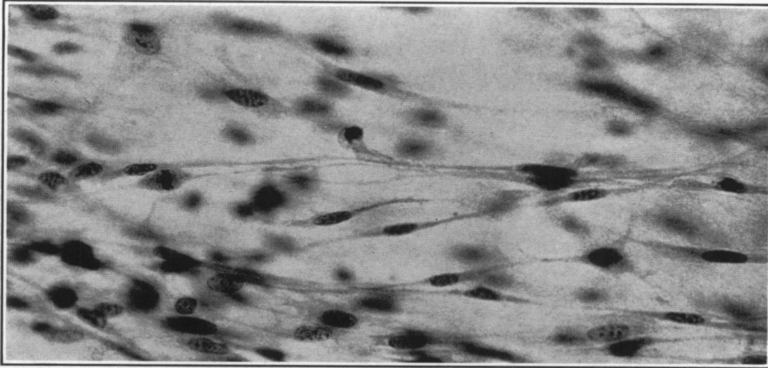

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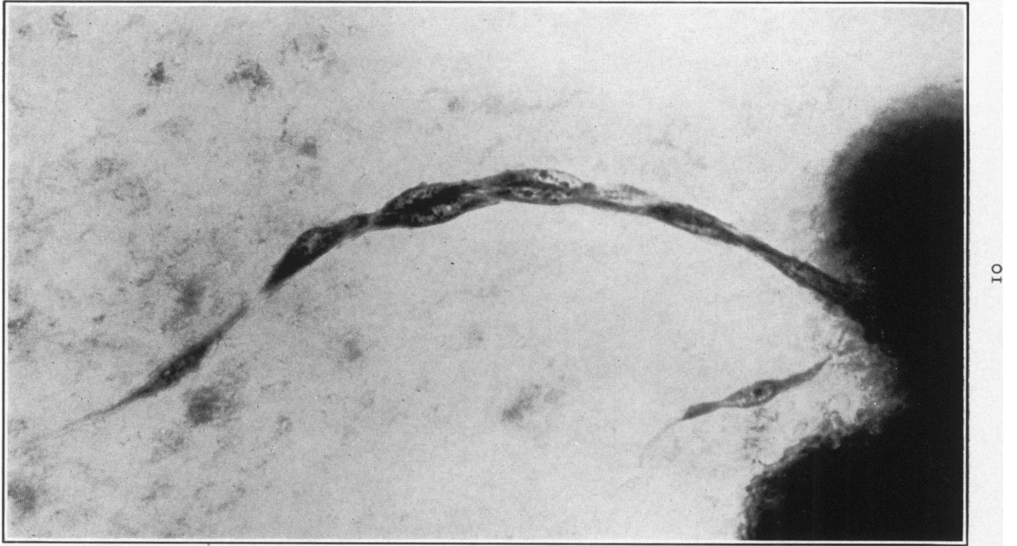
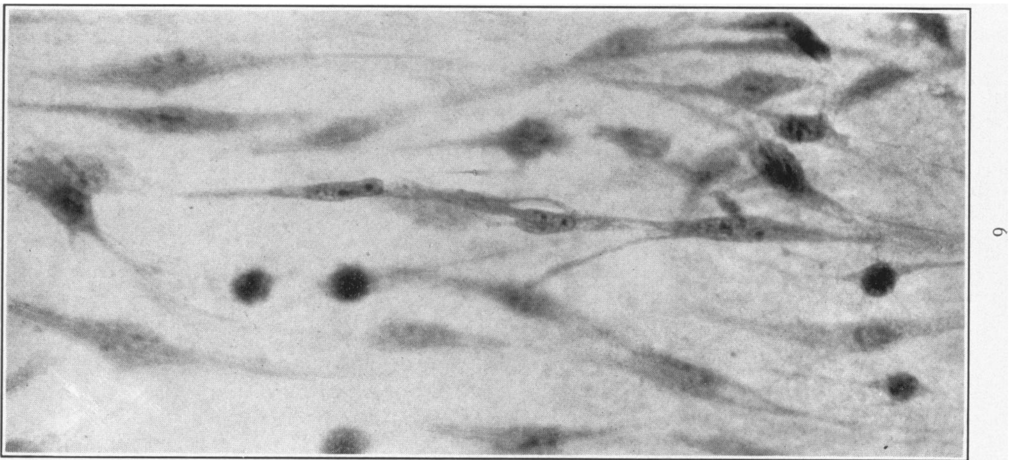
Murray and Stout Schwann Cell Versus Fibroblast

Plate 14

Fig. 9. Schwann cells from a distal amputation neuroma. Same procedure as in Figure 8.

Fig. 10. Double schwannian band from a distal amputation neuroma. Same procedure as in Figure 8.



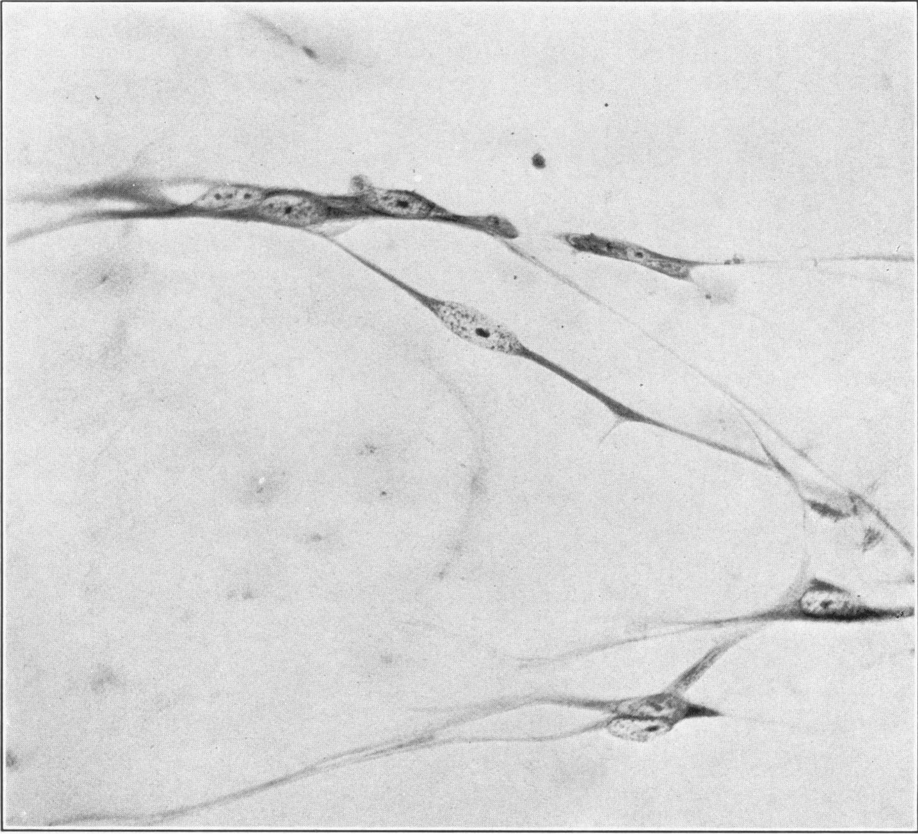


Murray and Stout Schwann Cell Versus Fibroblast

PLATE 15

FIG. 11. Outgrowth from A type area of neurilemoma, Case 1. Tissue culture 19 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.

FIG. 12. Another portion of the same culture shown in Figure 11.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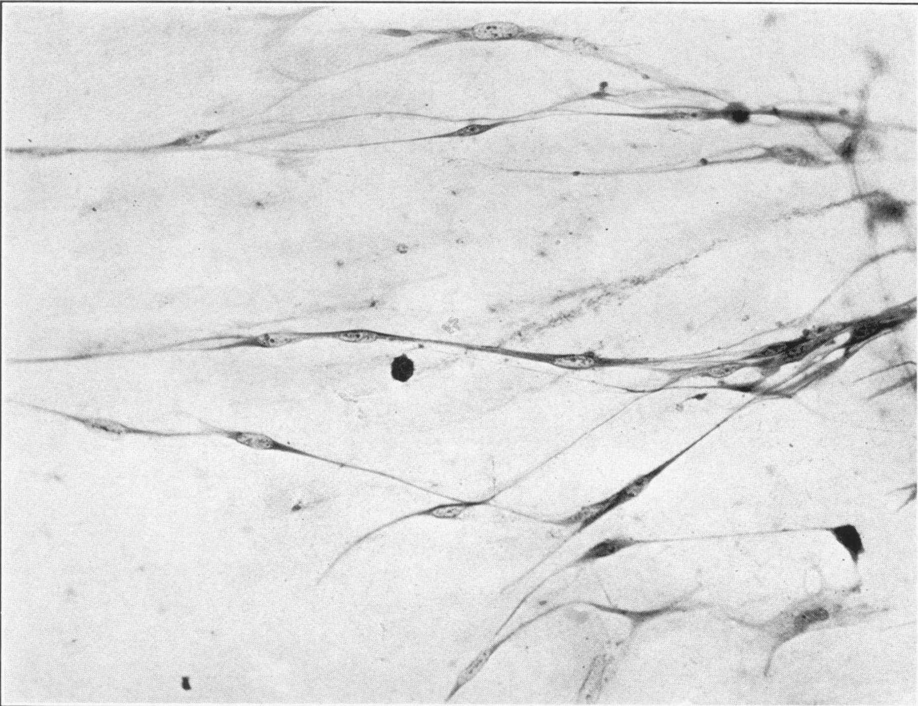
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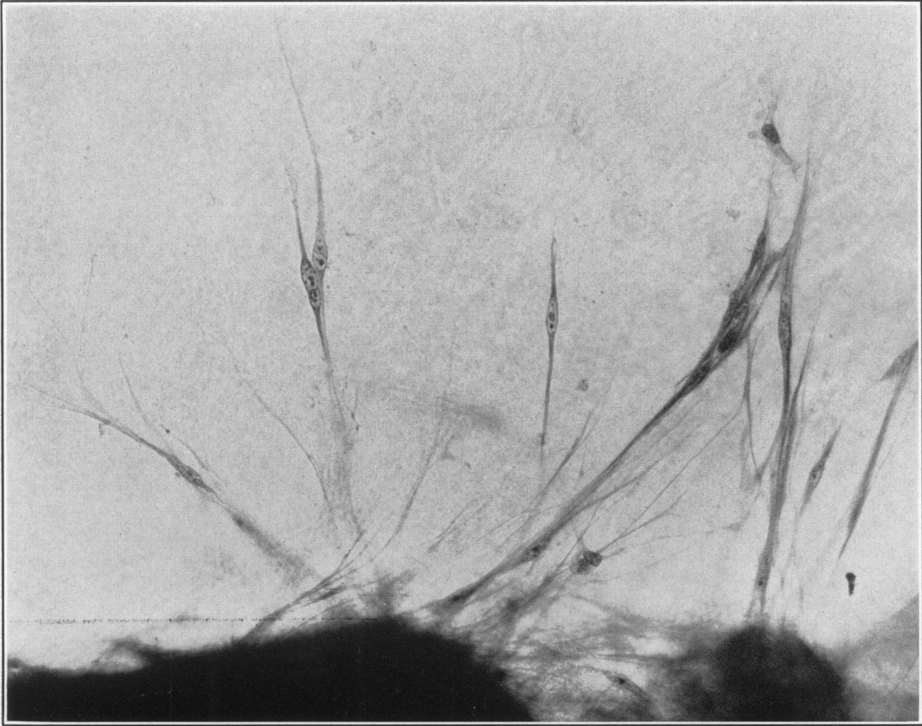
PLATE 16

FIG. 13. Outgrowth from A type area of neurilemoma from Case 1. Tissue culture 13 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.

FIG. 14. Outgrowth from a palisaded nodule, Case 1. Tissue culture 13 days *in vitro*. Bouin's fixation, phosphotungstic acid hematoxylin stain.



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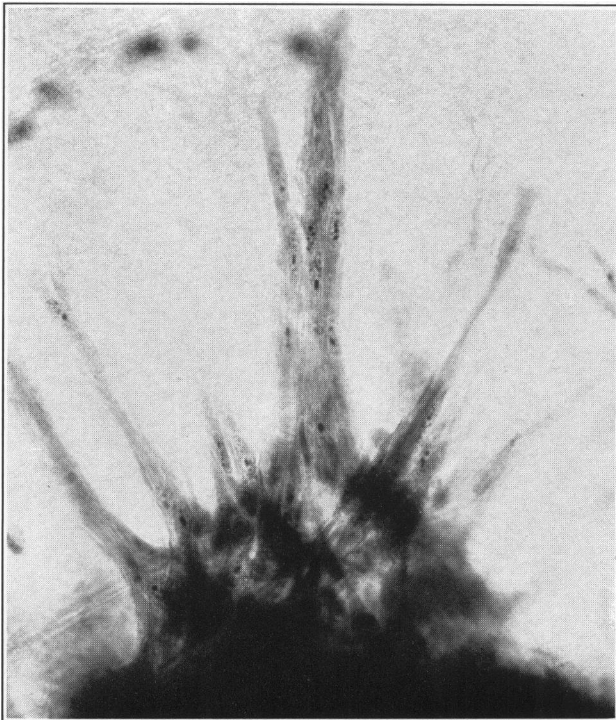

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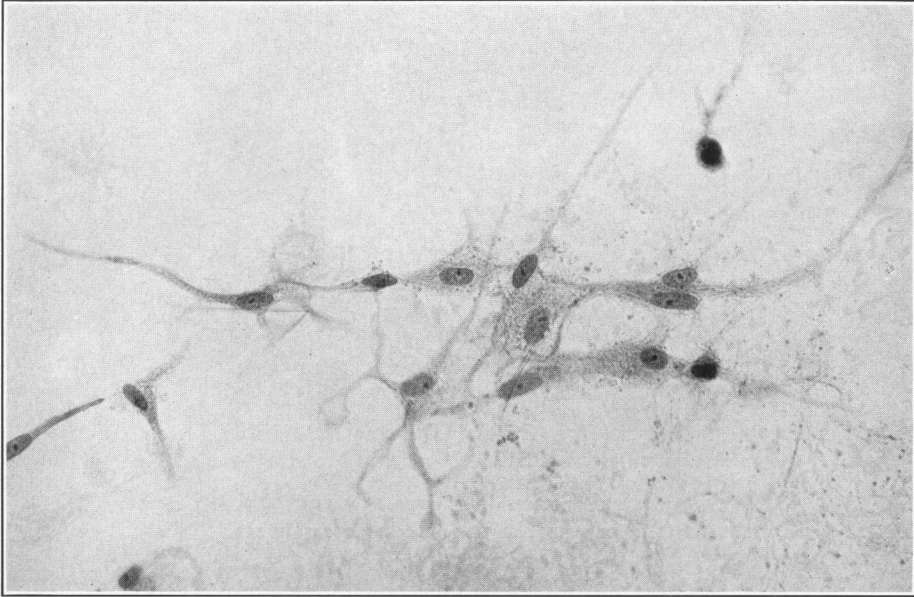
PLATE 17

FIG. 15. Outgrowth from B type area of neurilemoma, Case 1. Tissue culture 8 days *in vitro*. Bouin's fixation, phosphotungstic acid hematoxylin stain.

FIG. 16. From B type area, Case 1. Zenker's fixation, phosphotungstic acid hematoxylin stain.



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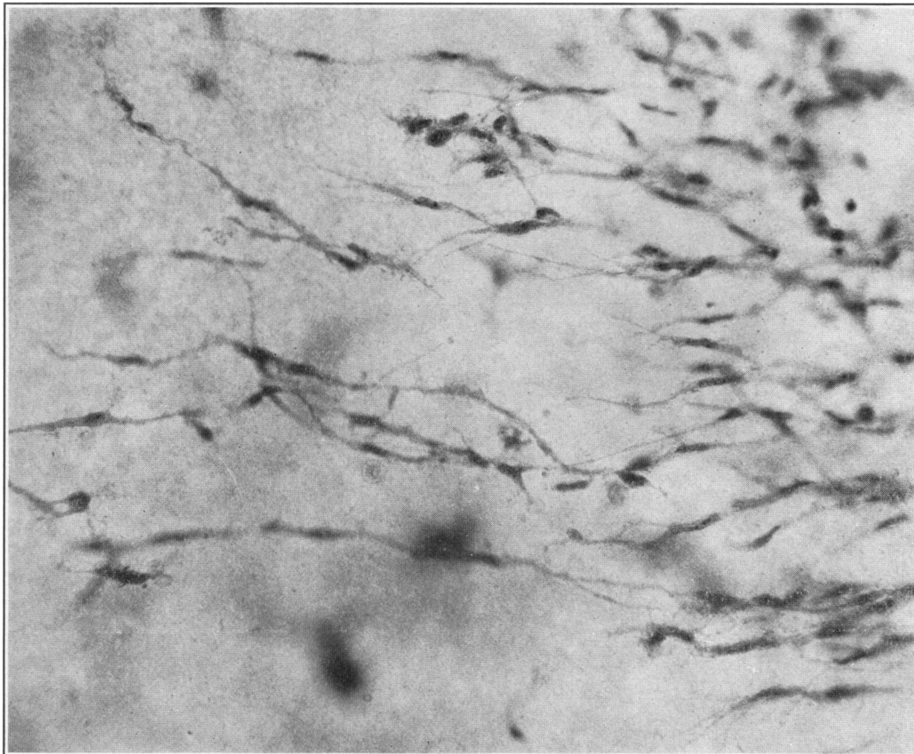

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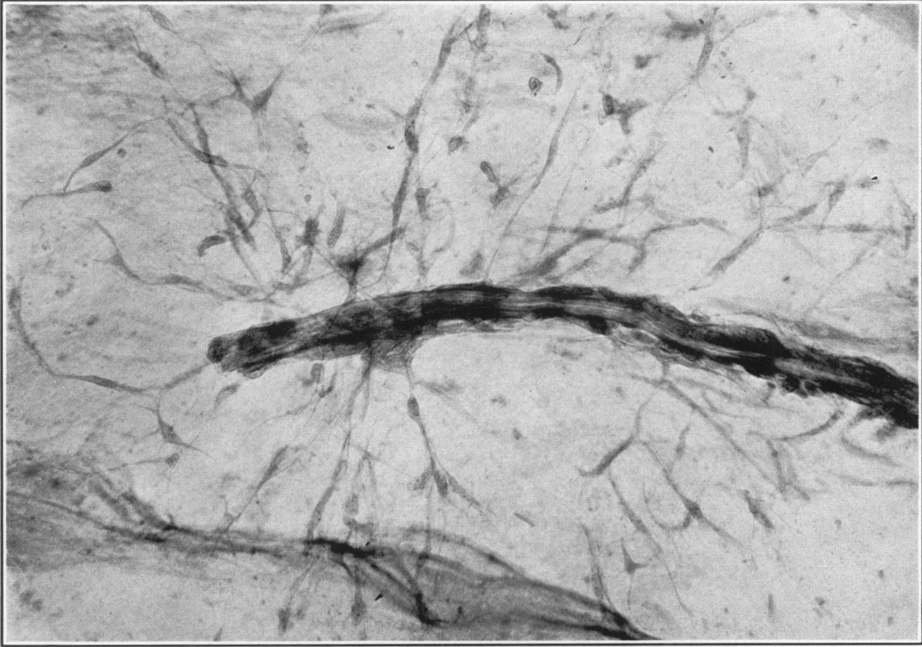
PLATE 18

FIG. 17. From B type area, Case 1. Extreme liquefaction. Tissue culture 8 days *in vitro*. Bouin's fixation, Delafield's hematoxylin stain.

FIG. 18. B type cells on a cotton fiber, Case 2. Tissue culture 7 days *in vitro*. Zenker's fixation, phosphotungstic acid hematoxylin stain.



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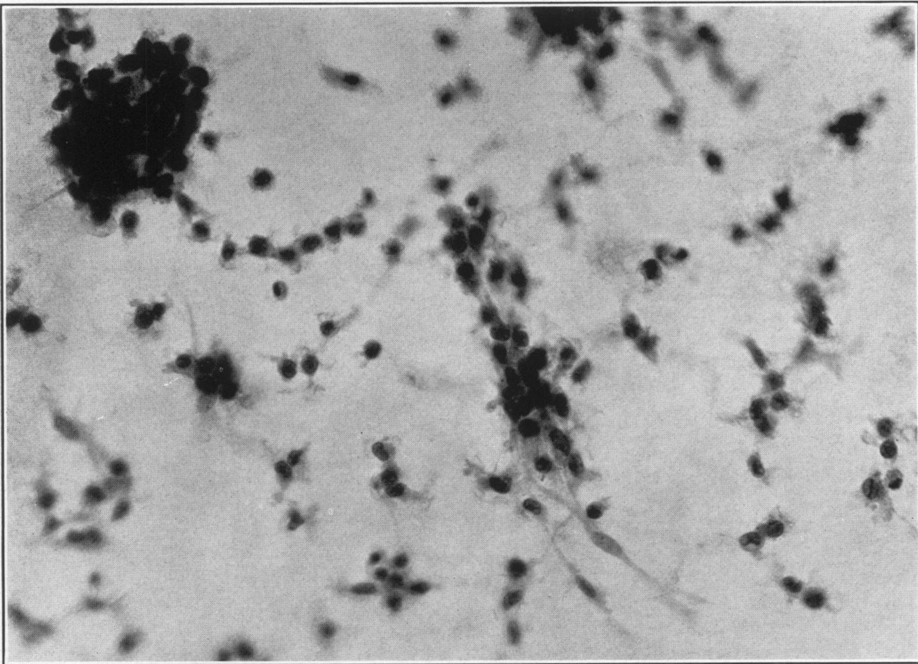

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