DEMONSTRATION OF THE FORMATION OF RETICULIN BY SCHWANNIAN TUMOR CELLS IN VITRO *

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In a recent communication we reported the *in vitro* cultural characteristics of Schwannian cells grown from normal nerves and from neurilemomas and demonstrated to our own satisfaction that neurilemomas are tumors composed of Schwannian cells. We inferred that the collagen and reticulin fibers in neurilemomas were formed by Schwannian cells, although at that time this had not been demonstrated *in vitro*.

More recently, by variations in technical methods, we have succeeded in demonstrating the formation of reticulin by Schwannian cells from a mediastinal tumor grown *in vitro*. This confirms the inference previously expressed and offers an entirely satisfactory explanation for the presence of connective tissue fibers in tumors of purely Schwannian origin.

The patient from whom the material was obtained was a female, 49 years old, who had had first interscapular pain and later paraplegia of the lower extremities for 4 years. A dumb-bell-shaped tumor was found which compressed the cord in the region of the fourth thoracic vertebra and extended through the intervertebral foramen to the right posterior mediastinum. The portion compressing the cord was first removed and 21 months later the mediastinal prolongation was excised. Two years after the first operation, and 3 months after the second, the patient was well and free from symptoms.

The tumor was encapsulated and the second portion, removed from the mediastinum, measured 7 by 5 cm. It was solid, with a firm, fibrous peripheral zone and a large softer center which was tinted yellow because of its large lipoid content. This tumor varied in its histological appearance in different parts. In the central area it had the characteristic appearance of a neurilemoma divided into A and B tissues with an unusually large number of phagocytic foam cells filled with lipoid. The Schwannian nuclei tended to be aligned in palisades but nowhere was there a definite organoid arrangement. Here there were extremely few reticulin fibers. In the peripheral zone there were few B areas and almost no lipoid. In this region the great bulk of the tissue was composed of Schwannian cells closely packed in

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masses and accompanied by many collagen and reticulin fibers. Some of these areas closely approached the appearance of neurofibroma. Chloral hydrate fixation and Cajal impregnation failed to show any neurofibrils. We were of the opinion that the tumor should be classified as a neurilemoma but it demonstrated what we have observed before—that neurofibroma and neurilemoma are in fact only different growth manifestations stemming from a single basic origin, namely, the Schwannian syncytium. Blood vessels consisting of endothelial lining and a rather thick collagenous sheath were scattered irregularly throughout the tumor.

The tissue used for explantation came from the periphery and included a larger proportion of the solid fibrous zone and a smaller amount of the lipoid-containing central area of characteristic neurilemomatous aspect.

Methods

The Maximow double-coverslip method of handling a hanging, or rather, a lying drop was used exclusively in the tissue cultures. These were explanted in a medium composed of 2/10 chicken plasma, 5/10 human placental serum, 1/10 chicken embryo extract and 2/10 serum ultra-filtrate, prepared by Dr. H. S. Simms from beef blood. The use of this medium reduced to a considerable extent the liquefaction of the clot which usually accompanies the growth of these tumors *in vitro;* so that it was generally possible to keep the cultures in their original situation on the coverslip without transferal throughout the period of cultivation. At intervals of 2 to 4 days they were washed in a buffered saline solution (Simms and Sanders) and the liquid components of the medium were subsequently renewed. Occasionally they were patched with fresh chicken plasma. For the washing it was found convenient to set the cultures vertically in small, covered Coplin jars designed to hold coverslips.*

At intervals during the 57-day period of cultivation, cultures were fixed in Zenker's or Helly's fluids and stained with Delafield's or phosphotungstic acid hematoxylin or with fuchsin-ponceau and aniline blue. Zenker's fluid was found to be the most favorable fixative for preceding silver impregnation for reticulin. For this the Bielschowsky method was used, as adapted for tissue cultures by McKinney from Foot and Mènard's modification. In all these procedures, coverslip Coplin jars were employed and the cultures handled in an upright position, which greatly facilitates drainage. The silver impregnations were counterstained lightly with toluidine blue, in 0.04 per cent aqueous solution.

* These jars, which are exceedingly useful in the various operations of tissue culture, are purveyed by the Arthur H. Thomas Co. of Philadelphia.

Growth Characteristics in Vitro

In vitro this mediastinal neurilemoma produced a characteristic Schwannian outgrowth of the same general type as that exhibited by other nerve sheath tumors which we have studied, and by normal adult nerves (Fig. 3). As already stated, the explanted tissue was composed chiefly of A-type tissue; this constitution was confirmed in the tissue cultures, which gave rise to almost no B cells.

Within 3 days after explantation the A type of Schwann cell began to grow. This cell at first appeared in single, filamentous formation resembling some forms of neuroglia, but was often multinucleate. Later it thickened, forming a ribbon or a bundle of parallel, anastomosing filaments (Fig. 2), which still later, after a month or so of cultivation, might develop into a bundle or tuft of ribbons (Fig. 1).

Characteristic of these bundles were their lateral anastomoses. In the tissue cultures there was an almost complete absence of the compression which usually obscures the relationships between these cells *in vivo*, and this permitted illuminating observations of their growth characteristics. By continuous observation, fasciculated bundles could be seen to form in essentially the same manner as postulated by Masson from the study of a tumor nodule distended by edema. Figure 2 might serve as the source of one of his diagrams. Palisading of the nuclei often occurs *in vitro*, but usually does not last over a long period, possibly because of the freedom of movement allowed the cells by their roomy environment.

After about 10 days *in vitro* some of the cultures produced, in addition to the A cells, a semimembranous outgrowth composed of broad, flat cells resembling endothelium which were usually to be found on the surface of the clot, as contrasted with the Schwannian cells which characteristically push their way through the clot. When treated with silver nitrate this semimembranous outgrowth was shown to form a mosaic of cells with blackened cement borders—characteristic of endothelium but not of Schwannian cells. It was therefore supposed that such cells were derived from the blood vessels of the tumor.

A 5-year study of human and animal, fetal and adult, normal and abnormal nerve sheath tissue *in vitro* has convinced us that Schwannian cells can be distinguished positively from fibroblasts in this medium, on the basis of growth characteristics and pattern, and on general morphology and physiology. We have no hesitation, therefore, in stating that some cultures, and many areas in all the cultures, were entirely free from cells of fibroblastic origin. Only such areas will be considered in the following discussion of fiber formation by Schwannian cells *in vitro*.

Reticulin Formation by Schwannian Cells

Reticulin formation in these cultures was slow, sparse and sporadic. The reticulin appeared first as a row of fine rods and granules which later coalesced to form long, single fibers running parallel to the long axis of the Schwann cell. The fiber always originated in close relationship to the cell, often appearing to lie in contact with the long-drawn-out cytoplasmic portion and making a curved detour around the lateral surface of the nucleus. Growth and migration of cell or bundle sometimes left these fibers behind without connection with any cell, but they did not so originate. They always appeared to develop singly, not as the mat of fibers described by McKinney in the mammalian lymph node, or as bundles of fibers described by Stearns in rabbit connective tissue.

In general, the reticulin fibers shown by silver impregnation were not in contact with fibers in the explant, but occupied a zone adjacent to the explant. Fibers which stained with aniline blue were always in contact with old fibers in a zone of dense outgrowth, so that it was difficult to be certain where the new growth of fibers began.

The first argyrophil fibers were observed in a 13-day culture. They were present in some older cultures and not in others. There was no orderly progress in fiber formation among the cultures as a whole. The speed and intensity of the process varied from culture to culture, as it appears to do in various areas of a tumor. By comparison, cultures of the stroma of an adenomatous human parathyroid gland, cultivated in the same medium under similar conditions, laid down reticulin fibers very much more densely, regularly and rapidly. Such fibers appeared first as criss-cross bundles in contact with the explant (Fig. 6).

In the neurilemoma cultures, reticulin was rarely seen in connection with macrophages or structures believed to be derived from blood vessels present in the explants. Great care was taken to exclude all such areas from consideration in the material used for the foregoing description of reticulin formation around Schwannian cells, and only cells and bundles which could be positively identified as Schwannian were included. Figures 4 and 5, showing reticulin, represent cell formations essentially similar to those of Figures 2 and 1, in which the cell structure and arrangement are brought out with hematoxylin staining.

Summary

Reticulin fibers have been shown to arise *de novo* in connection with Schwannian cells growing out from a mediastinal neurilemoma cultivated *in vitro*. The areas under consideration were devoid of cells of

fibroblastic derivation. The process of fiber production by Schwannian cells differs in certain respects from fiber production by cells of mesoblastic origin.

NOTE: We are greatly indebted to Irene Bokoff for technical assistance in the handling of the tissue cultures, and to Walter I. O'Neill for the photography.

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DESCRIPTION OF PLATES

PLATE 92

FIG. 1. Neurilemoma from mediastinum (no. 77393) after 24 days *in vitro*, showing Masson's fasciculated bundles of Schwann cells. Zenker's fluid, Delafield's hematoxylin stain.

FIG. 2. Neurilemoma from mediastinum, after 15 days *in vitro*, showing "aneuritic bundles" of Schwann cells, expanded in one plane. Helly's fluid, Delafield's hematoxylin stain.

FIG. 3. Schwannian outgrowth from small medullated fibers in normal human adult celiac nerve, after 24 days *in vitro*. Carnoy's fluid, Delafield's hematoxylin stain.


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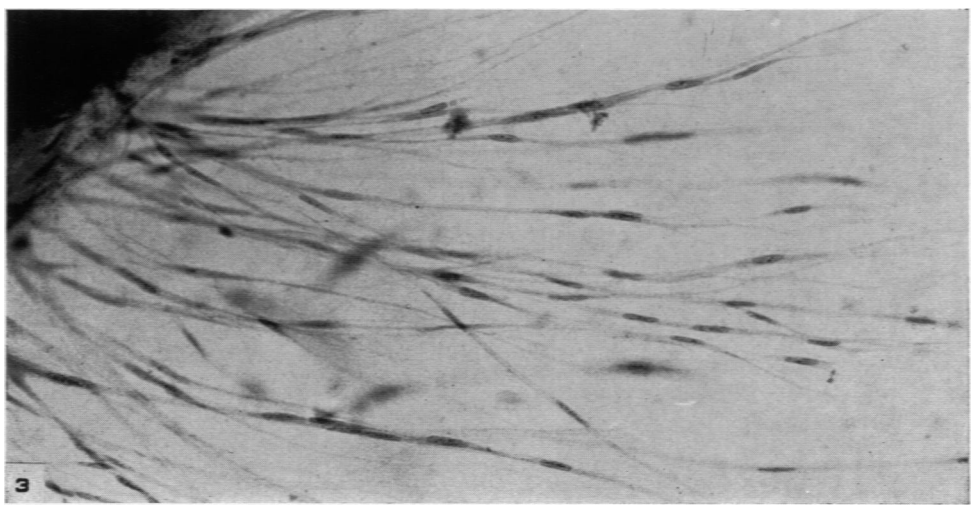
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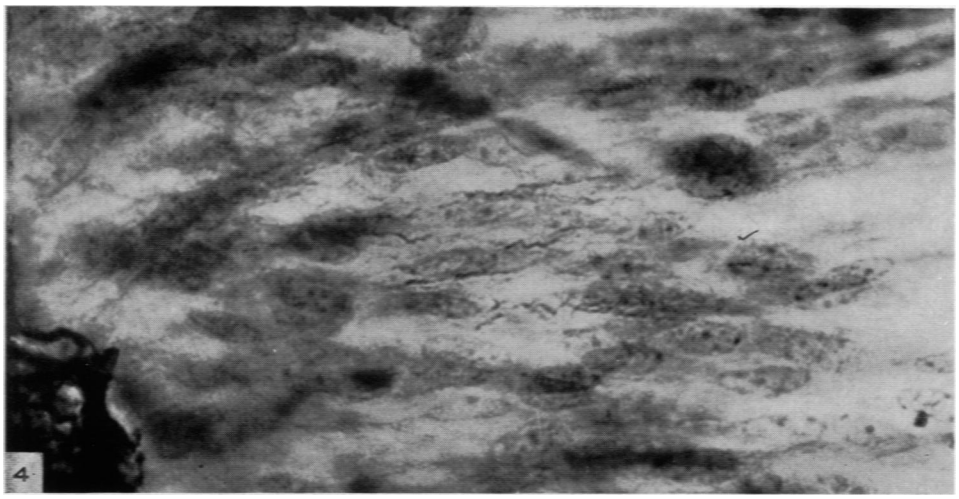
PLATE 93

(All three photomicrographs were taken at the same magnification, with high dry objective lens.)

FIG. 4. Neurilemoma from mediastinum (no. 77393) after 24 days *in vitro*, showing longitudinal reticulin fibers formed between Schwann cells. Zenker's fluid, Foot's silver impregnation.

FIG. 5. Similar to Figure 4, showing longitudinal fibers in a bundle of Schwann cells like those of Figure 1.

FIG. 6. Parathyroid adenoma (no. 76598) after 20 days *in vitro*, showing matted reticulin fibers formed among fibroblasts from the stroma of the tumor. Zenker's fluid, Foot's silver impregnation.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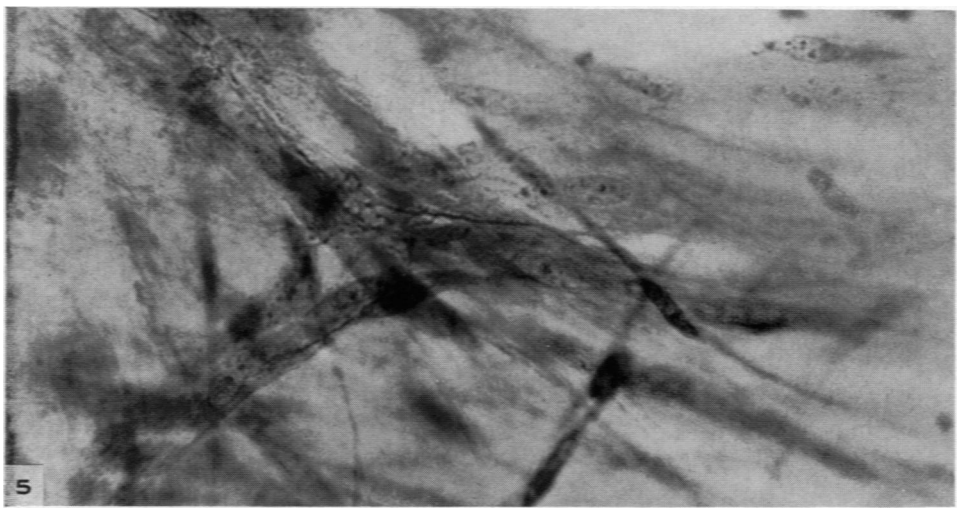
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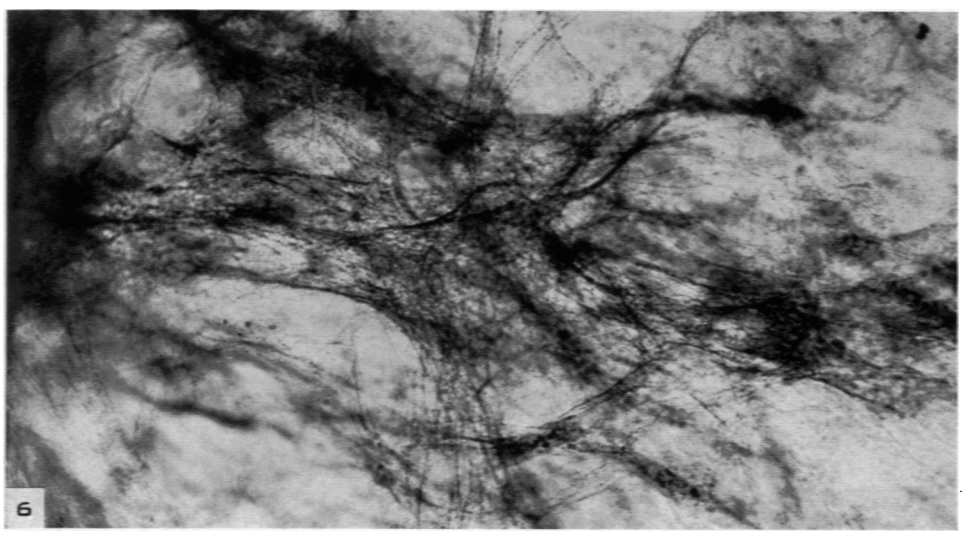
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