Technical Papers

The Electron Microscopy of Sectioned Nerve

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Nerve is one of the most important tissues for study under the electron microscope both because knowledge of its macromolecular structure is needed for an understanding of the mechanism of impulse propagation and because this knowledge is essential for any study of the neurotropic virus diseases. Until recently little progress in such a study has been possible on account of the large size and fragility of most nerve fibers. They can, however, now be examined to great advantage in thin section.

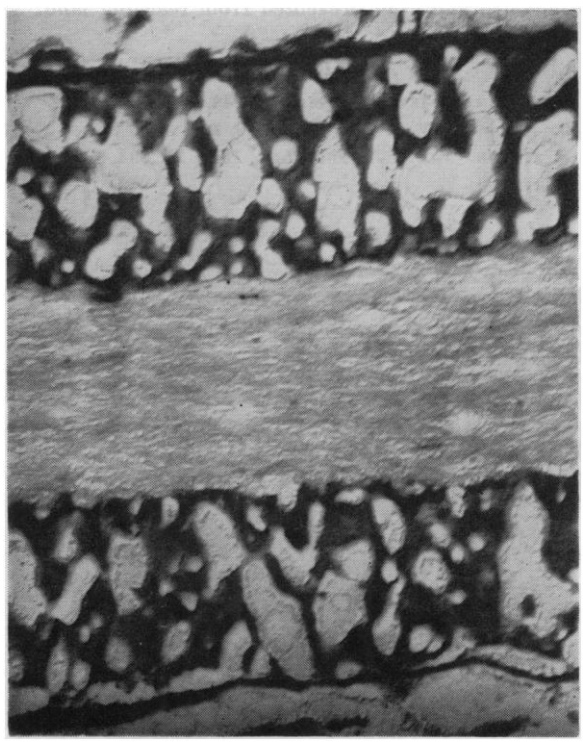

FIG. 1. An electron micrograph of a longitudinal section through a single fiber of myelinated nerve. The fibrous contents of the central axon are clearly seen, as are the denser lamellae of the surrounding myelin. The thin neurilemma enveloping the whole is visible at the very top and bottom of the photograph. Magnification, 5,000 ×.

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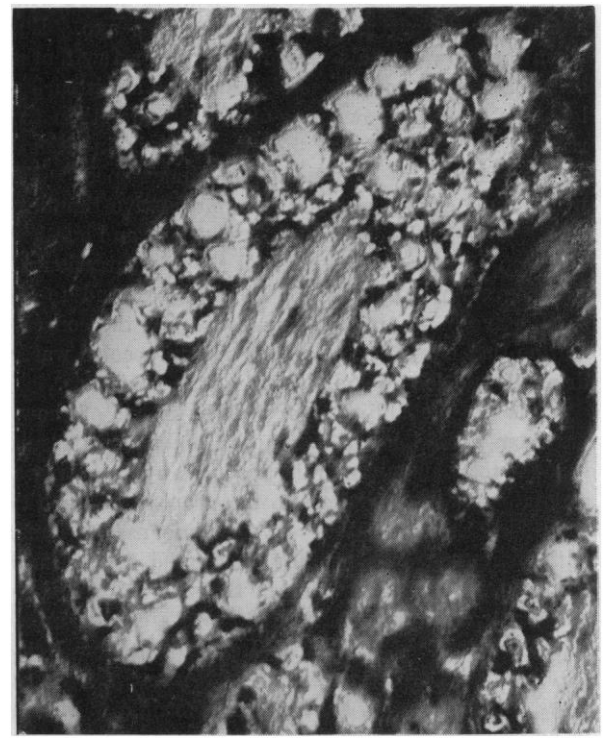

FIG. 2. A nearly transverse section through part of a nerve bundle. One fiber showing the same structures as Fig. 1 fills most of the picture. Small parts of two other fibers are in the lower right corner and the top, left of center. A band of connective tissue and the embedded sectioned tip of another fiber separate the lower fiber from the one in the center. Magnification, 5,000 ×.

Very recently preliminary electron micrographs have been published of sectioned nerve (*1*) but these photographs were of fibers that were so seriously damaged during preparation that their fine structures and the relation of these structures to one another were not evident. We have found that excellent sections can be obtained of nerve prepared and cut according to the techniques outlined by Newman, Borysko and Swerdlow (*2*). What is seen in such sections under the high resolution of the electron microscope depends in marked degree on the kind of fixation and dehydration used, and it will require much experimentation with many reagents to establish beyond doubt all aspects of the macromolecular texture of native nerve. Nevertheless, nerve can now be sectioned without disturbing its various components and consequently a detailed study can now be begun of its structure and of the changes caused by various preparative reagents.

Results of a first set of such experiments are being published elsewhere (*3*) but the accompanying photographs illustrate the kind of electron micrographs of sectioned nerve that can readily be prepared. These

show a longitudinal (Fig. 1) and nearly transverse (Fig. 2) section through the *nervus ischiadicus* of the adult rabbit fixed in 4% formalin and dehydrated in one case in ethyl alcohol and in the other with pyridine. In the longitudinal section one can see the filamentous fine structure of the central axon and the more "opaque" lamellar texture of the nonlipid portion of the enveloping myelin sheath. The neurilemma outside this and the absence of an axilemma of similar structure between axon and myelin are apparent. The same structures are visible in Fig. 2, which also gives an idea of the relation of a nerve fiber to the connective tissue elements with which it is associated in a complete nerve bundle. This photograph likewise brings out the fact that in many parts of nerve fibers the texture of the lamellae of the myelin is less coarse in the region immediately enveloping the axon. Other photographs we have made both at these and at higher magnifications show the fine details of the structures, the kinds of alteration in these details that result from the use of other fixatives, and the nature of such other optically recognized structures as the clefts of Schmidt-Lantermann and the nodes of Ranvier.

References

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Studies on Pituitary Adrenocorticotropin

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A considerable portion of the recent work on chemistry of the pituitary adrenocorticotropic hormone (ACTH) has indicated that it might have a relatively small molecular size. This is in contrast to earlier reports (*4*, *8*) of its protein nature and of the isolation of a "pure" protein having a molecular weight of approximately 20,000. Such possibilities are important in view of the limited supply of ACTH from animal sources, since synthesis of the hormone would provide a more adequate solution to the problem of supplying the clinical demand for the material.

Several preliminary reports on the preparation of active peptides from ACTH have appeared. The evidence provided in these preliminary reports does not definitely show that the activity observed is to be identified with a single peptide. Li (*5*) states that the *average* peptide size in a mixture of peptides following hydrolysis with pepsin of ACTH protein is eight amino acid residues.

Cortis-Jones *et al.* (*2*), employing ultrafiltration without partial hydrolysis, showed that biological activity will pass through cellophane membranes impermeable to molecules of 13,700 molecular weight but permeable to those of 8,000. Morris (*7*) indicated that the size of the ACTH molecule varies with pH and that this variation appeared to be a reversible phenomenon.

Brink *et al.* (*1*) reported the clinical activity of dialyzates following peptic hydrolysis.

Li (*6*), employing an acetic acid-butanol-water system on paper, separated six ninhydrin-positive spots, one of which was highly active in the adrenal ascorbic acid assay. Sedimentation and diffusion studies that were made on the peptide mixture led to an average molecular weight of 1200 for the peptide mixture that contained no free amino acids.

Some confusion is added to the picture by the use of ACTH from different species, but there is little evidence thus far to indicate major differences in the hormone from the pituitaries of the three species most widely used, namely, swine, sheep, and cattle.

TABLE 1

FRACTIONATION OF ACTH BY ADJUSTMENT OF pH IN AQUEOUS SOLUTION

Preparation	Initial potency × La-I-A	Fraction insoluble at pH	Fraction soluble at pH	Yield %	Potency × La-I-A
1	2.2	7.3		7.1	8
		6.3		6.2	6
		5.6		5.2	10
			5.2	70	1
2	2	7.3		5	8.6
		6.2		6	2.3
		5.2		5	11
			5.2	67.5	0.5
3	1.9	6.6		10.8	10
		6.0		3.3	6
		5.0		5.4	6
			5.0	74	1.5
4	1.9	7.2		10.5	4
		6.2		6	7
		5.2		6	7
			5.2	75	1.5
5	4	7.6		5.7	22
		6.3		3.5	21
		5.3		4.1	11
			5.3	75	1.6

Our studies on the properties of ACTH carried on over the past two years have been confined largely to that from pork pituitaries, which provide the best yield and upon which clinical experience is based. Although we have been able to concentrate the hormone and have obtained fractions with potencies of the order of 100–150 times standard or 100–150 times that of the previous reported pure ACTH proteins, these fractions appear to have molecular weights of from 2,500 to 10,000 rather than 1,000 or less. The general methods of preparation and characteristics of these fractions are described.

As starting material for preparation of more potent fractions, ACTH prepared for clinical use was employed. These preparations were obtained from fresh, frozen, whole hog pituitary glands by acid-acetone extraction followed by phosphate partition and ammonium sulfate