Missouri Section

St. Louis University School of Medicine, February 13, 1935.

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Chemical and Optical Properties of Nerve Proteins.

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Previous experiments on thermal shortening and on solvation and desolvation of medullated and non-medullated nerves indicated the presence of oriented protein primary valence chains in these nerves.[1] Similar oriented structures are present in most fibrous tissues.[2] To characterize these structures chemically and to localize them morphologically, experiments have been performed on the chemical and optical properties of normal nerves, of nerves which have been extracted in a variety of ways, and of fibers artificially spun from protein extracts of nerves. The present paper is a preliminary report of this work.

Because of certain difficulties inherent in the procedure of extraction in the case of medullated nerves, and in order to investigate axis cylinder material in as nearly pure form as possible we have turned to non-medullated nerves of the claws and legs of lobsters. The extractions indicate that essentially the same 3 nucleoproteins that McGregor[3] isolated from mammalian central nervous systems are to be found also in lobster nerves. Of particular significance in this connection is the third nucleoprotein extractable at pH 13 to 14. This appears to be the protein which has been called neurostromin by Shkarin.[4] After complete extraction of all proteins soluble below pH 13, the individual finely cut nerve bits still retain their original shape and general structure remarkably. Moreover, such

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1 Schmitt and Wade, *Am. J. Physiol.*, 1934, **109**, 93; 1935, **111**, 159, 169.

2 Meyer, K. H., *Biochem. Zeitschr.*, 1929, **208**, 1; 214, 253.

3 McGregor, *J. Biol. Chem.*, 1917, **28**, 403.

4 Shkarin, Inaugural Dissertation, St. Petersburg, 1902, **1**, 453, as quoted by McGregor, p. 421. In many respects neurostromin appears to be similar to the ''ellipsin'' of Bensley and Hoerr (*Anat. Rec.*, 1934, **60**, 251).

extracted nerve bits display thermal shortening to an extraordinary degree. Although it is impossible to state that other proteins are not involved in thermal shortening of lobster nerve, neurostromin must play the major rôle in the phenomenon.

While it has long been known that fresh lobster nerves show fairly strong positive birefringence we have found that after complete extraction in M/100 NaOH, the remaining neurostromin residue is equally if not more strongly positively birefringent. Extraction with stronger alkali attacks the neurostromin and brings about complete solution of the residue. Coincident with this destruction of the protein is the disappearance of birefringence, although thermal shortening persists as long as there is any visible structure.

Göthlin[5] observed that when lobster claw nerves are immersed in glycerin the birefringence rapidly changes from positive to strongly negative. We have confirmed and extended these observations and find that the reversal may be obtained equally strikingly with neurostromin. Soaking in alcohol prevents this reversal with neurostromin as well as with normal nerve.

It is well known that long asymmetric protein micelles or macromolecules tend to aggregate into fibers when spun from fine capillary tubes into precipitating media. Fibers spun from myosin for example, have been shown to have the same birefringence and to give the same X-ray diffraction pattern as normal muscle.[6] In the case of nerve proteins we have obtained fibers from both of the alkali-soluble nucleoproteins by forcing the alkaline solutions through fine capillaries into dilute acetic acid. Fibers thus made are weak and difficult to manipulate. However, by spinning the proteins into an acetic acid solution containing 30-50% alcohol, well formed fibers have been obtained. Denaturation does not occur under these conditions since the fibers remain soluble in dilute alkali and the proteins are precipitable again by acid. While the freshly formed wet fibers are only weakly birefringent, they show strong positive birefringency when dried. The ease with which these protein extracts may be spun into fibers substantiates the above thermal and optical evidence of the presence of long asymmetric micelles or macromolecules in the axis cylinder of normal nerve. It is not surprising that the thermal shortening, swelling, etc., of spun fibers is not comparable to that of normal nerve since the alkali treatment required for the extraction of the proteins probably has a destructive effect upon certain of the side chain linkages

[5] Göthlin, Kungliga, *Svenska Vetenskapsakademiens Handingar*, 1913, **51**, 1.
[6] Weber, *Ergeb. d. Physiol.*, 1934, **36**, 109.

which bind the primary valence chains laterally. How successfully methods can be devised for spinning fibers which shall have the same thermal and optical properties as normal nerve remains to be determined.

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Experimental Hypersensitiveness to Staphylococcus.*

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The occurrence of type-specific skin reactions to carbohydrates in patients with Staphylococcus infections[1] presented the opportunity for studying experimentally hypersensitiveness to Staphylococcus and its constituents. The recent demonstration that this organism is separable into two distinct immunological types,[2] A and B, dependent upon the presence of chemically and serologically different polysaccharides, has made it possible to consider the influence of type- and species-specificity on the reactions of increased tissue sensitivity. Both monkeys and rabbits were studied to observe evidence of hypersensitiveness to Staphylococcus and its derivatives.

Monkeys *(M. rhesus)* were given injections of Type A or Type B organisms. In one experiment 4 monkeys were given 9 injections intracutaneously of heat-killed bacteria, repeated at weekly intervals. The injections caused only small nodules at the site of inoculation, and the successive reactions following the repeated inoculations were of approximately the same size and severity. In a second experiment, 4 monkeys were inoculated with live bacteria into a subcutaneous agar focus. This was repeated at 3 different times after the effects of the succeeding inoculation had healed completely. The animals in both experiments were skin tested to Type A and B carbohydrates at different periods during the course of observation, but at no time was skin reactivity elicited despite the employment of graded dilutions of carbohydrate. At the termination of the experiment skin tests to carbohydrates were again repeated using 0.2 cc.

*Conducted under a grant from the Commonwealth Fund of New York, N. Y.

1 Unpublished data.

2 Julianelle, L. A., and Wieghard, C. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 947.