Reports

Migration of Proteins along the Axons of the Sciatic Nerve

Abstract. Soon after single or repetitive injections of leucine-H^3 in rats, an intense radioautographic reaction was seen over the nerve cell bodies, while the axons of the myelinated fibers of the sciatic nerve were insignificantly labeled. Later, however, these axons showed strong radioactivity, which at 4 days was located in a proximal and at 16 days in a distal region of the nerve. It was concluded that the protein synthesized in the nerve cell bodies of the sciatic nerve had migrated along its axons.

The "axonal flow" hypothesis states that the axoplasm of peripheral nerve fibers originates in, and continuously migrates away from the nerve cell body. This hypothesis was proposed by Weiss to explain the accumulation of axoplasm (*1*, *2*) and enzymes (*3*) on the central side of a constriction or transection of a nerve.

Several attempts were made to obtain supporting evidence from experiments on intact neurons. Radioactive substances were given, phosphate-P^{32} (*4*), glucose-C^{14} (quoted in *1*), and labeled amino acids (*5*), which, it was hoped, would be incorporated into the nerve cell body only and would then flow down the axon. Because the substances used are taken up not only by neurons but also by Schwann cells, connective tissue, and blood vessel cells present in peripheral nerves, the difference in radioactivity in successive segments of nerve trunks was so small that the results must "be rated as suggestive rather than conclusive" (*1*).

A different approach was an attempt to irreversibly inactivate the acetylcholinesterase present along the length of nerve fibers and to watch for its reappearance. The enzyme returned along the nerve uniformly, and, therefore, was presumed not to have migrated from the nerve cell body (*6*).

On the whole, the evidence in support of the axonal flow hypothesis is not conclusive, and there is a need for experiments that would either prove or disprove it. We decided to repeat the attempts at tracing amino acids along the axon, but with a tritium label. The high radioautographic resolution obtained with tritium might make it possible to show the presence of radioactivity in axons, as distinct from that in Schwann and connective tissue cells. Any migration along the axon might then be demonstrable.

Rats weighing 45 g were given nine injections of 3.3 μc of DL-leucine-4,5-H^3 per gram of body weight every 3 hours over a 24-hour period (total dose, 30 μc/g of body weight). One rat was sacrificed 27 hours, another 4 days, and a third 16 days after the first injection. Also, one animal received a single injection of 20 μc/g of body weight and was sacrificed 30 minutes later, in the hope of detecting the sites of protein synthesis. Spinal cord, spinal ganglia, and sciatic nerve were removed, fixed in Bouin's fluid, and radioautographed after histological processing (*7*). Longitudinal and transverse sections of the nerve were examined. One set of transverse sections was taken at 1.6 mm beyond the junction of the L_V-L_{VI} roots, that is, about 8 mm from the vertebral column, and will be referred to as "proximal." A second set of transverse sections was taken at 20 mm beyond the root junction, that is, approximately behind the knee joint, at a distance of about 26 mm from the vertebral column, and will be referred to as "distal." The silver grains were counted and the counts expressed as number of grains per 10 μ^2 of axonal section (Fig. 1).

In the animals sacrificed at 30 minutes and at 27 hours after the first leucine-H^3 injection, strong radioactivity was present in the nerve cell body of neurons of spinal cord and ganglia. Examination of the sciatic nerve showed silver grains over Schwann and connective tissue cells in both the proximal and distal regions (Fig. 2), but the number of grains over the axon was quite small and considered to be insignificant (Figs. 1 and 2).

Four days later the radioactivity in the nerve cell bodies of spinal cord and ganglia was weak, while that in Schwann and connective tissue cells had decreased only slightly. At this time, however, the axons in the proximal but not in the distal region were strongly radioactive (Fig. 1).

At 16 days there was a further decrease in the radioactivity of the nerve

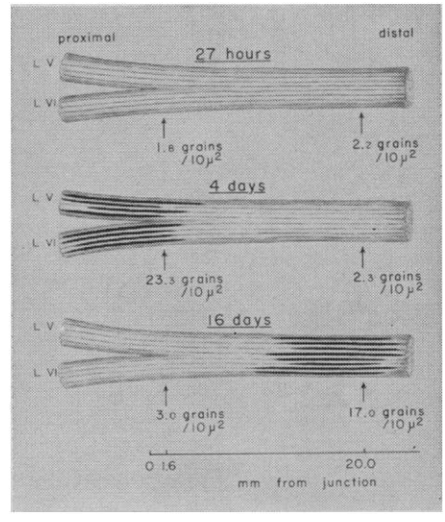

Fig. 1. Diagrams constructed from observations made on longitudinal sections of sciatic nerve. At left, the L_V and L_{VI} roots of the nerve and their junction. The heavy black lines in the second diagram (4 days after injection) and third diagram (16 days after injection) indicate the location of the axonal radioactivity at these times. Grain counts made on transverse sections at 1.6 and 20.0 mm from the junction and expressed per 10 μ^2 of axon are given below each diagram. It may be seen that axonal radioactivity is not significant at 27 hours, and appears in the proximal region at 4 days and in the distal region at 16 days after injection of leucine-H^3.

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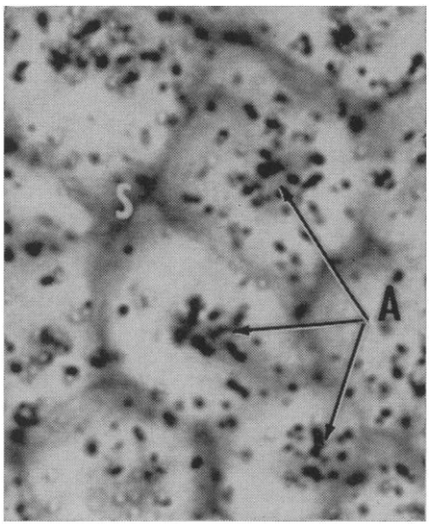
Type manuscripts double-spaced and submit one ribbon copy and two carbon copies.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each. Submit three copies of illustrative material.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].



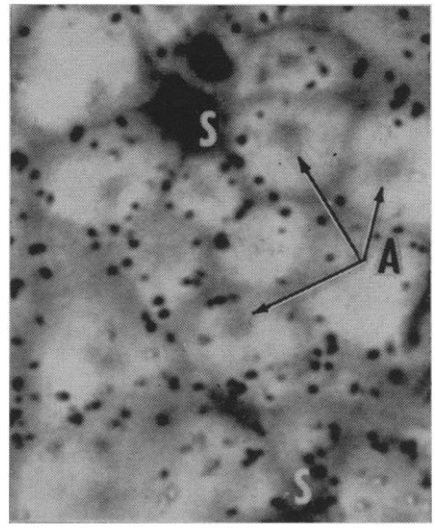


Fig. 2 (left). At 27 hours, that is, relatively early after injections of leucine-H^3, no significant radioautographic reaction was observed over the axons of the sciatic nerve (*A*), but silver grains were located over Schwann cell nuclei (*S*) and cytoplasm. Fig. 3 (right). After 16 days, silver grains were concentrated over the axons (*A*) in a distal region of the sciatic nerve, while the reaction over Schwann cell cytoplasm (*S*) is lessened.

cell bodies and of the cells located along the nerves. By this time, radioactivity was back to a low level over the axons in the proximal region (Fig. 1) but had reached a high level over those in the distal region (Fig. 3). It may be emphasized that the presence of silver grains over every single axon indicates that all nerve fibers, whether sensory or motor, were involved.

It is known that the radioactivity detected by radioautography in histological sections soon after leucine-H^3 injection consists of newly synthesized proteins (*8*). Therefore, the presence of radioactivity at the early time interval in the cells of spinal cord and ganglia indicates synthesis of protein [in confirmation of data showing continuous protein synthesis in nerve cell bodies (*9*)]; but there is no demonstrable synthesis in the axons of the sciatic nerve. Nevertheless, labeled proteins were detected in the axoplasm of the proximal region of the sciatic nerve 4 days, and in that of the distal region 16 days, after the first injection. Hence, the proteins synthesized in the nerve cell bodies must have migrated along the axons at a speed such that the proximal region was reached by 4 days and the distal region by 16 days (Fig. 1). The data permit only a very rough estimate of the migration rate of axoplasmic proteins—about 1.5 mm per day.

The present results offer the first direct evidence in support of the axonal flow hypothesis. Further direct evidence may be available in the near future, since a recent abstract by Weiss *et al.* (*10*) indicated that the migration of axoplasmic material may be seen in vitro in mouse peripheral nerves explanted with their ganglia. It is concluded from the present data that proteins migrate along the motor and sensory axons of the sciatic nerve of the rat (*11*).

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Chromatographic Validation of Two Morphologically Similar Hybrids of Different Origins

Abstract. The origin of natural hybrids of the plant *Baptisia* was determined by chromatographic analyses of leaf extracts of both hybrid and parent plants. This method demonstrated a pattern of inheritance in the hybrid of certain species-specific components of both parents.

After the first demonstration that chromatographic techniques are useful in validating the hybrid nature of suspected interspecific hybrids of *Baptisia* (*1*), the method was applied to the study of a trihybrid population to determine its composition (*2*) and also to validate a number of other combinations of suspected interspecific hybrids in the genus (*3*). One important generalization derived from these investigations is that hybrids of *Baptisia* tend to accumulate the sum of the species-specific components of both parents. Other investigators have reported similar results with different genera (*4*). This pattern of inheritance of species-specific components makes possible the determination of the origin of a particular hybrid, in some instances through chromatographic analyses alone and in other less favorable combinations through combined chromatographic and morphological analyses. Until now, it has not been possible to state unequivocally that a certain hybrid type could *only* be identified by means of a chromatographic analysis although some combinations bear a rather close morphological resemblance to each other. We can now report an example of hybridization involving three species, two of which are so similar in gross characters that it is only with great difficulty, if at all, that one can determine from external characters which of the two species is involved in hybridization with the third species in a particular instance. Chromatographic patterns can be used to establish the origin of these plants immediately, beyond doubt. Both of the nearly indistinguishable hybrid types have now been identified chromatographically from the same location.

In 1961 a putative hybrid between *Baptisia lanceolata* and *B. pendula* (*5*) was collected along state highway 56, 0.5 mile south of the Ogeechee River, Emanuel County, Georgia. Both *B. lanceolata* and *B. pendula* were observed in the area, and the former was seen in the immediate vicinity of the hybrid. A chromatographic analysis of