Myelin from Human Peripheral Nerves

QUANTITATIVE AND QUALITATIVE STUDIES IN TWO AGE GROUPS

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ABSTRACT Myelin in femoral nerve segments obtained at autopsy was isolated quantitatively by a series of discontinuous and continuous flotation procedures. The total amount of myelin isolated from these nerves was expressed as the sum of cholesterol, glycolipid, phospholipid, and protein and averaged 2.6±0.4 mg/g in a group aged 60–77 yr compared with 10.8±1.9 mg/g of nerve in a group aged 35–58 yr. The lower value in the older group remained apparent whether the myelin content was related to the whole nerve segment, its unit length or weight. This indicates that the decrease is an absolute one, not related to a change with aging in the nonmyelin content of nerve.

No qualitative differences in myelin lipids were found between the two groups. Protein content was, however, significantly higher in the older group (34 and 28.7% of the total myelin weight, respectively).

The decrease in myelin content with aging, observed by direct measurement in this study, may be the structural counterpart to age related alterations in peripheral nerve function-decreased conduction velocity, and impaired appreciation of vibration.

INTRODUCTION

It has been suggested on the basis of morphological studies that the amount of myelin in peripheral nerves can be affected by a variety of disease states (2, 3) and is decreased, in man, with aging (4). These estimates of myelin content have been based on measurement of the cross-sectional area with staining properties of myelin in whole nerves, or on measurement of internodal distances in isolated nerve fibers. They, at best, provide semi-quantitative information, are tedious to carry out, and are subject to considerable error related to fixation and the size and configuration of the nerve under study.

Methodological advances are now available that permit quantitative isolation of myelin from peripheral nerves. We have utilized such methodology to measure directly the amount of myelin in human peripheral nerve–the intra-abdominal portion of femoral nerves obtained at autopsy from persons in two age groups (35–58 and 60–77 yr). By utilizing this methodology we found the amount of myelin, measured after isolation was much lower in the older group. The composition of myelin, however, was similar in the two age groups and resembled that reported for other mammals.

METHODS

Subjects. Nerves were obtained from 13 people whose ages and diagnoses are listed in Table I. All were autopsied by the Medical Examiner of New York City within 36 h of death. In all cases, death was sudden and in the absence of debilitation. Persons known to have been narcotics abusers were not included and will be the subject of a separate report. Since the three persons with fatty liver and suspected of having used alcohol to excess did not differ from the rest of their age group, they were included.

Collection and handling of nerves. The portion of the femoral nerve from its origin at the lumbar roots to its exit from the abdomen at the inguinal ligament was chosen for study because it was readily available without extending the usual autopsy, and because it provided adequate material for analysis. Nerves from either the right or left side were used for this study. They were stored in preweighed screw cap containers at −5°C until being processed; the duration of storage was equivalent for specimens from both age groups.

Isolation of myelin. Nerves were thawed and cut into fragments about 1 cm in length and incubated for 24 hr in a glycine-triethylamine hydrochloride buffer as described by Adams, Abdulla, Turner, and Bayliss (5). This permitted removal of most of the neurolemma and yielded material that could be homogenized easily in a "Turbo-Shear" as-

This work was published previously in abstract form (1).
Received for publication 29 August 1972 and in revised form 30 October 1972.

TABLE I

Clinical Data in the 13 Cases

Age group	Age	Sex	Cause of death
	Yr		
I 35–58 yr	37	F	Accident-fire
	39	M	Found dead; fatty liver
	42	M	Homicide, gun shot
	45	M	Sudden death, rheumatic valvular disease
	55	M	Suicide, shooting
	58	M	Sudden death; fatty liver
II 60–77 yr	60	F	Automobile accident
	66	M	Sudden death; coronary occlusive disease
	66	M	Found dead, cancer of prostate gland
	72	M	Sudden death; fatty liver
	75	M	Smoke inhalation
	75	F	Homicide, shooting
	77	M	Sudden death; coronary occlusive disease

sembly (The VirTis Co. Inc., Gardiner, N. Y.). Myelin was isolated by a modification of the method of Autilio, Norton, and Terry (6) that we have used previously for isolation of myelin from mouse brain (7). This method includes a series of flotations in a discontinuous gradient; "osmotic shock" for the separation of myelin from other neuronal material, as well as the final isolation of myelin by flotation in a continuous cesium chloride gradient.

In order to determine the loss of myelin in the preparative steps, the nonmyelin material—both the heavy pellets and the fat-like cake that floated to the top of the 0.25 M sucrose phase—were pooled from each of the steps in the discontinuous gradient flotations and were then subjected to the entire isolation procedure. In no instance, using material from both age groups, could any material be recognized from these fractions that had the flotation characteristics of myelin.

Purity of the finally isolated material was evaluated by several criteria. A single compact band of myelin was obtained by flotation in the final continuous gradient of cesium chloride. By electron microscopic examination, the absence of nerve trunks as well as other nonmembranous material was verified. The preparation was completely soluble in chloroform: methanol (2:1). Myelin obtained by similar methods from the sciatic nerves of living rabbits was analysed for NADPH cytochrome C reductase activity and the specific activity of that enzyme in the myelin preparation was less than 10% of that in microsomes. Microsomal membranes, the most likely contaminant in the flotation steps was shown to separate clearly from the myelin fraction in the cesium chloride gradient.

Chemical determinations. Total proteins, cholesterol, glycolipids, and phospholipids and the individual phospholipid components were measured as reported previously (7).

RESULTS

Quantitative. The amount of myelin is reported as the sum of the protein, cholesterol, phospholipid, and glycolipid. In Fig. 1, the amount of myelin in the nerve fraction is depicted for the whole nerve segment, per gram of wet weight of nerve, and per centimeter of nerve length as related to age for the whole group. In the 35–58 y age group, the myelin content averaged 50.1±7.1 mg[1] in the nerve segment; 10.8±1.9 mg/g of nerve; and 2.6±0.4 mg/cm of nerve while in the older (60–77 y) group the averages were 9.9±2.8, 3.4±0.6, and 0.8±0.2, respectively. As indicated in Fig. 1, there was very little overlap between the two groups and differences were highly significant when analyzed by the rank order test.

Qualitative. Average values for the molar lipid composition are presented in Table II and fail to reveal any significant differences between the two groups. Protein content was, however, significantly higher ($P < 0.01$) in the older age group as compared to younger age group and was 34.0% and 28.7%, respectively. In general they have the composition of peripheral nerve myelin found in other mammals, as also indicated in Table II.

DISCUSSION

In this study we have determined for the first time the quantity of myelin in human peripheral nerves. This quantification was based on direct measurement of isolated myelin. The nerve segment utilized for study was the intra-abdominal portion (nerve root to inguinal ligament) of the femoral nerve, a mixed nerve with both sensory and motor fibres of varying degrees of myelinization.

Several methodological problems have to be considered in the interpretation of these data. It is difficult to be certain that the amount and composition of myelin is not appreciably altered during the period between death and removal of the nerves or during their storage after removal. In most instances, the nerves were removed within 24 h of death, and in several specimens obtained immediately after death, comparable values were obtained. Repeated studies of different samples from the same nerves stored at −5°C for different periods of time also failed to reveal any qualitative or quantitative alteration with duration of storage. Neither the time of storage nor the time between death and removal of nerves differed between the two age groups. In one subject in whom data was available for both the right and left nerve, myelin content was 18.19 and 18.54 mg/g and 2.58 and 2.47 mg/cm, respectively.

The possibility cannot be rigorously excluded that the lower value for myelin content in the older subjects reflects losses due to greater susceptibility of their myelin to injury by the incubation or other steps in the isolation procedure. This possibility is not supported by the demonstration of any important differences in com-

[1] Mean ± SE.

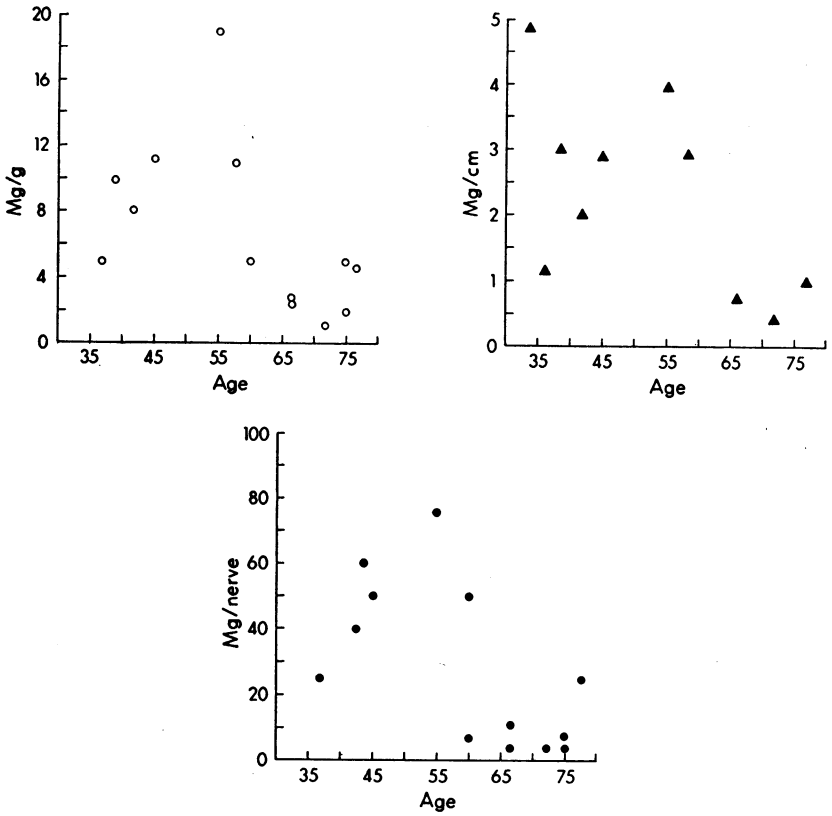

FIGURE 1 The amount of myelin (as the sum of protein, phospholipid, cholesterol, and glycolipid) as related to nerve weight (open circles), nerve length (triangles), and to the whole nerve segment (closed circles) according to age.

TABLE II

Composition of Peripheral Nervous System Myelin from Mammals

	Human femoral nerve: Present study*		Squirrel monkey brachial plexus (Horrocks [8])	Bovine intradural spinal roots (O'Brien, Sampson, and Stern [9])	Rat sciatic nerve (Evans and Finean [10])	Rabbit sciatic nerve (Singh, Spritz and Geyer‡)
	35–58 yr	60–77 yr				
Protein (*% of dry weight*)	28.7 (24.7–34.6)	34.0 (31.1–37.4)	30.5	24.2	—	22.3
				(*Mol/100 mg of lipid*)		
Cholesterol	38.0 (35.6–40.3)	34.8 (27.7–38.0)	43.1	40.9§	38.3	43.3
Glycolipid	16.7 (13.5–19.1)	18.9 (13.7–25.4)	18.2	11.5	15.3	11.0
Total phospholipid	45.3 (41.8–48.3)	46.3 (40.5–58.0)	38.7	42.3	46.4	45.7
Phosphatidylethanolamine	2.5 (1.8–3.1)	2.9 (2.0–4.6)	4.3	2.5	17.2‖	2.8
Phosphatidylcholine	6.6 (5.9–7.9)	6.6 (6.4–7.0)	5.1	10.0	11.1	7.5
Phosphatidylserine¶	7.8 (4.3–9.7)	7.3 (5.3–9.2)	7.5	6.9	8.8	6.2
Sphingomyelin	15.2 (12.7–17.6)	15.0 (12.9–17.5)	11.1	12.9	9.2	14.0
Phosphatidalethanolamine	13.2 (10.2–14.8)	14.4 (12.2–16.8)	10.7	10.0	—	14.9

* The data in parentheses for the data from the present study are the ranges of values. The data for the 35–58-yr old group is from five different nerves and that for 60–77 yr group from four.

‡ Unpublished results.

§ Contains in addition 3–5% uncharacterized components.

‖ Contains both phosphatidylethanolamine and phosphatadalethanolamine.

¶ All samples contained small amounts of phosphatidylinositol.

position that could account for such an increase in vulnerability for myelin from the older subjects. Also, if the isolation procedures had altered myelin in a way that interfered with its recovery, one might expect to find fragments with density changed from that of the original material. The finding of a single band in the continuous gradient, comparable with that of the myelin from the younger subjects and the absence of bands of other densities speaks against selective alteration of myelin from the older subjects.

Consideration was given to the question of the best base to which the myelin content should be related. We found that attempts to measure dry weights of the whole nerves led to inconsistent findings so that water content, which may have been affected by postmortem effects or aging could not be controlled. Also, apparent decreases in myelin content, when related to nerve weight, could actually reflect an increase in a nonmyelin component rather than a decrease in myelin. This question of a relative rather than absolute decrease in myelin in the older age group was resolved, however, by finding a similarly lower value for the older subjects when myelin was related to nerve length or to the entire, anatomically defined nerve segment, as indicated in Fig. 1.

There are no other data in man to compare with our quantitative findings. In the only other published account of myelin content determined in peripheral nerve by direct isolation, Rawlins and Smith (11) found that, in the rat, the content of sciatic nerve myelin increased up to the oldest age studied, 18 mo. Compositional studies of peripheral nerve myelin from other mammals are tabulated in Table II and show little variation among species. Recently Koeppen, Messmore, and Stehbens (12) during their studies on interstitial hypertrophic neuropathy analysed myelin isolated from lumosacral plexus from three normal humans. Compositional data in the present study agree with that reported by them except that we find considerably more sphingomyelin. Since, as these investigators point out, they recover only a small fraction of the peripheral nerve myelin, their findings might represent that of a fraction with different composition from the entire myelin. Also, since continuous gradient flotation was not used, contamination by another membrane with lower sphingomyelin content cannot be excluded.

The relationship between age-related alterations in peripheral nerve function and decreased myelin content remains uncertain. Our findings, as do those of Lascelles and Thomas (4), suggest that decrease in myelin content is not a continuous function of age, but occurs fairly abruptly in the seventh decade. It is of interest that the velocity of nerve conduction decreases with age and is a function of nerves that relates inversely to diameter of nerve fibers (13), i.e., the degree of myelinization. The time-course for decrease in conduction velocity has not been defined with certainty, but the findings of Mulder, Lambert, Bastron, and Sprague (14), Mayer (15), and Lascelles and Thomas (4) suggest that, particularly with proximal nerves, as used in the present study, the major decrease in velocity occurs beyond the age of 50. This raises the possibility that the decrease that we have found in myelin content of peripheral nerves in the older age group is the structural counterpart to age-related functional alterations.

ACKNOWLEDGMENTS

We are greatly indebted to the staff of the office of the Medical Examiner of New York City and to Miss Mara Feder and Marcia Adelson for their technical support.

This study was supported by the Veterans Administration and by the U. S. Public Health Service (AM-13525).

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