Decrease in Myelin Content of Rabbit Sciatic Nerve with Aging and Diabetes

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SUMMARY

Previous studies of the amount of peripheral nerve myelin have been based on histologic examination. In this study, myelin content was measured directly after quantitative isolation from sciatic nerve. There was a decrease in the amount of myelin beginning at nine months, the time of maximal myelin content in normal rabbits, and beginning at six months, the amount was decreased in diabetic as compared with control animals. Composition of myelin isolated from young (age three to four months) and old (age nine to thirteen months) rabbit sciatic nerves was also determined and is similar to that of other species. Although the composition was not affected by diabetes, with aging there was a significant decrease in the amount of cholesterol and an increase in glycolipid. DIABETES *24*:680-83, July, 1975.

Histologic and neurophysiologic studies suggest that, in man, the myelin content of peripheral nerve decreases with aging and in diabetes.[1-4] By utilizing a direct measurement of the amount of myelin in a standard segment of femoral nerve obtained at autopsy, we have been able to confirm and quantify these age-related changes in man.[5] We found that in the sixth decade there is a decrease in the amount of myelin such that the myelin content of the intraabdominal portion of the femoral nerve in a group of people sixty to seventy-seven years old averaged about one-fourth that found in a group thirty-five to fifty-eight years of age.

In order to study this phenomenon further and to ascertain the effect of experimental diabetes on the age-related loss of myelin shown previously by histologic and neurophysiologic studies,[6,7] we have now determined the amount of myelin in the sciatic nerves of control and alloxan-diabetic rabbits of age three to thirteen months. In these studies, the myelin in the nerve segment was quantitatively isolated by a series of flotation procedures, and its amount was determined chemically by measuring the components of this membrane. We found that there was a decrease in the amount of myelin beginning at age nine months, the time of maximal myelin content in normal rabbits, and beginning at age five months, the amount was decreased in diabetics as compared with control animals.

In the present investigation, we also studied the effects of aging and diabetes on the composition of this membrane. It was found that diabetes produced no change in composition but that significant effects of aging were noted in both diabetic and control animals.

MATERIALS AND METHODS

Male New Zealand rabbits were obtained as littermate pairs at age one month from Zartman Farms, Douglassville, Pennsylvania. One of each pair was made diabetic at age two months with a single intravenous injection of alloxan (175 mg./kg.). Blood sugar levels ranged from 500 to 640 mg./100 ml., and the animals were maintained on 5 to 7 I.U. of protamine zinc insulin given each day to prevent death in ketoacidosis. In both four- and eight-month-old animals, body weight was comparable in diabetic and control rabbits.

Isolation of myelin. Animals were killed with intravenous phenobarbitol, and both sciatic nerves were removed from their origin at knee; they were weighed, their lengths were measured, and the two nerves were pooled for each experiment. Myelin was isolated after homogenization of the nerves by a series of flotations in discontinuous gradient, "osmotic shock," and finally flotation in a continuous cesium chloride gradient. The details of the isolation procedure and evidence for its purity and its quantitative

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recovery are presented in two prior publications concerning human[5] and rat[8] nerves. Incubation of nerves with glycine-triethylamine hydrochloride buffer, necessary for isolation of myelin from human nerves, was not required for the rabbit nerves.[5]

Chemical determinations. Extraction of lipids and their separation and recovery by two-dimensional thin-layer chromatography as well as the methods for their quantification and determination of protein have been reported previously.[9] Total myelin content is estimated as the sum of protein, glycolipid, phospholipid, and cholesterol. The myelin content is presented in relation to nerve length (milligrams of myelin per centimeter nerve). As discussed previously,[5] this method of presentation was selected to obviate problems related to changes in water or nonmyelin components of nerve that might occur with aging and diabetes.

RESULTS

Myelin content of sciatic nerves. The quantitative data on the amounts of myelin in three-month-old rabbits is presented in figure 1. The amount of myelin in the six control animals ranged from 0.8 to 1.5 mg./cm.

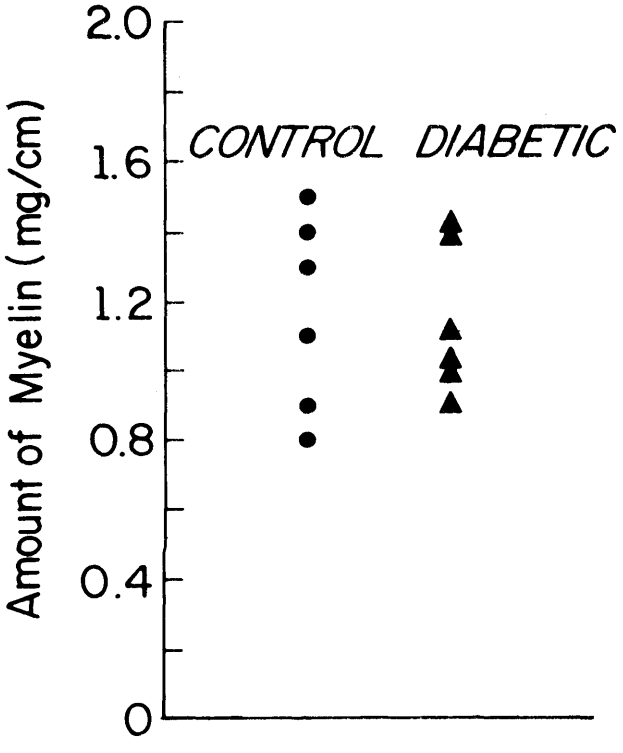

FIG. 1. Amount of myelin in six pairs of animals three months old. The diabetes was produced when animals were two months old.

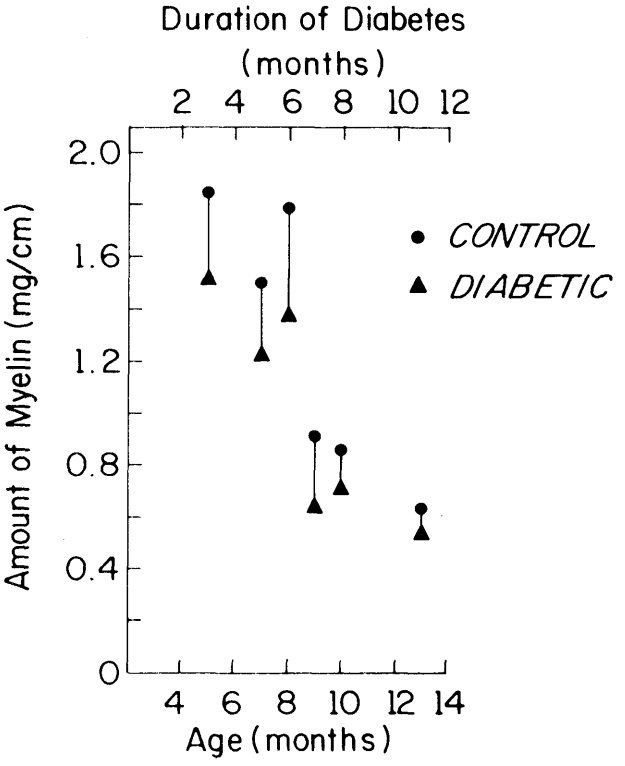

FIG. 2. Amount of myelin as function of age and duration of diabetes in six animals.

and did not differ from that of the diabetics. Figure 2 depicts the data from six pairs of older animals (five to thirteen months of age) and shows a decrease in myelin content related both to aging and to diabetes. From ages five to eight months, the amount of myelin in control (1.48 to 1.83 mg. myelin per centimeter nerve) is comparable or perhaps slightly greater than that of the three-month-old group, but at this age a lower value of myelin content can be seen in diabetics in each of the three comparisons. In three nondiabetic animals of age nine to thirteen months, a definite decrease in myelin is seen, and their values are now at the lower range seen for the three-month-old group. The lower values of myelin content for diabetics, first evident at five months, continues in the three comparisons for the older animals. Thus, the six diabetic animals from ages five to thirteen months (diabetes of three to eleven months' duration) were all lower in myelin than their paired controls, ranging from 24 to 16 per cent lower than in the nondiabetics.

Myelin composition. As indicated in table 1, rabbit sciatic nerve myelin has a composition like that described for other mammals, including man.[5] Protein constitutes less than 25 per cent of total weight, sphingomyelin is present in relatively high concentra-

TABLE 1

Composition of rabbit sciatic nerve myelin

	Age 3-4 Months		Age 5-13 Months	
	Control	Diabetic*	Control	Diabetic*
Protein (% dry weight)	23.0 (20.5–25.2)†	23.2 (21.0–25.3)†	21.8 (18.5–25.9)†	21.9 (18.5–26.5)†
			mole % of total lipid	
Cholesterol‡	42.2 (37.0–47.4)	39.3 (34.0–46.0)	36.4 (32.2–42.3)	35.3 (26.6–41.5)
Glycolipid§	13.0 (6.8–22.4)	14.7 (9.3–23.9)	18.3 (12.4–22.2)	19.6 (13.6–23.4)
Total phospholipid	44.8 (40.2–49.7)	46.1 (41.1–52.9)	45.3 (41.9–50.4)	45.1 (41.4–50.4)
Phosphatidylethanolamine	2.9 (2.2–3.6)	2.9 (2.7–3.2)	2.8 (2.0–3.9)	3.2 (2.3–4.5)
Phosphatidalethanolamine	14.2 (12.5–15.1)	15.3 (13.5–16.5)	14.8 (13.9–15.9)	13.7 (12.4–15.3)
Phosphatidylcholine	7.9 (7.2–8.6)	8.1 (7.2–8.9)	7.8 (6.7–9.4)	8.3 (7.1–10.2)
Phosphatidylserine //	6.2 (5.0–7.6)	5.7 (4.6–7.2)	6.7 (5.7–7.5)	6.4 (3.3–7.7)
Sphingomyelin	13.6 (12.5–14.4)	14.1 (13.3–14.9)	13.2 (10.5–15.1)	13.5 (11.1–15.7)

*Duration of diabetes. Three to seven weeks for three-to-four-month-old animals and fourteen to twenty-four weeks for five-to-thirteen-month-old animals.

†Mean (range). Two determinations on each of five different preparations for three-to-four-month-old animals and on nine different preparations for five-to-thirteen-month-old animals.

‡Cholesterol significantly different between two age groups ($p<0.05$). Data were pooled for control and diabetic animals.

§Glycolipid significantly different between the two age groups ($p<0.02$). Data were pooled for control and diabetic animals.

//All samples contain small amounts of phosphatidyl inositol.

tion, and the predominant glycerophospholipid is phosphatidalethanolamine, and the phospholipid:cholesterol molar ratio, especially in young animals, approaches 1:1.

No effect of diabetes on the composition of myelin is apparent in these studies. While the molar ratio of glycolipid to cholesterol or total phospholipid approaches 1:3 in the younger animals, it is closer to 1:2 in the older group. The decrease in cholesterol and the increase in glycolipid in the older group are significant ($p<0.05$ and $p<0.02$) when analyzed by the Wilcoxon rank-order sum test,[10] with data pooled for the diabetic and control animals in both age groups.

DISCUSSION

In these studies we have demonstrated by direct quantification that both aging and diabetes influence the amount of myelin in sciatic nerve in the rabbit. We have previously shown that, in man, the age-related decrease in myelin is present and is not a linear phenomenon but occurs fairly abruptly in the seventh decade.[5] Similarly, in the rabbit the decrease was apparent in animals nine to thirteen months old but was not seen earlier. The effects of diabetes on myelin content, as determined by direct isolation and measurement, has not been reported previously for any species, including man, but histologic[1,2] and neurophysiologic[3,4] studies suggest strongly that such a decrease does occur in the human disease.

The findings of the present study do not provide information concerning the mechanism by which this decrease in myelin occurs. The decrease could reflect either a loss of whole nerve fibers and/or a maintenance in the number of neuronal elements, but with a decrease in the average myelin content per nerve unit. We have shown that, in vitro, nerve obtained from rats with experimental diabetes shows impairment of incorporation of precursors into both the lipid and protein components of myelin,[8] and we have confirmed the findings of others that a similar decrease occurs with aging.[11] Since, in the peripheral nerve, the Schwann cell elaborates myelin and this membrane is an extension of that cell's plasma membrane, we have interpreted these findings as evidence of dysfunction of that cell with both aging and experimental diabetes. The segmental loss of peripheral nerve seen histologically in patients with diabetes[1,2] supports the concept that, in man, Schwann cell function is altered in diabetes.

The relevance of the findings in the present study to the effects on peripheral nerve of man in diabetes remains uncertain. While changes in peripheral nerve in experimental diabetes have been reported from histologic studies in several species,[6,7] segmental demyelinization, the hallmark of the human disease, has been documented only in the spontaneously diabetic Chinese hamster.[12]

Several authors have found a decrease in nerve conduction velocity in experimental diabetes[13,14] that is in some investigations reversible with insulin therapy. Winegrad has recently challenged the significance of these findings,[15] since in his investigations the con-

duction abnormality is transmitted and is corrected with oral myoinositol. In our own unpublished studies we find that in the streptozotocin diabetic rat, the conduction defect persists for at least a year in only mildly diabetic animals. In these animals, however, we have been unable to find changes in myelin content or composition with either aging or diabetes.

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