Nerve Conduction Defect in Galactose-fed Rats

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SUMMARY

Dulcitol accumulation associated with swelling occurs in the peripheral nerves of rats fed a 40 per cent galactose diet. These alterations are associated with a motor nerve conduction velocity defect which is reversible on withdrawal of galactose from the diet. These findings suggest that the accumulation of sugar alcohol plays an important role in the etiology of galactosemic neuropathy. DIABETES *21*:295-300, May, 1972.

Sugar alcohols have been shown to accumulate in the tissues of diabetic and galactosemic rats and humans.[2-7] In lens[2] it was demonstrated that the intracellular accumulation of sorbitol (glucitol) or dulcitol (galactitol) leads to osmotic swelling and other consequences culminating in the development of diabetic or galactosemic cataracts. Aldose reductase (alditol: NADP oxidoreductase E.C. 1.1.1.21) is the enzyme responsible for the conversion of glucose or galactose to their respective polyols. Although the "sorbitol pathway" has been demonstrated in many tissues, in diabetes or galactosemia only three, namely lens,[2] sciatic nerve,[5] and renal papilla,[7] have thus far been shown to contain sufficiently high concentrations of sugar alcohol to be of osmotic significance. In both in vitro and in vivo studies a role of sugar alcohol accumulation in cataract formation has been shown by the finding that an aldose reductase inhibitor, which blocks the increased formation of sugar alcohol in experimental diabetes and galactosemia, also prevents cataract development.[8-10] The role of sugar alcohol accumulation in the development of acute diabetic neuropathy has been postulated;[5,11] however, it has been difficult to isolate the effects of sorbitol accumulation from the many other associated metabolic alterations in diabetes.

In the galactose-fed rat the sugar alcohol dulcitol accumulates in peripheral nerve, thus providing a model to study the effects of sugar alcohol accumulation on the biochemistry and physiology of peripheral nerve. This report describes an in vivo, serially measured, reversible motor nerve conduction velocity (MNCV) decrease associated with increased dulcitol levels and swelling in the sciatic nerves of galactose-fed rats.

METHODS

Animals. Control and experimental male rats (CD strain, Charles River Breeding Laboratories) were age-matched. Control animals received regular chow pellets. Experimental animals received a diet of four parts D-galactose (Nutritional Biochemical Corp., Cleveland, Ohio) and six parts powdered rat chow. Water and diets were given ad libitum. In gavage experiments 10 ml. of a 30 per cent galactose solution was given daily to supplement the galactose diet.

Nerve conduction measurements. Serial measurements of motor nerve conduction velocity (MNCV) were made in rats by a method recently described by Ramerman, Honet and Jebsen.[12] Nembutal anesthetized rats (50 mg./kg. intraperitoneally) were placed on a specially built board which allowed the legs to be held in maximal extension. The proximal point of stimulation was over the sciatic nerve as it crossed behind the head of the femur and the distal point at the posteromedial aspect of the ankle. The muscle action potential was recorded by a monopolar recording electrode (Type MF 37M, TECA Corp., White Plains, N.Y.) inserted in the footpad. Ground and reference electrodes were lightly clamped on the footpad 10 mm. proximal to the recording electrode. The straight line distance between the two points of stimulation varied from 45 to 90 mm. depending on the age of the animal. Supramaximal stimulation was given using the stimulation unit

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and trigger section of a TECA Model B-2 electromyograph. The muscle action potential was amplified and photographed on a Tektronix 561B oscilloscope (Tektronix Corp., Beaverton, Ore.) with a time base of 1 msec./division. At least three superimposed action potentials were photographed at each stimulation point. MNCV was calculated as follows:

$$\text{MNCV} = \frac{\text{Proximal peak time} - \text{Distal peak time}}{\text{Distance between proximal and distal points}}$$

and the results are given as m./sec. ± SEM. Both right and left MNCVs were measured. The animals were acclimated for at least thirty minutes in a temperature controlled room maintained at 25 ± 0.5° C. before measurements were performed. Statistical tests of significance were carried out with Student's *t* test.

Chemical determinations. The animals, under Nembutal anesthesia, were decapitated and both sciatic nerves from the sciatic notch to the gastrocnemius tendon were removed and immediately weighed. In order to simultaneously determine both dulcitol content and water content on the same nerve, various metabolites including galactose, glucose and dulcitol were extracted by placing the nerve in 1 ml. water in a boiling water bath for ten minutes. After cooling, the nerve was removed and placed in a preweighed flask and dried in an oven at 90° C. for three hours and overnight in a desiccator over solid KOH before reweighing. The water content, representing the difference between the fresh wet weight and the dry weight, was expressed as mg. water/100 mg. nerve dry weight. Water contents determined by this method were reproducible although 2 per cent higher (N.S.) than values determined by direct drying of comparable normal or galactosemic nerves.

The extraction procedure for sugars and sugar alcohols gave comparable results to those obtained with barium hydroxide-zinc sulfate extraction of comparable nerves. One milliliter of a 10 per cent trichloroacetic acid (TCA) solution was added to the nerve water extract, and the resulting slightly turbid solution was centrifuged. The dulcitol content of the TCA extract was determined by an AutoAnalyzer (Technicon Corp., Tarrytown, N.Y.) using a periodate oxidation method described by Kraml and Cosyns[13] for the determination of glycerol. The method was modified by the use of 5 per cent TCA as rinse solution and a dulcitol standard curve in 5 per cent TCA (7.5 to 75 μg./ml.). The standard curve was linear over the range used. Glucose and galactose cause virtually no interference in this system at the levels encountered in normal and galactosemic nerves. Gas liquid chromatography[14] of the trimethysilyl derivatives of the carbohydrate of galactosemic nerve* (figure 1) identifies galactose, glucose, dulcitol and inositol as the main carbohydrates of the nerves of galactosemic animals. The periodate method for the determination of sugar alcohols gave higher values for sugar alcohol in normal nerve than the values obtained by gas liquid chromatography, presumably due to the presence of nonspecific periodate reactive substances in normal nerve.[15] Dulcitol levels in a few galactosemic nerves determined by the gas liquid chromatography method were similar to the levels obtained by the periodate method, and to the levels reported by Stewart et al.[6] Although normal sciatic nerve sugar alcohol levels reported here included nonspecific periodate reactive substance, this periodate method was deemed adequate and conveniently suited for determination of changes in the content of dulcitol in the sciatic nerves of galactose-fed rats.

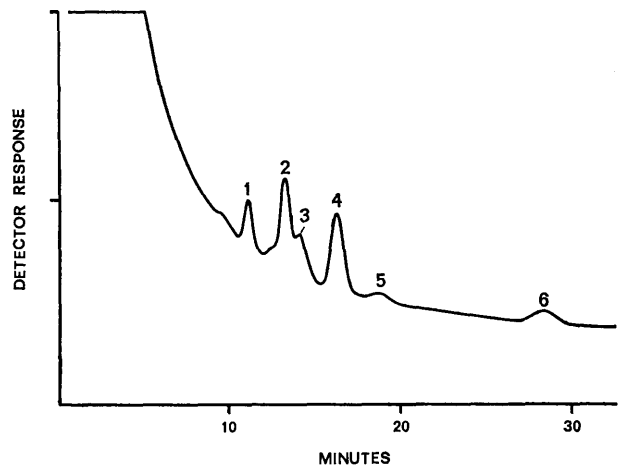

FIG. 1. Gas liquid chromatography of galactosemic nerve carbohydrates. Peaks are (1) α-galactose, (2) β-galactose, (3) α-glucose, (4) dulcitol, (5) β-glucose, (6) inositol.

RESULTS

Effect of age on MNCV and water content. Conduction velocity in rat nerves continues to increase up to one year of age, reaching a velocity of 53 m./sec.[16] In groups of rats varying in age from 36 to 140 days, figure 2, serially measured MNCV increases from 21.5 ± 1.0 to 40.3 ± 0.9 m./sec. ± SEM with a relative plateau reached at about three months of age. Water content, highest in the younger animals, decreases with age and

*GLC of TMS ethers were performed on an F and M gas chromatograph Model 5750 with a flame ionization detector. The extraction procedure and preparation of TMS ethers were previously described in Biochim. Biophys. Acta *128*:474, 1966.

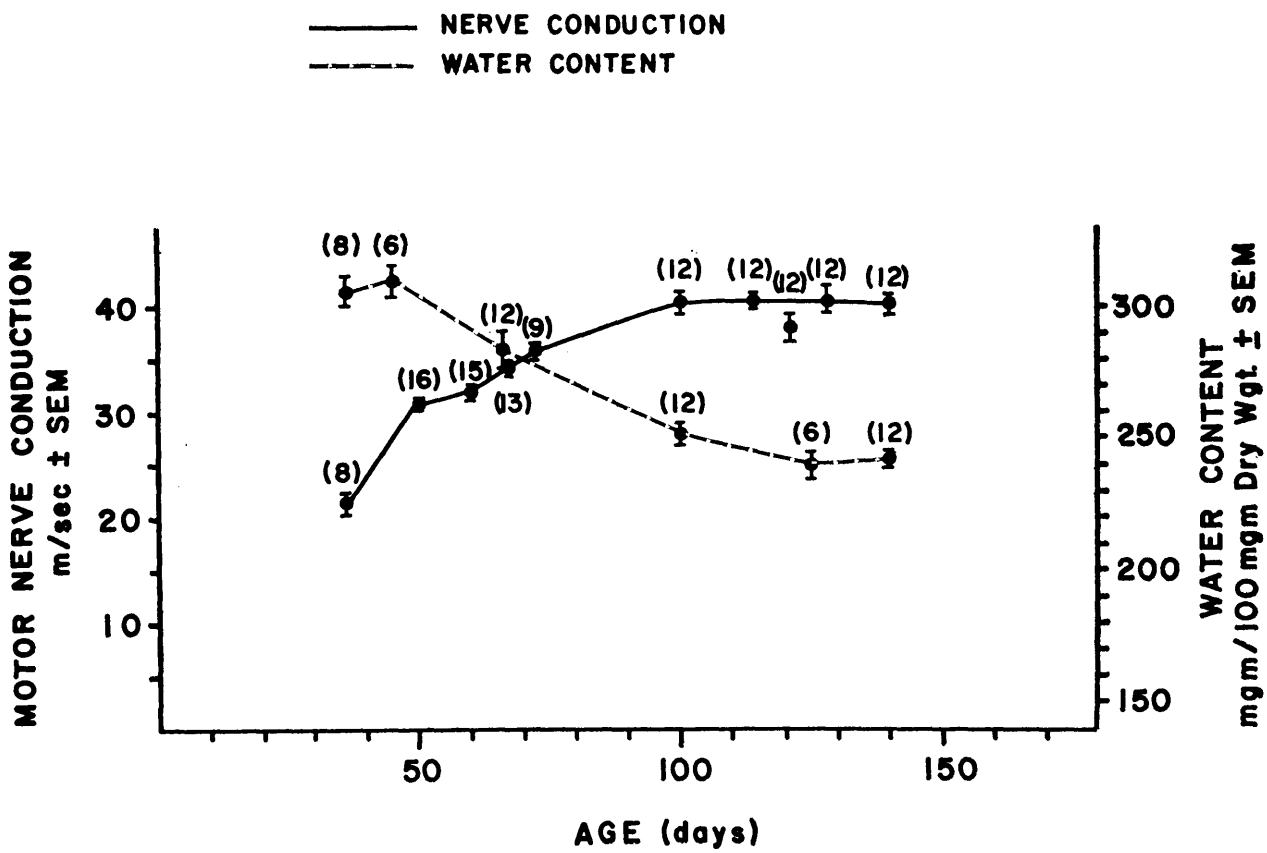

FIG. 2. Effect of age on normal rat MNCV and nerve water content.

stabilizes at about 100 days as previously described by Majno and Karnovsky.[17]

Effect of galactose feeding and gavage on MNCV. Serial measurements were performed on eight forty-day-old rats placed on the 40 per cent galactose diet for two months (figure 3). Mean MNCV for the control group was 42.4 ± 1.1 m./sec. while in ten galactose-fed rats it was decreased to 35.2 ± 1.1 m./sec. ($p < 0.001$). The galactose-fed rats were gavaged with a supplemental dose of 3 gm. galactose daily for one week, at which time the MNCV decreased further to 31.5 ± 1.1 m./sec., a significant decrease from the earlier measurements ($p < 0.05$); the control group MNCV was 40.5 ± 1.0 m./sec. Removal of galactose from the diet resulted in an increase of MNCV to 37.5 ± 1.9 m./sec. in two days and to 38.4 ± 1.2 in one week, vs. 40.5 ± 0.7 m./sec. for the control group ($p = NS$). The control group of rats was then placed on the 40 per cent galactose diet and the MNCV, as measured thirty-six days later, had decreased to 34.5 ± 1.1 m./sec.

Dulcitol and water content relationship to MNCV changes. Three groups of seventy-day-old rats were used to study the role of dulcitol and water accumulations in MNCV changes. The control and the two galactose-fed groups had an initial MNCV of 35.8 ± 0.9, 38.9 ± 2.1, and 36.8 ± 1.5 m./sec. respectively. The MNCV in the two galactose-fed groups decreased to 31.6 ± 1.4 and 30.7 ± 0.9 m./sec respectively after twelve days on the diet, while MNCV in the control group continued to increase. After fifty days of galactose feeding, animals in the control and in one of the galactosemic groups were sacrificed after MNCV measurement. Figure 4 shows that the dulcitol level increased to 11.3 μmoles/gm. and the water content increased from a control value of 241 mg. to 305 mg./100 mg. dry weight in the galactose-fed group. MNCV was 40.3 ± 0.99 in the control group and decreased to 31.7 ± 1.2 m./sec. in the galactose-fed group. The second group of galactose-fed rats had an MNCV of 31.3 ± 1.4 m./sec. A regular diet was administered to this group for two days, and the animals were studied again. Dulcitol had decreased to 3.4 μmoles/gm. and the water content correspondingly to 261.5 mg./100 mg. dry weight. MNCV returned to a normal value of 39.0 ± 1.4 m./sec.

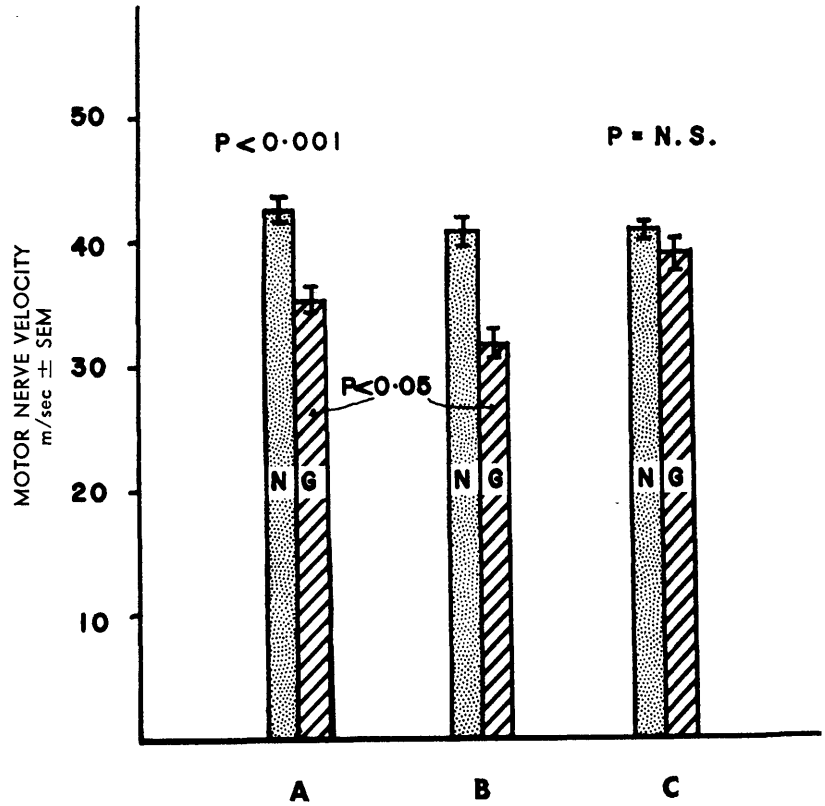

FIGURE 3

Effect of galactose feeding on MNCV. (A) Two months galactose feeding. (B) Effect of additional galactose gavage for one week. (C) Galactose withdrawal for one week.

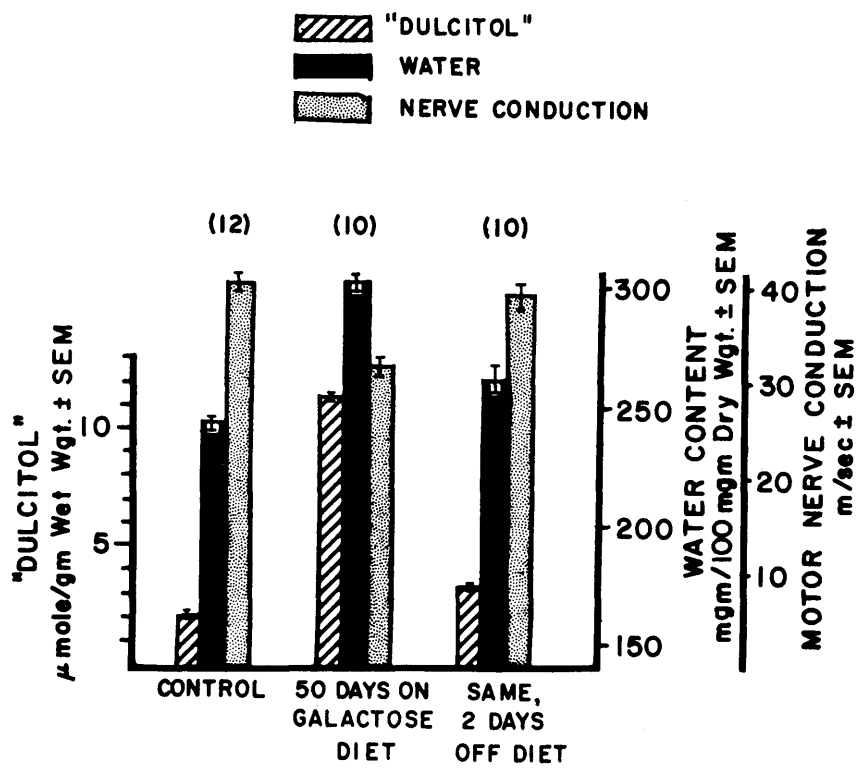

FIGURE 4

Relation of MNCV changes to nerve dulcitol and water content.

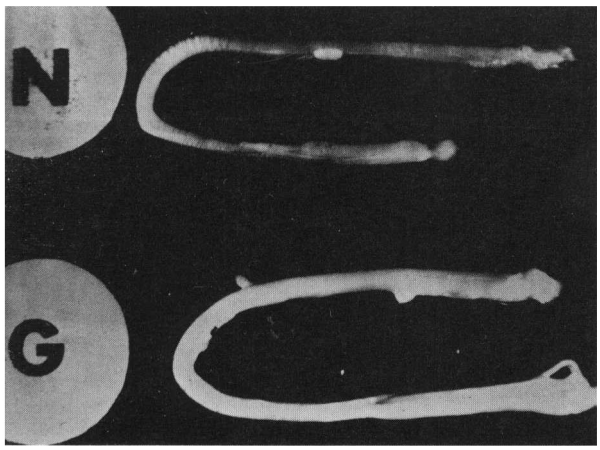

FIG. 5. Appearance of normal and galactosemic nerves (see text).

The increase in water content of galactosemic nerves can be seen grossly. Figure 5 shows a normal rat sciatic nerve with the typical cross striations of the collagenous nerve sheath. By contrast, the nerve in the galactose-fed rat appears taut and shiny, with loss of striations in the sheath secondary to the swelling. The nerve fibers bulge through the cut ends of the collagenous sheath.

DISCUSSION

Aldose reductase has a broad substrate specificity, converting many aldoses to their respective sugar alcohols. It possesses a high Km for glucose and galactose (70 to 100 mM), and, under conditions of hyperglycemia, such as in diabetes or excessive galactose intake, marked accumulations of sorbitol and dulcitol occur in a number of tissues. In contrast with diabetes, the magnitude of sugar alcohol accumulation is greater in the tissues of galactose-fed animals because of the inability of the second enzyme of the sorbitol pathway, namely sorbitol dehydrogenase, to further metabolize dulcitol. Once formed within cells, these sugar alcohols are unable to penetrate the cell membrane. As has been demonstrated in lens, this leads to intracellular accumulation and to the establishment of an osmotic gradient which results in cellular swelling and metabolic disturbances which culminate in cataract formation.[4]

We have previously demonstrated[11] that in peripheral nerves aldose reductase is localized in the Schwann cell, the cell responsible for the formation and maintenance of the myelin sheath.[18] Our finding of a dulcitol level of 11.3 μmoles/gm. whole nerve in the galactose-fed rat is a low estimate of the actual local concentration in the Schwann cell cytoplasm. Therefore it is not surprising that the galactosemic nerve water content is increased by 25 per cent over the control nerve values. The tautness of the collagenous sheath and the bulging of the nerve fibers through the cut ends of the sheath further demonstrate that the swelling is occurring in the nerve fibers. Thus, the accumulation of sugar alcohol and concomitant swelling in the peripheral nerve are analogous to the findings in lens during the process of galactose-induced cataract formation, and confirms previous findings by Stewart et al.[6]

As expected, the increase in MNCV in normal rats reached a relative plateau at about three months of age. This increase follows but is independent of growth in body weight.[16,17] Water content varied inversely with the MNCV increase reaching a plateau at about the same time. The galactose-fed rats developed the MNCV defect as early as twelve days following galactose feeding, and this defect was exacerbated by additional galactose intake (figure 3). The MNCV defect was quickly restored to normal within two days following galactose withdrawal. This improvement correlated with the decrease in dulcitol and water content in nerve, and would strongly suggest a relationship between the sugar alcohol accumulation and the MNCV defect.

Symptomatic neuropathy has not been described in galactosemic patients presumably because of the institution of immediate dietary control upon diagnosis. However, since nerve conduction measurements are a far more sensitive and objective index of nerve damage, an MNCV defect is more likely to be found in galactosemic patients, and such a study is currently in progress.

Similarly, the presence of dulcitol in human galactosemic peripheral nerves has not yet been described, although the presence of dulcitol in brain, lens, and urine of galactosemic patients has been previously demonstrated.[19-21]

Our finding that an MNCV defect develops in galactose-fed rats, and is associated with an increased accumulation of sugar alcohol and with swelling, suggests that this accumulation is indeed responsible for an acute and reversible metabolic neuropathy.

Bertrand and Lecoq[22] described severe demyelination and neurologic deficits in the peripheral nerves of pigeons occurring three days following the feeding of a 50 per cent galactose diet. Pigeons and chicks are known to be far more susceptible to the induction of neurological damage than is the rat.[23]

Gregersen,[24] in a study of untreated diabetic patients, showed a clear improvement in nerve conduction velocity following the regulation of hyperglycemia with

insulin therapy, and decompensation when diabetic control was discontinued, indicating an acute reversible component in diabetic neuropathy. Our findings with the galactose-fed rat support the hypothesis that the accumulation of sugar alcohol in peripheral nerves and the associated swelling can result in a neuropathy which is reversible on withdrawal of the provocative galactose feeding. As with cataracts,[8-10] definitive proof of this mechanism will await the demonstration that diabetic and galactosemic neuropathy can be reversed or prevented by treatment with an effective in vivo aldose reductase inhibitor.

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