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*Original Articles*

# Peripheral Neuropathy and Myopathy

## An Experimental Study of Rats on Alcohol and Variable Dietary Thiamine

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**Summary.** The effects of variable dietary thiamine concentrations (deficient, normal, surplus) on the development of alcoholic neuromyopathy in rats exposed for 36 weeks to 10–25% (v/v) ethanol or water (control group) as the sole drinking fluid were studied by histological and electrophysiological methods.

Abnormalities in the structure of the sciatic nerve (phagocytosis, myelin abnormalities, increase in nonspecific cholinesterase activity) and tibial muscles (angular atrophic fibers, group atrophy, fibre necrosis) developed more frequently in animals on diets deficient in thiamine than in animals on diets with normal or surplus thiamine, and more frequently in animals drinking alcohol and water than in those drinking water alone. No differences were observed between the different groups in the number of perivascular sympathetic nerves, in the motor nerve conduction velocities and in the muscle fibrillation potentials.

Thus, thiamine deficiency, established as a significant reduction of red blood cell transketolase activity, seems to have a deleterious effect on the peripheral nerves and muscles. The effect is enhanced by the simultaneous consumption of ethyl alcohol.

**Key words:** Alcohol – Thiamine – Peripheral neuropathy – Myopathy – Histochemistry – Electrophysiology – Rats.

## Introduction

The clinically well established occurrence of neuropathy and myopathy in chronic alcoholism (e.g. Victor, 1975; Behse and Buchtal, 1977) has recently been imitated experimentally in rats (Juntunen et al., 1978; Teräväinen et al.,

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1978). Neuromyopathy was produced by feeding rats daily on 20% (v/v) ethyl alcohol and a normal laboratory diet for about 40 weeks. A number of nutritional deficiencies often accompany alcoholism, but in the absence of experimental evidence their role in the pathogenesis of alcoholic polyneuropathy is not clear. For example, recent experimental studies concerning the role of thiamine in the development of peripheral neuropathy suggest that thiamine dietary deficiency alone can cause neuropathies in mammals (Kark et al., 1975; Pawlik et al., 1977).

In the present work, we have studied the role of thiamine in the development and prevention of alcoholic neuromyopathy, using rats as an experimental model.

## Materials and Methods

A total of 154 Sprague-Dawley rats, 2 to 3 months old, were initially included in the present study. The rats were divided into six different groups and maintained on thiamine deficient (0.9 mg/kg), normal thiamine (5 mg/kg) or surplus thiamine (60 mg/kg) diets, with or without alcohol. Rats in the thiamine deficient groups were originally maintained on a diet containing no thiamine, but owing to the high mortality, their diet was supplemented in the 9th week with 2 mg per kg of thiamine. An additional 15 animals were added to these groups.

The rats belonging to the alcohol groups were given ethanol in water ad libitum as their sole liquid. The content of ethyl alcohol in water was gradually increased; 15% (v/v) was used during the first four weeks, 20% (v/v) in the next 16 weeks and 25% (v/v) in the last 16 weeks. At the beginning of the forced ethanol drinking period the mean alcohol intake was 7.6 g/kg/day in thiamine deficiency, 7.4 in normal thiamine and 8.1 in surplus thiamine groups. The corresponding figures at the end of the period were 10.8, 9.5 and 10.5 g/kg/day. The differences in intake between the three groups were not statistically significant.

The animals were kept in stainless steel cages on metal grids without bedding material and fed on specially prepared food with controlled thiamine concentrations. The basic diet, deficient only in thiamine, was analyzed by the Technical Research Center of Finland and shown to be optimal for laboratory rats. The amounts of the main nutrients, trace elements, amino acids and vitamins, and the analytical data from raw materials have been published recently in connection with another study (Pekkanen et al., 1978). The rats were examined after 10, 19, 24, 33 and 36 weeks exposure to the different diets. The total number of animals analyzed by histological and electrophysiological techniques was 58.

*Histological Methods.* After decapitation, pieces of the distal part of the peroneal nerve were immersed in glutaraldehyde and postfixed in osmium tetroxide, embedded in Epon, cut to about 2 μ and stained with 1% methylene blue. Visual estimation and calculations of the number of myelinated fibres were performed from transverse sections.

Non-specific cholinesterase of the intramuscular nerves was demonstrated histochemically, after formalin fixation, according to a method based principally on the Gomori (1952) version of the Koelle (1951) technique. The mean number of fluorescing sympathetic perivascular nerves around the fascia of the tibialis anterior muscle was calculated after glyoxylic acid treatment under a fluorescence microscope according to the method of Teräväinen and Mäkitie (1976). Both histological staining and histochemical reactions were used to demonstrate the structure and chemistry of the tibialis anterior muscle. After removal, the muscle specimens were frozen in isopentane precooled in liquid nitrogen and sectioned at 10 micra in a cryomicrotome at −20° C for histological studies. Histological staining was performed with modified Gomori trichrome (Denny-Brown, 1958) in which Mayer's hemalum was substituted for Harris' hematoxylin. Sections were also incubated with NADH-diaphorase (Pearse, 1972) and ATP-ases, with ATP-disodium salt as substrate (Guth and Samaha, 1970). Preincubations of sections with acid (pH 4.3) or alkali (pH 10.2–10.4) were

carried out before incubation with the substrate at pH 9.4. Sections incubated with ATP-ase were also used for the calculation of fibre sizes.

*Electrophysiological Methods.* A Grass S 8 stimulator with an SIU 4678 isolation unit, a Tektronix 122 preamplifier and a Tektronix D15 storage oscilloscope with a 5B18N amplifier were used in the electrical recordings. Spontaneous electrical activity (fibrillation potentials) of the anesthetized rats was sought after inserting a concentric needle electrode into the distal end of the muscles of the tibialis groups at several points. On account of insertion activity, more than a minute was allowed to elapse before the interpretation. After decapitation, the sciatic nerve together with its distal branches was removed and studied at room temperature in vitro. The nerves were placed transversally on silver wires 1 mm in diameter and 2 mm apart in a plexiglass chamber for compound action potential measurements. The silver wires supported the nerve and simultaneously functioned as stimulating and recording electrodes. Monophasic recording was obtained by crushing the nerve between the two recording electrodes. The free length of the nerve between the stimulating and recording electrodes varied in different animals from 36–46 mm. The threshold for supramaximal stimulation with a current pulse of 0.1 ms duration was determined separately for each preparation and was 8–13V for large-diamter myelinated A-fibres and 44–55V for smaller myelinated B-fibres. Drying was prevented by mineral oil. The values for the conduction velocities at room temperature were recorded at 23–24° C (chamber temperature); they were found to be about 30% slower than those obtainable at 27° C. The values given in Table 5 are the measured values at 23–24° C and are not corrected to body temperature.

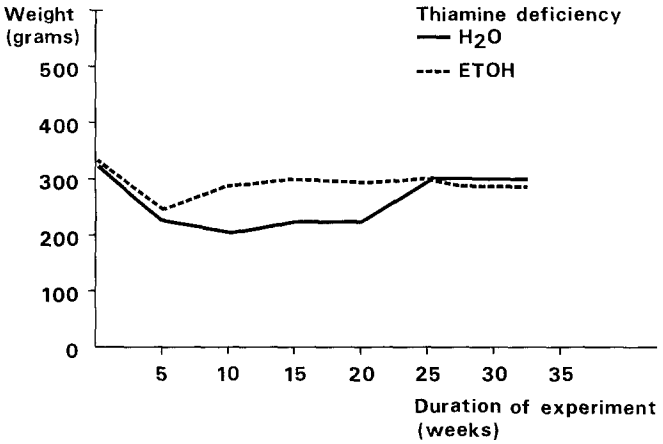
*Biochemical Methods.* To determine the thiamine status of the red blood cells, transketolase activity was assayed according to Nicholas et al. (1974) from animals killed after 24 and 33 weeks of treatment.

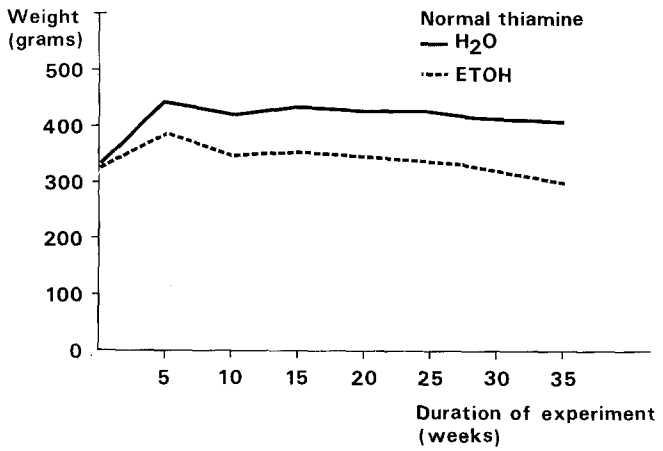
*Statistical Evaluation.* Statistical comparisons were made using student's t-test and the chi square test.

## Results

*General Condition of the Rats.* The rats were in relatively good condition at the end of the experiment with no external signs of neuropathy. An initial gain of weight occurred in all groups except those on the thiamine deficient diet. Animals on normal or surplus thiamine gained slightly less weight if drinking alcohol than if drinking only water (Fig. 1). Mortality of the rats during the first 21 weeks of the experiment was clearly higher in the thiamine deficient groups, and expecially in the thiamine deficient non-alcohol group, when compared with those on normal or surplus thimine (Table 1). Mean consumption of food in the different groups (kJ/day), alcohol excluded is depicted in Fig. 2. The figure illustrates that the consumption of food was generally somewhat less in the animals on alcohol, presumably due to the fact that alcohol served as a substitute source of energy. A marked decrease in the food consumption, concomitant with high mortality, was seen in the thiamine-deficient animals at the beginning of the experiment when they were receiving no thiamine at all (Table 1). The thiamine content of the food was increased to 2 mg per day in the ninth week, whereafter food consumption temporarily increased and mortality decreased.

*Transketolase Activity of Red Blood Cells.* A significantly lower level of red blood cell transketolase activity was observed in the thiamine-deficient animals than in other groups (Table 2), regardless of their alcohol state.



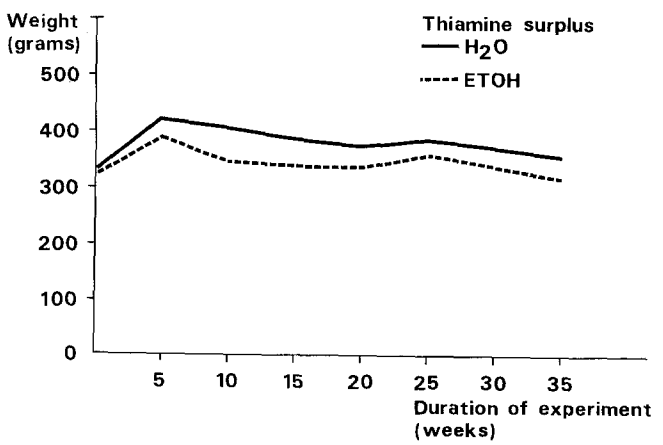




**Fig. 1.** Weights of the animals in the course of the experiment

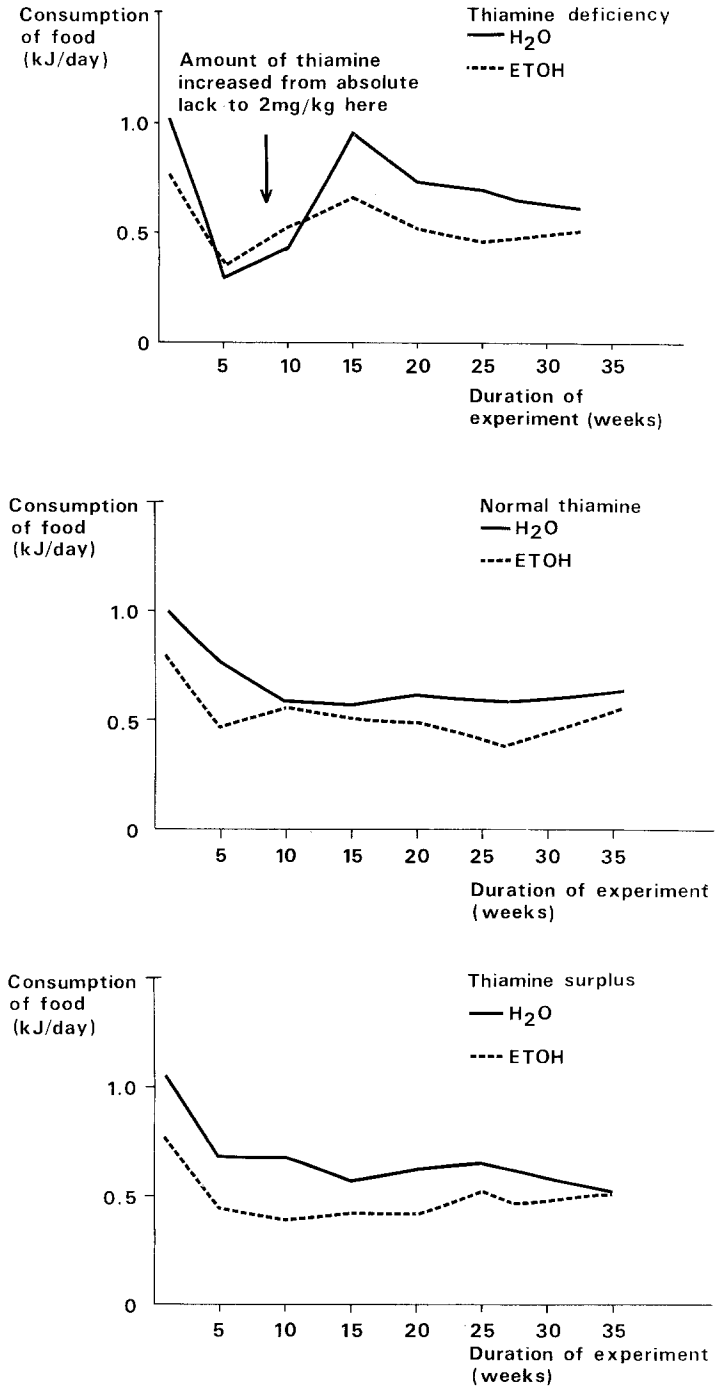



**Fig. 2.** Consumption of food of the animals in the course of the experiment

**Table 1.** Mortallity of rats during the first 21 weeks

| Group | Number of animals | Number of deaths | Mortality in % |
|---|---|---|---|
| 2mg/kg thiamine $H_2O$ | 26 | 13 | 50 |
| 2mg/kg thiamine ETOH | 26 | 6 | 23.1 |
| 5mg/kg thiamine $H_2O$ | 25 | 2 | 8.0 |
| 5mg/kg thiamine ETOH | 26 | 4 | 15.4 |
| 60mg/kg thiamine $H_2O$ | 25 | 3 | 12.0 |
| 60mg/kg thiamine ETOH | 26 | 2 | 7.7 |
| | 154 rats total | 30 | |

**Table 2.** Red blood cell transketolase activity

| | Thiamine deficiency | | Normal thiamine | | Thiamine surplus | |
|---|---|---|---|---|---|---|
| | $H_2O$ | ETOH | $H_2O$ | ETOH | $H_2O$ | ETOH |
| Transketolase (μmol/min/l) mean ± SD | 12.3 ± 2.4 ($n=3$) | 14.6 ± 5.1 ($n=3$) | 35.2 ± 3.9 ($n=2$) | 25.3 ± 5.0 ($n=3$) | 33.7 ± 7.0 ($n=2$) | 51.9 ± 7.0 ($n=3$) |

*Histochemistry and Electrophysiology of the Nerves.* The structure of the distal sciatic nerve was estimated under light microscopy from epon-embedded transverse sections. The presence or absence of Schwann cell hypertrophy, myelin abnormalities, phagocytosis and the number and diameters of myelinated nerve fibres were estimated visually. The mean number of the perivascular fluorescent nerves in the fascial arteries of the tibialis anterior muscle was calculated (Fig. 5). The findings from the nerves are summarized in Table 3. Although the observed changes from normal were quantitatively less than in a previous study involving a longer period of alcohol use (Juntunen et al., 1978), there was a similar tendency for the pathological changes to occur more frequently in rats on alcohol, especially if the diet was deficient in thiamine. The presence of nonspecific cholinesterase (Ns.Che) activity along the distal intramuscular nerves was

**Figs. 3 and 4.** Examples of pathological (Fig. 3) and normal (Fig. 4) muscle histochemistry. The figures illustrate transverse sections of the tibialis anterior muscles of the rat on alcohol and deficient thiamine for 36 weeks (Fig. 3) and of the control rat (Fig. 4), incubated for NADH-diaphorase. Note the presence of some angulated fibres with intense enzyme activity and (arrowheads) typical for neurogenic atrophy. ×200

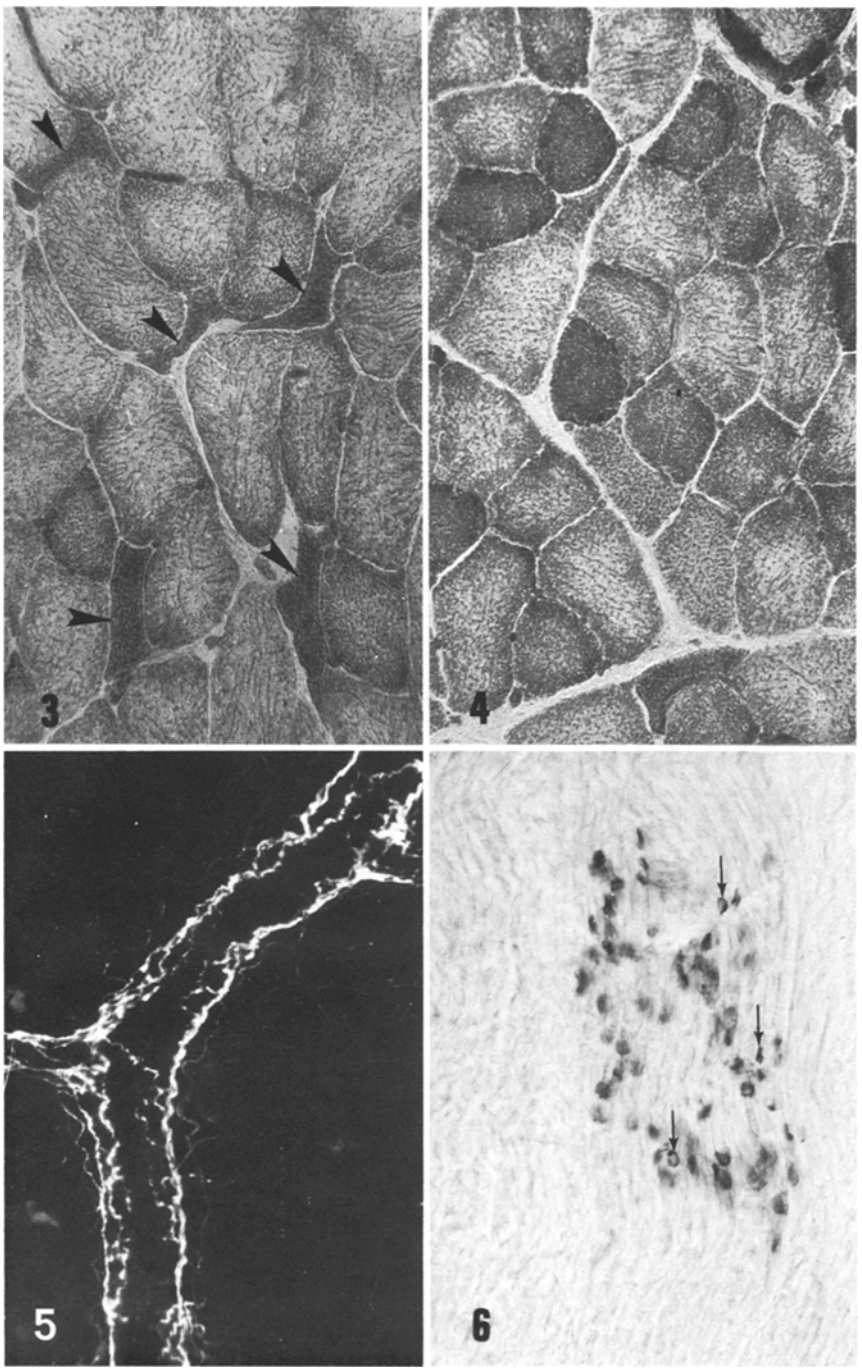



**Fig. 5.** An example of the glyoxylic acid-induced fluorescence of the peripheral sympathetic nerves in stretch preparation of muscle fascia from the animal on alcohol and thiamine deficient diet after 36 weeks of treatment. Neither the intensity nor the number of the fluorescent fibres differed from those of the controls. ×450

**Fig. 6.** Non-specific cholinesterase of the tibialis anterior muscle of the same animal as in Fig. 5. The activity of this enzyme is restricted to the myoneural junctions (arrows show some individual myoneural junctions) and only occasionally can staining of the intramuscular preterminal axons be seen. ×90

**Table 3.** Light microscopy of sciatic and sympathetic nerves

| Duration of experiment | | Thiamine deficiency | | Normal thiamine | | Thiamine surplus | |
|---|---|---|---|---|---|---|---|
| | | $H_2O$ | ETOH | $H_2O$ | ETOH | $H_2O$ | ETOH |
| 10 weeks | neuropathy | 1[a] | 1 | 0 | 0 | 0 | 0 |
| | symp.nerves | 3.9[b] | 4.0 | 4.3 | 4.1 | 4.2 | 4.3 |
| | | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) |
| 19 weeks | neuropathy | 0 | 1 | 0 | 1 | 0 | 1 |
| | symp.nerves | 4.4 | 4.2 | 4.4 | 4.6 | 4.3 | 4.1 |
| | | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) |
| 24 weeks | neuropathy | 0 | 0 | | | | |
| | symp.nerves | 4.0 | 4.1 | | | | |
| | | ($n=3$) | ($n=3$) | | | | |
| 33 weeks | neuropathy | | | 0 | 0 | 0 | 0 |
| | symp.nerves | | | 4.2 | 4.4 | 4.0 | 4.1 |
| | | | | ($n=3$) | ($n=3$) | ($n=2$) | ($n=3$) |
| 36 weeks | neuropathy | | 0 | 0 | | | |
| | symp.nerves | | 4.3 | 4.1 | | | |
| | | | ($n=4$) | ($n=1$) | | | |
| all animals | neuropathy | 1 | 2 | 0 | 1 | 0 | 1 |
| ($n=58$) | symp.nerves | 4.1 | 4.2 | 4.3 | 4.4 | 4.2 | 4.2 |
| | | ($n=9$) | ($n=13$) | ($n=10$) | ($n=9$) | ($n=8$) | ($n=9$) |

[a] refers to the number of rats with light microscopic changes in the nerve
[b] mean number of the perivascular fluorescing nerves

**Table 4.** Analysis of muscle biopsies

| Duration of experiment | | Thiamine deficiency | | Normal thiamine | | Thiamine surplus | |
|---|---|---|---|---|---|---|---|
| | | $H_2O$ | ETOH | $H_2O$ | ETOH | $H_2O$ | ETOH |
| 10 weeks | atrophic fibres | 1[a] | 2 | 0 | 1 | 0 | 0 |
| | group atrophy | 0 | 0 | 0 | 0 | 0 | 0 |
| | myopathy | 0 | 0 | 0 | 1 | 0 | 1 |
| | | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) |
| 19 weeks | atrophic fibres | 0 | 2 | 0 | 2 | 0 | 0 |
| | group atrophy | 0 | 0 | 0 | 1 | 0 | 0 |
| | myopathy | 0 | 0 | 0 | 0 | 0 | 0 |
| | | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) | ($n=3$) |
| 24 weeks | atrophic fibres | 1 | 1 | | | | |
| | group atrophy | 0 | 0 | | | | |
| | myopathy | 0 | 0 | | | | |
| | | ($n=3$) | ($n=3$) | | | | |
| 33 weeks | atrophic fibres | | | 1 | 0 | 1 | 1 |
| | group atrophy | | | 0 | 0 | 0 | 1 |
| | myopathy | | | 0 | 1 | 0 | 0 |
| | | | | ($n=3$) | ($n=3$) | ($n=2$) | ($n=3$) |
| 36 weeks | atrophic fibres | | 1 | 0 | | | |
| | group atrophy | | 0 | 0 | | | |
| | myopathy | | 0 | 0 | | | |
| | | | ($n=4$) | ($n=1$) | | | |
| all animals | atrophic fibres | 2 | 5 | 1 | 3 | 1 | 1 |
| | group atrophy | 0 | 0 | 0 | 0 | 0 | 1 |
| ($n=58$) | myopathy | 0 | 0 | 0 | 2 | 0 | 1 |
| | | ($n=9$) | ($n=13$) | ($n=10$) | ($n=9$) | ($n=8$) | ($n=9$) |

[a] refers to he number of animals

**Table 5.** Nerve conduction velocities (NCV)

| Duration of experiment | Measured variable (mean ± SD) | Thiamine deficiency | | Normal thiamine | | Thiamine surplus | |
|---|---|---|---|---|---|---|---|
| | | $H_2O$ | ETOH | $H_2O$ | ETOH | $H_2O$ | ETOH |
| 10 weeks | Weight (g) | 181 ±19 | 221 ±13 | 331 ±21 | 265 ±11 | 311 ±12 | 282 ±14 |
| | NCV I (m/s) | 33.5± 2.2 | 31.9± 3.2 | 34.0± 3.2 | 32.3± 3.4 | 38.9± 1.6 | 35.2± 2.7 |
| | NCV II (m/s) | 9.4± 0.8 | 9.6± 1.0 | 9.8± 0.4 | 9.7± 1.0 | 10.6± 0.7 | 11.0± 1.3 |
| | | ($n$=3) | ($n$=3) | ($n$=3) | ($n$=3) | ($n$=3) | ($n$=3) |
| 19 weeks | Weight | 227 ±13 | 232 ±35 | 294 ±28 | 259 ±31 | 292 ±15 | 255 ±41 |
| | NCV I | 32.7± 1.5 | 33.5± 1.2 | 35.2± 2.2 | 32.6± 1.5 | 35.8± 1.8 | 32.1± 0.9 |
| | NCV II | 9.9± 1.2 | 9.7± 0.4 | 10.0± 0.9 | 9.5± 0.3 | 9.6± 1.1 | 9.8± 0.7 |
| | | ($n$=3) | ($n$=3) | ($n$=3) | ($n$=3) | ($n$=3) | ($n$=3) |
| 24 weeks | Weight | 305 ±46 | 278 ±16 | | | | |
| | NCV I | 33.3± 1.1 | 34.1± 3.5 | | | | |
| | NCV II | 8.9± 0.6 | 8.6± 0.9 | | | | |
| | | ($n$=3) | ($n$=3) | | | | |
| 33 weeks | Weight | | | 449 ±107 | 239 ±21 | 364 ±86 | 338 ±43 |
| | NCV I | | | 32.7± 1.5 | 34.1± 0.9 | 39.6± 3.0 | 37.1± 3.3 |
| | NCV II | | | 9.1± 1.1 | 8.5± 1.0 | 9.4± 1.2 | 9.5± 1.2 |
| | | | | ($n$=3) | ($n$=3) | ($n$=2) | ($n$=3) |
| 36 weeks | Weight | | 253 ±56 | 308 | | | |
| | NCV I | | 33.3± 2.6 | 33.0 | | | |
| | NCV II | | 8.5± 0.4 | 9.1 | | | |
| | | | ($n$=4) | ($n$=1) | | | |
| Mean of all animals ($n$=58) | Weight | 238 ±60 | 246 ±39 | 363 ±80 | 255 ±23 | 323 ±57 | 291 ±48 |
| | NCV I | 33.2± 1.7 | 33.2± 3.2 | 33.9± 2.2 | 32.7± 2.1 | 38.0± 3.3 | 35.0± 3.5 |
| | NCV II | 9.4± 0.9 | 9.1± 0.8 | 9.6± 0.8 | 9.3± 0.9 | 9.9± 1.0 | 10.1± 1.0 |
| | | ($n$=9) | ($n$=13) | ($n$=10) | ($n$=9) | ($n$=8) | ($n$=9) |

also estimated. There were no differences in the distribution between any of the groups except after 33 weeks exposure when some animals on the combined alcohol and thiamine deficient diet showed pathological changes in the Ns.Che activity in 9% (mean) of the peripheral motor axon branches. Rats on the thiamine deficient non-alcohol diet showed pathology in six percent, which was about the same as in the control groups (Fig. 6). The results of the nerve conduction velocity measurements are summarized in Table 4. The mean conduction velocity of the large myelinated nerve fibres (NCVI), calculated from the beginning of the stimulus to the peak of the action potential, was somewhat slower in rats on alcohol (33.6 ± 2.9 m/s) than in those on water (35.0 ± 2.4 m/s). The alcoholic rats were also smaller in size. The conduction velocities of the small myelinated fibers (NCV2) were indentical in the two groups: 9.5 ± 6 m/s in animals on alcohol and 9.6 ± 9 m/s in animals on water. The velocities of small myelinated fibres tended to be higher in the rats on surplus thiamine, even though these animals were not larger in size than those on normal thiamine; differences were not found between the other groups (Table 4).

*Histochemistry and Electrophysiology of the Muscles.* A summary of the light microscopic analysis of muscles of animals in the different groups after application of the various histochemical reactions (see Methods) is presented in Table 5. Pathological structural changes were somewhat more prevalent in the animals on alcohol; i.e. 29% of the animals showed some degree of pathology while only 14.8% of those drinking water were abnormal. The changes were more prominent in animals on the thiamine deficient diet (38.5%) than in those on normal or suplus thiamine (22.0%). The changes observed: phagocytosis of the muscle fibers, single fiber and group atrophy, and changes in the distribution of NADH diaphorase were as previously reported (Terävänen et al., 1978) but quantitatively less (Figs. 3 and 4). There were no significant differences in the mean diamter of the type 1 and type 2 muscle fibers in the different groups. Sporadic fibrillation was observed in a few animals on alcohol after 36 weeks treatment, but not in other groups. The fibrillation was not considered significant.

## Discussion

Recent studies on the effects of chronic alcohol feeding on the development of neuropathy and myopathy in rats have demonstrated an unequivocal histological and electrophysiological pathology of peripheral nerves (Juntunen et al., 1978) and muscles (Terävänen et al., 1978) after 40 weeks of exposure. A mild distal neurophathy was seen in rats when polydipsia of 12 g of absolute alcohol per kg body weight per day was schedule-induced during 16 weeks (Bosch et al., 1978). This dosage of alcohol was about double that used by us. In the present experiment, the changes in individual muscles were quantitatively slight, possibly due to the relatively short exposure time. The high initial mortality of the rats on the thiamine deficient diet was correlated to the absolute thiamine deficiency, since their general condition greatly improved and their mortality rate markedly decreased after addition of 2 mg per kg thiamine to the diet. The mortality rate also correlated with the marked weight loss, as shown by the contrasting weight gain of the other groups and of the thiamine deficient groups in the first weeks after the addition of thiamine. Further, the mortality rate and weight loss were clearly related to the marked decrease in the food consumption in the thiamine deficient groups. Presumably we could have produced neuropathic changes somewhat sooner by continuing with an absolute lack of dietary thiamine (cf. Kark et al., 1975), but the possibility of serious misinterpretation would have existed, for a sudden weight decrease in a developing animal, combined with greatly decreased food intake, may imply general malnutrition. Interestingly, with the addition of ethyl alcohol to the basic diet, mortality was greatly reduced (Table 1) and less diminution in the consumption of food occurred even though the alcohol was acting as an extra source of energy (Fig. 2). The life-preserving capability of alcohol under these circumstances has been previously documented (Westerfeld and Lawrow, 1953). Ethanol may provide useful energy through metabolic pathways less affected by thiamine deficiency than pathways for other nutrients because ethanol can be metabolised

by the Krebs' cycle without the thiamine-requiring pyruvate carboxylase reaction. The situation is very different when there is thiamine in the diet. Under these conditions, the amount of available thiamine is expected to be less than that in the controls (Kiessling and Tilander, 1960). In any case, an absolute lack of dietary thiamine is presumably rare even in alcoholics, and relative deficiency, as modelled in the present study, serves better for a comparison of the combined effects of the use of alcohol and dietary factors. Increased food consumption and gain of weight occurred in animals on the diet deficient in thiamine after the first nine weeks whether they were on alcohol or not. Thereafter, however, the weight and the food consumption of the animals on alcohol was less than those on water. The same differences were found in the normal and surplus thiamine groups throughout the test period.

Although there has been previous experimental evidence showing that a diet deficient in thiamine (Kark et al., 1975) does produce neuropathy in nonalcoholics, the combined roles of relative thiamine deficiency and alcohol consumption have not been previously tested experimentally. This was done in the present study both by controlling the amount of dietary thiamine and demonstrating, by transketolase activity measurements, that the animals in fact developed a thiamine deficiency. The relatively high value of transketolase in the rats on alcohol and surplus thiamine is due to great variation within the group. The evidence presented suggests the tendency of myopathy and peripheral neuropathy to occur more frequently in the groups drinking alcohol than water, especially where the diet is deficient in thiamine (e.g. Table 4). The effects found cannot be explained by the possible effects of the thiamine content of the diet on ethanol elimination or acetaldehyde formation, however, because dietary thiamine apparently has no effect on these functions in the rat (Eriksson et al., 1978). The actual biochemical mechanisms underlying the development of alcoholic neuropathy and myopathy and the reasons why some individuals are more susceptible to these diseases than others remain to be determined.

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