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Myopathy Associated with Chronic Alcohol Drinking

Histological and Electrophysiological Study*

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Summary. Muscles of the lower legs of rats given 25% ethanol in water ad libitum for up to 9.5 months were studied using histological, histochemical and electrophysiological techniques. Ethyl alcohol was substituted for about 20% of the total calorific input of the animals. The observations were compared with the structure of the gastrocnemius muscle of five alcoholics with clinical neuropathy. Fibrillation potentials and angulated atrophic fibers were observed in the muscles of animals on alcohol for 9.5 months. No fiber type grouping was present. There was also phagocytosis of the muscle fibers and changes in their internal structure, as reflected by the distribution of NADH-diaphorase. The observed muscle changes in the alcoholics and those in the experimental animals on alcohol differed mainly quantitatively, the only exception being the presence of fiber type grouping in the biopsies from the alcoholics.

Key words: Alcohol – Myopathy – Histochemistry – Electrophysiology – Human – Rat.

Introduction

The extended use of alcohol is often combined with the occurrence of peripheral neuropathy (Victor and Adams, 1953; Schneeman and Kunze, 1973) and sometimes of myopathy (Ekbom et al., 1964). The presence of neuropathy has often been considered to result from nutritional deficiencies (Strauss, 1935; Jolliffe et al., 1936; Victor and Adams, 1953; Fenelly, 1964; Walsh and McLeod, 1970) but in other instances this is not true (Spillane, 1947; Denny-Brown, 1958; Behse and Buchthal, 1977). It has also occurred in alcoholics whose nutrition was considered to be good (Walsh and McLeod, 1970). However dietary defi-

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ciency of both proteins (Sachdev et al., 1971) and vitamins (Erbslöh and Abel, 1974) can result in polyneuropathy and myopathy.

An experimental model is necessary for controlled tests on the effects of different nutrients, in combination with the use of alcohol, on the development of neuromuscular symptoms. We have performed preliminary studies by feeding rats ethyl alcohol for several months under controlled nutrition. From our results it is evident that histologically and electrophysiologically demonstrable neuropathy can be induced by peroral alcohol feeding (Juntunen et al., 1978). We therefore studied the muscle histochemistry of these rats and compared the observations with findings in muscles from 5 alcoholic patients with clinical neuropathy. The results indicate that both neuropathy and myopathy can be produced in the rat by the long-term use of alcohol, though the changes are somewhat different from those in human alcoholics.

Material and Methods

Experimental Animals. Twenty-two male Spraque-Dawley rats about 2.5 months old were used in the study. Fourteen of the rats were given ethanol in water ad libitum as the sole source of liquid. The alcohol content was gradually increased. Solutions 10–15% (v/v) were used during the first two months, and 15–20% (v/v) during the next four months, and 25% (v/v) during the last three and half months. Eight of the rats were used as controls (age-matched).

The rats were kept in a stainless steel cage and given Astra-Ewos® standard laboratory food ad libitum. The diet was analyzed by the Technical Research Center of Finland to assess any dietary deficiencies; there were none. When the ethanol content was 25% the amount of ethyl alcohol consumed was estimated to be 8–9 g of absolute alcohol/kg per day. The mean food consumption of the alcohol group during the follow-up period was estimated to be about 105 cal/kg (430 kJ/kg) compared with about 130 cal/kg (540 kJ/kg) for the control group. Ethyl alcohol was thus substituted for about 20% of the total caloric input of the animals.

Patients. For a comparison with results obtained from the experimental animals, gastrocnemius biopsies were made under local anesthesia with 1% lidocaine. Five patients with clinical neuropathy, one female of 52 and four males between 46 and 54 years of age (mean 50.7), were chosen because of the absence of tendon reflexes and diminished distal cutaneous sensation, combined with heavy drinking for more than 8 years. Four of the patients complained of weakness of the legs and one had pain; none had swollen or tender muscles.

Methods. All rats were examined under pentobarbital anesthesia between 2.5 and 9.5 months after the beginning of the diets. Spontaneous electrical activity (fibrillation potentials) was sought after inserting a concentric needle electrode into several positions of the distal ends of the muscles of the tibial group (EDL and TA). Due to insertion activity, more than a minute was allowed to elapse before interpretation. The rats were then killed, the proximal ends of the same muscles were removed and processed for light and electron microscopy. A piece of the sciatic nerve was also removed and similarly processed. The opposite sciatic nerve, together with its distal branches, was removed for compound action potential measurements. The present paper reports histological and electrophysiological observations on the muscles. The structural and electrophysiological observations on the sciatic nerves have been reported separately (Juntunen et al., 1978).

A Grass S 8 stimulator with an SIU 4678 isolation unit, a Type 122 preamplifier and a Tektronix D15 storage oscilloscope with a 5B18N amplifier were used in the electrical measurements. The observations were documented by taking photographs from the storage oscilloscope screen. After removal, the muscle specimens were frozen in isopentane precooled in liquid nitrogen, and sectioned at 10 μ in the cryomicrotome at −20° C for histological studies. The sections were thawed and dried on glass slides. 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 for NADH-diaphorase (Pearse, 1972) and ATP-ases with ATP-disodium salt as a substrate (Guth and Samaha, 1970). Preincubations with both acid (pH 4.3) and alkali (pH 10.2–10.4) were used for separate sections before incubation with the substrate at pH 9.4. Sections incubated with ATP-ase were used for the calculation of the diameters of type I and type II fibers using the smallest diameter measurement (Brooke and Engel, 1969). The sizes were measured from photographs taken at random at standard magnification together with a standard micrometer reading.

Results

The control animals gained more weight than did the animals with ethyl alcohol in their diet (Fig. 1).

Sporadic fibrillation was observed in one animal after 2.5 months and after 7 months on alcohol. Marked fibrillation was present in the animals on alcohol after 9.5 months (Fig. 2). Fibrillation was not seen in the controls, nor in the animals on alcohol for 4.5 months.

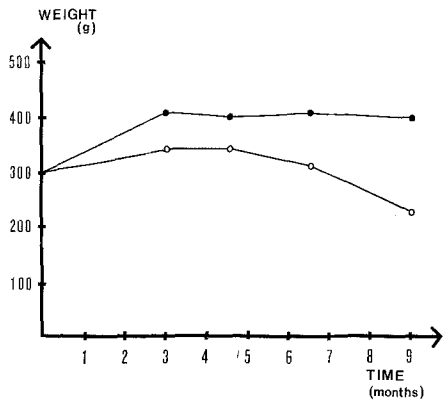


Fig. 1. Mean weight of the control animals (closed circles) and that of the animals on alcohol (open circles)

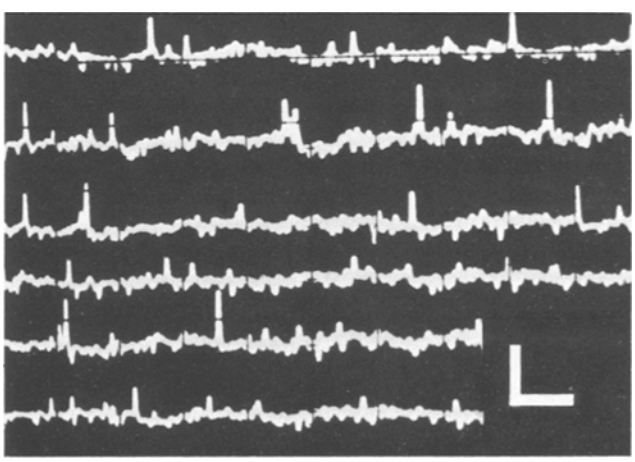


Fig. 2. Fibrillation recorded from the tibial muscles of an experimental animal after 9.5 months on alcohol. The readings are 2 μV and 20 ms

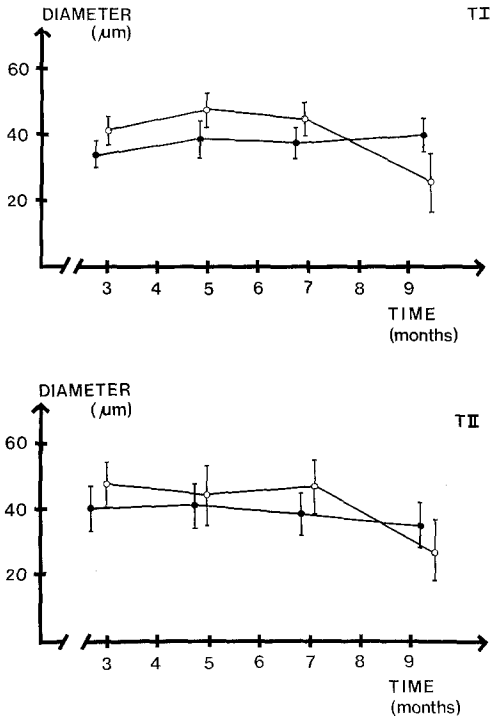


Fig. 3. Mean diameter of type I and type II muscle fibers of the rats on alcohol (open circles) and of those of age-matched controls (closed circles)

In most of the experiments the mean size of type I and type II muscle fibers in the alcohol group was larger than in the controls. After 9.5 months on alcohol the mean size of type I fibers, in particular, had diminished (Fig. 3).

Only after 9.5 months on alcohol was fibrillation correlated with histological evidence of muscular damage (Fig. 4). An examination of the muscle structure after the histochemical and histological reactions showed fiber size variation with small angular atrophic fibers sometimes in small groups. This variation in the fiber size can also be seen as an increase in standard deviations in Figure 3. There was no large group atrophy, nor was there fiber-type grouping in the sections incubated with ATP-ase. The distribution of NADH-diaphorase was abnormal in a limited number (less than 2%) of the large fibers (Fig. 4). This is in contrast to the often marked changes in the selected patients with alcoholism and neuropathy (e.g. Fig. 5).

Muscle fibers undergoing phagocytosis were present in the biopsy specimens after 9.5 months on alcohol, though they were not very frequent. Rough calculations of their number during microscopy gave an estimation of about 1% of all the fibers. Similarly about 6% of the muscle fibers contained one or more central nuclei, compared with less than 1% in the controls.

In all five biopsies from the alcoholics with clinical neuropathy, fiber size variation and small atrophic angulated fibers were present. In three of the specimens small-group atrophy was also present to a variable extent. The specimens least and most affected are illustrated in Figure 5. Sporadic fibers undergo-

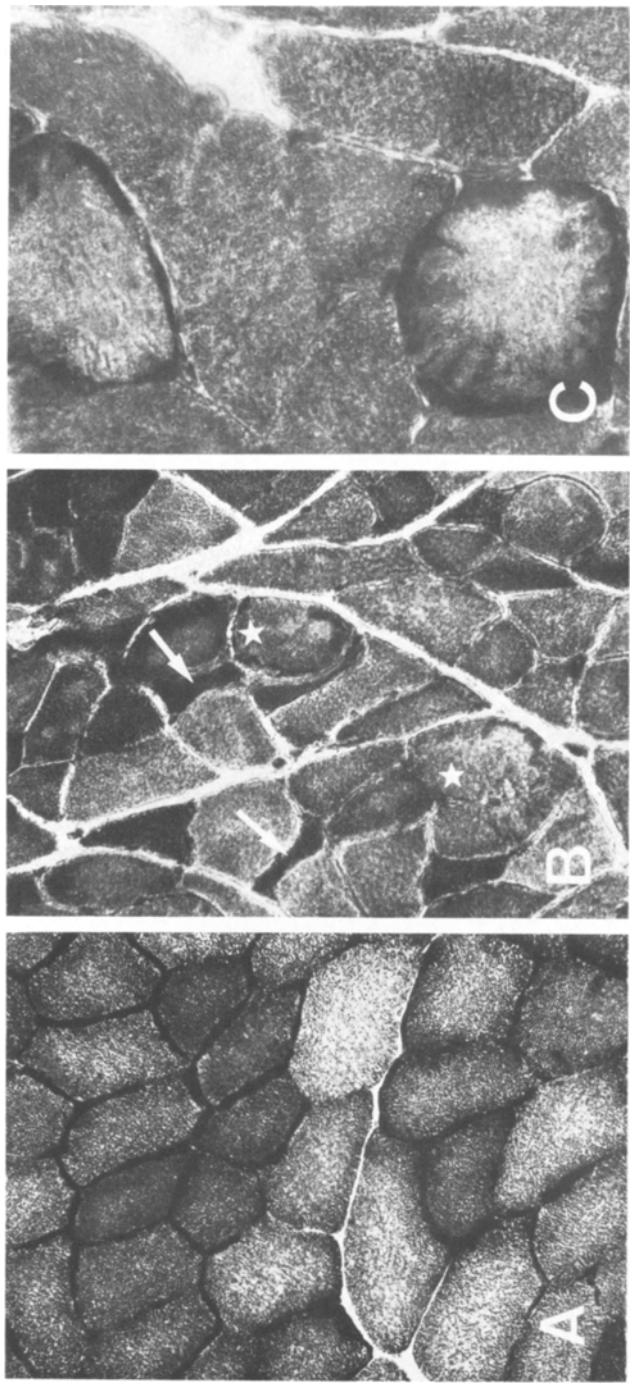
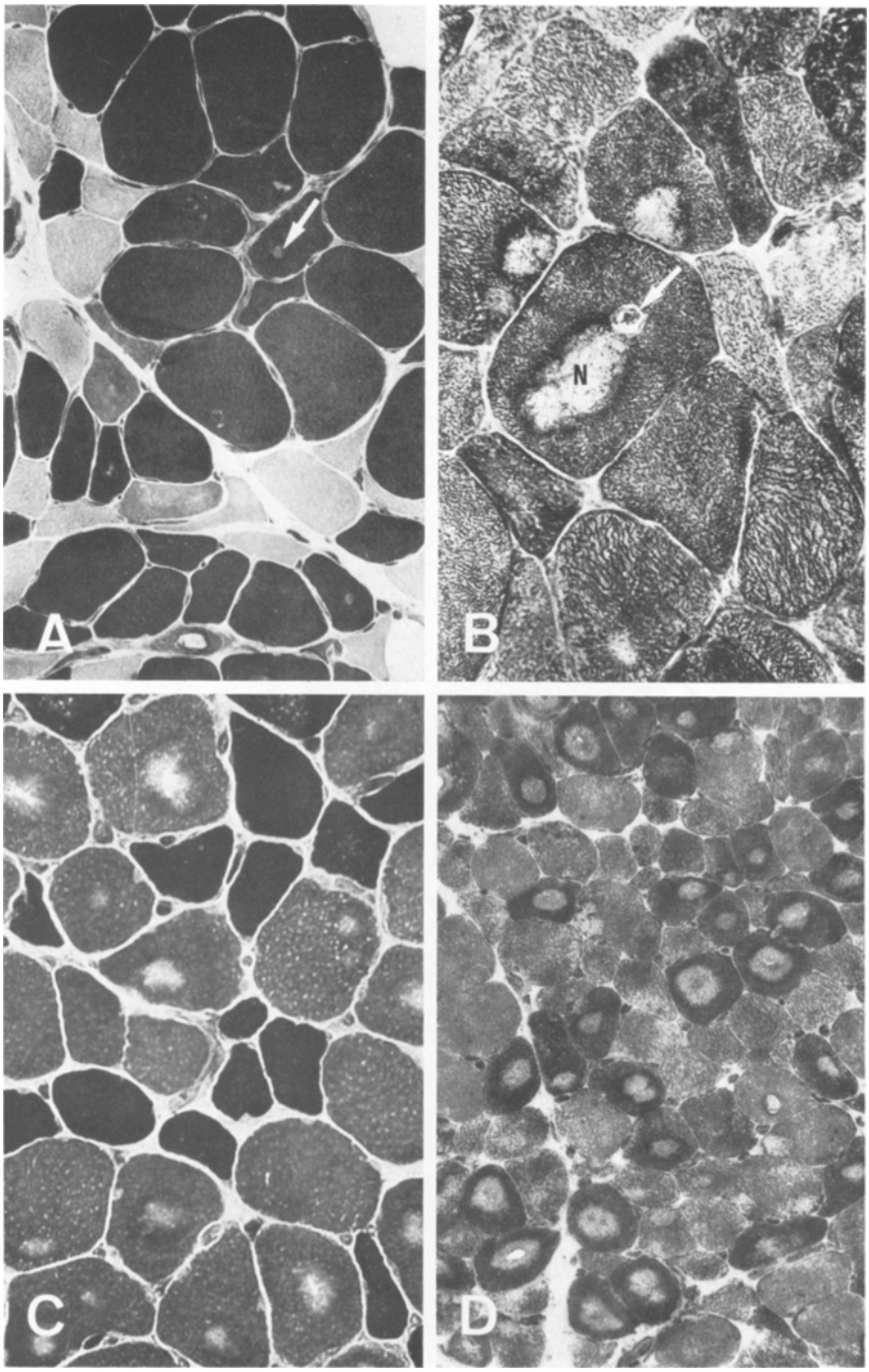


Fig. 4. **A** illustrates a transverse section of the muscle from a control animal, incubated for NADH-diaphorase. Muscle fibers with varying intensities of the enzyme activity are present, and the muscle mitochondria are evenly distributed within individual muscle fibers. ×240. The transverse section of the EDL muscle incubated for NADH-diaphorase from an animal on alcohol 9.5 months is illustrated in **B**, ×190. Note the presence of fibers with intense enzyme activity (*arrows*), typical for neurogenic muscle atrophy. There are also some fibers in which the distribution of mitochondria is irregular (*stars*) differing from that seen in the control muscles illustrated in **A**. **C** shows two abnormal fibers in higher magnification. ×400


A
B
N
C
D

ing phagocytosis were seen in two of the biopsies. In all cases there were numerous muscle fibers with abnormal distribution of mitochondria as illustrated in NADH-diaphorase sections (Fig. 5). Fibers with predominantly oxidative metabolism seemed to be more affected but irregularities were also present in the fibers with lower NADH-diaphorase activity. Some of the areas devoid of NADH-diaphorase also showed diminished ATP-ase activity of myosin ATP-ases (Fig. 5). These areas of diminished ATP-ase activity were present in type I fibers.

Discussion

Fibrillation combined with atrophy suggests neurogenic damage of muscle fibers in the groups of rats fed with ethyl alcohol for 9.5 months; a finding consistent with the neurological changes observed (Juntunen et al., 1978). The fact that fiber type grouping was not present may be due to progressive degeneration without regeneration either by axonal regrowth or by terminal sprouting. In addition to the neurogenic changes there was also phagocytosis, as well as some changes in the internal structure of the muscle fibers, suggestive of a direct effect on the muscle. The changes were somewhat different from those seen in the alcoholics with clinical neuropathy. In all the patients alterations in the internal structure of the muscle fibers were more often marked than in the experimental animals. In addition, fiber-type grouping was present in the humans, indicative of previous denervation and reinnervation of muscle fibers. Thus the changes in the alcoholics and in the experimental animals differed from each other mainly in quantitative terms, and the only exception was that there were signs of reinnervation in the alcoholics, but not in the experimental animals. It seems therefore, that the rat might be used, under the conditions described, as a satisfactory experimental model for alcoholic neuromyopathy.

There have been short-term experiments in man to evaluate whether alcohol alone, or combined with nutritional factors, can cause structural or functional

Fig. 5A–D. The figure is a composite from transverse sections of the gastrocnemius muscle of two alcoholics with clinical evidence of neuropathy. The section illustrated in **A** was incubated for ATP-ase after acid preincubation and the figure illustrates a small piece of the muscle with damage of neurogenic type, i.e. fiber size variation, type grouping and small angular atrophic fibers of both type I and type II. The small non-reactive areas (*arrow*), seen better in the dark type I fibers, only partially correspond to central nuclei seen in Gomori trichrome-stained sections, ×120. **B** is from the same patient but incubated for NADH-diaphorase, ×160. Irregularities in the NADH distribution are best seen as non-reactive "moth-eaten" areas (N). The arrow points at a probable autophagic vacuole. The changes in the internal structure seen in the muscles of this 52 year old female were much slighter than those present in the most affected specimen from a 46 year old male alcoholic, illustrated in Figures C and D, ×120, ×60. **C** was photographed after pre-incubation for ATP-ase at pH 10.2. Type I fibers appear lighter than type II fibers. There are non-reactive areas in type I fibers which correspond to the regions with diminished NADH-diaphorase activity illustrated in **D**. Note that there are also irregularities in the mitochondrial distribution in the fibers with lower NADH-diaphorase activity

disturbances in muscles and nerves (Mayer, 1966; Perkhoff et al., 1966). The only long-term study on muscle structure known to us was performed with Sprague-Dawley rats by Jordö and his coworkers (Jordö et al., 1975). They added ethanol, red wine, whiskey, brandy or gin to a diet with varying fat, carbohydrate and protein content and observed no changes in the muscle when stained with hematoxylineosin after paraffin embedding. It is difficult to try to explain the differences in the results they obtained. We used more sensitive methods, but this is only a partial explanation. The difference in the amount of alcohol consumed may be important. Changes in the heart muscle and capillaries have been reported in alcoholics and in experimental animals (Hibbs et al., 1965; Sohal and Burch, 1969; Christmann, 1972; Herrlinger et al., 1974).

Previous studies on muscles in alcoholic patients have been performed by clinical, electrophysiological, electron microscopical and histological means (Hed et al., 1962; Ekbom et al., 1964; Perkoff et al., 1966; Klinkerfuss et al., 1967; Kahn and Meyer, 1970; Mayer and Garcia-Mullin, 1972). Histochemical techniques were applied by Martinez, Hoosmand and Faris (1973) to study acute alcoholic myopathy in five patients. They described patchy loss of oxidative enzymes in type I fibers. The histochemical tests used in the present work, especially NADH-diaphorase, showed that a relatively large proportion of individual muscle fibers of type I and, to a somewhat lesser degree, of type II were devoid of NADH-activity in places, and are presumably not properly functioning parts of the muscle fibers. These occurred in patients devoid of clinical signs of myopathy and to a greater extent (e.g. Fig. 4) than can be expected to occur in peripheral neuropathies or spinal muscular atrophies (Dubowitz and Brooke, 1973). Some of these changes observed resemble those seen in central core disease, in particular the unstructured cores described by Gonatas et al. (1965). However, many of the irregularities in mitochondrial distribution were not correlated with altered ATP-ase activity and were different from both structured and unstructured cores. Our observations indicate that some of the muscle fibers in patients with alcoholic neuropathy are abnormal as judged by histochemical evidence of alterations in the contractile elements and by the abnormal distribution of cell organelles responsible for their energy metabolism.

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