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BIOCHEMICAL AND MORPHOLOGICAL FEATURES OF EXPERIMENTAL CHRONIC ALCOHOLIC MYOPATHY

(With 1 Table and 4 Figures)

By

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العضلات الإراديه في الضمور العضلي الناجم عـــن الكحـــــــــــول

تحية سليـــم ، علام نفـــــــادي

تم إحداث ضمور عضلي كحولي في ١٠ فئران بيضاء من فصيلة السبراج داولي عن طريـــق إعطاء الكحول في جرعة تقدر بـ ٥ر١٣ جرام لكل كيلو جرام من وزن الفئران لمدة ١٠ أسابيـــع وتم أخذ عضلة التبيالـــز الأمامية وتم طحنها في المحلول المعادل وقدرت نشاطات أنزيمـــــات الجليكوليـــس بها ووجد أن معدل الجليكوليـــس قد نقص نقصانا واضحا كما اتضح من نقـــص نشاط أنزيمات الالدولاز والفوسفوهكسوزأيزومبراز والفسفوجلكوميتاز وكذلك اللاكتيـــــــات ديهدروجيناز بالمقارنة بعضلات الفئران الطبيعيه وبدراسة التغيرات الهستولوجية المصاحبـــة وجد أن هناك تغيرات واضحة في الألياف العضلية وأظهرت ضمورا من نوع ٢ ب . ويمكـــن أن نستخلص من هذا البحث أن سبب تثبيط أنزيمات الجليكوليـــس في الأشخاص المتعاطين للكحول هو هذا النوع من التغيرات .

SUMMARY

Experimental alcoholic myopathy was induced in 10 male Sprague Dawly rats by a combination of prolonged alcohol intake (mean 15.3 g ethanol/ Kg/day for up to 10 weeks) and a short fast. Histological and biochemical studies were combined to characterize the type of change in alcoholic myopathy. Therefore, muscles (tibialis anterior) were homogenized while cold in TED buffer and the supernatant after centrifugation was used to determine the activity of the glycolytic enzymes: aldolase, phospho-glucomutase, phosphohexose isomerase and lactate dehydrogenase. Tibialis anterior muscules were histologically examined. The biochemical and morphological data were compared to a control group formed of 7 normal rats. There is significant panglycolytic enzymes decreased activities ($P/0.05$, $P/0.001$, $P/0.001$ and $P/0.05$ respectively) compared to controls. The predominant findings were degeneration, necrobiosis and atrophy which partly accounts for the depressed glycolytic enzyme

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activity. The role of oxygen free radicals generated by alcohol in attacking the proteins and membranes can not be ignored.

INTRODUCTION

Alcholol is a drug characterized by its deleterious effects. Although death from acute ethanol poisoning itself is uncommon, yet chronic alcoholic intoxication is common. Alcoholic muscle disease is divided clinically into two distinct myopathic syndromes, the rare syndrome of acute rhabdomyolysis (GROSSMAN et al., 1974) and the common syndrome of chronic proximal wasting which develops to some degree in almost all severe alcoholics (MARTIN et al., 1982). Type .II b fibre atrophy has been demonstrated as the major histological abnormality in chronic alcoholic myopathy, observed in vastus lateralis (HANID et al., 1981). More definite degenerative changes are found in tibialis anterior (LANGOHR et al., 1983). These changes are keeping with the associated mild distal neuropathy (HALLER, 1984) but not with a frank denervation similar to the situations in disuse atrophy, polymyalgia rheumatica and osteomalacia (TROUNCE et al., 1987).

The possibility that a toxic effect on glycolytic or mitochondrial metabolism related to ethanol or its major metabolite acetaldehyde, may be important in the pathogenesis of chronic alcoholic muscle wasting. Studies of skeletal muscle glycolytic function have recorded a lowered level of several enzymes in tibialis anterior (LANGOHR et al., 1983) and a transient fall in lactate dehydrogenase after an alcoholic binge (SUOMINEN et al., 1974). Normal activities were found in one study in abstinent alcoholics (SUOMINEN et al., 1974). In another study of three patients a depression of all glycolytic enzyme activities were found, although clinical information was not provided for correlation (HALLER and KNOCHEL, 1984). since these data, concerning glycolytic function in "alcoholic myopathy" in humans are more or less difficult to interpret because of the limited information with regard to the patient's clinical statuses and the duration of their abstinence from alcohol (TROUNCE et al., 1987), the aim of the present work is to carry out a detailed study of glycolytic enzymes as well as morphological changes in skeletal muscles of wasted alcoholic rats.

MATERIAL and METHODS

Male Sprague-Dawley rats (17 animals, 150-250 g at begining of experiment) fed ad lib with a standard rat diet were used. Experimental alcoholic myopathy was induced in 10 animals by adding ethanol to the drinking water, begining at 2.5% w/v and increased to 25% over four weeks which corresponds to a 15.3 g/Kg/day. Animals were weighed regularly and total calorific intake and percentage calorific intake obtained from ethanol were calculated by monitoring solid food and liquid consumption. When progressive weight loss was noted, solid food was withheld from the controls and alcoholics for 24 h and the animals anaesthesized with ether and killed (TROUNCE et al, 1990).

Muscles (tibialis anterior) were homogenized while cold in TED buffer (Tris, 10 mM, EDTA disodium 1.5 mM and dithiothreitol, 1 mM pH 7.4 mM pH 7.4 at 4°C). The muscles were centrifuged at 800 xg for 5 minutes. The supernatants were used for the determination of aldolase by the method of PINTO et al. (1969); phosphohexose isomerase by the method of HORROCKS et al. (1963); phosphoglucomutase by the method of LVIE (1964) and lactate dehydrogenase by the method of WROBLEWSKI et al. (1955). Protein was determined by LOWRY et al. method (1951).

Histomorphological study :

Immediately after killing the rats, several blocks of 2 x 2 mm size from the chosen muscles were taken and fixed in cold 5% glutaraldehyde. These samples were embeded in Epon 112 as usual. semithin sections (1u in thickness) were prepared and stained with toludin blue. Also ultrathin sections were prepared by using LKB ultratom and contrasted by uranil acetate and lead citrate. By using Jeol 100 C X II electron microscope, the contrasted ultrathin sections were examined and photographed.

RESULTS

Muscle glycolytic enzyme activities for control rats and those with induced alcoholic myopathy are shown in Table (1) and Fig. (4). The activities ofthe 4 enzyme studied were significantly decreased in wasted alcoholic group with reference to normal control. Light microscopy of muscles of control rats revealed that the muscular fibres are closely attached to each other and nearly of the same size in cross sections (Fig. 1-1). Ultrastructurally, the muscle fibres of the control rats (Fig. 1-2) contained symmetrical myofibriles, as somewhat few cellular organelles especially mitochondria. The myofibriles showed that some of the muscle fibres were rich in cell organelles, white and dark bands as well as Z disk. In contrast, the muscles of alcohol treated rats were slightly decreased in size and paler in colour on gross appearance compared to control group. Moreover, muscles of alcohol treated rats showed myopathic changes. These myopathic changes were in the form of degeneration and necrobioses of the muscle fibres. The myofibrils appeared widely separated from each other with vriation in their size. Vacuolation of the mitochondria and numerous fat globules were detected (Fig. 2-1 and 2-2). Numerous muscle fibrils were destructed where they were smaller and showed marked vacuolation and destruction of the cell organelles especially the mitochondria (Fig. 3-1 and 3-2). These necrotic or degenerative changes were more marked in muscle fibres which were poor in cellular organelles than those rich in cell organelles. These cells which are poor in cell organelles are known as type II b atrophy (JONES and HUNT, 1983).

DISCUSSION

The present work showed that a significant myopathy in muscle fibres which were poor in cell organelles (type II b fibres) (CARPENTER and KARPATIY, 1984) was the striking histomorphological finding in this animal model. This keeps with the

changes observed in rats by TROUNCE et al. (1990), and in humans by MARTIN et al. (1984). This morphological change was associated with significant depletion of the glycolytic enzymes aldolase, phosphohexose isomerase, phosphoglucomutase and lactate dehydrogenase (Table 1 and Fig. 1). The morphological and biochemical findings in the model were not as striking as in patient studies observed by TROUNCE et al. (1987) and was similar to that reported by TROUNCE et al. (1990) in the same model. This could be related to the relatively short duration of animal experiments relative to human disease (TROUNCE et al., 1990). Itis clear that a significant glycolytic impairment develops in chronic alcoholic myopathy. While the level of impairment seen would not be functionally significant if spread equally across all muscle fibres, the patchy fibre involvement seen histomorphologically may indicate that some fibres are more severely impaired than the whole muscle (TROUNCE et al., 1990).

The panglycolytic enzyme reduction is in part related to type II b fibre atrophy, where positive correlation beteen type II b fibre atrophy and decreased activity for most glycolytic enzymes was observed by TROUNCE et al. (1987). Such correlation supports the contention that a loss of volume of the II b fibres, which are richest in glycolytic enzymes, at least partly explain the decreased activity levels of these enzymes found in alcoholics (TROUNCE et al., 1987).

There is a growing interest in the role of oxygen free radicals in the pathology of alcohol abuse. The metabolism of ethanol raises free radical levels through activation of cytochrome p-450 and increased free radical attack on proteins, and membranes (TROUNCE et al., 1990) leading to decreased glycolytic enzymes.

This persistent reduction in total glycolytic capacity in chronic alcoholic muscle wasting may render the patient more susceptible to an acute toxic ethanol or ethanol metabolite effect on glycolysis (TROUNCE et al., 1987). In conclusion, among the hazardous sequelae of chronic alcoholism is the alcoholic muscle disease with the chronic proximal wasting as the common form characterized by type II b atrophy with panglycolytic muscular deficiency.

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Table 1: Glycolytic enzymes specific activity levels (mean±S.E.) in normal and alcoholic rats muscles.

	Normal rats muscles (n = 7)	Alcoholic rats muscles (n=10)	t test
Aldolase mU/mg protein	86.9±7.2	62.96± 7	P < 0.05
Phosphohexose Isomerase mU/mg protein	93.05±4.7	46.8±7.8	P < 0.001
Phosphogluco-mutase mU/mg protein	51.2±6.8	20.9+6.1	P < 0.001
Lactate dehydro-genase U/mg protein	687.5±45.3	566.9+23	P <0.05

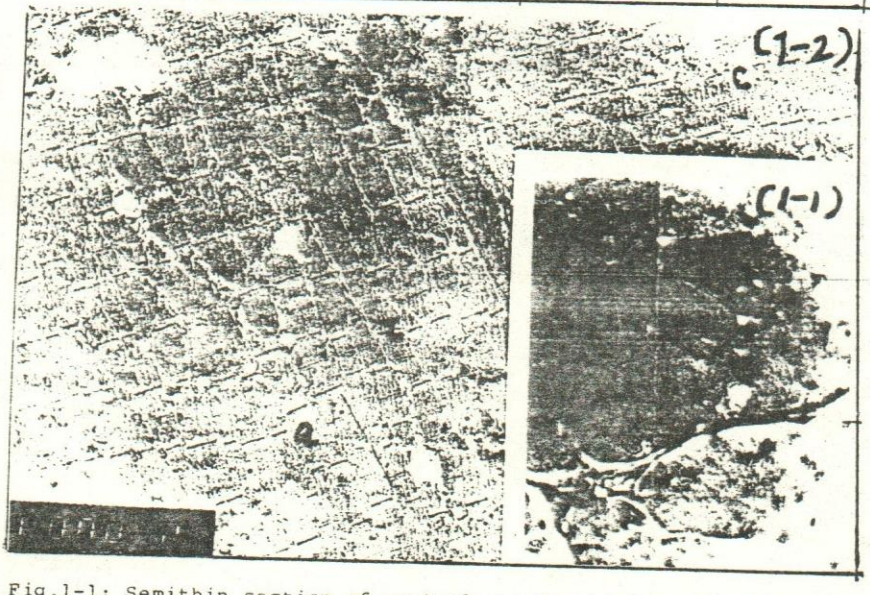

Fig.1-1: Semithin section of control muscle showing the muscle fibre closely attached to each other. T & B stain Mag. (100 X 10).

Fig.1-2: Electron micrograph of the control muscle illustrate the normal structure:
a) myofibrils
z) Z-disk
c) mitochondria
Mag. (2700X2.7).

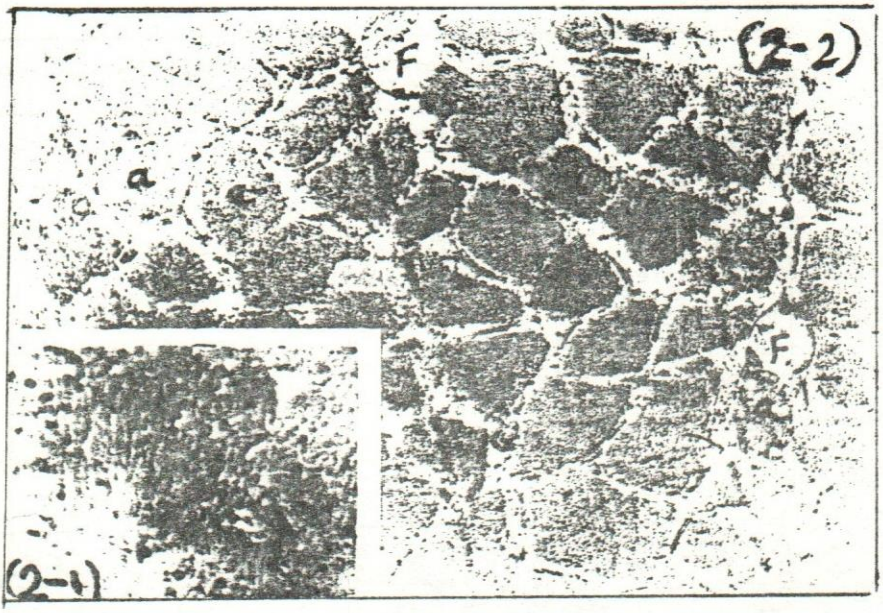

Fig.2-1: Semithin section of alcohol treated muscle showing vacuolation of the muscle fibres T & B stain Mag.(100X10).

Fig.2-2: Electron micrograph staining showing diminution and separation of the muscle fibrils:
a) Presence of fat globules (F) and vacuolation of mitochondria (C) .

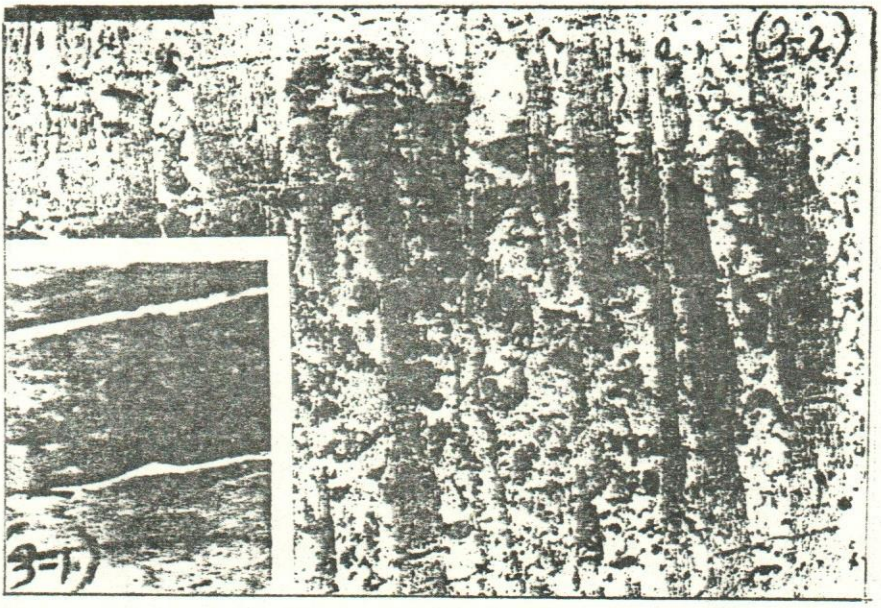

Fig.3-1: Semithin section of treated muscle staining with degeneration of the muscle fibre. T & B stain (Mag. 100X10).

Fig.3-2: Electron micrograph of treated muscle illustrating picture of necrobiosis:
1- destruction of the muscle fibrils (a).
2- great variation in the size of the muscle fibrils (b)
3- vacuolation even destruction of the mitochondria (c).

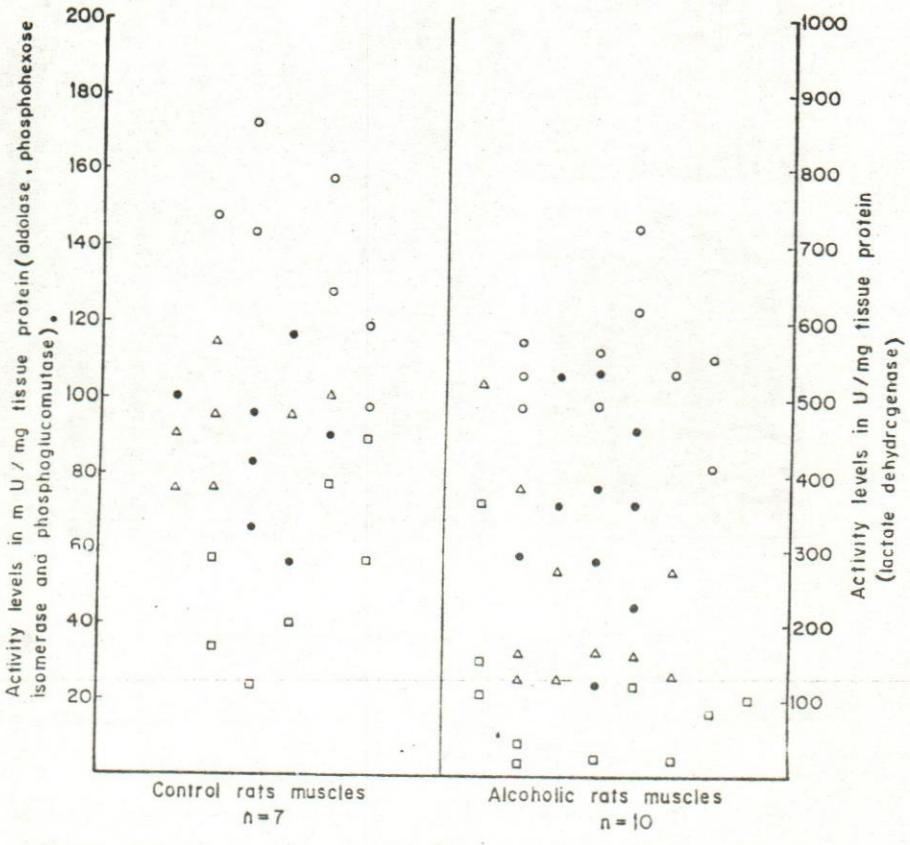

Fig. 4 · Scatterogram of specific activity levels of aldolase (•) Phospho_hexose isomerase (△) Phosphoglucomutase (□) Lactate dehydrog_enase (○) in control and alcoholic rats muscles.