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SKELETAL MUSCLE LESIONS IN RATS WITH ACUTE ALCOHOL INTOXICATION

I. Ya. Britvan

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Skeletal muscle damage is found in 20-60% of cases of alcohol intoxication [3, 5] and it is a special and significant component of alcohol disease. Experimental studies of the skeletal muscle, however, could not be found in the literature. It was accordingly decided to study lesions of the gastrocnemius muscles of rats with acute alcohol intoxidation.

EXPERIMENTAL METHOD

Experiments were carried out on 40 female Wistar rats weighing 200-230 g, divided into three groups. Animals of groups 1 and 2 (18 and 14 rats, respectively) were given a single dose of 1.2 ml/100 g body weight of 50° ethanol by gastric tube. Rats of group 2 were kept on a starvation diet for 48 h before the beginning of the experiment. In group 3 (control) eight rats were kept under similar conditions. The animals were killed by decapitation under ether anesthesia 24 h after the beginning of the experiment. Besides histological stains, histochemical and enzyme-histochemical methods were used to study glycogen, lipids, ribonucleoproteins, and oxidation-reduction and hydrolytic enzymes. Activity of oxidation-reduction enzymes and concentrations of lipids were determined by measuring the optical density of the reaction product with the LYUMAN-I-2 cytophotometer. Cytophotometric parameters of myocyte metabolism (in optical density units) are given in Table 1.

EXPERIMENTAL RESULTS

In response to administration of alcohol 12 rats of group 1 become apathetic and lethargic, and six became restless and excited. Toward the end of the first hour of the experiment all the animals fell asleep. In group 2, after starvation for 48 h, the animals sat still, in close contact with each other. After administration of alcohol and food for 2 h they were active. Examination 24 h after alcohol administration revealed a fixed posture, untidiness of the hair, and rapid respiration in 13 rats of group 1 and seven of group 2; the remaining animals moved actively around the cage. At autopsy moderate congestion of the internal organs was found, with petechial hemorrhages beneath the pleura and on the gastric mucosa. The liver was yellowish brown in color, sometimes with a nutmeg pattern at the edge. The skeletal muscle was pale brown in color and of the ordinary consistency.

Microscopic investigations of the skeletal muscles showed that most muscle fibers were polygonal in shape and the rest were round. Cross-striation in most fibers was well-marked. The nuclei were rod-shaped and distributed, 7-10 in number (normally 8-12), beneath the sar-

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TABLE 1. Cytophotometric Parameters (conventional units) of Activity of Oxidation-Reduction Enzymes and Lipid Concentrations in Gastrocnemius Muscle Fibers of Rats with Acute Alcohol Intoxidation (M ± m)

Parameter	Muscle fibers	Group of animals		
		1	2	3 (control)
SDH	White	0,183±0,007***	0,196±0,004***	0,194±0,010
	Red	0,314±0,012***	0,322±0,006***	0,322±0,007
LDH	White	0,344±0,011***	0,327±0,015***	0,252±0,006
	Red	0,508±0,011*	0,436±0,014*	0,473±0,008
NAD-diaphorase	White	0,286±0,009	0,321±0,007*	0,309±0,012
	Red	0,384±0,007	0,435±0,004***	0,397±0,009
NADP-diaphorase	White	0,274±0,008***	0,211±0,006***	0,159±0,006
	Red	0,418±0,011***	0,271±0,007**	0,301±0,007
G6PDH		0,242±0,014	0,194±0,010	0,210±0,011
Sudan		0,082±0,004**	0,052±0,002**	0,067±0,003

Legend. *p < 0.05; **p < 0.01, ***p < 0.001 compared with control.

colemma. On average, in every 15th fiber in the rats of group 1 and in every 10th in the animals of group 2, one or two nuclei were juicy and had shifted into the center of the fiber. The glycogen content was reduced, more especially in the animals kept on a starvation diet. Fatty infiltration was intensified in the animals of group 1 ($p < 0.001$), whereas in the rats of group 2 it was reduced statistically significantly ($p < 0.001$) compared with the control.

In the skeletal muscle of rats of both groups palely stained segments of fibers with ill-defined or completely absent cross striation, and with fewer (2-3) pale nuclei, sometimes vacuolated, than in the control, were observed in every 10th-15th fiber in the animals of group 1 and in every 7th-10th fiber in the animals of group 2. Necrotic muscle fibers with intact sarcolemma and proliferating nuclei also were found. Occasionally in the place of muscle fibers a fine-grain mass could be seen containing a few lymphocytes, macrophages, and solitary polymorphs. The foci of myocardial damage were more extensive in the animals of group 2.

Determination of activity of oxidation-reduction enzymes in viable muscle fibers of the rats of group 1 revealed a statistically significant decrease in SDH activity compared with the control in white and red fibers ($p < 0.001$). In the animals of group 2 the SDH content was unchanged in muscle fibers of both types. A cytophotometric study of ODH revealed increased activity in white and red muscle fibers ($p < 0.001$) of the animals of group 1. The character of distribution of LDH diformazan granules in the rats of group 2 corresponded to higher activity than in the control ($p < 0.001$) in white fibers, and of activity at virtually the control level in the red fibers.

Investigation of NAD-diaphorase in muscle fibers of the rats of group 1 showed that the tendency for its activity to decrease was not statistically significant ($p > 0.05$). NAD-diaphorase activity in the animals of group 2 was increased only in red muscle fibers ($p < 0.001$). In the animals of both experimental groups NADP-diaphorase activity was increased in fibers of types I and II ($p < 0.001$), except in red fibers of the rats of group 2, in which it was reduced ($p < 0.001$). Meanwhile glucose-6-phosphate dehydrogenase (G6PDH) activity in the animals of group 1 was higher than in the control, but the decrease in its activity found in muscle fibers of the rats of group 2 was not statistically significant ($p > 0.05$). Weak acid phosphatase activity was discovered in the muscle fibers of the rats of group 1, but it was higher in the fibers of the rats of group 2.

In the rats of experimental groups 1 and 2 the endomysium and perimysium consisted of loose connective tissue with fat cells in the perimysium, and with congested and sometimes gaping capillaries numbering 3-6 per field of vision. The walls of some vessels were thickened and infiltrated by lymphohistiocytes and solitary polymorphs. Their endothelium was swollen and juicy, and gave a positive reaction for alkaline phosphatase. Macrophages (up to 2-5 per field of vision under a magnification of 126) with high acid phosphatase activity in their cytoplasm, were found in the interstitial tissue, and mast cells up to 2-6 per field of vision in the endomysium and up to 14-20 per field of vision in the perimysium also were present; these were large cells, with metachromatic granules, and sometimes degranulating, by contrast with the single, densely packed, orthochromatic cells in the control. Some mast cells gave a positive RAS reaction.

Administration of ethanol caused a combination of varied metabolic disturbances in the muscular system. The deficiency of catecholamines due to the action of acetaldehyde led to their increased synthesis and release into the blood stream. Subsequent stimulation of catecholaminergic receptors led to glycogen deprivation as a result of conversion of ATP into cyclic AMP followed by conversion of inactive phosphorylase into active, with consequent decomposition of glycogen into glucose. Accumulation of lipids in the muscle fibers of the rats of group 1 may have occurred through several mechanisms. Disturbance of intramitochondrial oxidation of NADH formed during metabolism of ethanol and acetaldehyde, and increased NADPH-diaphorase activity leads to stimulation of lipogenesis [1]. Additionally, under the influence of acetaldehyde and its conversion into acetate, lipid peroxidation is initiated, and in turn, this damages the myocyte membranes, leading to inactivation of the enzymes and damage to nucleic acids [24]. It is generally known that catecholamine release also leads to the formation of nonesterified fatty acids. Reduction of SDH activity is evidence of inhibition of oxidation in the tricarboxylic acid cycle, and it may be linked with the direct inhibitory action of ethanol or acetaldehyde on the enzyme. Activation of LDH is evidence of stimulation of anaerobic degradation of glucose and it depends on an increase in the intracellular concentration of incompletely oxidized products. Dystrophic changes, sometimes turning into necrotic, are associated not only with the direct action of ethanol and its metabolites on myocytes, but also with disturbances of the microcirculation. Morphological confirmation of this fact is given by the foci of injury, the number of which is increased by starvation, the circulatory disturbances with the development of capillaritis, the microfocal perivascular and interstitial infiltration with lymphocytes, macrophages, and polymorphs, and the increase in number of mast cells.

In acute alcohol poisoning changes in the skeletal muscles are thus characterized by metabolic disturbances, which are represented by a decrease in the glycogen content, infiltration by microdroplets of fat, reduced activity of enzymes of aerobic and increased activity of enzymes of anaerobic glycolysis, and dystrophic and focal necrotic changes in the myocytes. Acute alcohol poisoning against a background of starvation is associated with more marked dystrophic and necrotic changes in the myocytes and by a decrease in the content of glycogen and lipids.

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