CHANGES IN LIPID METABOLISM, OXYGEN BALANCE, AND ULTRASTRUCTURE OF MUSCLES IN EXPERIMENTAL TETANUS

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As a result of continuous spasma a muscle does an enormous quantity of work which is associated with utilization of sources of energy and oxygen, reflected in the intensity of their metabolism [2, 4]. Under these circumstances lipid peroxides are formed [1, 3], changes take place in the blood formula [7] and enzyme activity [9], and the erythrocyte count and hemoglobin concentration in the blood fall [13]. In tetanus the greatest load falls on the skeletal muscles and the oxygen supplying system of the body. Under the conditions thus created the utilization not only of carbohydrates and lipids stored in depots, but also of the structural elements of cells and, in particular, their membranes, as energy-yielding materials cannot be ruled out.

The aim of this investigation was to compare the resistance of erythrocyte membranes and the ultrastructure of myocytes with lipid metabolism, the oxygen balance, cytochrome oxidase (CCO) activity, and the lipid peroxide level in the course of experimental tetanus.

EXPERIMENTAL METHOD

An experimental model of tetanus was produced in 50 conventional albino rats of both sexes weighing 180±10 g and in 20 dogs weighing 12-25 kg, by injecting them with 1 MLD of native tetanus toxin, not containing active tetanohemolysin. The toxin was injected into the thigh muscles, where it caused ascending tetanus. The animals survived on average 6± 1 days.

The partial pressure of oxygen (PO_2) in the skeletal muscles was determined at various stages of tetanus in the rats by means of a platinum needle electrode on an LP-9 polarograph (Czechoslovakia). In addition blood was taken from the dogs at the same times to determine the lipid concentration in their plasma and erythrocytes and to study the resistance of the latter. In rats, PO_2 and CCO activity were determined in the thigh muscles and aspartate aminotransferase (AAT) activity in the blood. Total lipids were extracted from the blood plasma [10], purified with 0.74% KCl solution, and concentrated on a rotary evaporator. The erythrocytes were washed with 0.15 M NaCl solution to remove plasma proteins, and after osmotic shock and washing with solutions No. 1 (0.5 ml of 0.05 M Tris-HCl, 6 mg NaCl, 5 mg KCl, 20 mg sucrose, water up to 1 liter) and No. 2 (0.1 ml 0.05 M Tris-HCl, 3 mg NaCl, 10 mg EDTA, 4 mg KCl, water up to 1 liter) to remove hemoglobin, the lipids were extracted with a mixture of chloroform and methanol (2:1). Lipids were determined by the method in [11] and phospholipids by the method of Lowry and Lopez in Skulachev's modification [6], triglycerides by the Carlson—Ignatovskaya method in Panchishina's modification [5], and nonesterified fatty acids by the method in [12]. Lipid peroxidation was estimated from the malonic dialdehyde (MDA) level in the reaction with thiobarbituric acid. AAT was determined by the reaction of pyruvic acid with α-ketoglutarate and with 2,4-diphenylhydrazine.

Pieces of freshly removed muscles from decapitated rats were placed in a 2% solution of osmium tetroxide in 0.1 M cacodylate buffer, pH 7.36, fixed for 2 h in the cold, dehydrated in alcohols and acetone and embedded in a mixture of Epon and Araldite. Sections were cut

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TABLE 1. Lipid Concentration in Blood Plasma and Erythrocyte Membranes, Resistance of Erythrocytes, and Their Sedimentation Rates in Dogs in the Course of Tetanus

Parameter studied	Stage of poisoning				
	Control	Incubation period	Local tetanus	Generalized tetanus	Terminal stage
Total lipids					
Plasma, mM	449±45	490±51	592±53	585±57	619±58*
Membranes, mg/g	629±60	573±58	524±53	482±51	446±43*
Phospholipids					
Plasma, mM	143±13	149±15	172±19	218±20*	223±21*
Membranes, mg/g	342±34	313±35	282±31	255±26	232±22*
Triglycerides					
Plasma, mM	200±20	177±19	156±19	142±16	135±14*
Membranes, mg/g	131±13	129±15	148±15	160±16	165±17
Cholesterol					
Plasma, mM	26,8±2,7	24,8±3,5	26,9±2,8	29,2±2,9	29,0±3,0
Membranes, mg/g	36,0±3,5	40,8±4,2	38,7±3,6	35,5±3,7	43,1±4,0
Plasma FFA, mM	5,0±0,4	5,1±0,5	5,6±0,5	6,4±0,6	6,9±0,7*
Plasma MDA, mM	19,8±2,8	25,5±4,2	29,9±4,9	35,5±5,0*	37,7±5,3*
ARE, conventional units	6,8±0,69	6,71±0,65	6,51±0,62	5,08±0,52	4,63±0,47*
ESR, mm/h	12±2	17±4	20±5	25±5*	36±6*

Legend. Here and in Table 2: *P < 0.005 compared with control.

TABLE 2. Oxygen Balance in Thigh Muscles of Albino Rats during Tetanus Poisoning

Experimental conditions	Parameter studied	Stage of poisoning			
		Control	Local tetanus	Generalized tetanus	Terminal stage
Hyperoxia	Initial O_2 level, kP	7,67±1,53	9,99±1,06	10,77±1,19	1,12±0,15*
	Latent period, sec	14±3	9±3	11±4	19±5
	1 min of oxygenation, kPa/sec	9,61±1,61	10,86±2,17	16,98±3,4	1,45±0,35*
	Rate of oxygenation, kPa/sec	0,032±0,006	0,116±0,019*	0,082±0,012*	0,005±0,002*
	Maximal level, kPa	10,76±2,15	17,46±3,5	18,85±3,71	1,47±0,37*
	1 min of utilization, kPa	8,42±1,67	10,82±2,16	11,31±2,25	1,35±0,31*
	Rate of utilization, kPa/sec	0,039±0,007	0,047±0,018	0,119±0,015	0,002±0,001*
	Ratio between velocities oxygenation: utilization	0,82	1,38	1,05	2,5
Ischemia	Half utilization time of O_2 reserves, sec	29±6	39±9	48±10	56±8
	Rate of utilization, kPa/sec	106±12	148±24	161±25	173±21
	Latent period, sec	0,072±0,012	0,067±0,009	0,067±0,011	0,008±0,002*
	Recovery time of pO_2, sec	8±2	20±5	30±6*	35±7*
	Rate of recovery, kPa/sec	120±15	113±14	127±18	196±34
	Final O_2 level, kPa	0,067±0,011	0,074±0,012	0,066±0,013	0,011±0,004*
	Ratio of velocities of utilization/recovery	8,08±1,15	8,33±2,08	8,5±2,12	2,26±0,45*
		1,07	0,9	1,01	0,72
	Activity				
	Cytochrome oxidase, mmole/g	2,8±0,2	2,8±0,4	2,3±0,3	1,8±0,2*
	Aspartate transaminase, mmole/liter	9,2±1,2	14,1±2,3	25,4±4,2*	30,8±5,1*

from blocks on a UMPT-3 ultramicrotome, stained in a 2% solution of uranyl acetate and lead citrate by the methods of Reynolds [14] and Tempak and Ward [15], and examined in the UÉKV-100K electron microscope with accelerating voltage of 75 kV. CCO was determined in muscle homogenates colorimetrically in the reaction with dimethylparaphenylenediamine hydrochloride.

EXPERIMENTAL RESULTS

The data in Table 1 show an increase in the concentration of total lipids in the blood plasma and a fall in their level in erythrocyte membranes, mainly due to elution of the phospholipids. Their deficiency is partially compensated by triglycerides, the concentration of which falls in the plasma and rises in the membranes. There is also a substantial increase in the concentration of free fatty acids (FFA) and of lipid peroxides. Definite correlation is observed between the phospolipid concentration in the membrane and acid resistance of the erythrocytes (ARE), and also between the concentration of lipid peroxides and the erythrocyte

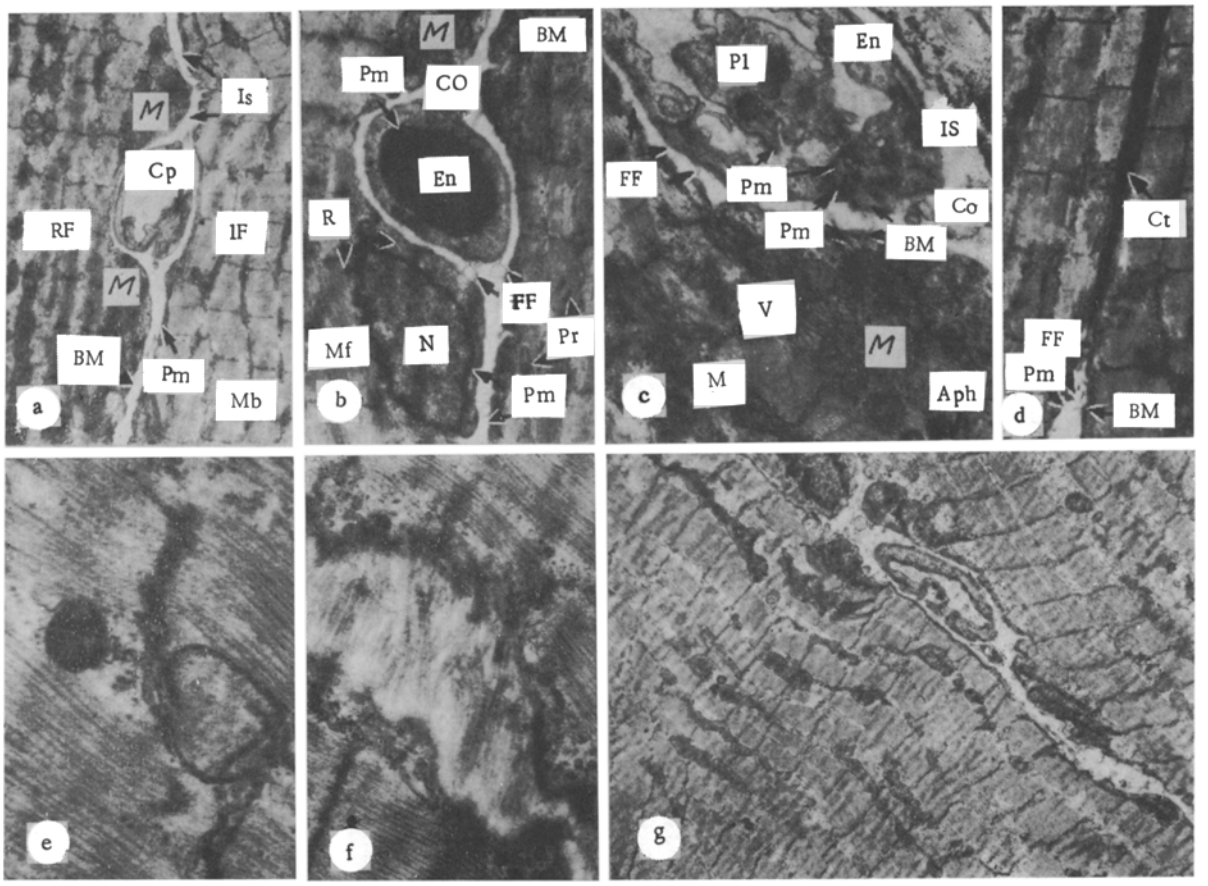

Fig. 1. Ultrastructural organization of thigh muscles of rats with local tetanus: a) increased heterogeneity of muscle fibers, associated with nonuniform increase in electrical density of ultrastructures of neighboring cells (3000 x); b, c) loss of outline, loosening of structure, and separation into layers of cell ultrastructures and noncellular components, widening of intercellular space (b, 3500 x; c, 5000 x); d) formation of electron-dense components of coagulum type (4000 x). Aph) Autophagolysosomes, BM) basement membrane, V) vacuole, CF) collagen fibers, RF) red muscle fiber, Ct) coagulum layer type of contact, Cp) capillary, Co) coagula, L) lysosome, M) mitochondria, IS) intercellular space, Mb) myofibrils, Mf) myofilaments, FF) filamentous formations, Rb) residual bodies, Pm) plasmalemma, IF) intermediate muscle fiber, Pr) precipitates, R) ribosomes, Pl) platelets, En) endothelial cell, N) nucleus.

sedimentation rate (ESR). These two correlations were found to be inversely proportional.

Data on pO_2 and oxygen metabolism in the course of experimental tetanus in rats are given in Table 2. At the local tetanus stage, the oxygen utilization time after application of a tourniquet to the thigh was increased relative to the control and the latent period after its removal was lengthened; in all probability this was due to edema of the limb, which could be observed visually and is typical of most animals at this stage of tetanus poisoning. At the state of generalized tetanus the changes described above were intensified. In the terminal stage nearly all parameters of the oxygen balance showed statistically significant changes and edema of the test limb was considerably reduced.

The electron-microscopic investigations showed that the ultrastructural organization of the muscles under normal conditions was the same as that described previously [8]. However, the number of intermediate fibers was much greater than the number of red and white fibers. At the local tetanus stage a sharp increase in heterogeneity of the muscle fibers was observed on account of the irregular formation of electron-dense fibers against the background of a reduction in the number of intermediate fibers (Fig. 1a). The plasma membrane of most cells was loose in structure, detached from the basal layer, and it formed a single entity with the appearance of a cloud, with filamentous formations arising from it and running into

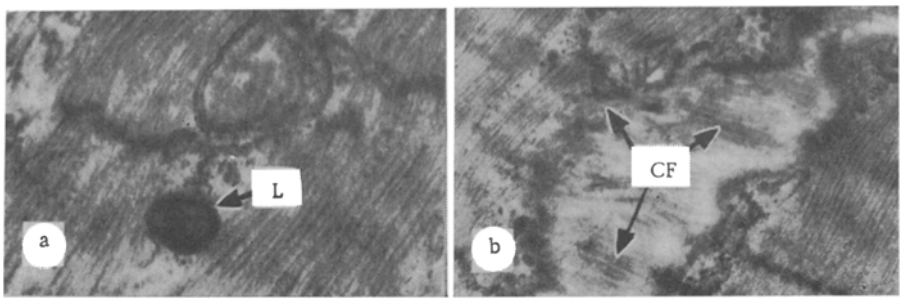

Fig. 2. Ultrastructural change in thigh muscles of rats with generalized tetanus: a) decrease in electron density of cytoplasm of muscle fibers with simultaneous appearance of lysosomes with disturbed integrity (30,000x); b) filling of intercellular space with collagen fibers and absence of plasmalemma in muscle fibers (15,000x); CF) collagen fibers, L) lysosome. Remainder of legend as to Fig. 1.

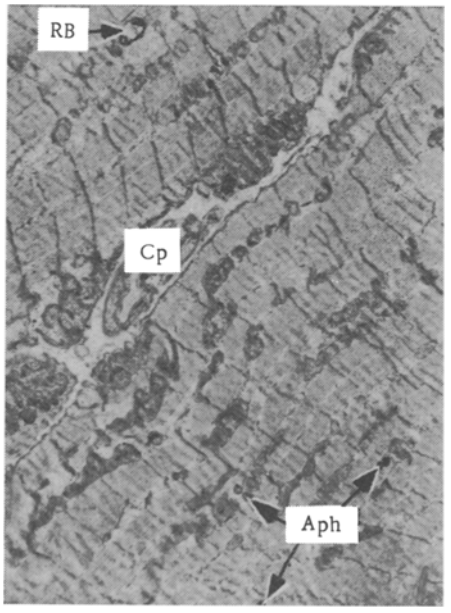

Fig. 3. Ultrastructural changes in thigh muscles of rat in terminal stage of tetanus. Autophagolysosomes and residual bodies present, accompanied by reduction of functioning structures in muscle tissue (2500x). RB) Residual bodies. Remainder of legend as to Fig. 1.

the intercellular space (Fig. 1a-d). In some cases the muscle fibers appeared to be joined by their lateral surfaces, to form an electron-dense layer of coagulum type at the site of contact (Fig. 1d). Changes in some ultrastructures were observed predominantly in the red fibers. For instance, against the background of marked contraction of the myofibrils the density of the components of the cytoplasm was increased, but this was accompanied by their disorganization. The number of mitochondria in such cells, and also their size, were increased (Fig. 1a-c). They were distributed mainly at the periphery of the cells, especially in that part of them which lay next to the capillary.

The nuclei were irregular in shape and their chromatin was distributed mainly at the periphery. The nuclear membrane in many places was loose in structure or absent altogether. Near such nuclei ribosomes were concentrated. The number of ribosomes diminished away from

the nucleus (Fig. 1b). Regions of increased electron density (precipitates and coagula of microfilaments) appeared in the myofibrils (Fig. 1 a-d).

The intercellular space was widened and marked heterogeneity was observed in the form of alternation of electron-dense and electron-translucent zones. The former consisted mainly of disorganized connective-tissue structural elements, the latter of liquid contents (Fig. 1b, c). These features are evidence of edema. Similar changes took place in the capillary lumen, but they were more pronounced. Both in erythrocytes (Fig. 1b) and in platelets (Fig. 1c) the integrity of the plasma membranes was disturbed, so that they adheared to endotheliocytes. Evaginations and microvilli were formed on the surface of the latter, and their cytoplasm contained many vesicles.

In the stage of generalized tetanus a reduction in electron density of all component elements of the muscle tissue was observed with the appearance of many primary electron-dense lysosomes with disturbed integrity of their membranes (Fig. 2a). The intercellular spaces were filled with collagen fibers, with direct connection with the cytoplasm of the adjacent myocytes. Plasma membranes were absent in such areas (Fig. 2b).

In the terminal stage of tetanus the changes described above intensified and gave way to total destruction of the intracellular structures through stages of formation of autophagolysosomes and residual bodies (Fig. 3). In many cells this process led to the almost complete disappearance of ultrastructures. The considerable increase in AAT activity in the blood also indicated cell destruction.

Appreciable changes in lipid metabolism with disturbance of the structures of the erythrocyte and myocyte membranes thus take place in tetanus. Judging by the oxygen balance and CCO activity, metabolism in the muscle tissue is initially enhanced, with the formation of an excess of peroxides, which cause disturbance of the integrity of the cell membranes and of the ultrastructure of the myocytes, but later it is depressed because of exhaustion of the compensatory mechanisms, and it terminates with death of some of the cells, and later, of the animals themselves.

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