Morphological Study of Somatic Muscles in Alimentary-Toxic Paroxysmal Myoglobinuria

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The development of acute focal metabolic lesions of somatic muscles is shown in alimentary-toxic paroxysmal myoglobinuria. Two principal universal reactions of striated muscles are traced in the genesis of this pathological process, namely, contracture damage and intracellular myocytolysis. The functional asynchronism and structural-metabolic heterogeneity of muscle fibers are reflected in the stagewise and typical heterogeneity of the morphological picture, which preserves the entire spectrum of stereotypical pathological reactions regardless of the severity of the illness.

Key Words: *alimentary-toxic paroxysmal myoglobinuria; skeletal muscles; metabolic damage; electron and polarization microscopy*

The unpredictability of sudden outbreaks of alimentary-toxic paroxysmal myoglobinuria (ATPM) which occurs on the banks of closed reservoirs when the fish dwelling in them become toxic due to an unknown concatenation of natural conditions [2,4,8] hampers the use of many clinical and experimental methods [1]. It is not surprising that the pathomorphology is one of the least understood aspects of this illness. Morphological studies have been few and for the most part have been carried out on sectional material with the use of only a light microscope [2].

The goal of the present investigation was to study the morphogenesis of the main pathological process in muscle tissue with the use of a set of modern methods, including light, polarization, and electron microscopic analysis, on biopsy, autopsy, and experimental material obtained during the last major outbreak of ATPM (1984-1985, Lake Ubinskoe, Novosibirsk Region).

MATERIALS AND METHODS

Twelve observations of ATPM comprised the clinical part of the study including 8 *m. gastrocnemius* biopsies and 4 autopsies of skeletal muscles of different localization.

An experimental study was carried out on 2 male and 10 female random-bred cats with an initial weight of 770-4900 g. The test animals were fed boiled and raw fish suspected of being toxic. Control cats received nontoxic boiled fish. Depending on the manifestation of the clinical picture of disease, the animals were divided into groups with mild and pronounced clinical signs and a group of cats in a terminal state. Animals were sacrificed under Nembutal anesthesia as soon as signs of disease became apparent. Tissue pieces were sampled from *m. biceps* and *m. soleus*, the diaphragm, and in some animals, in addition, from *m. intercostales* and *m. tibialis anterior*. Somatic muscles were fixed in a state of slight distension by being pinned to wooden blocks.

Tissue samples were fixed in 12% neutral formalin for light microscopy. Histological preparations were stained with hematoxylin-eosin in combination with Perls' reaction for revealing iron ions, after van Gieson with additional resorcin-fuchsin staining after Weigert to visualize the elastic fibers, and with colloid iron-PAS-hematoxylin; the PAS-reaction was also performed.

For electron-microscopic study the samples were fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, and, after routine processing, embedded in

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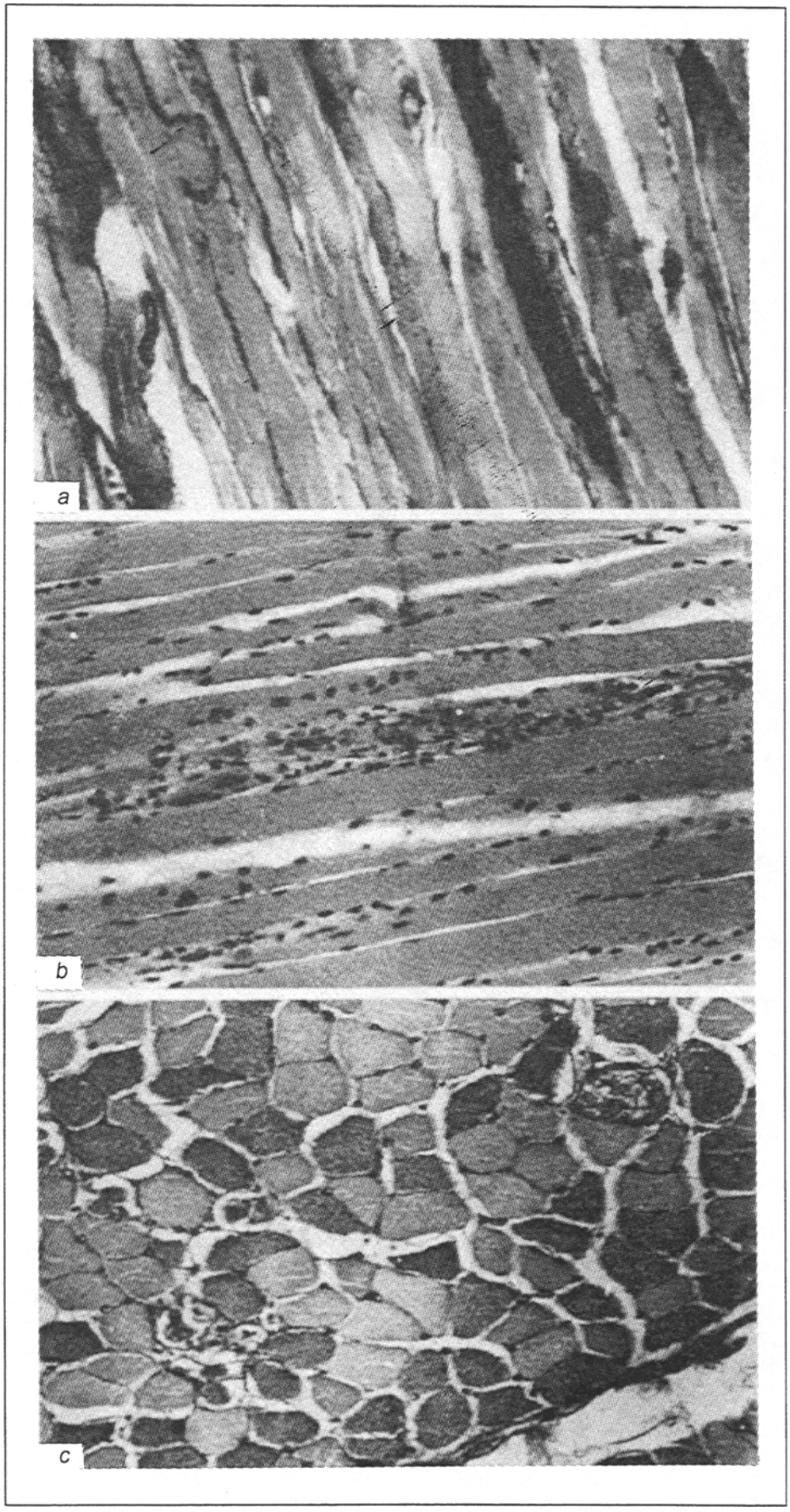

Fig. 1. Light microscopy features of bioptates from skeletal muscles in patients with alimentary-toxic paroxysmal myoglobinuria. *a*) focal metabolic lesions: contractures, lumpy degeneration, and myocytolysis. Hematoxylin and eosin staining. ×300; *b*) cellular infiltrate in a ribbonlike necrotic focus. Hematoxylin and eosin staining. ×350; *c*) mosaic morphological picture due to varying extent of muscle fiber damage (cross section). PAS-reaction. ×320.

Epon-Araldite. The semithin sections were stained with 1% Azure II and PAS and then examined under the light microscope. After contrasting with uranyl acetate and lead citrate they were examined with a JEM 100B electron microscope.

For polarization microscopy paraffin sections were used, stained with hematoxylin and eosin.

RESULTS

Damage of somatic muscles, attended by paroxysmal violent pain in muscles, pareses, and paralyses, was found to be the main clinical ATPM syndrome [2,5]. In accordance with this, the most marked changes of the pathological picture were revealed in the striated musculature in various groups of muscles.

The heterogeneity and multiformity of the general picture struck us when we were examining the biopsy and autopsy material. This heterogeneity was evident both on individual slides, where groups and even individual fibers with deep-seated pathological changes were found among preserved muscle fibers (MF), and when a comparison was made for different sections from one muscle, various groups of somatic muscles, and, of course, for different observations. But in all cases two types of foci were clearly defined, namely, contracture lesions of MF of varying severity and foci of myofibril disintegration, or intracellular myocytolysis (Fig. 1, *a*). The tissue of a severely damaged MF consists of a homogenous isotropic waxy mass devoid of structured myofibrils. Then the homogenous substrate begins to break down into lumps, at first large and then smaller ones, after which cellular infiltration of the focus occurs, consisting predominantly of macrophages which resorb the necrotic masses (Fig. 1, *b*).

The distribution of contracture lesions along the MF is of a total or segmental nature and embraces the entire fiber or separate regions (Fig. 1, *c*). The ribbon-like necrotic foci forming in the first case stain intensively and diffusely with Schiff reagent. Penetration of cellular elements of infiltrate into the focus both from the poles and from the sarcolemma edges may be indirect evidence of damage and increased permeability of MF membrane structures. In the second case the necrotic foci are PAS-positive cone-shaped expansions; the cells of the infiltrate "invade" the fragment, moving toward its center from the poles, leaving the sarcolemma edges free. This probably attests to the relative preservation of the sarcolemma.

Ultrastructural changes in the first stages of this process are expressed in a shortening of the I-disks, the formation of contractile bands, and the discomposition of myofilaments (Fig. 2, *a*). The disappearance of glycogen granules, lysis of myofibrils, and destruction of mitochondria are the most prominent ultrastructural manifestations of the lytic processes, which are poorly demonstrated with light microscopy. Lightening of the sarcoplasm between myofibrillar bundles and in the perinuclear region results from the lysis (Fig. 2, *b-d*). Products of lysis in the form of amorphous substance fill the intermuscular layers and capillary lumens. Plasmatic impregnation and a macrophagal reaction are absent in lytic foci.

It should be stressed that according to the data of ultrastructure analysis, lytic processes are largely responsible for the MF changes in ATPM as well as in some other conditions of the "myoglobinuric myopathy" type [13,15,16]. A key role in the pathogenesis of this disease is played by massive lysis of the sarcoplasmic proteins as well as lysis and destruction of myofibrils and mitochondria. Subsequently, myoglobin and other toxic products are released into the bloodstream, protein synthesis decreases, and thus regeneratory processes decline. Evidence of this is seen in changes of the nucleolar apparatus, which shows a prevalence of fibrillar material - a sign of a slowing down of ribosome assembly.

Metabolic damage develops against the background of disturbances in hemodynamics expressed in capillary plethora and edema (which is sometimes significant). However, obstructive changes of vessels, including capillaries, which could cause ischemia of muscle tissue are absent, confirming our assumption that the toxic agent acts directly on MF metabolism.

The goal of our experimental studies was to verify the toxic genesis of the pathological process and analyze its special pathomorphological features in relation to the degree of expression of the clinical picture.

Somatic muscle fibers stained evenly and moderately with eosin in control cats. Dustlike glycogen granules were visualized by the PAS-reaction along isotropic disks of myofibrils. Uniform cross-striation is clearly seen in MF under polarized light.

Heterogeneity of pathological reactions, primarily due to contractures and lumpy degeneration of MF, remains one of the main features of the morphological picture in the groups of cats with weakly expressed and pronounced clinical signs and in terminally ill animals (Fig. 3, *a*). An increase in the fraction of more serious forms and a greater spread of MF damage, attended by an increase of illness severity (coagulation necrosis and inflammatory infiltration) reflect certain clinical-morphological correlations (Fig. 3, *b-d*).

Two main well-defined forms of reactions are noted in the genesis of metabolic damage to muscles in ATPM. A wide diversity of injurious factors and metabolic disturbances - hereditary and acquired, of exogenous and endogenous origin - result in ATPM, attesting to the nonspecific nature of these reactions [3,6,9-16]. The mosaic combination of contracture lesions

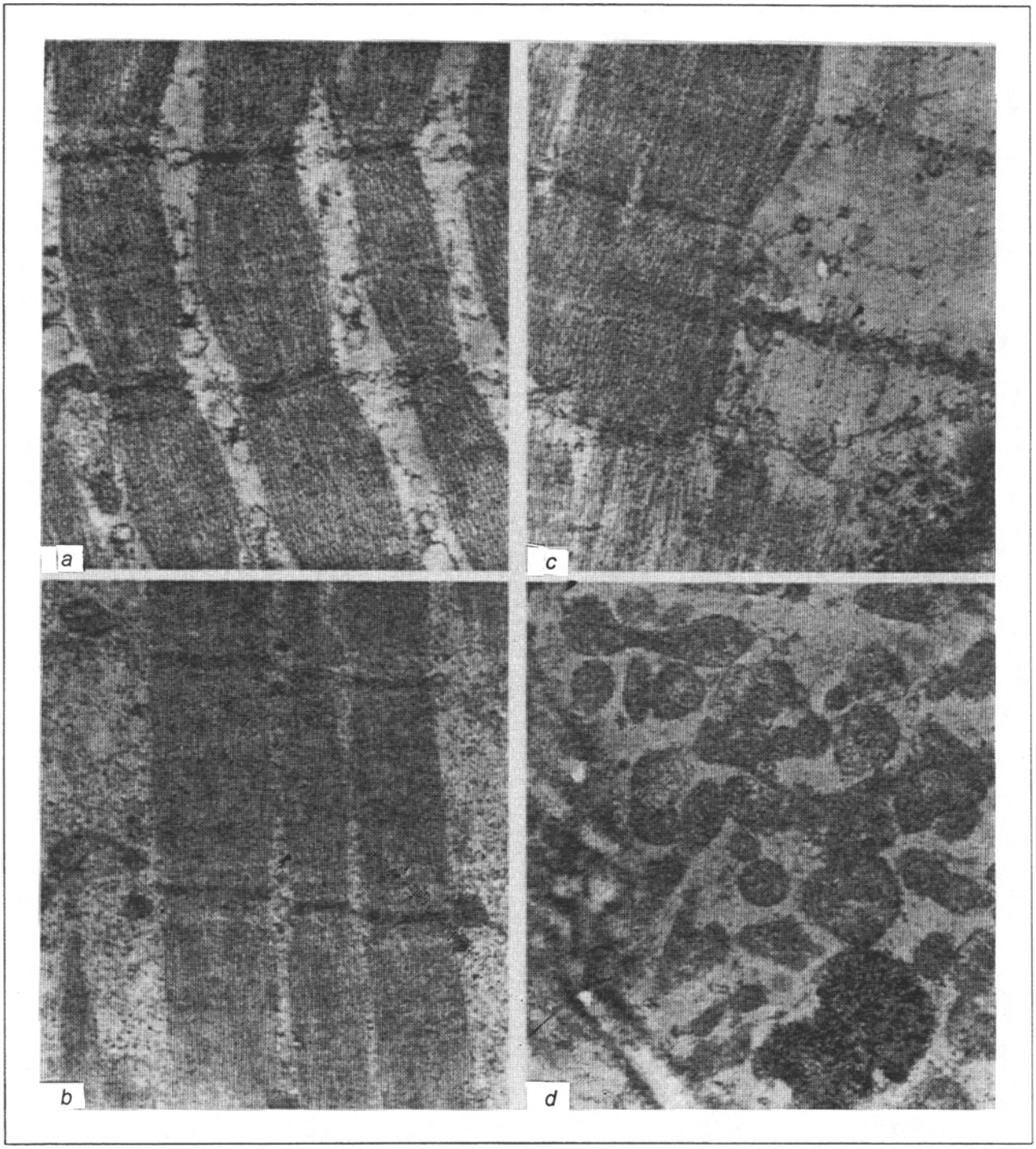

Fig. 2. Ultrastructural changes in bioptates of skeletal muscles from patients with alimentary-toxic paroxysmal myoglobinuria. *a*) contracture changes of myofibrils: shortening of I-disks. Lysis of sarcoplasmic matrix, destruction of mitochondria and transverse tubules of sarcoplasmic reticulum. ×9000; *b*) lysis of myofibrillar structures. Contracture changes in myofibrils, thickening of Z-disks. Glycogen granules in sarcoplasm. ×8300. *c*) marked lysis of myofibrillar bundles in subsarcolemmal zone with exposure of T-system tubules. ×10,000. *d*) destruction of cristae and lysis of matrix of mitochondria in perinuclear zone. Secondary lysosomes are large with osmiophilic inclusions. ×9300.

and lysis in a single preparation, plus individual "nuances" in morphological pictures are mainly the result of unique properties of MF - their structural and functional heterogeneity. The length of a damaged region and the permeability of the cell membrane are associated with the type of fiber in the damaged region. The "choice" between contracture and myocytolysis is determined by the state of a given portion of fiber at the moment of damage (by its contraction or relaxation), and the stagewise heterogeneity of the destruction foci probably depends on the fact that working fibers are the most vulnerable to damage [7].

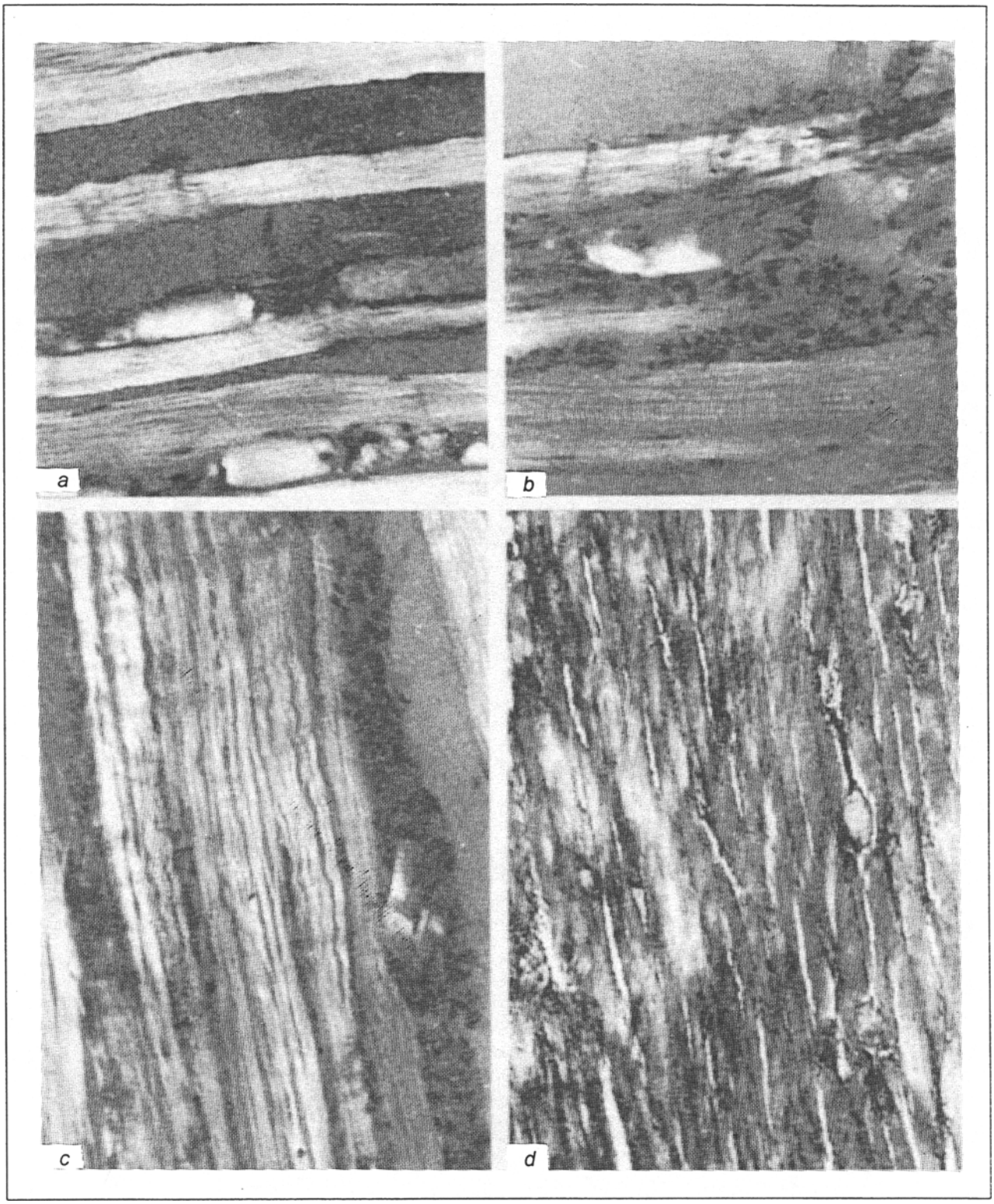

Fig. 3. Structural changes in skeletal muscles from cats in modeled alimentary-toxic paroxysmal myoglobinuria. Hematoxylin and eosin staining. *a*) contractures and lumpy degeneration of muscle fibers (MF); *b*) coagulation necrosis and inflammatory perifocal infiltration; *c*) macrophagal infiltration of necrotized MF; *d*) multiple focal necroses and infiltration in MF. *a-c*) photographed in polarized light, ×320; *d*) ×125.

Thus, analysis of the clinical and experimental materials suggests that the pathological changes in somatic muscles in ATPM are acute focal metabolic lesions which belong to the arsenal of universal MF reactions to different impacts. Focal injuries presumably develop in response to myotropic toxin-induced

disturbances of cell metabolic processes which, as in the case of other "myoglobinuric myopathies" have to do with energy production and/or ion transport.

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