MORPHOLOGY AND PATHOMORPHOLOGY

General Pathological Processes in Somatic Muscles in W/SSM Rats with Genetically Determined Metabolic Myopathy

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Hypertrophy, atrophy, and dystrophy, which reflect the balance between alterative and compensatory-adaptive processes at all levels of structural organization, predominate among structural changes in W/SSM rats with metabolic myopathy under conditions of progressive chronic disorders of cellular homeostasis. Primary universal reactions of striated muscles to damage (myofibril contractures and intracellular myocytolysis) are observed at the ultrastructural level. Increased incidence of focal changes and the time course of morphological and stereological parameters in a constantly functioning organ (diaphragm) indicate that working muscle fibers are most susceptible to injury.

Key Words: *somatic muscles; metabolic myopathy; W/SSM rats; polarization and electron microscopy*

Somatic muscular tissue is highly sensitive to injury; the symptoms of neuromuscular dysfunction have been observed in many diseases [3,10]. The mechanisms and morphological manifestations of muscle fiber (MF) damage are little known, particularly in nonspecific disorders of cellular homeostasis.

The total-system oxidative stress and lipid peroxidation play an important role in the development of myopathy [9,11,13,14]. A new strain of Wistar rats (W/SSM) with hereditary hyperproduction of free radicals was created by selection and inbreeding [4]. Biochemical and genetic studies showed that this hyperproduction results from intense glycolysis and autooxidation of hexoses, which are excessively accumulated because of mutation of glucose transporter of the plasma membranes [4,6]. The signs of skeletal muscle involvement are observed among numerous manifestations of polyorgan abnormalities [4,5].

We investigated the morphogenesis of changes in somatic muscles of W/SSM rats with chronic nonspecific disorders of cellular metabolism caused by hereditary hyperproduction of free radicals.

MATERIALS AND METHODS

Structural reactions of somatic muscles in genetically determined metabolic myopathy were studied in 38 male W/SSM rats weighing 130-420 g. Fifteen male Wistar rats weighing 120-400 g were the controls. Pathological changes are accumulated with time; therefore, experiments were carried out on rats aged 2 and 9 months.

Preparations of the crural muscles "strained at rest" [7] were obtained as follows. After decapitation, the left hind paw was amputated at the level of the hip joint, the skin was rapidly detached where necessary, and the limb was put in fixing solution

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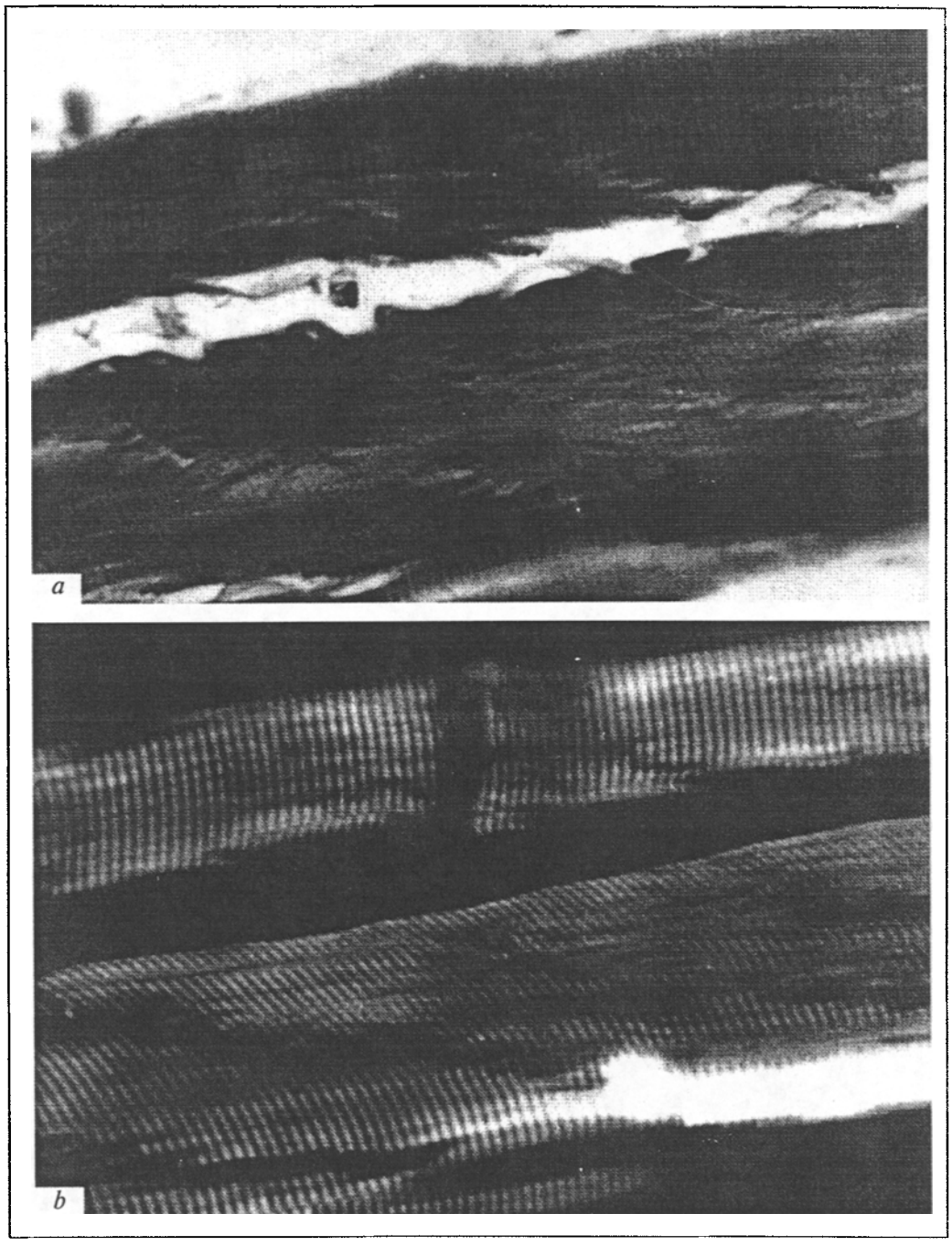

Fig. 1. The gastrocnemius of a 9-month-old W/SSM rat in normal (*a*) and polarized (*b*) light. Contraction node: second-degree contracture. Hematoxylin and eosin staining, ×1200.

(4% paraformaldehyde in Millonig's phosphate buffer, pH 7.4, 4°C).The diaphragm was fixed together with the costal ring. After a 24-h fixation, fragments of the gastrocnemius muscle and diaphragm were cut from macropreparations for further treatment.

For light microscopy, fragments of tissues were fixed in 12% neutral formalin. Histological preparations were processed as follows: hematoxylin and eosin staining with Perls' test for iron ions, Van Gieson's staining and Weigert's resorcin-fuchsin staining of elastic fibers, staining with colloid iron and Schiff-iodine acid hematoxylin, and Schiff-iodine acid test.

For electron microscopy, the fragments were fixed in 4% paraformaldehyde and in 1% osmium tetroxide, and after standard processing were embedded in Epon-Araldite. Semithin sections were stained with 1% Azur II and by Schiff-iodine acid,

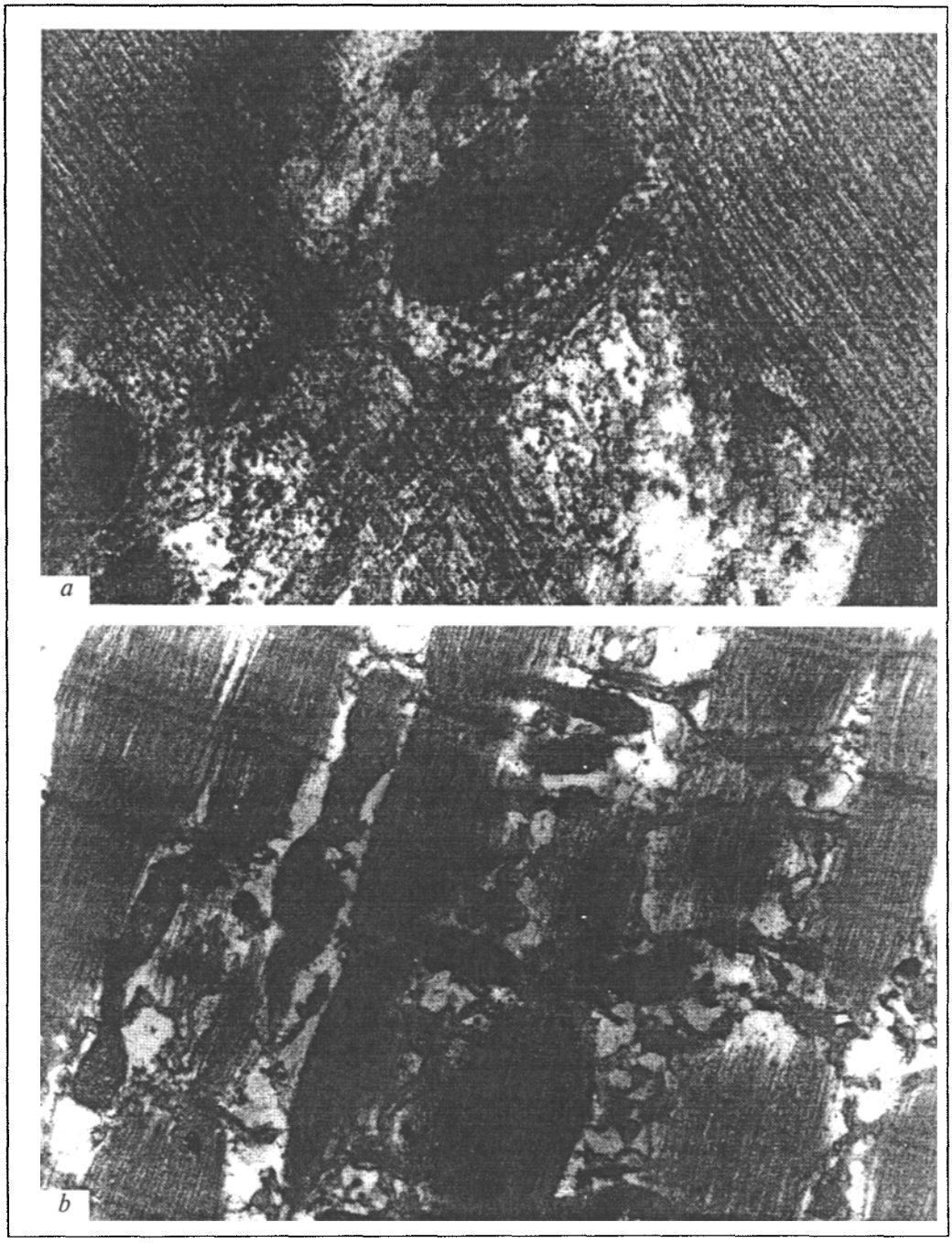

Fig. 2. The diaphragmatic muscle of a 2-month-old W/SSM rat. *a*) focus of myofibril disaggregation and lysis, destruction of mitochondria, ×25,000; *b*) contracture changes and lysis of myofibrils, sarcoplasmic edema, exposed T-tubules, dilatation of sarcoplasmic reticulum cisterns, ×10,000.

and examined in a Docuval optic microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate and were examined in a JEM-100B electron microscope.

For assessing the fibrillar system of muscle cells, paraffin stained and unstained sections with transversely oriented MF were examined in polarized light [2]. Phase-contrast studies were supplemented by polarization microscopy.

The mean MF diameter was measured on transverse semithin sections using a MOV-1-15^{x} ocular manometer, at least 60 measurements per animal. Stereological analysis of tissue structure of the gastrocnemius and diaphragm was carried out using an ocular multipurpose test system of short pieces. The primary stereological parameters were surface and volume compactness of MF, volume compactness of the stroma; then surface-volume and volume ratios

of the studied compartments were estimated, as well as the area of transverse section of MF and the capillarization index [12].

Morphometric and stereological findings were analyzed using Student's t test; the differences were considered to be significant at $p<0.05$.

RESULTS

Histological studies of somatic muscles in both age groups revealed two groups of changes: universal focal reactions of striated muscles to damage [1,7,8] and diffuse integral changes in muscular tissue.

Focal changes consisted in eosinophilia of muscle segments with an increase in anisotropy of A-disks, their approximation, and sometimes fusion: first-third degree contractures (Fig. 1); the presence of few sites with clarified sarcoplasma, lack of transverse strips, and the signs of focal myofibril disaggregation were observed.

Indirect and residual signs, such as small cords of decayed sarcolemma with individual mononuclear cells and fibroblasts, focal infiltration with segmented and mast cells at short pieces of MF, signs of intracellular regeneration along some MF, indicate focal changes.

These focal changes were observed in the control (only in the diaphragm) and in experiment (more frequently, involving the limb muscles) and could be regarded as the background. The older age group was characterized by a higher "focal background".

In the absence of severe injuries, variously directed diffuse changes, which reflect the balance of alterative and compensatory processes in somatic muscles, predominated in this experimental model. Their manifestations at a light optic level were indefinite and nonspecific: variability of diameters, decreased density of myofibril packing, longitudinal cleavage of MF and uneven distribution of glycogen granules in them. The younger animals were characterized by a simpler structure of the diaphragm in comparison with the control, consisting in a lower degree of ramification and twisting of MF. In adult animals the connective tissue carcass was often coarse, because of accumulation of collagen fiber bundles.

The main sign of the ultrastructural picture was heterogeneous intracellular arrangement with less orderly myofibril packing, frequent cleavage of myofibril bundles, irregularity, dislocations, and transverse deformations of Z-disks.

Small sites of lythic changes (no more than 1-2 sarcomers) were often seen, in which Z-strips disappeared; thin and thick filaments disaggregated and were disoriented (Fig. 2, *a*). Larger zones of pronounced edema and lysis of sarcoplasma proteins with a wider space between the bundles, dilatation of the sarcoplasmic reticulum profiles, and exposed T-tubules were observed in some cases (Fig. 2, *b*). Sites of overcontracted myofibrils (decreased height of I-disks) were often detected. Mitochondria varied in shape and size, some of them were characterized by destructive changes (vacuolation, partial or complete reduction of crysts, and sharp clarification of the matrix). The signs of regeneration were observed: myofilament synthesis on polyribosomes with formation of small protofibril bundles.

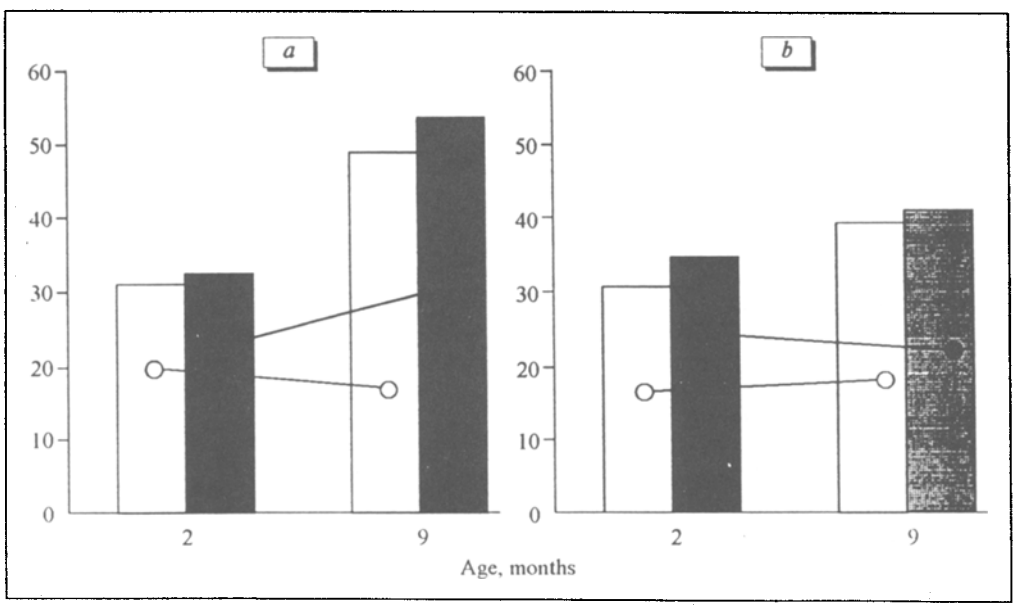

Fig. 3. Changes in the diameter of muscle fibers (μm, bars) and coefficient of variations (%, curves) in experimental (W/SSM) and control (Wistar) rats with aging. *a*) gastrocnemius; *b*) diaphragm. Light bars and markers: control; dark bars: experiment.

TABLE 1. Morphostereological Parameters of Tissue Organization of MF in Genetically Determined Metabolic Myopathy (*M±m*)

Age, months	Group	MF surface compactness, m^2/cm^2	MF volume compactness, cm^3/cm^3	MF surface/ volume ratio, m^2/cm^3	Stromal volume compactness, cm^3/cm^3	Stroma/parenchyma volume ratio, cm^3/cm^3	Area of MF transverse section, mm^2	Capillarization index
Gastrocnemius								
2	Control	0.081±0.016	0.700±0.098	0.116±0.012	0.252±0.017	0.360±0.022	817±12	1.16±0.07
	Experiment	0.092±0.023	0.710±0.051	0.130±0.021	0.263±0.063	0.370±0.006	936±18*	1.18±0.10
9	Control	0.053±0.006	0.618±0.012	0.086±0.017	0.301±0.012	0.487±0.010	1975±31	1.50±0.08
	Experiment	0.056±0.008	0.691±0.013*	0.081±0.009	0.284±0.037	0.411±0.011*	2385±86*	1.53±0.07
Diaphragm								
2	Control	0.079±0.012	0.655±0.075	0.121±0.059	0.196±0.021	0.299±0.012	879±21	1.21±0.10
	Experiment	0.076±0.006	0.668±0.059	0.114±0.017	0.202±0.013	0.302±0.010	1062±48*	1.15±0.02
9	Control	0.064±0.010	0.599±0.061	0.107±0.016	0.226±0.017	0.377±0.032	1675±36	1.48±0.01
	Experiment	0.061±0.009	0.603±0.085	0.101±0.029	0.231±0.008	0.383±0.018	1789±32*	1.44±0.05

Note. *$p<0.05$ *vs.* control.

According to quantitative structural analysis, the variability of MF size increased in all age groups, as a manifestation of compensation/decompensation processes and, consequently of hypertrophy and atrophy of MF. However, the time course of these changes and their degree are different in different muscles (Fig. 3). In the gastrocnemius muscle the mean MF diameters increased more gradually and progressively (by 6% in 2-month-old and by 11% in 9-month-old animals *vs.* the reference of the same age), while the coefficient of variations sharply increased in the older group (almost 2-fold in comparison with the control). In the diaphragm, the signs of chronic disorders of cellular metabolism were manifested earlier: at the age of 2 months the mean MF diameter increased by 13% and the coefficient of variations by 1.5 times, while in the older group these values approximated the control level, which may indicate decreased compensatory hypertrophy of MF in which dystrophic processes were intensified. The capillarization index (the number of capillaries per MF) was slightly decreased in the diaphragm or was almost the same in the gastrocnemius muscle, while MF decreased in size in comparison with the control. This lagging behind was particularly pronounced in 2-month-old animals (Table 1).

Therefore, under conditions of progressing chronic disorders of cellular homeostasis in W/SSM rats with metabolic myopathy, when chronic exposure is insufficiently intense to cause deep acute damage, the morphological picture is characterized by hypertrophy, atrophy, and dystrophy, reflecting a combination of alterative and compensatory adaptive processes at all levels of structural organization. This increases the sensitivity of MF to stress stimuli (increased background incidence of acute contracture and lythic injuries) and affects the morphostereological parameters (a general tendency to MF hypertrophy combined with a notable increase in the variability of their size). Primary alterative changes in MF developing in response to chronic disorders of cellular homeostasis are clearly discernible at the ultrastructural level.

Increased background focal changes and peculiar time course of morphostereological parameters, occurring in W/SSM rats with metabolic myopathy in a constantly functioning organ (diaphragm) indicate that MF under conditions of exercise is the most sensitive to a damaging exposure.

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