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Physical and Biochemical Characteristics of Biological Fluids in Rats with Modeled Thermal Injury

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Lactate dehydrogenase activity, crystallogenic and initiator characteristics of biological substrates were evaluated by enzymological and crystalloscopic analysis of rat serum and urine. Changes in these characteristics of biological media in combined thermal injury were shown. This approach is effective for evaluation of the metabolic status in rats with experimental burn disease.

Key Words: *thermal injury; simulation; crystallogenesis; lactate dehydrogenase; biological fluids*

Modern combustiology, a branch of high-technology medicine, is to be based on clear-cut notions on the pathogenetic, sanogenetic, and adaptive mechanisms which are activated during significant thermal exposure [6]. These studies, in turn, are impossible without creation of adequate experimental models of burn and cold injuries. An important aspect of this problem is the search for new diagnostic approaches to integral evaluation of the status of the biological object (*e.g.*, an animal) subjected to thermal injury [1,2,7]. However, relevant tests for evaluation of the metabolic profile are scanty and are based mainly on evaluation of the pro- and antioxidant status [3]. Since diagnostic potentials of these methods are limited, evaluation to the informative value of other methods is desirable.

The integral approaches to studies of the metabolic status include primarily enzymological methods, but the key factor here is the choice of the indicator enzyme. Biocrystalloscopic studies are now winning the recognition of scientists. These methods are used for the diagnosis of some diseases [1,8]; studies on animals are rare [1,2,7]. On the other hand, by the present time there are no data on the crystallogenesis patterns of normal biological media of laboratory animals, specifically, rats [2,7].

We studied activity of lactate dehydrogenase (LDH) and the crystallogenic and initiator potentials of rat biological media in health and experimental thermal injury.

MATERIALS AND METHODS

Experiments were carried out on 68 Wistar rats (160-180 g). Combined thermal injury, contact thermal burn of the posterior surface of the body (IIIAB-IV degree, 20% body surface) and thermoinhalation injury was inflicted to ether-narcotized animals.

Erythrocyte LDH activity was measured as described previously [3], protein concentration was measured by modified [9,10] Lowry's method.

Crystallogenic characteristics of the serum and urine were studied by the classical crystalloscopic method, the initiator capacity of biological medium was studied using differential tesigraphy [1,4,5]. Saline (0.9% NaCl) served as the basal substance

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in the tesigraphic test. Crystalloscopic and tesigraphic facies were described using a system of quantitative tests [4,5]. Micropreparations were examined in 3 non-overlapping visual fields.

The data were processed by methods of variation statistics using Primer of Biostatistics 4.03 software.

RESULTS

Combined thermal injury led to a decrease in LDH activity in comparison with its level in normal animals. This decrease was much more pronounced in reverse reaction ($p<0.01$), which was actively inhibited by thermal exposure (Fig. 1). Let us note, that normal lactate:pyruvate proportion is 10:1 [3]. A drop of LDH activity in reverse reaction with unchanged LDH activity in the direct reaction leads to accumulation of pyruvic acid, which indicates inhibition of anaerobic glycolysis. Pyruvate conversion to acetyl coenzyme A is essential for understanding of the role of pyruvate; this coenzyme, in turn, is metabolized in the Krebs cycle (tricarbonic acid cycle) with subsequent oxidative phosphorylation with the formation of the main universal energy source (ATP) [3]. Pyruvate can be also utilized for glucose regeneration by conversion into oxaloacetate. In our case, the most significant cause of reduced utilization of pyruvate is tissue hypoxia.

The next step in the evaluation of the metabolic status is the study of free and induced crystal formation of animal's liquid media. Study of the serum crystalloscopic facies showed that the pattern of free crystallogenesis of this biological medium of rats subjected to thermal injury differed significantly ($p<0.05$) from normal pattern by structural index, crystal formation capacity, degree of facies destruction, and clear-cut pattern of the marginal zone (Fig. 2). Characterizing these changes, we see that experimental burn promoted a significant increase in the crystallogenic potential of the biological fluid with a trend to the formation of more intricate crystal structures, paralleled by their pronounced fragmentation. In addition, thermal exposure led to a drastic narrowing of the marginal zone, presumably because of reduction of the protein component of the medium.

Analysis of serum samples prepared by the differential tesigraphic method showed that the parameters of induced crystallogenesis exhibited trends similar to those observed for crystalloscopic facies. After the trauma was inflicted, the initiator potential of biological material sharply increased, which was seen from the level of the main tesigraphic coefficient; zonal coefficient indicating the range of molecular weights of the biological liquid components also sharply increased. These shifts were paralleled by an increase in the degree of element destruction and thinning of the marginal protein zone, which was in a good agreement with the results of crystalloscopic analysis of the serum.

Trends similar to those detected in serum micropreparations were detected also in dried crystalloscopic and tesigraphic specimens of the urine (Fig. 3). Urinary crystallograms of normal animals are formed by solitary crystal elements with a low level of destruction, while thermal injury results in a sharp increase of the crystallogenic and initiator potentials of the biological medium. It is remarkable that the facies were formed by numerous structures, including those with dendritic pattern. In addition, a modeled burn aggravated proteinuria,

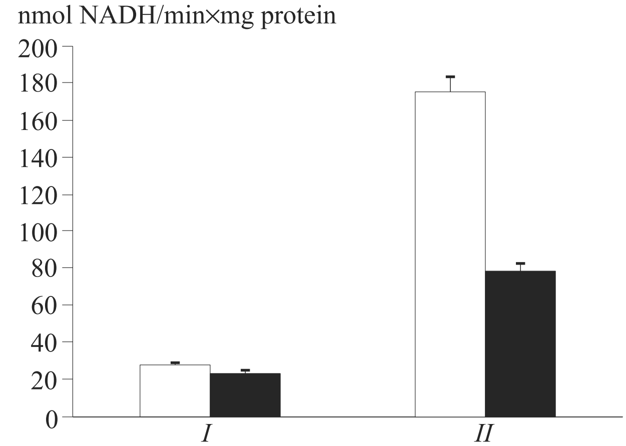

Fig. 1. Activity of LDH in rat blood erythrocytes. *I*: direct reaction; *II*: reverse reaction. Here and in Figs. 2, 3: light bars: normal (n=38); dark bars: burn (n=30).

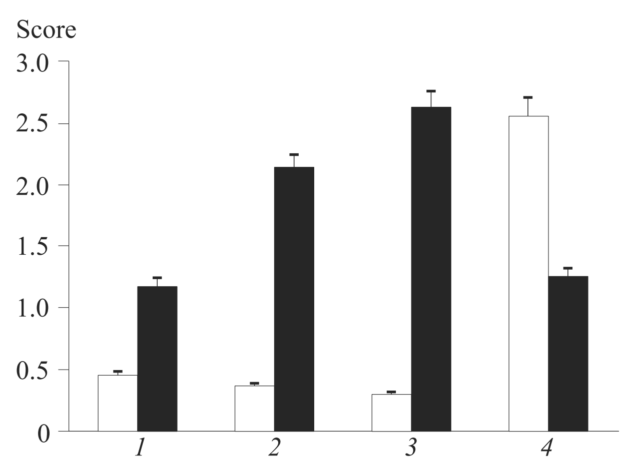

Fig. 2. Free crystallogenesis of the rat serum after thermal exposure. Here and in Fig. 3: *1*) structural index; *2*) crystal formation capacity; *3*) degree of facies destruction; *4*) clearness of marginal zone.

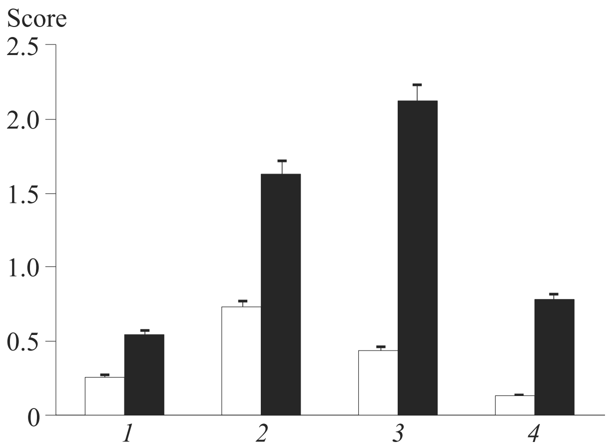

Fig. 3. Transformation of free crystallogenesis of the rat urine in experimental combined thermal injury.

which was seen from a significant ($p<0.05$) widening of the marginal zone of the preparation.

Hence, modeled thermal injury promoted significant inhibition of catalytic activity of rat erythrocyte LDH mainly in the reverse reaction, this indicating tissue hypoxia. A burn injury is a factor transforming free and induced crystallogenesis of the serum and urine into a specific tesiocrystalloscopic pattern. Study of the crystallogenic and initiator potentials of biological fluids of the body can serve as a test for detection and evaluation of the depth of metabolic shifts associated with thermal exposure.

REFERENCES

1. A. V. Vorobyov, A. K. Martusevich, and S. P. Peretyagin, *Crystallogenesis of Biological Fluids and Substrates for Evaluation of Body Status* [in Russian], Nizhnii Novgorod (2008).
2. I. P. Gromova, *Gig. San.*, No 2, 66-69 (2005).
3. G. A. Kochetov, *Handbook of Enzymology* [in Russian], Moscow (1980).
4. A. K. Martusevich, *Quantitative Evaluation of Results of Free and Induced Crystallogenesis of Biological Substrates* [in Russian], Nizhnii Novgorod (2008).
5. A. K. Martusevich and N. F. Kamakin, *Klin. Lab. Diagn.*, No. 6, 21-24 (2007).
6. B. A. Paramonov, Ya. O. Porembskii, and V. G. Yablonskii, *Burns. Manual for Physicians* [in Russian], St. Petersburg (2000).
7. N. N. Trizno, I. A. Bednov, and A. A. Rezaev, *Vestn. Nov. Med. Tekhnol.*, Nos. 1-2, 23-24 (2003).
8. V. N. Shabalin and S. N. Shatokhina, *Morphology of Human Biological Fluids* [in Russian], Moscow (2001).
9. J. M. Dawson and P. L. Heatlie, *Anal. Biochem.*, **140**, No. 2, 391-383 (1984).
10. J. Kostir, *Chemicke Listy*, **79**, No. 9, 989-991 (1985).