between the time points. Moreover, the amplitude of the circadian rhythm of ICE and blood pH rises and falls, just as in the case of the other parameters, but the interindividual dispersion does not change in the first case and falls continuously in the second. Amplitudes of circadian rhythms of blood glucose and pyruvate, however, start to fall before interindividual dispersion increases.

The statements made above do not rule out the possibility that changes in amplitudes are to a definite degree connected with desynchronization between animals. However, its contribution to this process is exceedingly small.

If we speak of increasing desynchronization between animals with increasing distance from the adult age, besides the phase shift, changes in mesors and amplitudes, which also determine the increase in interindividual dispersion, must also be taken into account. Only an individual approach can determine the contribution of individual changes to the general picture. However, the following conclusion can already be formed from the data given above together with analysis of the calculated acrophages: some improvement in internal and external synchronization can be observed in early ontogeny, with their worsening in late ontogeny, but changes take place primarily and most strongly in the amplitude of circadian rhythms, and this may provide important material in respect of biological age, especially in the final stages of ontogeny.

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EFFECT OF IMMOBILIZATION ON MYOCARDIAL MITOCHONDRIAL ENERGY METABOLISM AND ULTRASTRUCTURE IN RATS OF DIFFERENT ZOOSOCIAL RANKS

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KEY WORDS: immobilization stress; zoosocial ranks; mitochondrial respiration; ultrastructure.

The writers' previous investigations showed that a single exposure to immobilization stress causes changes in the ultrastructural organization of heart muscle cells of rats, the intensity of which depends on the animal's zoosocial rank in the group. The most marked disturbances of myocardial ultrastructure are observed in rats belonging to the zoosocial rank of dominants [1].

There is reason to suppose that the cause of the lower resistance of the heart of dominant rats to immobilization stress is the greater energy dependence of their myocardium. To test this hypothesis we studied mitochondrial metabolism in the myocardium of rats belonging to different zoosocial ranks under normal conditions and after immobilization.

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TABLE 1. Effect of Immobilization on Parameters of Function of Myocardial MCh of Dominant and Outcast Rats ($M \pm m$)

Exptl. conditions	V_2		V_3		V_4		RC		V_p		P/O	
	A	B	A	B	A	B	A	B	A	B	A	B
Dominants	43,9±4,6	92,7±7,9**	88,4±15,4	178,8± ±11,7**	36,8±5,5	?	1,71±0,04	?	2,12±0,06	?	1,84±0,23	?
Outcasts	60,6±1,5*	76,3±1,88**	86,6±5,4	132,8± ±8,9***#	65,5±0,9*	92,6±0*#	1,27±0,06*	1,29±0	1,61±0,10*	1,13±0,08*#	1,13±0,10*	0,53± ±0,04***

Legend. A) Control, B) immobilization. Isolation medium with MCh: sucrose 0.32 M, Tris 0.02 M, EDTA 0.001 M (pH 7.5). Incubation medium: sucrose 0.24 M, $MgCl_2$ 0.005 M, KH_2PO_4 0.01 M, KCl 0.15 M. Oxidation substrate 0.005 M sodium succinate. Rate of respiration of MCh expressed in nA O_2/min/mg protein of MCh. ?) V_4, RC, V_p, and P/O could not be calculated exactly in MCh of the dominant rats after immobilization because the MCh very quickly exhausted all the oxygen before the beginning of the recovery state. *$p < 0.05$ compared with dominant rats; **$p < 0.01$, ***$p < 0.02$, ****$p < 0.05$ compared with control.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. Immobilization stress was induced by fixing the animals to a board in the prone position for 6.5 h [5]. Preliminary subdivision of the animals into zoosocial ranks was carried out in groups of three rats on the basis of differences in food-getting behavior and its productivity under conditions of competition for food [2]. By means of this method the rats were divided into dominants, subdominants, and outcasts. The animals were killed by decapitation. Pieces of myocardium were taken from the anterior wall of the left ventricle for electron-microscopic investigations. The tissue was fixed in 4% paraform in phosphate buffer, pH 7.4. Ultrathin sections were cut on an LKB-8800 Ultrotome, stained with lead citrate and uranyl acetate, and examined in an electron microscope of Philips type. Mitochondrial (MCh) were isolated from the myocardium of the immobilized and unimmobilized animals by the usual method with minor modifications [7]. Respiration of MCh was recorded on an LP-60 polarograph (Czechoslovakia) at 20°C. The rate of oxygen consumption of MCh was studied in different metabolic states: at rest — in the presence of only the oxidation substrate in the incubation medium (V_2), in a state of activity — after addition of adenosine diphosphate (ADP) in a concentration of 200 μM (V_3), and in a state of recovery — after phosphorylation of ADP (V_4). The quantity of oxygen utilized for phosphorylation of the added ADP (ΔO_p), and its phosphorylation time (t_p) were measured. The rate of phosphorylation (V_p = [ADP]/[t_p/mg protein of MCh]), the efficiency of phosphorylation: P/O = [ADP]/ΔO_p, and the respiratory control (RC = V_3/V_4) were calculated. The protein concentration in the suspension of MCh was determined by the method in [6].

EXPERIMENTAL RESULTS

The investigation showed that the ultrastructure of the myocardial mitochondria of rats under normal conditions did not depend appreciably on rank. Only in individual cases were swelling of MCh and some dilatation of the cisterns of the sarcoplasmic reticulum observed in the cardiomyocytes of the dominant rats. Despite the absence of any significant differences in the ultrastructure of MCh, the functions of these organelles differed in representatives of the different zoosocial ranks under normal conditions (Table 1). During oxidation of succinate in the myocardial MCh of the outcast rats, rates of respiration at rest and during the recovery period were higher but the rate and efficiency of phosphorylation, and also the respiratory control, were lower than in the dominant rats. A similar pattern was also observed during oxidation of sodium β-hydroxybutyrate. Thus, the MCh of the dominant rats under normal conditions were more highly energized and the energetic regulation of respiration was more efficient in them.

Under the influence of immobilization the respiration rates in the myocardial MCh of the dominant and outcast rats were increased in all metabolic states, during oxidation of both succinate and of β-hydroxybutyrate. Under these circumstances the rate and efficiency of phosphorylation were significantly depressed. However, as will be clear from the data given in Table 1, immobilization caused much more marked changes in the functions of MCh in the dominant rats than in the outcasts. The respiration rates V_2 and V_3 in MCh of the dominant rats increased almost twofold after immobilization, but the P/O ratio in them was reduced by at least threefold, and V_p twofold (see the footnote to Table 1). This indicates maximal strain on energy metabolism in the myocardial MCh of the dominant rats and a decrease in the efficiency of phosphorylation. An electron-micro-

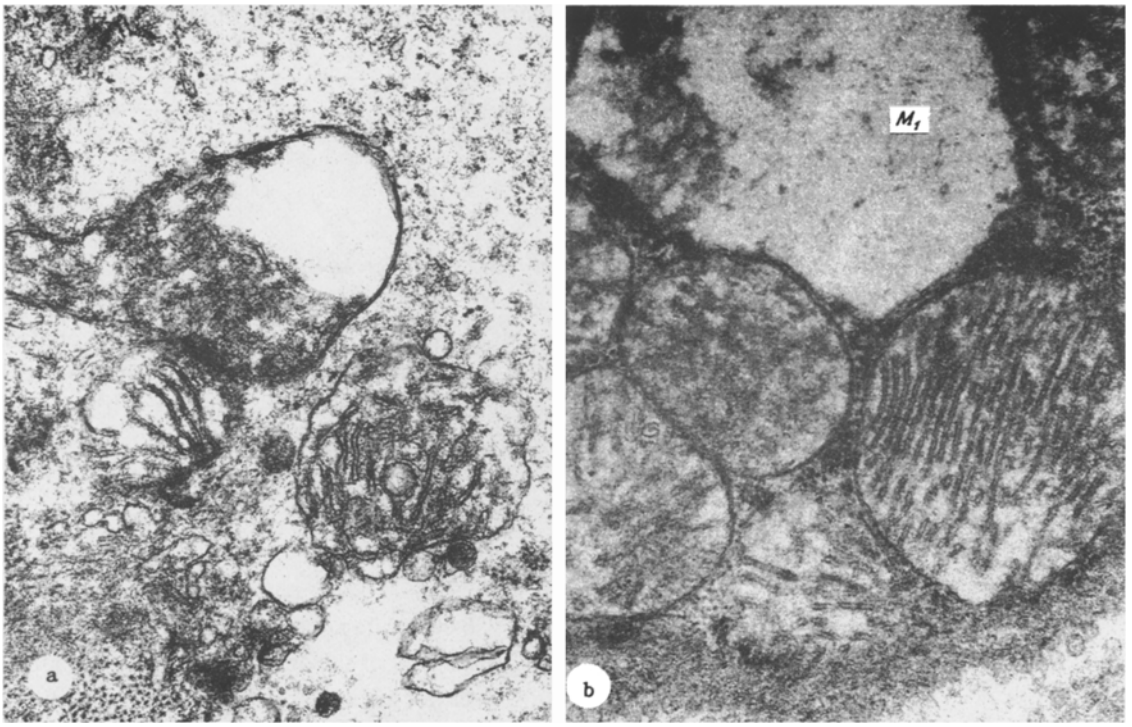

Fig. 1. Parts of cardiomyocytes of dominant rats after immobilization stress: a) peripheral part of cardiomyocyte. Destruction of outer membrane of cardiomyocytes and release of MCh into intercellular space. Vacuolation of MCh. 45,000×; b) lysis of cristae of MCh (M_1). 50,000×.

scopic study showed that the MCh of the cardiomyocytes of the dominant rats had undergone swelling, and changes were particularly marked in MCh located at the periphery of the cardiomyocytes. Their matrix was pale and their cristae partly destroyed. In some cases the formation of zones of microlysis, rupture of the outer membranes of the cardiomyocytes, and release of MCh into the intercellular space were observed (Fig. 1a, b). MCh of the outcast rats were more resistant to the action of immobilization stress. In these animals swelling of MCh was less marked and the formation of areas of cytolysis and the release of MCh into the intercellular space were found in only two cases (Fig. 2a, b).

MCh of the subdominant rats had the greatest resistance to immobilization stress. For instance, after immobilization neither the energetic regulation of their respiration (RC before immobilization was 1.24 ± 0.07, after $1.14 \pm 0, p > 0.25$) nor the efficiency of phosphorylation (P/O before immobilization was 0.51 ± 0.08, after 0.51 ± 0, $p > 0.5$) showed any change after immobilization, although the latter was lower before immobilization than in the dominant and outcast rats. Their ultrastructural organization as a rule was indistinguishable from normal (Fig. 3). According to data in the literature [3, 4] it is the processes of energy transformation in MCh that ultimately determine the resistance of the organism to extremal conditions. From this standpoint our results indicating more severe damage to the cardiomyocytes of the dominant rats than of the outcast and subdominant rats, can be understood.

The investigation thus showed that animals belonging to different zoosocial ranks differ in the level of their myocardial energy metabolism and in its resistance to stress. The facts given above are evidence of close correlation between behavioral activity, determined by the higher levels of the brain, and the energy supply of the cardiomyocytes. Individuals occupying a leading position in the population have a more powerful and well regulated intracellular system of energy production and individuals tending toward relative isolation. Meanwhile, rejection of a leading position and avoidance of active dominance in the population endow the myocardium with greater resistance to a single exposure to immobilization stress. Conversely, active dominance and increased activity associated with stabilization of the state of leadership enhance the sensitivity of the myocardium to this particular stress-inducing factor.

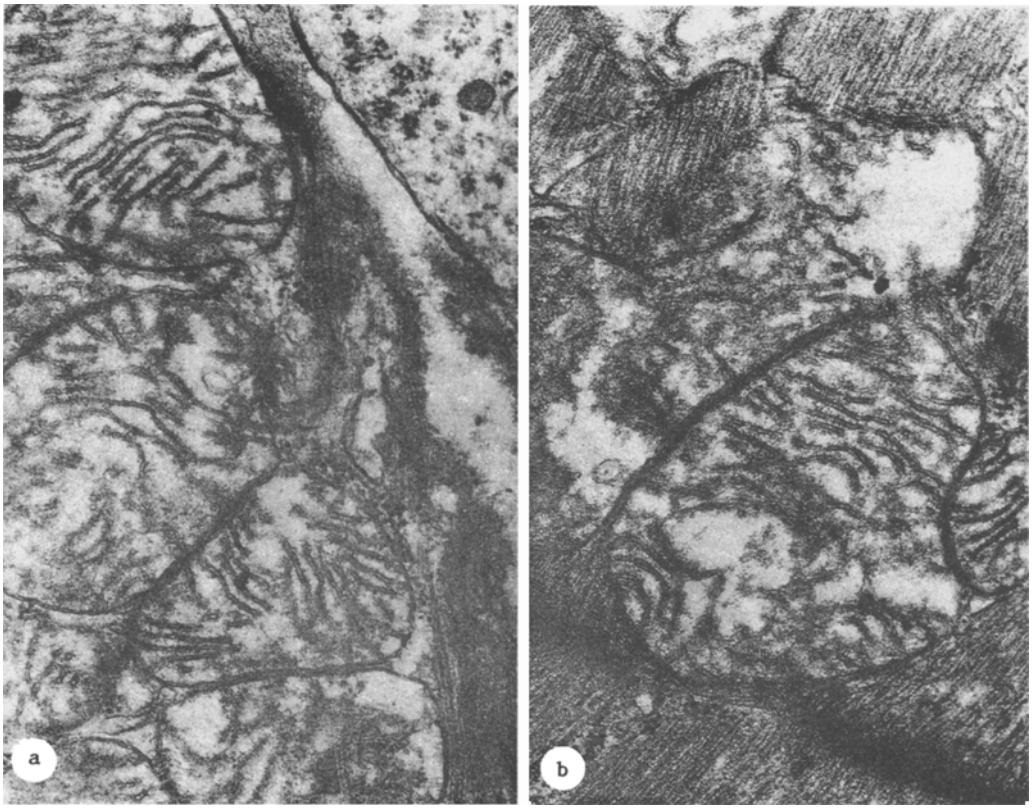

Fig. 2. Parts of cardiomyocytes of outcast rats after immobilization stress: a) peripheral part of cardiomyocyte. Its outer membrane is intact. Matrix of MCh translucent. 50,000×; b) local translucency of matrix of MCh. 45,000×.

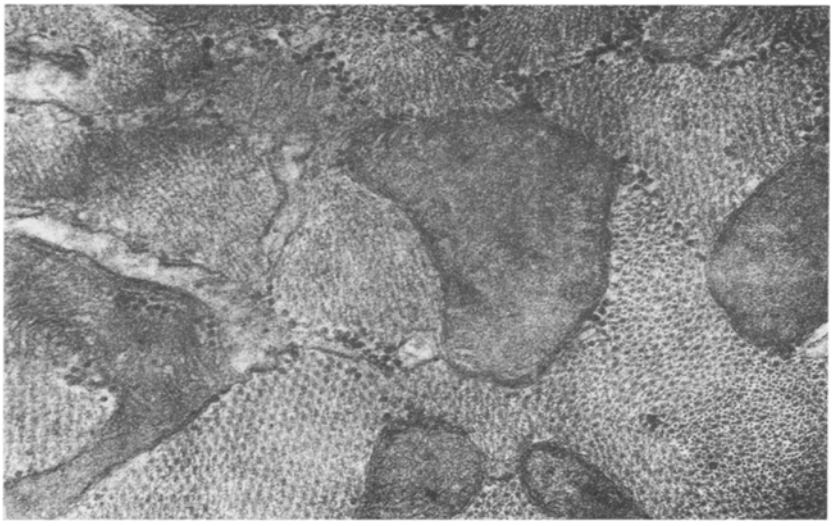

Fig. 3. Transverse section through cardiomyocyte of subdominant rat. MCh contain dense matrix and densely packed inner membranes. 35,000×.

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EFFECT OF BRONCHIAL MUCUS OF BRONCHIAL ASTHMA PATIENTS AND CHRONIC BRONCHITIS ON CILIARY ACTIVITY OF CILIATED EPITHELIAL CELLS

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Defects of ciliary motility of ciliated epithelial cells in the bronchi may be primary in character and may arise as a result of organic changes in the contractile apparatus of the cilia (the tubulin-dynein complex), as is found in Kartagener's syndrome [4]. Meanwhile, disturbance of ciliary motility may be secondary and due to functional (reversible) changes arising as a result of inflammatory or allergic processes, increased viscosity of the mucus, and other pathological conditions. The possibility of the appearance of various factors influencing ciliary motility in the ciliated epithelium of patients with bronchopulmonary pathology has been widely discussed in the literature. The presence of glycoprotein factors, inhibiting ciliary motility in the ciliated epithelium in vitro in biopsy material taken from animal tissues has been observed to be present in the serum [2, 13, 14], blood plasma [5], and lymphocytes and mononuclear leukocytes [15] of patients with mucoviscidosis, bronchial asthma, and various respiratory and autoimmune diseases. In mucoviscidosis, the patient's saliva also possesses inhibitory activity [2]. Some species of microorganisms, which are agents of bronchopulmonary diseases, can produce compounds inhibiting ciliary motility [7]. Among the list of substances capable of disturbing normal ciliary functions may be mentioned the major basic protein (MBP) of eosinophils, which accumulates in the blood and sputum of patients with lung diseases [8]. Nevertheless, the information given on factors disturbing ciliary motility in the ciliated epithelium is contradictory in character and has not always been confirmed by analysis of biopsy material from the human mucosa [11]. This may indicate that the inhibitory effect observed is partly the result of species-specific incompatibility [10]. The most encouraging results have been obtained by the investigation of the inhibiting activity of sputum. The sputum of patients with bronchial asthma [5, 6] and bronchiectasis [12] has been found to contain factors other than MBP and the blood glycoproteins which reduce ciliary motility of the ciliated epithelium in vitro in experiments with biopsy material both from animal tissues and from the human mucosa. The cilioinhibitory action of sputum in bronchial asthma is reversible, it does not cause ultrastructural changes in the cilia, and the intensity of its manifestation depends on the patient's clinical state [5, 6]. As an original clinical material, sputum corresponds more closely than others to the medium in which the

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