tent (in percent) in the cells of these patients under the influence of exogenous antioxidants also was greater than in normal subjects.

Disturbances found in TS are evidence of a shift of equilibrium in the LPO system of the neutrophils, which is probably a sign of their functional insufficiency. The test with loading of the neutrophils with adrenalin revealed that activation of LPO takes place in TS, and the functional activity of endogenous antioxidants is probably depressed. This fact is in agreement with previous observations showing a decrease in the activity and thermostability of myeloperoxidase, one component of the neutrophil antioxidant system [1]. It can also be postulated that the sensitivity of the neutrophils to the exogenous antioxidant adrenalin is undisturbed in TS.

It follows from these results that the MDA level in blood neutrophils, in the test system used, was much higher in patients with TS than normally. The altered response of the cells to addition of the antioxidant in patients with TS confirms the writers' hypothesis of a functional defect of the neutrophils. The hormonal imbalance characteristic of TS, and due to an anomaly of the sex chromosomes, may perhaps have an aggravating action on this function of the blood neutrophils.

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EFFECT OF TEMPERATURE *IN VIVO* AND *IN VITRO* ON OXIDATION AND PHOSPHORYLATION IN ALBINO RAT MYOCARDIAL MITOCHONDRIA

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It is now known that cold has a significant effect on the energy metabolism of the myocardium [1, 2]. It has been suggested that this effect is based on changes in functional activity of the mitochondria as the main energy-producing structures. For instance, it has been found in experiments on mitochondria isolated from the liver of albino rats exposed to acute chilling that the rate of respiration in them in all metabolic states and the rate of phosphorylation throughout the period of cooling were raised by 24-50% during oxidation of succinate and certain other substrates [5]. Meanwhile direct cooling of heart muscle, its homogenates, or isolated mitochondria was accompanied by a considerable decrease in the rate of oxidation substrates of the Krebs cycle in all metabolic states and by inhibition of phosphorylation [8]. Dependence of respiration and phosphorylation on temperature has been studied in greater detail in liver mitochondria, and changes in that dependence have been found in muscle mitochondria during adaptation to cold [6, 7, 9-11]. As regards cardiac mitochondria, no such information is available.

The aim of this investigation was to measure oxidation and phosphorylation in mitochondria of the myocardium both when subjected to the direct action of temperature on them and during acute and chronic exposure of the whole animal to cold.

EXPERIMENTAL METHOD

Experiments were carried out on mature noninbred male albino rats weighing 250-270 g. The animals were adapted to cold at 3-4°C for 4-5 weeks. Control and cold-adapted animals

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TABLE 1. Effect of Short-Term Hypothermia and Restoration of Normal Temperature on Oxidation and Phosphorylation in Cardiac Mitochondria of Control and Cold-Adapted Albino Rats (M ± m)

Parameter	Temperature, °C	Control			Adaptation		
		normal	hypothermia	recovery	normal	hypothermia	recovery
V_2	25	47,4±4,4	73,3±6,1*	66,7±4,0*	80,8±6,3	54,4±3,8*	53,4±1,8*
V_3	25	87,4±8,8	123,4±9,9*	120,4±8,8*	128,4±10,1	100,5±8,0*	91,6±4,9*
V_4	25	53,0±6,4	61,8±5,4	73,7±7,3*	69,8±6,3	49,0±4,3*	55,4±3,0
V	25	151,0±14,6	196,4±20,6	203,0±19,7*	237,9±16,6	197,1±15,9	148,5±7,6*
DK	25	1,70±0,05	1,73±0,05	1,65±0,07	1,88±0,05	2,03±0,06	1,68±0,04*
ADP / O	15	1,72±0,10	1,75±0,04	1,68±0,07	1,84±0,10	1,88±0,18	1,66±0,09
	15	1,72±0,04	1,39±0,08	—	1,53±0,08	1,66±0,13	1,43±0,18
$Q_{10}V_2$		1,43±0,05	1,44±0,13	1,30±0,09	1,52±0,09	1,54±0,06	1,41±0,13
$Q_{10}V_3$		1,69±0,07	1,57±0,12	—	1,62±0,06	1,61±0,05	1,56±0,12
$Q_{10}V_4$		1,70±0,06	1,60±0,19	—	1,54±0,07	1,72±0,12	1,57±0,22
$Q_{10}V_p$		1,72±0,13	1,98±0,12***		1,96±0,07 **	1,82±0,09	1,89±0,19

Legend. *P < 0.05-0.01 compared with normal; **P < 0.05 compared with 25°C; ***P < 0.05 compared with oxidation. Number of animals shown in parentheses.

were subjected to brief hypothermia (cooling at −20°C to a rectal temperature of 22-25°C) followed by reheating to room temperature until the normal body temperature was restored. Preparations of mitochondria were obtained before cooling, during hypothermia, and after rewarming. The composition of the isolation medium was: sucrose 0.1 M; KCl 0.18 M, EDTA 0.01 M, $MgCl_2$ 0.005 M, ATP 0.001 M; bovine serum albumin 0.5%, Tris 0.1 M; pH 7.4, at 0°C. The intensity of respiration of the mitochondria was measured in two cells with open revolving platinum electrodes at 25°C and 15°C simultaneously. The composition of the incubation medium was: mannitol 0.3 M, HCl 0.01 M; KH_2PO_4 0.01 M; Tris 0.01 M; pH 7.4. Succinate (0.006 M) was used as the oxidation substrate. ADP was added in a dose of 200 nmoles. The following parameters were determined: rate of respiration in three metabolic states according to Chance (V_2, V_3, V_4), the rate of phosphorylation (V_p), and also the temperature coefficients Q_{10} (the ratio between velocities at 25 and 15°C), and the phosphorylation coefficient ADP/O, and the value of Chance's respiratory control (RC). Rates of oxygen consumption were expressed in nanomoles O_2/mg protein/min, and the velocity of phosphorylation in nanomoles ADP/mg protein/min. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

Acute cooling of the control animals accelerated respiration of isolated myocardial mitochondria, particularly intensively in metabolic states 2 and 3 (Table 1). The energy coupling parameters ADP/O and RC were unchanged. Consequently, myocardial mitochondria of hypothermic albino rats not only remained capable of functioning effectively, but they exhibited this ability at a high level. The response of the cardiac mitochondria to acute cooling thus revealed resembles the response of the liver mitochondria [5].

Mitochondria isolated from the myocardium of animals rewarmed after hypothermia maintained the increased rates of respiration in metabolic states 2 and 3, and the values of V_4 and V_p were increased by 39 and 34% respectively (Table 1).

Dependence of oxidation and phosphorylation on temperature in the control normothermic animals was identical, so that a fall of temperature *in vitro* caused no change in the values of ADP/O and RC. Acute cooling of the animals revealed no significant changes in the values of Q_{10} of respiration (Table 1). Absence of temperature compensation of tissue respiration in the internal organs of endothermic animals, by contrast with heterothermic and poikilothermic animals, has been observed by other workers also [3]. As regards phosphorylation in the myocardial mitochondria, its dependence on temperature was changed by a short exposure to cold, as a result of which the coefficient of phosphorylation fell with a fall of temperature *in vitro* ($P < 0.01$; Table 1).

Prolonged but moderate exposure to cold considerably increased the rate not only of respiration, but also of phosphorylation. Parameters of energy coupling were increased somewhat in this case (Table 1). All this can probably be regarded as an adaptive reaction of the mitochondrial apparatus of the myocytes, a response to considerable strain on cardiac activity during prolonged exposure to cold. Meanwhile, with a fall of temperature, the mitochondria demonstrated potential ability to lower their coefficient of phosphorylation. Consequently, there were some differences in the changes in the rate of respiration and phos-

phorylation with a fall of temperature *in vitro* in animals exposed to both acute and chronic cold. Greater sensitivity of phosphorylation on temperature also has been observed in mitochondria of skeletal muscles [7]. Changes in dependence on temperature were less marked in myocardial mitochondria. The reason for this may perhaps be the different levels of temperature constancy of these organs *in situ*. The response of the mitochondria, for instance, both to short-term acute cooling and to long-term exposure to cold, thus consists of intensification of functional activity, which is indirect in character in response to factors acting *in vivo*, and modification of temperature dependence during exposure *in vitro*.

In cold-adapted albino rats acute cooling and subsequent rewarming revealed a response of the myocardial mitochondria that differed from the response in control animals. The rates of respiration and phosphorylation were reduced in hypothermia (Table 1). Rewarming led to an even greater decrease in V_2, V_3, V_4, and V_p. RC was increased during hypothermia, but decreased considerably during rewarming. This fall in the rates of oxidation and phosphorylation, and also in the parameters of energy coupling in the mitochondria, can probably be explained by modification of the functional activity of mitochondria in cold-adapted animals exposed to acute cooling, the result of the unfavorable influence of prolonged exposure to cold on the cardiovascular system [4].

The results are thus evidence of an increase in the oxidative and phosphorylating capacity of mitochondria of the albino rat myocardium in response to exposure to cold or different duration and intensity, while parameters of energy coupling remained unchanged. Adaptation to cold modified the response of the mitochondria to short-term hypothermia and to subsequent rewarming. With a fall of temperature *in vitro*, the rates of respiration and phosphorylation decreased. The sensitivity of phosphorylation to changes of temperature was higher than that of respiration.

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