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Tolerance of Altitude-Acclimatized Rats to Exercise in the Cold (38873)

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The hypothesis that long-term exposure to one adverse environment may lead to increased or decreased tolerance to other adverse environments not previously experienced, has been advanced by a number of investigators (1–4). For example, residence at high altitude can be used as a method for training pilots, astronauts, and sportsmen to raise their resistance to the action of unfavorable factors in the environment such as hypoxia, acceleration, high temperature, and heavy physical exercise (4). Recently it was stated that "studies on cross-adaptation should deal not only with single-agent environments, but also multiple-agent environments" (5). No previous detailed studies have been reported in which altitude-acclimatized animals were simultaneously exposed for the first time to multiple stresses. Accordingly, we have studied the effects of simultaneous exposure of altitude-acclimatized rats to exercise and cold at ground level. Continuous exposure to a simulated altitude of 18,000 ft was chosen to induce altitude acclimatization, since it was found that no pathological changes were found in the vital organs at this altitude. In addition to determining exercise tolerance in the cold, changes in body temperature, blood lactic acid, serum glucose, and structure of the heart, striated muscle, and liver were studied.

Methods. Male Sprague–Dawley rats were housed in a well-ventilated decompression chamber 9 × 20 ft maintained at 23°. The rats were exposed continuously for 4 wk to a simulated altitude of 18,000 ft (bp 380 mm Hg, pO_2 79 mm Hg). The chamber was brought to ground level (elevation 300 ft referred to as ground level) for 1 hr twice weekly for resupplying food and water and for cage cleaning. The rate of ascent and descent was approximately 2000 ft/min. The exercise experiments were conducted in a large cold room maintained at 5.0 ± 2.0° at ground level. The exerciser was similar to one previously described (6), except for accommodating only four rats. The rats were exercised at rate of 6.9 m/min for 9 hr with a 5-min rest period at 30-min intervals. With the onset of fatigue all four limbs became immobile. Usually, after a rest period of 5 min, tiring rats resumed walking and if, within a few minutes, they again ceased to walk, they were then considered severely fatigued, and were promptly removed from the exerciser. Groups of four rats (two altitude-acclimatized and two unacclimatized) were exercised simultaneously and were sacrificed under chloroform anesthesia immediately or at 24, 48, or 96 hr after exercise. Hematocrits were determined by use of tail blood and microhematocrit tubes. For other studies heart blood was obtained and the serum was separated within 30 min. Serum glucose was measured with glucose oxidase using Glucostat Reagent (Worthington Biochemical Corp., Freehold, NJ). Blood lactic acid was determined by the method of Barker and Summerson as modified by Natelson (7). Body temperatures were obtained by inserting a probe of a Yellow Springs Telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) 10–14 cm into the rectum. Body temperatures after 3- and 6-hr exercise were obtained during the rest intervals. Sections of the heart, liver, and thigh muscles were fixed in buffered (pH 7.0) formalin. Frozen sections were stained with oil red O to study fatty changes in the tissues. Paraffin sections were stained with azure-eosinate for routine histopathologic studies. Portions of the heart, liver, and thigh muscle were fixed in acetic–alcohol–formalin and stained for glycogen by the periodic acid–Schiff procedure.

Results. Physiological changes. Severe fatigue during exercise in the cold occurred within 2–½ hr in altitude-acclimatized and within 5 hr in unacclimatized rats. More acclimatized then unacclimatized rats fatigued during 9-hr exercise (Fig. 1).

The mean body weight of 18 rats increased

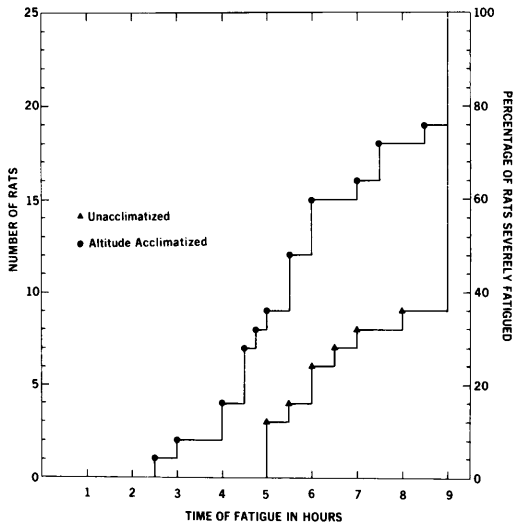

FIG. 1. Time of onset of severe fatigue in rats exercised at 5° at ground level.

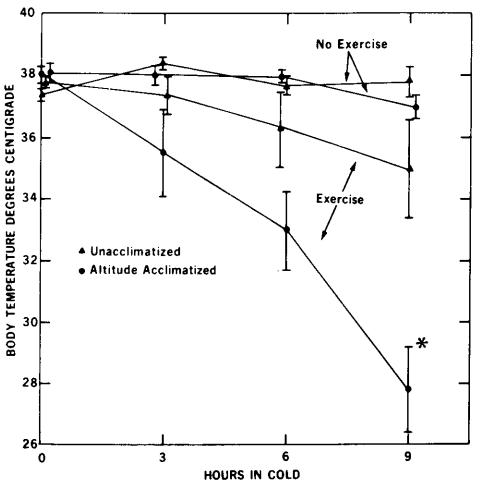

FIG. 2. Mean body temperatures of unacclimatized and altitude-acclimatized rats at intervals after exposure to 5° with and without exercise. * Significantly different from unacclimatized rats at 9 hr. $P < 0.05$ (Student's t test).

from 315 ± 6 (SE) to 379 ± 8 g during 4-wk exposure to 18,000 ft. The mean body weight of 19 unacclimatized rats increased from 326 ± 8 g to 405 ± 5 g during the 4 wk prior to exercise in the cold. The rats in both acclimatized and unacclimatized groups lost about 8% of their body weight during a 9-hr period of exercise in the cold. Preexposure body weights were largely restored in both groups within 4 days. No significant difference in body temperature was found between acclimatized and unacclimatized rats after 3-, 6-, and 9-hr exposure to the cold without exercise. However, with cold exposure, the rats in both groups became hypothermic during exercise, with acclimatized rats showing the greatest decline in body temperature (Fig. 2). The mean hematocrit for 14 rats acclimatized to 18,000 ft for 4 wk was 69 ± 1.1 and for eight unacclimatized rats 48.2 ± 0.9.

The mean serum glucose concentration of eight acclimatized and eight unacclimatized rats not exercised was 155 ± 9 and 147 ± 5 mg/100 ml, respectively. Immediately after 9-hr exercise in the cold the serum glucose concentration of eight acclimatized and eight unacclimatized rats was significantly reduced to 80 ± 7 and 113 ± 11 mg/100 ml, respectively ($P < 0.05$ and < 0.01, Student's t test). The decline in serum glucose was significantly greater in the altitude-acclimatized rats ($P < 0.05$). Normal serum glucose concentrations were regained in both groups of rats from 1 to 4 days after exercise in the cold. The mean blood lactic acid concentration of 15.2 ± 1.2 mg/100 ml for unacclimatized rats was not changed significantly immediately or at 1 and 4 days after exercise in the cold (eight rats/group). The mean lactic acid concentration of altitude-acclimatized rats was reduced from 20.5 ± 3.9 to 8.6 ± 0.5 mg/100 ml ($P < 0.01$, Student's t test) immediately after exercise in the cold A normal concentration, 16.1 ± 1.8 mg/100 ml, was regained within 1 day (eight rats/group).

Tissue changes. Practically all of the rats in both acclimatized and unacclimatized groups showed complete depletion of liver glycogen after exercise in the cold. Normal liver glycogen levels were regained within 1 day. Approximately one-half of the rats in both groups showed moderate glycogen depletion in the heart and in the striated muscle of the thigh. Restoration of cardiac glycogen was complete within 4 days in unacclimatized rats but required more time in acclimatized rats. Normal thigh muscle glycogen levels were regained within 4 days.

No significant fatty changes or necrosis were found in the heart, muscle, and liver of 26 rats immediately and at 1–4 days after 4-wk exposure to 18,000-ft simulated altitude. Fatty changes were found in striated

TABLE I. INCIDENCE (%) OF TISSUE CHANGES IN UNACCLIMATIZED (UEC) AND ALTITUDE[a]-ACCLIMATIZED (AEC) RATS EXERCISED 9 HR AT 5°.

Treatment	Time after treatment (days)	No. rats	Fatty changes						Focal areas of necrosis					
			Muscle		Heart		Liver		Muscle		Heart		Liver	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	—	7	0	0	0	0	0	0	0	0	0	0	0	0
UEC	0	8	2	25	0	0	4	50	0	0	0	0	0	0
	1	10	7	70	0	0	9	90	0	0	2	20	0	0
	4	11	3	27	0	0	4	40	3	27	2	18	0	0
	Total	29	12	41	0	0	17	58	3	10	4	14	0	0
AEC	0	16	2	12	0	0	8	50	2	12	3	19	0	0
	1	9	6	66	3	33	9	100	5	56	3	33	0	0
	4	16	2	12	0	0	19	87	2	12	10[b]	62	0	0
	Total	41	10	24	3	7	31	76	9	22	16[c]	39	0	0

[a] Continuous exposure to 18,000 ft for 4 wk.
[b] Significantly different from 4-day unacclimatized rats (UEC), $P < 0.01$, chi-square test.
[c] Significantly different from total of unacclimatized rats (UEC), $P < 0.05$, chi-square test.

muscles of about two-thirds of both acclimatized and unacclimatized rats 1 day after exercise in the cold (Table I). Such changes were less frequent at 4 days. One-third of the altitude-acclimatized rats showed scattered fatty changes in the myocardium of the left ventricle 1 day after exercise in the cold and none thereafter. The highest incidence of fatty changes occurred in the liver of rats 1 day after exercise. These changes persisted in many rats in both groups for at least 4 days.

Scattered areas of necrotic thigh muscle fibers were found in 22% of acclimatized and 10% of unacclimatized rats exercised in the cold (Table I). The necrotic muscle fibers resembled those found after exercise as described previously and were classified as mild since only 3 to 15 fibers were affected in a single tissue section (6, 8). Necrosis of muscle fibers was first observed in two acclimatized rats immediately after exercise, but they were not found until 4 days after exercise in unacclimatized rats. The percentage of rats with muscle cell necrosis was similar in both groups. No necrotic lesions were found in the liver of any of the rats used in this study.

Scattered small necrotic lesions were found in longitudinal sections and subapical cross sections of the left ventricular subendocardium of the heart. The lesions consisted of single or multiple areas of inflammatory foci showing necrosis similar to that found after exposure to cold as described previously (9). Occasionally there was loss of myocardial fibers with replacement by loose fibrous tissue and infiltration with mononuclear cells and replacement by granulation tissue. There was a significantly greater increase in the incidence of cardiac necrosis in the altitude-acclimatized rats (Table I).

Discussion. This study shows that rats acclimatized by 4 wk of continuous exposure to 18,000-ft simulated altitude fatigued faster than unacclimatized rats when exercised in the cold. Previously it was found that rats acclimatized by 5-hr intermittent daily exposures for 6 wk to 18,000 ft, showed no adverse effects after exercise at a comfortable temperature of 23° (10). In a study of lowland native athletes trained and acclimatized for 6 wk to 14,160 ft, physical performance was actually improved for a few days after they returned to normal atmospheric conditions. This result was attributed largely to improved pulmonary ventilation due to physical training at altitude (11). Since no temperatures were given, it is likely that the experiments were conducted at a comfortable temperature. A comparison of these studies indicates that the addition of cold adversely affected the rats' ability to exercise. Such evidence supports the hy-

pothesis that available homeostatic mechanisms become exhausted more readily in multiply stressed than in singly stressed rats as advanced previously (12). This provides evidence of a negative cross-adaptation between altitude and other stresses such as cold and exercise.

There are a number of factors which may have contributed to the poor exercise tolerance of the altitude-acclimatized rats, such as development of severe hypothermia, hypoglycemia, increased susceptibility to tissue damage, and polycythemia. It is well known that the fall in rectal temperature in animals exposed to acute hypoxia is due to a reduction in heat production (13). The heat loss in animals exposed to altitude has been attributed to a possible decrease in thyroid function and an increased capillary area/unit area of skin and muscle (14). Altitude-acclimatized rats in this study and altitude-acclimatized rabbits in another study (15) did not develop hypothermia while at rest or when mildly restrained during exposure to cold (5°). However, Fregly (14) noted that rats acclimatized to 19,000 ft for 3 wk cooled rapidly when immobilized at 5°, and in this study severe hypothermia was found in altitude-acclimatized rats exercised in the cold. These findings suggest that the alteration in the regulation of body temperature induced by exposure to altitude persists even after altitude acclimatization, but that development of hypothermia occurs during exposure to cold only after the addition of another stress such as exercise or immobilization. In cases of severe hypothermia the metabolic requirements of tissue declines and the need for oxygen is correspondingly reduced (16). Apparently the blood carries sufficient oxygen to the heart during hypothermia to meet basic requirements for survival, but as reported before, metabolic imbalances in H ions, potassium, sodium, and calcium levels occur, and normal contractile processes in the heart are not maintained (17). Such physiological alterations may have contributed to decreased exercise performance in this study.

The low serum glucose, lactic acid concentration, and loss of liver glycogen in altitude rats exercised in the cold, indicates that the supply of these substances was greatly reduced. This reduction may have affected ability to sustain exercise in the cold. Ordinarily, after exercise at room temperature (23°), serum lactic acid concentration is either above or near control levels (18). However, in this study, the blood lactic acid levels were abnormally low in altitude rats after exercise in the cold. This low concentration may be due to increased oxidation and exhaustion of available metabolites.

The increased incidence of focal myocardial necrosis in the altitude-acclimatized rats after exercise in the cold, compared with exercised unacclimatized rats in the cold, indicates that some change induced by altitude was responsible for the difference. Although no histopathological changes were observed prior to exercise, it is possible that the altitude acclimatization may have induced undetected submicroscopic changes, which made the cardiac tissue more susceptible to damage by additional stress. It is known that chronic hypoxia produces focal, moderate, mitochondrial swelling in the heart (19).

Altitude-induced polycythemia may have played a role in reducing exercise performance. It is well known that an increased erythrocyte volume increases blood viscosity. The rats became severely hypothermic during exercise in the cold, which may have increased the blood viscosity above that produced at normal body temperatures. Such a change could have increased the cardiac work load and hastened fatigue. Uncomplicated polycythemia vera (hematocrit 55) does not increase the work of the heart in man at rest or after exercise, but when complicated by heart disease or hypertension, the capacity for exercise is reduced (20). Likewise, uncomplicated altitude polycythemia (hematocrit 76), induced by intermittent exposure to 18,000 ft for 6 wk (10), had no adverse effect on exercise performance, but in this present study, with a moderately severe polycythemia (hematocrit 69) complicated by hypothermia, exercise performance was reduced.

Summary. The tolerance of altitude-acclimatized (18,000 ft 4 wk) and unacclimatized rats to exercise at 5° was determined. Fewer

unacclimatized than acclimatized rats became fatigued during 9 hr of exercise in the cold. Normal body temperatures were maintained in both groups during 9 hr in the cold at rest, but after exercise unacclimatized rats became mildly hypothermic (body temperature 35°) and acclimatized rats severely hypothermic (body temperature 27.9°). Polycythemia (hematocrit 69) was produced during the altitude acclimatization. Altitude-acclimatized rats developed more severe hypoglycemia and lower liver glycogen and serum lactic acid concentrations after exercise than did controls. No pathological changes were found in resting altitude-acclimatized rats, but after exercise in the cold, a higher percentage of acclimatized than unacclimatized rats developed focal myocardial necrosis within 4 days. Reduced exercise tolerance is attributed to severe hypothermia with associated decreased metabolism, polycythemia, hypoglycemia, and a higher incidence of pathological changes in the cardiac and striated muscles.

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