Swimming Compared to Cold for Eliciting Maximal Oxygen Uptake in Mice[1] (37674)

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Mice are frequently used in experiments which may alter their maximal oxygen uptake ($\dot{V}_{O_{2max}}$), and thus, physical work capacity. Swim time to exhaustion is one means of evaluating their fitness (1), but the measurement of $\dot{V}_{O_{2max}}$ during swimming has been shown to be more reliable (2). However, exercise tests in small animals involves technical and motivational difficulties which raise questions as to whether true physiological $\dot{V}_{O_{2max}}$ is achieved (2–4). Rats exposed to cold have been shown to have oxygen uptake values approaching that of exercise (5, 6). Therefore, the purpose of this study was to determine in mice: (a) if a technically simpler cold exposure could substitute for swimming in eliciting $\dot{V}_{O_{2max}}$; (b) whether the $\dot{V}_{O_{2max}}$ measured during the tests was a true physiological limit of aerobic metabolism as evidenced by the respiratory exchange ratio (R); (c) whether $\dot{V}_{O_{2max}}$ could finely differentiate between normal animals and those suffering from an impairment of aerobic function (as simulated by hypoxia).

Methods. Ten week old male albino mice (Swiss/Cox, av 35 g) were used for this study. Swimming was performed in 36° water with a 5% body load weight attached to the tail (2). This water temperature (T_w) was presumed to be thermoneutral (3, 7). For cold stress, the mice were lightly restrained by the tail and immersed to the neck in either 28 or 20° water while sitting quiescent on a movable platform. Controls were similarly restrained and immersed in 36° water. Air (21% O_2) or a hypoxic gas mixture (12% O_2), supplied from compressed gas cylinders, was breathed during the tests.

Oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide output ($\dot{V}_{CO_2}$) were monitored by open circuit spirometry. The metabolism chamber and related apparatus was identical to that previously described by Glaser, Gross and Weiss (2) except that in addition to the Servomex model OA-150 paramagnetic O_2 analyzer, a Beckman Model LB-1 infrared CO_2 analyzer was added in series in the gas line from the metabolism chamber. Outputs from these analyzers recorded on separate channels of a Grass polygraph for continuous readout. With compressed gas entering the chamber at 1 liter/min, expanded scale calibration ranges of 1% for O_2 and CO_2 (between 21–20% and 12–11% for O_2, and 0–1% for CO_2) were adequate for all tests.

Maximal oxygen uptake and maximal carbon dioxide output were taken at their respective peak values during a 5 min test. $\dot{V}_{O_{2max}}$ is reported in relative terms (ml/kg/min, STPD). The respiratory exchange ratio was computed as $R = \dot{V}_{CO_{2max}}/\dot{V}_{O_{2max}}$. Because of its high solubility, some CO_2 diffused into the water along its gradient, requiring all measured CO_2 values to be corrected upward. The correction factor was determined to be 1.024 by measuring the CO_2 reference gas (0.86%) as it was fed directly into the analyzer, and then noting the difference in concentration when the gas first passed through the metabolism chamber. Rectal temperature (T_r) was detected by a thermistor probe (YSI no. 402) inserted 4 cm into the rectum and displayed on a Digitec

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TABLE I. Maximal Oxygen Uptake and Respiratory Exchange Ratio of Mice During Swimming and Exposure to Cold Water.[a]

O_2 concn breathed	Stress applied		$\dot{V}_{O_{2max}}$ (ml/kg/min, STPD)		Respiratory exchange ratio
	Type	T_w[a]	Mean ± SE	Coefficient of variation (%)	(mean ± SE)
21% (air)	Swim	36	131.2 ± 3.17	7.6	0.92 ± 0.020
	Cold	28	124.7 ± 6.28	15.9	0.94 ± 0.029
	Cold	20	121.5 ± 3.52	9.1	0.96 ± 0.020
	Control	36	79.9 ± 3.47	13.7	0.86 ± 0.021
12% (hypoxia)	Swim	36	91.5 ± 1.75	6.0	1.05 ± 0.015
	Cold	28	91.8 ± 2.46	8.5	0.95 ± 0.026
	Cold	20	94.4 ± 1.57	5.2	1.04 ± 0.031
	Control	36	71.5 ± 2.68	11.9	0.89 ± 0.032

[a] Ten different mice (av 35 g) were used for each treatment.

model 501. Each mouse was used only once in any phase of the study.

Results. Table I gives the $\dot{V}_{O_{2max}}$ for mice while swimming in 36° water or exposed to 28 and 20° water, and breathing either 21 or 12% O_2. In 21% O_2, all three stresses produced highly significant increases in $\dot{V}_{O_{2max}}$ ($p < 0.01$), of the order of 50–60% greater than controls exposed to 36° water. Analysis of variance indicated that the three stressed groups were not significantly different from each other. The overall mean for the 30 stressed animals was 126 ml/kg/min. In 12% O_2, the three stressed groups again had significantly higher $\dot{V}_{O_{2max}}$ than the controls ($p < 0.01$), but only of the order of 30% greater. There were again no differences among the three stressed groups, and the overall mean for the 30 mice was 93 ml/kg/min. Mean $\dot{V}_{O_{2max}}$ for the stressed groups in 12% O_2, was a highly significant 26% lower than in air ($p < 0.01$), but for the controls the 10% lower $\dot{V}_{O_{2max}}$ in 12% O_2 was not statistically significant ($p > 0.05$). Coefficients of variation (CV), presented as a measure of variability in the data, tended to be higher in 28° water than in the other stressed groups.

Respiratory exchange ratios ($\dot{V}_{CO_{2max}}/\dot{V}_{O_{2max}}$) are also shown in Table I. R was significantly higher for the stressed groups than for the controls in both 21 and 12% O_2 ($p < 0.01$). There were no significant differences in R for the 3 groups stressed in 21% O_2 (mean $R = 0.94$). In 12% O_2, however, the R of 0.95 for the 28° group was significantly lower ($p < 0.05$) than the 1.04 and 1.05 R values for 20° and swimming groups, respectively. The mean R of 1.01 for these animals stressed in hypoxia was significantly higher than those stressed in normoxia ($p < 0.01$), but R for controls did not statistically differ between 12 and 21% O_2.

$\dot{V}_{O_2}$ values recorded in 21% O_2 during each min of the 5 min cold stress test is shown in the upper graph of Fig. 1. The lower graph shows the change in T_r of 6 additional mice at each T_w. Control animals in 36° water remained at a relatively low metabolic level which decreased slightly with time while their T_r tended to increase slightly. In 28° water, $\dot{V}_{O_{2max}}$ occurred during the second minute and plateaued for the remainder of the test as T_r fell at a relatively slow rate of 0.2°/min. In 20° water, $\dot{V}_{O_2}$ also peaked during the second minute, but thereafter metabolism declined steadily as T_r fell at a relatively high rate of 1.2°/min. $\dot{V}_{O_{2max}}$ during swimming also occurred after about 2 min (2).

Discussion. The similarity in time occurrence and magnitude of $\dot{V}_{O_{2max}}$ between mice swimming in thermoneutral water or sitting quiescent in cool water (Table I) suggests that the simpler cold stress may be substituted for exercise. Undoubtedly the rapid dissi-

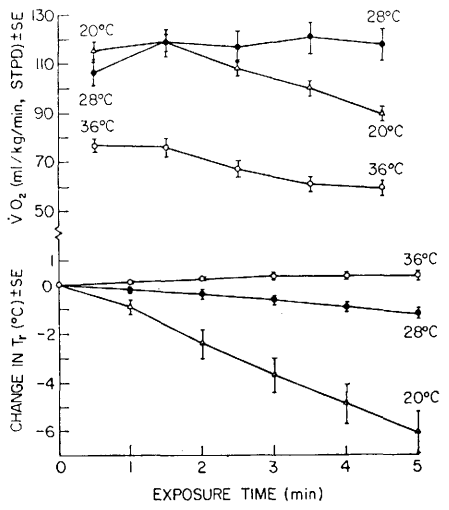

FIG. 1. Time course for the 5 min cold stress test in 21% O_2. The upper graph shows mean oxygen uptake for 10 mice at each water temperature, whereas the lower graph shows mean change in rectal temperature of six mice at each water temperature (av starting T_r was 37.1 ± 0.3°).

pation of heat caused by the large surface area to volume ratio in mice makes the cold stress more effective in these species than might be expected in larger animals. Skill and/or motivation seem to account for individual variation in swimming performance (2–4). These factors should be eliminated during cold stress. However, the coefficient of variation is no less for cold stress than for swimming indicating that similar individual variation exists for thermoregulatory responses.

Some clues are provided as to the optimal water temperature at which to carry out the cold exposure. 20° water is clearly an overwhelming stress, resulting in a rapid and continuous fall in T_r and a fall in $\dot{V}O_2$ after the second minute (Fig. 1). At peak aerobic power, T_r was already almost 2° below normal. The measured $\dot{V}_{O_{2max}}$ in 20° water may thus represent a lower than true value because of the effects of hypothermia on slowing metabolism (5, 8, 9). 28° water appears to be about the upper limit of T_w for accomplishing a cold stress. Although a high $\dot{V}O_2$ level was reached in the presence of an almost normal T_r, the relatively large coefficient of variation associated with this $\dot{V}_{O_{2max}}$ suggests that some mice fail to react fully to the stress. Whether water at a temperature 1 to 2° lower could elicit a more uniform response remains to be determined. With respect to the controls, the slight rise in T_r and decline in $\dot{V}O_2$ during the 5 min exposure suggests that under the conditions of this test, thermoneutrality may be slightly lower than 36°.

The evidence from the respiratory exchange ratios suggests that neither swimming nor cold produced a true physiological maximum in O_2 uptake. Mean R was below 1.00 for all stress measurements made in 21% O_2. It would be expected that R would increase to 1.00 or above if the aerobic energy system was taxed to its capacity, presumably from a shift toward carbohydrate metabolism and a buffering of lactic acid (10). By contrast, while breathing 12% O_2, R rose to 1.05 for swimming and 1.04 in 20° water, suggesting that the additional stress of hypoxia had depleted aerobic reserves, and increased the anaerobic energy component. The failure of R to rise above 1.00 in 28° water during hypoxia possibly stems from the mildness of this stress. All coefficients of variation for $\dot{V}_{O_{2max}}$ were reduced in hypoxia, as might be expected when aerobic limits are approached. From these data, it appears that tests of submaximal aerobic stress may be changed into tests of maximal aerobic stress simply by lowering the P_{O_2} in the gas breathed.

Even if the $\dot{V}_{O_{2max}}$ induced by swimming or cold is not a true physiological maximum it can nevertheless be used to differentiate between normal animals and those with some defect in aerobic metabolism. In this case, a defect was simulated by hypoxia. The 12% O_2 environment, which was estimated to be equivalent to about 4300 m altitude (84 mm Hg tracheal P_{O_2}) was sufficient to produce a 26% decrease ($p < 0.01$) in $\dot{V}_{O_{2max}}$ in the stressed mice. On the other hand, $\dot{V}_{O_{2max}}$ in the unstressed controls failed to differentiate significantly between the normal and hypoxic animals.

Summary. Exposure of mice to water of either 28 or 20° stimulated in about 2 min a maximal oxygen consumption value (120–130 ml/kg/min) similar to swimming in ther-

moneutral (36°) water. 20° water was considered an excessive stress because of a relatively rapid decline in rectal temperature of 1.2°/min which resulted in metabolic depression. Respiratory exchange ratios during stress in 21% O_2 averaged 0.94 suggesting submaximal loads on the aerobic system. During the additional stress of hypoxia (12% O_2) R rose above 1.00 possibly indicating a true maximal aerobic stress. The ability of these tests to detect aerobic defects was demonstrated by the 26% lower $\dot{V}_{O_{2max}}$ in mice breathing 12% O_2 during stress as compared to those in 21% O_2. It seems both the swimming and cold stress tests may be used interchangeably. Each is of short duration and neither require training.

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