Thirst Immediately Following Removal of Rats from Graded Levels of Hypoxia[1] (35917)

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Earlier studies from this laboratory showed that rats exposed chronically to varying degrees of hypoxia become relatively dehydrated (1). Thus, exposure to an atmosphere containing 12.0% oxygen for 35 days increased both serum osmolality and sp gr significantly and induced a thirst within 1 hr after return to normal oxygen concentration (20.9%).

The objective of the experiments described here was to determine whether the magnitude of the water intake immediately following return from hypoxia to normoxia was a function of the percentage of oxygen to which the rats were previously exposed.

Methods. Four separate experiments were performed. Each used 12 male rats of the Blue Spruce Farms Strain. For 2 weeks prior to beginning each experiment, all rats were kept in stock cages in a room maintained at 26 ± 1° and illuminated from 8 a.m. to 6 p.m. The food was finely ground Purina laboratory chow and was available *ad libitum* in spill-proof feeders (2). Tap water was also available *ad libitum* in infant nursing bottles containing cast aluminum drinking fountains as described by Lazarow (3).

Each experiment began with a 5 day control period during which the rats were caged individually and food and water intakes and body weight were measured daily. During the 8 day experimental (hypoxic) period, intakes and body weight were measured every other day. The length of the experimental period was arbitrarily chosen. A 5 day period followed the experimental period during which intakes and body weight were again measured daily. The single exception to this is in the first (17.0% oxygen) experiment in which no post-hypoxic measurements were made.

The chamber for exposure of the rats to hypoxia was located in the animal room and was the same as that described by Sisson and Fregly (4). The method of exposure was essentially a gas dilution technique at atmospheric pressure. During the experimental period, the chamber was opened every second day to measure food and water intakes, weigh the rats, replenish carbon dioxide and water absorbers and to clean the cages. This operation required approximately 1 hr. Thus, the experimental rats were in 20.9% oxygen for 1 hr out of every 48. In the first experiment, 6 rats were exposed to an atmosphere containing 17.0% oxygen while 6 others served as controls and were maintained at ambient oxygen tension (20.9%). Three additional experiments, each using a total of 12 rats, were performed exactly as described above, except exposed to an atmosphere containing 16.0 ± 0.2, 15.0 ± 0.2, or 12.0 ± 0.2% oxygen. Six controls for each group were maintained in 20.9% oxygen.

At the end of the 8 day exposure to hypoxia, the experimental rats were returned to the normoxic (20.9%) control environment and measurement of spontaneous water intake began immediately for both control and treated rats. Intakes were measured during the first and entire (cumulative) 3 hr after removal from hypoxia.

Comparison of data among groups was made by means of the *t* test for the 95% confidence limit (5) as well as by an analysis of covariance using the IBM 360/50 computer and the Biomed Series program BMD04V.

Results. Food and water intakes and body weights of control and treated groups prior to, during and following exposure to hypoxia

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TABLE I. Effect of Exposure to Hypoxia for 8 Days on Mean Water and Food Intakes and Mean Body Weight.

Hypoxic exposure	No. of rats	Control period			Hypoxic period			Posthypoxic period		
		Body wt (g)	Water intake (ml/day)	Food intake (g/day)	Body wt (g)	Water intake (ml/day)	Food intake (g/day)	Body wt (g)	Water intake (ml/day)	Food intake (g/day)
17% Oxygen										
Control	6	366 ±10[a]	35.1 ±1.9	21.7 ±0.8	378 ±11	34.9 ±1.3	22.5 ±0.7	—	—	—
Hypoxia treated	6	339 ±12	32.9 ±1.1	20.4 ±0.8	347 ±12	33.2 ±0.9	19.3 ±0.6	—	—	—
16% Oxygen										
Control	6	186 ± 4	25.0 ±1.8	17.0 ±0.9	213 ±10	28.0 ±2.0	19.7 ±1.0	238 ±9	29.8 ±1.6	20.6 ±0.9
Hypoxia treated	6	184 ± 6	28.9 ±0.8	18.1 ±0.5	226 ± 7	26.6 ±2.0	17.5 ±1.1	249 ±9	31.2 ±1.3	19.8 ±1.1
15% Oxygen										
Control	6	230 ± 5	30.8 ±0.9	21.1 ±3.9	245 ± 9	32.9 ±1.1	23.4 ±0.9	261 ±8	35.3 ±1.1	23.6 ±0.7
Hypoxia treated	6	247 ± 5	27.8 ±1.7	19.8 ±1.7	259 ± 5	27.6 ±1.7[b]	17.2 ±1.4[c]	283 ±6[b]	35.2 ±1.7	23.1 ±1.1
12% Oxygen										
Control	6	289 ± 3	30.9 ±1.4	22.7 ±0.4	306 ± 2	33.1 ±0.9	22.8 ±0.5	321 ±2	32.8 ±1.5	24.0 ±0.8
Hypoxia treated	6	293 ± 3	27.8 ±1.8	21.7 ±1.7	276 ± 3[c]	19.9 ±1.6[c]	14.4 ±1.4[c]	293 ±3[c]	29.9 ±1.6	21.4 ±1.1

[a] One standard error of mean.

[b] Significantly different from control: $p < .05$; [c] $p < .01$.

are shown in Table I. No significant differences between groups were observed during the control, hypoxic, or posthypoxic periods for any of the measurements made during the experiments in which the treated group was exposed to either 17.0 or 16.0% oxygen. An analysis of covariance was also performed on data from these, as well as that from the other experiments (15.0 and 12.0% oxygen), to aid in determining the oxygen fraction at which changes in these measurements first occurred. Exposure to 15.0% oxygen significantly reduced both food ($p<.01$) and water ($p<.05$) intakes but did not affect body weight significantly over the 8 day treatment period. Exposure to 12% oxygen reduced significantly ($p<0.01$) all measurements made, suggesting that the critical oxygen concentration affecting these measurements is somewhere between 12.0 and 15.0%. Both food and water intakes returned to control level after removal from hypoxia (Table I). However, mean body weight of the rats exposed to 12% oxygen was still significantly less than that of controls during the posthypoxic period.

Water intakes during the first, the first three, and the total 24 hr following removal from hypoxia are shown in Table II. Although water intake of the treated group was greater than that of the control group during the first hour following removal from 16.0, 15.0, and 12.0% oxygen, it was significantly ($p<.01$) greater only following removal from 12.0% oxygen. The cumulative 3 hr water intakes of treated rats following removal from the four test environments were greater than that of their simultaneous controls but differences were significant only after removal from 17 ($p<.05$) and 12.0% ($p<.01$) oxygen. No significant differences were observed between the cumulative 24 hr water intakes of treated and control rats at any of the percentages of oxygen used.

The difference in water intake between control and treated groups during the first hour after removal from hypoxia increased in a linear fashion with decreasing oxygen percentage to which the rats were previously exposed (Fig. 1). These results suggest that the difference reached zero at 16.5% oxygen. When the difference in water intake during the entire 3 hr following removal from hypoxia was plotted in the same fashion, the difference reached zero at approximately 19.3% oxygen. Since the water ingested during the first hour represented from 40 to 75% of the total 3 hr intake, greater emphasis is placed on the results obtained during the first hour as more nearly representative of the posthypoxic thirst.

Discussion. A clear concept of the state of water balance of animals exposed to hypoxia is difficult to grasp from data available in the literature. For example, Silvette (6) observed that daily exposure for 3 hr to 15,000 and 25,000 ft simulated altitude increased urinary flows in rats 150 and 300%, respectively, above control levels. The increased urinary flow was maintained for the 27 days of the experiment and returned to control level when daily exposure to hypoxia ceased. Stickney (7), using daily exposures for 3.5 hr to simulated altitudes from 400 to 28,000 ft, concluded that the increase in both urinary flow and insensible water loss was roughly proportional to the altitude to which the rats were exposed. The increase in urinary flow during intermittent exposure to hypoxia occurred in association with increases in kidney weight (6, 8–10). Increased urinary flow may also be related to renal pathologic changes, particularly at altitudes of 25,000 ft or more (8). In spite of the increased urinary flow and insensible water loss, Lawless and Van Liere (11) could find no consistent changes in the water content of cerebrum, kidney, liver, muscle, skin, and adrenal glands of rats subjected daily for 3.5 hr to approximately 8000, 18,000, and 28,000 ft altitude. Swann *et al.* (12) and Swann and Collings (13) also called attention to the increased insensible water loss and negative water balance of rats exposed for 6–23 hr to 18,000 ft altitude (380 mm Hg pressure). However, urinary flow failed to show a consistent increase and actually decreased below that of controls during the 23 hr exposure. In addition, water intake measured during these studies also failed to show consistent changes.

It is apparent that the changes in water

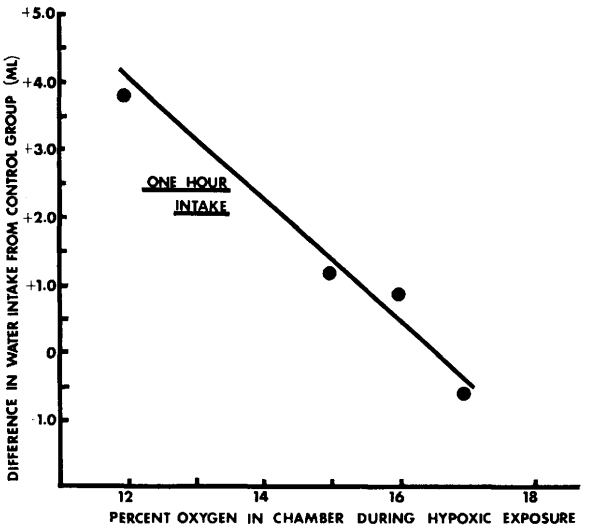

FIG. 1. Difference in water intake from the control group is plotted against the concentrations of oxygen to which the rats were previously exposed. Water intake was measured during the first hour after removal from hypoxia.

metabolism induced by acute exposure to hypoxia, including increased urinary flow, increased insensible water loss, and no increase in water intake, could not be maintained during chronic exposure to hypoxia. The question as to whether the changes occurring during acute exposure to hypoxia, even discontinuous exposures over many days' duration, are representative of those taking place in animals exposed chronically and continuously is worthy of consideration. Picon-Reategui *et al.* (14), using tritiated water, measured body water content of rats exposed chronically to a simulated altitude of 15,000 ft. The results suggested that body water depletion was greatest from days 4 to 7 of exposure to this altitude. Measurements made during this period indicated that 20% of the body water content had been lost. With time at altitude, both body weight loss and body water loss diminished such that by 30 days of exposure to altitude, about 5 g of water was lost/100 g original body weight.

Earlier experiments from this laboratory showed that exposure of rats to 12.0% oxygen at atmospheric pressure for 32 days was accompanied by an increase in serum osmolality and specific gravity (1). In addition, the experimental rats increased their spontaneous water intake within the first hour after returning to control environment (20.9% oxygen). The objective of the present experiments was to determine whether the magnitude of the water ingested following return from hypoxia to normoxia was a function of the degree of hypoxia to which the rats had been exposed. Rats were exposed to 17.0, 16.0, 15.0, and 12.0% oxygen for 8 days and spontaneous water intake was measured hourly during the first 3 hr after removal from hypoxia. The difference in water intake between treated and control groups during both the first and the entire 3 hr after removal from hypoxia increased linearly with decreasing oxygen concentration (Fig. 1). Whatever factors are responsible for the post hypoxic thirst in rats they do not appear to be activated by exposure to 16.5% oxygen or higher for 8 days. These results are the first to suggest that the extent of the posthypoxic thirst is an inverse linear function of the degree of hypoxia to which the rats were exposed. The results also suggest, but do not prove, that the extent of dehydration induced in rats may also be an inverse linear function of the degree of hypoxia to which the animals were exposed, at least within the period of exposure used here.

Measurements of mean daily food and water intakes prior to and during exposure to

TABLE II. Effect of an 8 Day Exposure to Hypoxia on Spontaneous Water Intake During the First 3 hr After Return to a Normoxic Environment (20.9% oxygen).

Group	(hr)	No. of rats	Percentage of oxygen in chamber			
			17.0	16.0	15.0	12.0
			Water intake (ml)			
Control	1	6[a]	2.38 ± 0.58	0.67 ± 0.19	0.62 ± 0.21	0.13 ± 0.02
Treated	1	6	1.83 ± 0.60	1.80 ± 0.74	1.93 ± 0.76	3.92 ± 0.54[c]
Control	0–3	6	3.23 ± 0.53	1.97 ± 0.23	1.23 ± 0.19	0.66 ± 0.36
Treated	0–3	6	4.68 ± 0.38[b]	4.07 ± 1.17	3.37 ± 0.92	5.33 ± 0.80[c]
Control	0–24	6	35.07 ± 0.77	31.35 ± 2.01	38.02 ± 2.34	30.05 ± 1.54
Treated	0–24	6	36.28 ± 1.21	29.50 ± 3.28	36.70 ± 1.97	35.48 ± 3.23

[a] Six different control and treated groups were exposed to each oxygen percentage listed. One standard error is set off at each mean.

[b] Significantly different from control: $p < .05$; [c] $p < .01$.

hypoxia suggested that the effects of the hypoxic environment were first manifested at 12% oxygen. Since the next higher oxygen fraction used was 15%, the effect of hypoxia on food and water intakes would be expected to manifest itself at some fraction of oxygen between 12 and 15%. The fact that a lower oxygen fraction is required to show an effect on food and water intake (12.0 to 15.0%) than is required to show an effect on water intake during the first hour following removal from hypoxia (16.5%) suggests that the latter may be a more sensitive measure of the dehydration induced.

It is difficult to explain the thirst following removal from hypoxia. In spite of the increase in serum osmolality and specific gravity during exposure to hypoxia reported earlier (1), the rats failed to ingest sufficient water to prevent a relative dehydration from occurring. Experiments of others show that the extent of water ingested during a drinking test can be correlated with serum osmolality and deficit of body water prior to the test (15). The failure of the usual response to a normally adequate stimulus points up the interesting question posed by Swann and Collings (12) concerning lack of thirst during exposure to hypoxia. The point at which the stimulus–response system fails is unknown because little is known about the receptors for thirst, as well as the afferent and efferent pathways concerned with it. Central areas of the brain mediating thirst sensations have been described (16). Hypoxia could affect water intake by reducing receptor sensitivity, by reducing sensitivity of central areas of the brain, or conversely, by increasing the sensitivity to factors affecting satiation of thirst.

The importance of the relative dehydration occurring during chronic exposure to hypoxia for the process of acclimatization to altitude is unknown. It does not appear, however, that acclimatization is necessary for the posthypoxic drinking response to occur since it was observed in an earlier study after only 5 days of exposure to 12.0% oxygen (1).

Sundstroem and Michaels (17), in their comprehensive study of the physiological effects of exposure of rats to hypoxia, reported that plasma specific gravities increased during the first day of exposure to 14,000; 24,000; and 26,000 ft simulated altitude. Plasma specific gravity returned to control levels after 7 days of exposure. Earlier studies from this laboratory reported increases in plasma specific gravity and osmolality after 32 days of exposure to 12.0% oxygen (1). This suggests that the relative dehydration induced by hypoxia may continue for longer periods then were observed by Sundstroem and Michaels (17). The relative contributions of renal and extrarenal water loss to the dehydration occurring during hypoxia cannot be assessed at present. Nor can one assess the contribution to dehydration of the apparent failure to drink sufficient water to maintain serum osmolality at control levels. Further study will be needed to deter-

mine these.

Summary. The objective of these experiments was to determine whether the magnitude of the water intake measured immediately following return from hypoxia to normoxia was a function of the percentage of oxygen to which the rats had been exposed. Rats were exposed to an atmosphere containing 17.0, 16.0, 15.0 or 12.0% oxygen in nitrogen for 8 days. Food and water were available at all times both prior to and during exposure to hypoxia. Immediately following removal from hypoxia the spontaneous water intake of each rat was measured during the first, and the entire first 3 hr after removal from hypoxia. The difference in water intake between treated and control groups during both the first and the entire 3 hr after removal from hypoxia increased in a linear fashion with decreasing oxygen percentage to which the rats were previously exposed. The difference in water intakes between treated and control groups (posthypoxic thirst) measured during the first hour after removal from hypoxia reached zero at 16.5% oxygen. These results are the first to suggest that the extent of the posthypoxic thirst, and perhaps the relative dehydration induced, are inverse linear functions of the degree of hypoxia to which the rats were exposed.

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