Metabolic Depression in Animals Exposed to Air After Living In a He-O_2 Environment.* (31694)

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Although narcosis is a well documented phenomenon associated with high partial pressures of gaseous nitrogen (N_2), at one atm N_2 is generally considered to be physiologically inert(1). In spite of this, a few studies in which N_2 was replaced by other inert gases can be interpreted to mean that metabolism may be decreased by the N_2 present at sea level. For example, Schreiner *et al*(16) exposed the fungus *Neurospora crassa* to 5% O_2 and 95% of either He, Ne, N_2, Ar, Kr or Xe at 760 mm Hg, and found that growth rate was inversely proportional to molecular weight: higher in He, lowest in Xe with N_2 intermediate. Higher metabolism and/or metamorphosis in He-O_2 than in air has also been reported for the meal worm, *Tenebrio molitor,* the fruit fly, *Drosophila melanogaster,* and mouse tissue slices(4). In these poikilothermic situations, stimulation of metabolism by greater heat loss in He should not be a factor, as it may be in the intact homeotherm(5).

In related studies, Wright *et al*(18) observed a depression in O_2 uptake below that of air controls in homogenates of chick embryos from eggs which had been incubated for 8 days in a 79% He-21% O_2 environment, but only if the homogenates were prepared in air before being gassed with 100% O_2 in the Warburg flasks. The authors speculated that the period of incubation in a N_2 free environment (*i.e.,* He-O_2) might have resulted in increased sensitivity to the depressant effects of N_2. Our preliminary studies with intact rats and chicks(13) seemed to bear out the possibility of a depression in O_2 uptake on exposure to air following a period of maintenance in He-O_2. Further support came recently when Bond(3) reported that in high pressure-underwater work, men going from a He-O_2 mixture in which they had been living into air at the same pressure experienced an immediate, dangerous nitrogen narcosis beyond that expected for the depth involved. Bond suggested that once the body is denitrogenated, susceptibility to N_2 narcosis may be increased.

Methods and material. To study the possibility of an increased sensitivity to N_2 depression, we followed in detail the metabolic response of intact animals to air at one atm after they have lived for a period of time in a N_2 low environment. Mice, chicks, and rats were maintained for 10-22 days in a 79% He-21% O_2 environment using sealed, recycling, polyvinyl isolators in which gas composition, temperature, and relative humidity were controlled(17). N_2 was kept below 2% by flushing with He-O_2 when necessary. An identical control isolator was filled with air.

Resting oxygen consumption ($\dot{V}_{O_2}$) was measured in individual, unrestrained animals using a ten-place, closed-system metabolimeter in which each animal chamber could be flushed independently with any desired gas(12). Precautions were taken to avoid artifacts in $\dot{V}_{O_2}$ due to premature exposure to N_2 or from time differences in the measurements. Four to five animals were transferred into individual He-O_2 flushed animal chambers while still within the experimental isolator, thus avoiding any contact with air. An equal number of controls were placed in the remaining air filled chambers, all 8-10 units placed simultaneously in a common water bath and connection made to the metabolimeter. For rats and mice, measurements were carried out at 28C, and for day-old chicks, 38C.

To reduce the possibility of artifacts from differences in gas solubilities, diffusabilities,

* Supported by NASA grant NsG 295-62.

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etc., the gases which surrounded the animals while metabolism was measured were changed in a 3-step sequence as follows: 1) $\dot{V}_{O_2}$ was determined in the gas in which they were raised, 79% He-21% O_2 or air, then 2) both the experimentals and the controls were measured in 100% O_2. The purpose of this sequence was to evaluate the acute and sustained effects of the experimental atmosphere. The 100% O_2 step should have eliminated most of the residual inert gas dissolved in the tissues and also provided a common base of O_2 consumption which would be independent of any direct He stimulating or N_2 depressing effects. Finally 3) both groups were measured in air, marking the first time the He-O_2 animals were exposed to N_2. The time course for each sequence was 55 minutes; 10 minutes for flushing, 5 min for temperature equilibration and 40 minutes for measuring V O_2.

The measurements of $\dot{V}_{O_2}$ consisted of 8 consecutive 5-min O_2 uptakes for each animal, the last 4 of which were averaged and converted to ml/kg/min, STPD. The effect of first exposure to N_2 was tested by the paired difference in $\dot{V}_{O_2}$ between the air step and the preceding 100% O_2 step (*i.e.*, $\Delta \dot{V}_{O_2}$, air-100% O_2). To determine statistical significance, the average $\Delta \dot{V}_{O_2}$ calculated for the He-O_2 animals was compared with $\Delta \dot{V}_{O_2}$ for the simultaneously exposed controls.

Results. In Table I are listed $\dot{V}_{O_2}$ for 26 g (young adult) mice, 1-3-day-old chicks and 160 g rats which had lived for an extended time in He-O_2 (ten days for mice and rats, 21-22 day incubation period for chicks). The results are pooled data from 2-3 separate trials, each on 8-10 animals, for each species. All 3 species showed higher metabolism while in the He-O_2 in which they were raised compared to air, ranging from 23% for rats, to 11% for mice, to 6% for chicks. The mouse and rat differences were statistically significant. When the groups were flushed and measured in 100% O_2, most of the differences in $\dot{V}_{O_2}$ between He and air groups disappeared, due primarily to a decrease in the He animals. Finally, when the groups were measured in air, $\dot{V}_{O_2}$ for the animals raised in He-O_2 fell even further, and was actually below that of the air controls for mice and chicks. The difference here was statistically significant only for the chicks.

In Fig. 1 are plotted the averages and standard errors of the paired differences in O_2 uptake, $\Delta \dot{V}_{O_2}$, between the final air step and the preceding 100% O_2 step, which marks the

TABLE I. Oxygen Consumption in Relation to the Inert Gas Composition of the Environment. Mice and rats maintained in 79% He-21% O_2 for 10 days and chicks for a 21-22 day incubation period before determining $\dot{V}_{O_2}$.

Gas surrounding animals during metabolism	Oxygen uptake ($\dot{V}_{O_2}$), ml/kg/min ± S.E., STPD					
	Mice (18/group) avg wt 26 g		Chicks (27/group) avg wt 38 g		Rats (16/group) avg wt 160 g	
	Experimentals (He-O_2 raised)	Controls (Air raised)	Experimentals (He-O_2 raised)	Controls (Air raised)	Experimentals (He-O_2 raised)	Controls (Air raised)
Gas in which raised	77.4 ± 2.55*	70.0 ± 2.00	26.1 ± .79	24.7 ± .77	33.0 ± .85*	26.7 ± .76
100% O_2	71.2 ± 1.28	67.8 ± 1.53	23.0 ± 1.01	23.6 ±1.09	28.8 ± .65	27.0 ± .58
Air	61.0 ± 2.40	64.0 ± 2.12	19.6 ± 1.04*	22.6 ± .86	26.8 ± .59	26.7 ± .57

* Significantly different from controls at the 5% level.

first exposure of the He-O_2 raised animals to N_2. Although there is a general decrease in $\dot{V}_{O_2}$ for both experimentals and controls, the decrease is significantly greater for the He-O_2 raised animals in all 3 species. In relation to the controls, the decrease in $\Delta \dot{V}_{O_2}$ was 10% greater for the He mice, 11% greater for the He chicks, and 7% greater for the He rats.

Discussion. The factor which appears most relevant to the significant decrease in O_2 consumption of the experimental animals on their first exposure to air is their past history of having lived for an extended period of time in a He-O_2 environment. Conceivably, it could be due to some direct residual effect of He, or indirectly to the absence of N_2. Actually, the expected He effect is present in the elevated metabolism of the experimentals shown when the measurements were made in the gas in which they were raised (Table I). This elevation in $\dot{V}_{O_2}$ presumably is in response to increased heat loss because of higher thermal conductivity of He(9,10). However, since this elevated metabolism is of about the same magnitude as we have found with acute (1-2 hr) exposures to He-O_2 there is no indication of any unusual effects from the extended (10-22 day) exposures. Furthermore, when the He-O_2 and air were replaced by 100% O_2, metabolism of the experimentals fell to that of the controls, again indicating that the He effect was temporary.

Although some decrease in O_2 uptake with time is suggested by the progressive decline in $\dot{V}_{O_2}$ in the control mice and chicks during

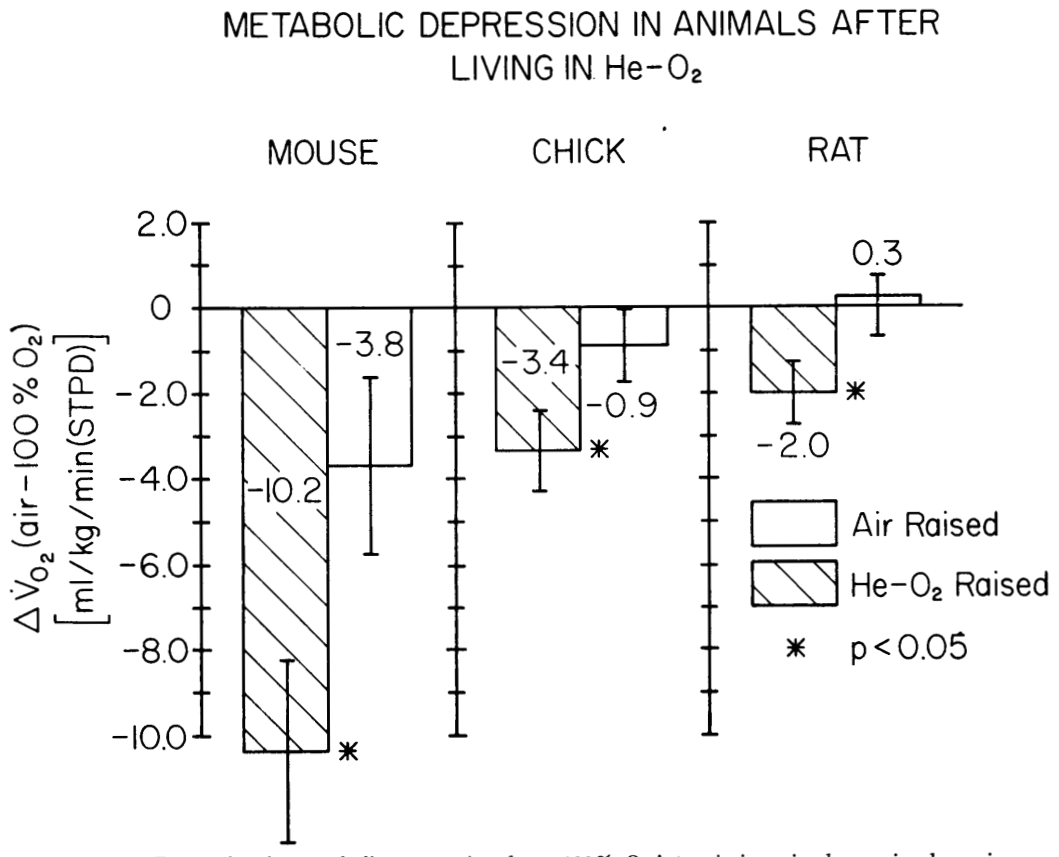

FIG. 1. Depression in metabolism on going from 100% O_2 into air in animals previously maintained in a He-O_2 environment for 10 days (mice and rats) or a 21-22 day incubation period (chicks).

the measurement sequence, it is difficult to see how this could explain the difference between the simultaneously exposed He-raised and air-raised groups. It would be necessary to speculate that the fall in $\dot{V}_{O_2}$ with time, both absolutely and relatively, is much greater in the He animals than the controls, and this would still require attributing some unusual persistent effects to the extended exposure to He-O_2. Furthermore, when the 40-minute measurement periods for experimentals and controls in either 100% O_2 or air were examined for difference in rate of change of $\dot{V}_{O_2}$ with time, none were found.

It does not seem likely that the short exposure to 100% O_2 was responsible for the metabolic difference observed in the last step of the gas sequence. Froese(7) and others(8) have shown that breathing pure O_2 for 1-3 hours does not alter O_2 uptake in rats and mice, and this seems pretty well confirmed by the small changes which were observed in the controls between air and 100% O_2 (Table I). Since experimentals and controls were exposed to 100% O_2 simultaneously, it would be necessary to postulate that the animals which had lived in He-O_2 reacted differently to 100% O_2 than those in air. However, there was no difference between experimentals and controls while in 100% O_2, leaving only the remote possibility that 100% O_2 interacted uniquely with the He animals to set the stage for a subsequent depression in metabolism upon tranfer to air.

The 100% O_2 step was used in part to wash out residual inert gas from both experimentals and controls in order to reduce the possibility of artifacts from different solubilities and diffusabilities. Conceivably the 15 minutes of flush failed to accomplish washout, leaving different amounts of He and N_2 dissolved in the animal times. Theoretically then, in the last air step He could continue to evolve and might show up as a decrease in O_2 uptake. However, even the maximum volumes of dissolved gas seem far too small to explain the observed differences. For example, the total volume of He which might dissolve in a 160 g rat, assuming it is approximately 60% water, and using a solubility coefficient for He in water of 0.009, would be less than 1 ml, whereas the decrease in $\dot{V}_{O_2}$ during the 40 minutes of measurement in air was of the order of 10 ml per rat.

In a similar vein, if for some unexplained reason, more O_2 dissolved in the tissue of the He animals while they were in 100% O_2, and/or more O_2 was released from their tissues in the air step, it could result in an artifactual indication of a greater fall in $\dot{V}_{O_2}$ between the 100% O_2 and air steps. On theoretical grounds this would seem unlikely, since Froese(7) has indicated that any excess O_2 evolved would be matched by N_2 dissolved. On practical grounds, the maximum volume involved seems far too small to account for the observed differences. For example, in the mouse, the maximum O_2 which might be dissolved or released is well below 1 ml (using a solubility K of 0.023) whereas the decrease in $\dot{V}_{O_2}$ over the controls is greater than 6 ml during the 40 min of the measurements.

In view of these considerations, we are inclined to the idea that the decrease in metabolism in the experimentals on first exposure to air was due to an increased sensitivity to the depressant action of N_2 which developed during the period of time that they lived in its absence.

Little information is available on the period of denitrogenation required for this apparent increased sensitivity to N_2 to develop, or for the onset and duration of the depression. In a limited series of exposures lasting from 2 to 48 hours, a consistent depression in the air step of 2-4% was seen, but this was far too small for statistical significance. A comparison between the first and last 5-min interval measurement of O_2 uptake showed the depression to be essentially unchanged between 15 and 55 minutes after first introduction to air.

The approximate 10% depression in metabolism found in this study is probably too low to produce subjective effects and probably too small to be shown objectively without

special technical precautions. This may account for the lack of reports of signs or symptoms in man or animals upon return to sea level following extended periods in 100% oxygen at reduced pressures or He-O_2 mixtures(2,6,11,14,15), assuming that denitrogenation *via* 100% O_2 or He-O_2 produces the same effect. Nevertheless, it remains a matter of some interest that N_2 may exert any depressant effect at all at sea level pressures. If in fact we are dealing with an aspect of N_2 narcosis, the increased sensitivity to N_2 should show up much more dramatically, as the observation of Bond(3) would imply, if the first exposure to N_2 were to take place at partial pressures higher than those at sea level.

Summary. Mice, rats and chicks were maintained in a 79% He-21% O_2 environment for 10-22 days. When measured in He-O_2, their metabolism was higher than that of controls maintained and measured in air. On transfer to 100% O_2, metabolism of the controls remained relatively unchanged, but that of experimentals fell to control levels. On first exposure to air, the animals maintained in He-O_2 showed a significant 7-11% depression in O_2 consumption below controls. The suggestion is made that the period of denitrogenation preceding the measurements may have sensitized the He-O_2 animals to the depressant properties of N_2.

We thank Susie Kreglow and Joe Pitt for technical assistance.

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Received August 25, 1966. P.S.E.B.M., 1967, v124.

Influence of Growth Hormone, Steroids and Relaxin on Acid Phosphatase Activity of Connective Tissue. (31695)

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The pubic joint of the mouse offers an interesting model for the study of hormonal influences upon connective tissue. At this site cartilage may be transformed to ligamentous connective tissue under the influence of estrogens and relaxin(1, for review). This process, which is accompanied by "depolymerization" of acid mucopolysaccharides of the ground substance(2) and erosion of pubic bone by osteoclasts(3) has been termed "pubic symphyseal relaxation". The process is of interest not only because it represents a reversion from an adult to a more embryonic form of connective tissue but because many hormones have been implicated in the reversible transformations which occur. Thus,