Protein Metabolism During Exposure to Hyperbaric Oxygen[1] (34204)

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The mechanism of toxicity due to O_2 under high pressure (OHP) may be either destruction of enzymes by direct oxidation, or destruction by reaction with oxidizing free radicals formed by O_2 at high pressure or possibly both of these (1). These enzymes include those with essential –SH groups (2), those involved in the oxidation of glucose (3, 4) and others (5). Energy-linked pyridine nucleotide reduction is inhibited at high pressure (6), although this does not occur at 3 atm (7). In any event, inhibition of energy transfer (8) and decline in ATP levels in tissue (9) have been reported. The possibility that protein synthesis might be affected by OHP is, therefore, to be expected, but until recently this matter has been given little attention. Ford *et al.* (10) observed reduction in incorporation of lysine-^{3}H into protein in tissues of rats exposed to 3 atm of O_2. Mice have been studied after exposure to 3 atm of an air–O_2 mixture containing 27% O_2 (11). Incorporation of ^{14}C-lycine into protein was investigated.

Materials and Methods. A compression chamber of 35 ft^2 capacity was available for this study. A mixture of 27% O_2 was achieved by first introducing compressed air, then O_2, to achieve a pressure of 45 lb, above atmospheric pressure. A pan with KOH pellets was used to control CO_2 levels and silica gel dehumidifier to absorb moisture.

In routine use, mice were placed individually in plastic cages 5 $\times$ 7 $\times$ 2.5 in. containing sawdust with 40 g of food pellets (Purina rat chow). The water bottle contained approximately 1/5 of its capacity of water. Compression was maintained for 72 hr and the decompression period was 6 hr. During the compression period, the animals lost 3–6 g in body weight.

The conditions of compression were the most severe which would result in virtually no mortality during the period or the ensuing recovery period of 4 days. With increase in concentration above 27% O_2, mortality increases rapidly. Control animals were likewise maintained in individual boxes outside of the chamber.

During the experiment 1 μCi of ^{14}C-lysine (241 mCi/mmole) in 0.1 ml of saline was injected intraperitoneally into control and experimental mice. The latter were compressed immediately. In some cases the animals were compressed, then after 24 hr rapidly decompressed, injected, and recompressed immediately; in other cases, this was done after 48 hr. No obvious harm resulted from the treatment. Control animals were injected at the same time.

After completion of the 72-hr compression period, the mice were rapidly decompressed and killed by cervical fracture. The pelt, head, paws, and tail were removed and discarded and the remaining carcass was homogenized with 10% ice-cold trichloroacetic acid in a Virtis homogenizer. The homogenate was centrifuged and the residue was treated again in this manner, then extracted with acetone, ether, and dried. The protein preparation was dissolved in 50 ml of concentrated formic acid in an autoclave at 14 lb pressure for 1.05 hr. The protein solution was then used to measure total nitrogen content by the Kjeldahl procedure and ^{14}C was measured in a gas flow counter.

Results. Fifty experimental animals of mean starting weight of 31.2 g lost an average of 5.1 g (16.3%) of body weight during

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TABLE I. Total cpm in Carcasses of Experimental and Control Animals.

Injection time[a] (hr)	n	Controls	n	Exptl.
24	21	151,500 ± 5430	21	151,130 ± 8680
48	15	125,060 ± 6930	13	130,820 ± 8390
72	24	104,980 ± 4700	23	112,160 ± 4630

[a] Prior to completion of 72-hr compression period.

the compression period. A regression was derived correlating body weight for normal mice with carcass nitrogen determined as described above: mg of nitrogen $= 0.9906$ (body wt)$^{1.754819}$ ($r = .81$, $p < .001$). With the use of this equation, it was possible to determine the nitrogen content of each mouse from the weight at the initiation of compression. At the end of the compression period, the protein preparation was obtained as described above and the nitrogen content was measured. Thus by comparing the value calculated from the weight and the observed value, any change in nitrogen content resulting from the exposure could be determined for each mouse. It was observed that a mean loss of 12.5% in carcass nitrogen resulted among 56 mice during the 72-hr exposure period.

The total cpm in the carcass protein of experimental and control animals is shown in Table I. The experimental values for mice which were injected 24 hr before completion of the 72-hr compression period were no different from the controls. Those injected at 48 hr before completion of compression exhibited 4.5% greater values in total cpm. Those injected at the initiation of the compression period had 6.9% more cpm.

The specific activities of the protein are shown in Table II.

The sp act values were 1.10, 1.13, and 1.14, respectively, times greater than the controls of each group.

It was determined that mice consumed an average of 6 g of food during the 72-hr compression period. Noncompressed mice of similar starting weights lost 14.1% body weight and 13.9% carcass nitrogen after they were fed 2 g of food/day for 3 days.

In order to achieve greater understanding of the findings, the following experiments were carried out with noncompressed mice: (i) A group of mice were injected with 1 μCi of ^{14}C-lysine, were given 2 g of food daily for 3 days and killed 72 hr after injection. (ii) A group was injected and starved for the duration of this period. The results are shown in Table III.

Discussion. Since energy metabolism is affected by OHP, it is pertinent to inquire into the possibility that effects on protein metabolism may result. Loss of weight occurs and nitrogen loss is to be expected. In the present experiments, 12.5% loss of carcass nitrogen resulted during 72 hr of compression under the stated conditions. This was determined by estimating carcass nitrogen prior to compression using the regression equation. This loss could have resulted from: (a) impaired synthesis resulting from reduced energy sources or some effect on enzymes and organelles involved in protein synthesis, (b) increased breakdown, e.g., resulting from re-

TABLE II. Specific Activities of Protein in Experimental and Control Animals.

Injection time (hr)	n	Controls	n	Exptl.
24	20	360.7 ± 18.3	21	398.6 ± 22.2
48	15	310.1 ± 19.3	13	351.7 ± 21.4
72	24	288.3 ± 12.4	23	328.0[a] ± 14.7

[a] Significant difference.

TABLE III. Total cpm in Carcass and sp act in Underfed and Starved Mice.

Group	No.	Treatment	(cpm)	(sp act)
1	11	Injected, 2 g of food daily for 3 days	116,777 ± 2041	345.9 ± 8.8
2	10	Starved	116,955 ± 5785	429.3 ±39.1

lease of enzymes from damaged lysosomes (12), although it has been claimed that O_2 in sufficient concentration may prevent disruption of lysosomal membranes which would prevent leakage of enzymes into cytoplasmic compartments (13), or (c) it may merely reflect decreased food intake from reduction in appetite due to general malaise which is part of the OHP syndrome.

The greater sp act values in the experimental animals are subject to three possible interpretations: (a) decreased turnover rate, which is absurd in the face of large losses in nitrogen, (b) nonrandomized breakdown in protein, *i.e.*, "older" protein is preferentially broken down relatively to the young, more highly labelled protein, for which there is no known basis, or (c) breakdown with more efficient reutilization in the starved animals. The last possibility seems to be the most reasonable.

The data of Table III reveal that the findings of underfed animals did not differ significantly from experimental animals injected at the initiation of the compression (difference of 4617 cpm, sp act difference of 17.9, neither significant). In the starved animals, cpm were 4795 greater and sp act 101.3 greater. The last difference was significant ($p<.05$). The findings suggest that increase in sp act under these circumstances may be correlated with degree of nitrogen lost.

Thus, in resolving the question whether there occurs a specific effect of OHP on protein metabolism, or whether the findings result from reduced food intake, the data suggest that the latter is the case. This differs from the results of Ford *et al.* (10). However, these investigators used 3 atm of O_2 over periods no longer than 60 min, whereas in the present study, O_2 tension in the chamber was approximately 1 atm for 72 hr. These workers studied tissues which were discarded in this experiment, *i.e.*, brain as well as adrenals, cardiac muscle, liver, and thyroid. No specific pattern of change was noted in skeletal muscle, whereas the bulk of the labeled protein in the present experiment was muscular protein. Young (14) observed inhibition of protein synthesis in *Pseudomonas saccharophila* under high O_2 tension. This investigator proposed that high O_2 tension interfers with the transfer process of nutrients and that this is the primary site of damage by OHP in bacteria. If the situation were similar in the mouse, one would expect reduced rather than increased reutilization of radioactive components.

Summary. Mice were exposed to compression with 3 atm (rel.) of an air O_2 mixture containing 27% O_2 for 72 hr. They were injected with 1 μCi of ^{14}C-lysine at the initiation, 24 and 48 hr after compression was started. The cpm and sp act values of the protein of the carcass were determined. The sp act values were 10, 13, and 14% greater than that of the controls in the three groups. This appeared to be related to reduced food intake rather than any specific effect on nitrogen metabolism.

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