Hyperoxia: Influence of Food Deprivation on Protein Synthesis by Lung (37375)

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In vivo exposure of rats to >98% O_2 at 1 atm for 24 or 48 hr decreases *in vitro* protein synthesis as measured by the incorporation of radioactive leucine into protein (1). In addition, rats exposed to oxygen eat less than rats exposed to compressed air, and lose weight. Since food deprivation alone decreases *in vitro* protein synthesis by lung tissue (2), it is possible that the decreased amino acid incorporation into protein by lung slices from rats exposed to hyperoxia is the result of food deprivation alone rather than to some other effect of oxygen exposure. To examine this possibility, I measured the influence of food deprivation on the *in vitro* incorporation of radioactive leucine into protein by lung slices from rats exposed to oxygen or compressed air.

Materials and Methods. I used Dublin-Sprague–Dawley derived rats (Flow Research Animals, Inc., Dublin, VA). They were allowed water *ad libitum* but were deprived of food for the 48 hr during which they were exposed to oxygen or compressed air. The cages were designed to exclude the possibility of coprophagia. For exposure to oxygen they were maintained in plastic cages and supplied with humidified oxygen from a wall outlet at 10 liters/min. The O_2 and CO_2 concentrations were measured (1) and were never found to be lower than 98% or higher than 0.4%, respectively. Control rats were kept in identical cages which were supplied by humidified compressed air from a wall outlet at 10 liters/min. The rats were sacrificed by exsanguination following the intraperitoneal injection of sodium pentobarbital (30 mg/kg).

Incubation of lung slices, assay for radioactivity and measurements of protein were performed as previously described (2) except that lungs were sliced at 1 mm thickness using a McIlwain tissue chopper (Brinkman Instruments Inc., Westbury, NY) and incubated in Waymouth medium (Grand Island Biological Co., Grand Island, NY). DNA was measured on the hot TCA-soluble material (2) using calf thymus DNA (Mann

TABLE I. Influence of Hyperoxia and Fasting on the Chemical Composition of Lung and Body Weight of Rats.[a]

Parameter	Air	Oxygen	p
DNA (mg/100 mg dry tissue)	4.1 ± 0.2 (5)	3.0 ± 0.2 (5)	<.01
Protein (mg/100 mg dry tissue)	55.8 ± 2.3 (5)	48.5 ± 2.8 (5)	<.05
Leucine (nmoles/100 mg dry tissue)	25.7 ± 4.4 (4)	28.1 ± 1.9 (3)	.50
Animal wt (g)			
Initial	269 ± 6 (5)	279 ± 3 (6)	>.1
Final	233 ± 5 (5)	240 ± 3 (6)	>.1

[a] The values in parentheses indicate the number of animals. Mean ± SEM are given.

TABLE II. Influence of *in Vivo* Hyperoxia on Leucine-^{14}C Incorporation into Protein by Lung Slices from Fasted Rats.[a]

cpm/mg Protein			cpm/mg DNA		
Air	O_2	p	Air	O_2	p
798 ± 62 (5)	517 ± 60 (6)	<.001	8460 ± 740 (5)	5607 ± 747 (6)	<.05

[a] In each experiment three replicate flasks each containing 100 mg of sliced lung tissue, 0.01 ml of L-leucine-U-^{14}C (9.5 × 10^{-4} μmoles; sp act 291 μCi/μmole) and 2.5 ml of Waymouth medium were incubated at 40° for 60 min. Values in parentheses indicate the number of animals. Values are means ± SEM.

Research Laboratories, Inc., New York, NY) as standard (3). Free leucine in the lung tissue was determined as previously described (2).

Results and Discussion. In the present study rats were fasted to eliminate differences in food intake noted between animals exposed to hyperoxia compared to those exposed to compressed air (1). This resulted in an equal weight loss in the two groups (Table I). There was no difference in the free leucine content of the lung tissue between the two groups. The DNA and protein content was lower in the lungs of the rats exposed to hyperoxia than in those exposed to air (Table I). The difference in DNA content is consistent with the evidence that hyperoxia inhibits lung DNA synthesis (4).

In spite of the same degree of food deprivation and weight loss, amino acid incorporation into protein by lung slices was less in rats exposed to oxygen than in rats exposed to compressed air (Table II). The differences in specific radioactivity between the two groups is similar to that noted in rats of similar size allowed food *ad libitum* during exposure to oxygen or compressed air (1). The present study, by eliminating differences in food intake and producing an equal weight loss, indicates hyperoxia decreases protein synthesis by a mechanism unrelated to food deprivation alone.

Summary. Rats which are deprived of food and exposed for 48 hr either to >98% oxygen or compressed air lose an equal amount of weight. Under these conditions the incorporation of leucine-^{14}C into protein is less in lung slices from rats exposed to hyperoxia than in lung slices from rats exposed to compressed air.

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1. Gacad, G., and Massaro, D., J. Clin. Invest. **52**, 559 (1973).

2. Gacad, G., Dickie, K., and Massaro, D., J. Appl. Physiol. **33**, 381 (1972).

3. Schneider, W. C., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. 3, p. 680. Academic Press, New York (1956).

4. Northway, W. H., Jr., Petriceks, R., and Shahinian, L., Pediatrics **50**, 67 (1972).

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