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Energy Metabolism of Rats Born and Raised in a Low Pressure Pure Oxygen Environment.* (32191)

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Little work has been done concerning energy balance in low pressure normoxic environments. Dines and Hiatt(1) reported normal growth rates, oxygen consumption and food consumption in rats maintained 24 days in pure oxygen at 196 mm Hg absolute. On the other hand, Agadzhanyan *et al*(2) reported that rats kept in an oxygen environment at 198 mm Hg absolute for 100 days lost up to 35% of their body weight the first half of the experiment. Increased growth rate during the second half, however, resulted in greater total gains than in the controls.

Carbon dioxide production of humans was reported decreased in an oxygen environment at 226 mm Hg absolute(3). Oxygen consumption of humans was also decreased in an oxygen environment at 380, 226, and 155 mm Hg absolute with mice showing a similar decrease at 170 mm Hg absolute(4). In more recent work, however, no decrease in oxygen consumption could be detected in humans breathing pure oxygen at 380 or 266 mm Hg absolute(5), or in mice breathing pure oxygen at 226 mm Hg absolute(6).

The present experiment was designed to measure food intake, growth rates, digestibility, and to obtain an estimate of the metabolic rate of rats during a 6-week period in a normoxic nitrogen free environment.

Methods. Charles River CD* strain male rats were used as experimental subjects. All experimental animals were born and maintained in the low pressure normoxic environment until sacrifice. Breeding and care of animals at altitude have been described earlier (7).

Experimental animals were maintained in an altitude chamber having an interior volume of about 300 cubic feet. The chamber was equipped with a man lock, to allow entry without a change in environment. Total pressure was automatically controlled at 210 mm Hg absolute. Humidity ranged from 40-50% with $CO_2 < 2$ mm Hg. Chamber and room temperature was maintained at 76 $\pm$ 1°F. Chamber oxygen levels were $\geqq$97% of the total dry gas. Control animals were born and maintained at ground level in the same room as the altitude chamber.

Six altitude and 6 control rats were sacrificed at 21 days of age to provide an estimate of whole body energy at start of the experiment. Ten experimental and 10 control male rats were placed individually in metabolism cages at 21 days of age and remained there for 6 weeks. The chamber was entered twice weekly, during which time the rats were transferred to clean cages, and the old cages removed for collection of feces, urine and

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TABLE I. Body Composition of 21-Day-Old Rats Born and Maintained in 100% Oxygen at a Pressure of 210 mm Hg Abs.

Variable	Control*	Std. dev. mean	Experimental*	Std. dev. mean	Sig.
Body wt, g	62.9	4.65	56.0	3.25	NS
% Dry matter whole body	34.4	.4	31.9	.9	<.05
Fat % dry matter whole body	26.0	1.5	22.7	1.7	NS
Protein % dry matter whole body	49.6	1.2	52.5	1.8	NS
Kcal./g dry body wt	6.21	.07	6.04	.09	NS

* N = 6 for all groups.

uneaten food. The animals were sacrificed at the end of the experiment. Purina laboratory chow was fed *ad libitum*.

Total energy of all rat carcasses, feed, feces, and urine was determined by bomb calorimetry. In addition, total nitrogen and total fat were determined in all rats, food, and feces. Intestinal contents were removed from all rats prior to analysis.

Nitrogen analysis was carried out on a Coleman nitrogen analyzer, and fat was determined using a chloroform ethanol extraction.

Results. All data on animals sacrificed at 21 days of age is listed in Table I and all data on animals living for 6 weeks in metabolism cages and sacrificed at 63 days of age are listed in Table II.

Carcasses of 21-day-old control rats contained 2.12 kcal. per gram of wet body tissue as compared with 1.93 for altitude rats ($P < .05$). To balance both groups isocalorically at the start of the metabolism experiment, slightly larger experimentals were selected although limited numbers did not allow perfect balance: 118.6 kcal. per animal for controls as compared with 114.3 for experimentals (Table II). These differences, however, were not significant.

The altitude exposed rats increased in weight from 61.6 g to 302.3 g between 3 and 9 weeks of age while controls increased from 55.8 to 312.5 g during the same period (Table II). An unexplained decrease in food consumption by 2 of the altitude rats resulted in a slightly decreased average weight gain during the final week. The growth curve for experimentals between 3 and 9 weeks of age was fitted to the regression line, weight in grams $= -63.9 + 41.3 \times$ age in weeks compared to $-80.4 + 44.0 \times$ age for controls. Variance of the slope Sb^2 was 1.32 for experimentals and 0.72 for controls. The slopes did not differ significantly nor did a comparison of average weekly weights.

Twenty-one day control rats contained a greater number of calories per gram on a wet weight basis, and a higher percent of dry matter. On a dry weight basis, calories per gram did not differ significantly. At 63 days of age, neither percentage of dry matter nor calories per gram showed significant differences.

Experimental rats passed more energy into the feces ($P < .05$) and digested their food less efficiently ($P < .001$), particularly in the case of protein. This was offset by a tendency toward greater food intake, resulting in only a small, insignificant difference between the two groups with respect to digestible or metabolizable energy.

No differences were found in the increase in total number of calories per animal, metabolizable energy, caloric increase per gram of metabolizable energy, or energy given off as heat.

Body content of fat and protein on a dry matter basis did not differ significantly from controls in the 21- or 63-day-old animals.

Discussion. In the past it has been assumed that nitrogen gas plays no role in the metabolic functions of animals. Since nitrogen at 2 atmospheres begins to interfere with normal function of the nervous system, the question has been raised as to whether 0.79 atmosphere of nitrogen also exerts some effects(8).

The metabolic rate of humans was reported depressed in 100% oxygen at 380, 226 and 155 mm Hg, as were mice at 170 mm Hg absolute(3,4). In more recent work, no significant differences in oxygen uptake could be detected in humans breathing pure oxygen

TABLE II. Energy Metabolism and Body Composition of Rats Born and Maintained in 100% Oxygen at a Pressure of 210 mm Hg Abs. and Kept in Metabolism Cages from 21-63 Days of Age.

Variable	Ground level control*	Std. dev. mean	Altitude experimental*	Std. dev. mean	Sig.
Wt, g 21 days	55.8	2.3	61.6	2.9	NS
Total kcal. 21 days†	118.6	3.9	114.3	5.5	NS
Wt, g 63 days	312.5	12.5	302.3	7.1	NS
kcal./g dry body wt 63 days	5.79	59.5	5.69	63.7	NS
Whole animal					
Total kcal. 63 days	581.8	22.7	550.8	20.5	NS
Whole animal					
Caloric increase 21-63 days	462.7	19.2	436.4	18.8	NS
Food intake/animal, g 21-63 days	744.6	20.3	800.5	34.2	NS
Total caloric intake/animal 21-63 days	3,067.6	99.3	3,258.6	151.9	NS
Feces kcal./animal 21-23 days	626.2	21.4	832.4	37.2	<.05
(Total energy intake-feces energy)					
Total digestible energy/animal 21-63 days	2,441.5	88.7	2,426.1	126.1	NS
Percent digestibility	79.51	.67	74.34	.86	<.001
Fat digestibility	81.4	2.8	75.6	5.3	NS
Protein digestibility	74.6	1.1	65.5	1.6	<.001
Urine kcal./animal 21-63 days	133.6	5.3	147.9	14.1	NS
(Total kcal. intake-energy of feces & urine)					
Metabolizable energy/animal 21-63 days	3,037.9	85.4	2,283.5	114.8	NS
Caloric increase/kcal. of metabolizable energy	.2013	.0070	.1964	.0138	NS
(Metabolizable energy-whole body caloric increase)					
kcal./animal given off as heat 21-63 days	1,845.2	75.1	1,847.1	123.8	NS
Whole body					
% dry matter 63 days	34.5	1.2	33.5	.4	NS
Dry wt basis					
Whole body % fat 63 days	17.5	1.7	16.3	1.5	NS
Dry wt basis					
Whole body % protein 63 days	58.3	1.1	60.7	1.2	NS

* N = 10 for all groups.
† Estimates, using kcal./g of animal sacrificed at 21 days.

at 380 or 226 mm Hg absolute(5).

In the preceding reports, metabolic rate was estimated by measuring oxygen consumption or carbon dioxide production for periods of a few minutes to several hours. In the present experiment an attempt was made to determine energy expenditures for a period of 6 weeks.

Total heat production was calculated by subtracting the energy increase of the whole animal from the metabolizable energy (energy intake minus energy in the feces and urine). The answer obtained is an overall estimate of metabolic rate since it includes not only basal heat production, but also heat production resulting from activity of the animal, and the work of digestion. Since very similar amounts of food were digested, the specific dynamic effect should differ very little between the two groups. At present, there is no evidence that activity levels differ in a low pressure normoxic environment. In as much as heat production was almost the same for both groups, it would appear unlikely, therefore, that the two groups differed in basal metabolic rate.

The most important difference found was the decreased efficiency of digestion in the altitude group ($P<.001$), particularly in the digestion of protein ($P<.001$). This decreased digestibility could be due to several causes: Changes in the absorbing capacity of the intestinal mucosa, an increased rate of passage, or a decrease in the level or activity of proteolytic enzymes in the small intestine.

Further work along these lines is needed to determine the reasons for the lowered digestibility.

Summary. Ten rats born and maintained in a low pressure, pure oxygen environment did not differ in growth rates from ground level controls. However, overall digestibility was lowered in experimentals, 74.3% *vs* 79.5% for controls ($P<.001$), with even greater differences in protein digestibility, 65.5% *vs* 74.6% ($P<.001$). Although less fat was digested by experimentals, 75.6% *vs* 81.4% for controls, these differences were not significant. Net caloric intake was similar due to a slightly greater food intake by experimentals. Calories given off as heat did not differ, suggesting similar metabolic rates.

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Connective Tissue XV. Amino Acid Composition of Soluble Collagen of Pig Uterus. (32192)

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Variations in amino acid composition of collagen, both from species to species and from tissue to tissue in a given species have been reported by a number of investigators (1-9). The importance of total pyrolidine amino acid, (*i.e.*, proline plus hydroxyproline) to the stability of the collagen structure has been discussed by Harrington *et al* (10). A possible role of lysine in the formation of intra- and inter-molecular cross linkages in collagen was reported by two independent groups(11,12). To understand alterations of collagen in pathological states, the complete elucidation of the normal composition of collagens from the various tissues is necessary. In the past few years, soluble collagen has been prepared from skin, tendon, and fish swim bladder(7,8,9) and the heat denatured products and their amino acid compositions have also been determined. Recently we reported the preparation and amino acid composition of collagen from human uterus(13). The present report describes (a) methods of preparing 1 M NaCl- and 0.5 M acetic acid-soluble collagens from pig uterus; (b) the separation of the α- and β-chains of these collagens after heat denaturation; and (c) the determination of the amino acid composition of the α- and β-chains of these pig uterine collagens.

Methods. Uteri from 6- to 12-month-old pigs, obtained from a local slaughter house, were cut into 1 inch squares and ground with dry ice in a meat grinder. All procedures were performed at 5°C. All extractions were done with a magnetic type stirrer and the extracts were cleared at 30,000 rpm in a Spinco Model L centrifuge for 1 hour before precipitation.

Preparation of 1M NaCl soluble collagen. Uterine tissue was first extracted with 1M NaCl and collagen was precipitated by the addition of solid NaCl to 20% concentration. In one instance this precipitation was