ANTIOXIDANT REQUIREMENTS OF RATS EXPOSED TO 100 PERCENT OXYGEN

II. EFFECTS OF DIETARY ASCORBIC ACID*

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Running Title: Ascorbic Acid in 100 Percent Oxygen

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If the adverse effects of elevated oxygen tensions are due to increased formation of oxidizing free radicals, as suggested by Gerschman,[1] they ought to be considerably ameliorated by increasing the antioxidant capacity of the animal by dietary or other means. There is considerable evidence of such an effect of antioxidants in animals exposed to oxygen at pressures of 1 atmosphere or more.[2-4] However, at oxygen tensions below 1 atmosphere the effects of antioxidants are not so clear cut. It has been shown previously that supplying an excess of vitamin E in the diet did not significantly improve growth rates or food efficiency ratios of rats exposed to oxygen at 600 mm Hg.[5] The vitamin A status of the animal also appeared to be unaffected by oxygen, even though vitamin A is one of the most readily oxidized vitamins. Nevertheless, *in vitro* peroxidative swelling studies on kidney lysosomes indicated that the antioxidant reserves of exposed rats may be lower than those of their pair-fed controls. In addition, blood ascorbic acid was significantly depressed in oxygen-exposed rats relative to controls, suggesting that aqueous phase compounds with antioxidant or synergistic properties may be important to the oxygen-exposed animal.

Although ascorbic acid may act synergistically with α-tocopherol to increase its antioxidant capacity,[6] it also has a significant prooxidant role in many systems. Ascorbic acid has been frequently used alone or with ferrous ion to initiate *in vitro* peroxidative swelling in mitochondria and microsomes.[7] Similarly, homogenates from certain tissues of scorbutic guinea pigs produce fewer peroxides *in vitro* than those of normal animals, and the addition of ascorbic acid *in vitro* to normal tissues increases peroxidation.[8]

Although ascorbic acid is normally synthesized by the rat, it is possible that synthesis is inhibited in rats exposed to oxygen, particularly if their antioxidant reserves are low. Kitabchi et al.[9] and McCay[10] found complete inhibition of oxidation of gulonolacetone to ascorbic acid by liver microsomes from tocopherol deficient rats and rabbits. Inhibition was accompanied by the production of a substance which reacted with 2-thiobarbituric acid to form a red pigment, and was prevented by the addition of tocopherol, EDTA, or certain cations (Mn^{++}, Co^{++}). Stirpe et al.[11,12] also found that ascorbic acid synthesis by rat liver in vitro was inhibited by prior X-irradiation, starvation, or carbohydrate deprivation of the animal.

The possible pro-oxidant activity of ascorbic acid should not be overlooked in considering the ascorbic acid requirements of animals exposed to 100 percent oxygen. Gerschman et al.[13] found that the protective effect of substances generally considered to have antioxidant activity varied markedly with oxygen pressure, being detrimental at some pressures while protective at others.

In the present experiments oxygen-exposed and air control rats were fed diets containing various levels of ascorbic acid, in some cases in the absence of tocopherol, to determine any beneficial or detrimental effects of increased dietary ascorbate on the animal's adaptation to oxygen at a pressure of 600 mm Hg.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin, 5,5' dithiobis (2-nitrobenzoic acid) (DTNB), L-ascorbic acid, and reduced glutathione were obtained from Sigma Chemical

Company. 2,4 Dinitrophenyl hydrazine and spectroquality heptane were obtained from Matheson, Coleman, and Bell. All trans retinyl acetate was obtained from Distillation Products Industries. All other chemicals were of reagent grade and all solutions were made with deionized distilled water.

Experimental animals, exposure system, and diets

Male Sprague-Dawley rats, weighing about 80 to 100 grams, were divided into four groups and fed the experimental diets 2 to 5 days before beginning exposure. The rats in each group were paired by body weight, one member of each pair to be exposed to oxygen, the other to serve as a pair-fed air control. Details of oxygen exposure, pair-feeding, weight and food intake records, and basal diet composition have been given previously.[5] All diets in the present experiments contained 2000 IU vitamin A/kg. α-tocopheryl acetate and ascorbic acid were added to the basal diet in the quantities indicated in Table 1. In each of the present experiments an additional group of 6 rats was fed the complete diet (Diet IV) ad libitum, and housed in metabolism cages, exposed to air.

Analytical methods

After 29 to 30 days exposure the rats were returned to ambient pressure, anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital (nembutal), and sacrificed. Blood was taken into a heparinized tube from the jugular vein following decapitation. A sample was removed immediately for determination of reduced glutathione, and stabilized by addition of precipitating reagent. A second sample was centrifuged at 2400 rpm for 20 minutes to obtain plasma for vitamin A determinations. The remainder was frozen for later analysis of total

ascorbic acid. The liver was perfused with ice-cold isotonic saline through the portal vein, weighed, minced. A 1-1.2 gram portion was weighed, frozen immediately in liquid nitrogen, and kept in liquid nitrogen until analysis of liver sulfhydryl groups could be performed (3-4 hours). Additional portions for liver catalase and vitamin A determinations were weighed, frozen in liquid nitrogen, and stored at -20°C.

Liver non-protein and total sulfhydryl groups were estimated using the reaction of -SH with DTNB at pH 6.8 and pH 7.6.[14] In the present experiments liver samples were thoroughly homogenized in phosphate-saline and made to a final volume equivalent to a 3-4 percent homogenate. So that the determinations could be done rapidly enough to minimize loss of labile sulfhydryl groups, the whole homogenate was used in the determination immediately after preparation without centrifuging. Turbidity due to the homogenate was determined in a separate sample. μ Moles of sulfhydryl groups per sample were calculated using the extinction of a glutathione standard at each pH. Values for liver sulfhydryl groups were expressed as μmoles -SH per 100 mg protein to account for difference in liver composition and degree of perfusion.

Determinations of blood reduced glutathione,[15] blood total ascorbic acid,[16] liver and plasma vitamin A,[17] liver catalase,[18] and protein,[19] and statistical analysis of the data were carried out with the modifications previously described.[5]

RESULTS

In contrast to previous work[5] analysis of variance showed no significant difference between the oxygen-exposed and pair-fed air control

rats in the average mean weights of the animals for the experimental period (calculated by summing the weights recorded for each individual animal and dividing by the number of weighings). In the present experiments a modification of the food cup used for the oxygen-exposed animals allowed more accurate measure of food consumed, and may account for the difference from results of previous work. However, in confirmation of previous results, all diets not containing both vitamins E and C showed a significant group-time interaction; i.e., the weight versus time curves of oxygen-exposed and pair-fed air control rats diverged, the oxygen-exposed animals showing the smaller gains ($p < 0.05$). When the diet contained both vitamins E and C there was no significant group-time interaction.

In contrast to the pair-fed controls, controls fed diet EC or 1500 C ad libitum showed a highly significant group difference in mean weight and in group-time interaction when compared to oxygen-exposed rats fed the same diets ($p < 0.005$). Figure 1 summarizes the average weight and food intake data for oxygen-exposed rats and pair-fed and ad libitum controls fed diet 1500 C. The oxygen-exposed rats showed a depression in food intake between 4 and 9 days exposure, which is accompanied by a weight plateau in both the oxygen-exposed and pair-fed control groups. The ad libitum fed controls showed no depression in food intake and no weight plateau. Their superior weight gain appeared to be due mainly to their consistently elevated food intake.

Figure 2 summarizes the total weight gain, food intake, and food efficiency ratios obtained on the various diets in the two experiments. There was no significant difference in total food intake among the oxygen-exposed groups in either experiment. In the first experiment the oxygen-exposed rats showed significantly lower food efficiency ratios than their

pair-fed air controls, and there was no significant improvement of the FER of the exposed rats by addition to the diet of either ascorbic acid (250 mg/kg) or vitamin E (500 mg/kg) or both. In the second experiment the average FER's of oxygen-exposed rats were lower than those of pair-fed air controls on all the diets, but the difference was significant only when the diet lacked ascorbic acid. However, comparison of the FER's of oxygen-exposed rats indicates that ascorbic acid tended to improve the FER when fed at a level of 750 mg/kg diet, but when fed at 250 mg/kg caused no improvement and at 1500 mg/kg tended to lower the FER. In both experiments the ad libitum fed controls exhibited higher gains, higher total food intakes, and higher food efficiency ratios than the oxygen-exposed rats fed the same diet.

Table 2 shows the liver and plasma vitamin A levels, blood reduced glutathione, and total ascorbic acid values found in oxygen-exposed and air control rats in these experiments. In agreement with previous work there was no significant difference between the exposed animals and their pair-fed controls in the amount of vitamin A found in the liver. Ad libitum fed controls also showed about the same amount of liver storage. There was no significant difference in plasma vitamin A levels in the first experiment, but all the exposed rats fed diets containing ascorbic acid in the second experiment had lower plasma vitamin A levels than the air controls, equivalent to a 10 to 20 percent depression.

There was no significant difference between oxygen-exposed and pair-fed control rats in blood glutathione levels. However, in agreement with the results of Brooksby et al.[20] in both experiments glutathione levels were significantly higher in the ad libitum fed control rats when compared to oxygen-exposed rats fed the same diets. In contrast

blood ascorbic acid levels were significantly lower in the oxygen-exposed rats in the first experiment compared to pair-fed or ad libitum fed air controls. In the second experiment oxygen-exposed rats fed the -C or 250 C diets continued to show a depressed blood ascorbate level, although the difference was not significant. Increasing the dietary ascorbic acid level to 750 mg/kg significantly increased the blood ascorbic acid in the oxygen-exposed animals ($p < 0.05$).

The catalase activity found in the livers of oxygen-exposed and air control rats is shown in Table 2. Only the oxygen-exposed rats fed the 750 C diet showed a significant increase in liver catalase activity compared to their pair-fed air controls. Although the exposed rats consistently tended to have slightly higher average catalase activities than their pair-fed controls, this response was not always the same from one experiment to another, even though the same diets were used. In addition the ad libitum fed controls in both experiments showed a slightly higher catalase activity than did exposed rats fed the same diet. Thus there is no consistent evidence that catalase levels are increased on prolonged exposure to oxygen.

The results of the liver sulfhydryl groups analysis are presented in Table 3. There was very little difference between the oxygen-exposed rats and the controls in either non-protein or protein sulfhydryl groups. It is interesting that increasing the ascorbic acid in the diet to 750 mg/kg in the second experiment caused a significant increase in non-protein sulfhydryl groups in the exposed rats, compared to exposed rats fed either no ascorbate or 250 mg/kg ($p < 0.05$). The non-protein sulfhydryl group concentration in the exposed rats fed the 1500 C diet was even somewhat higher than that of the rats fed diet 750 C ($p < 0.10$).

These increases were also found in the pair-fed air control groups, although not in the ad libitum control group fed the 1,500 C diet.

DISCUSSION

Although the growth depression of the exposed rats compared to their pair-fed air controls was less striking than that previously observed, the exposed rats fed diets lacking either vitamin E or C still showed significantly lower gains and food efficiency ratios than did their pair-fed controls. When both vitamins E and C were supplied in the diet, the gains and food efficiency ratios of the exposed rats were no longer significantly different from pair-fed controls, although they were still significantly lower than those of air controls fed the same diet ad libitum. The results obtained here confirm those of Brooksby et al.[20] who found significantly higher gains, food intake, and FER's in ad libitum fed control rats, but not in pair-fed control rats compared to oxygen-exposed rats fed a commercial chow diet.[21] Presumably the lower food intake of the exposed rats caused the decreased FER since the animals were forced to use a greater proportion of the food consumed for energy, leaving less to increase body weight.

Actually, adding ascorbic acid to the diet did not significantly improve the gain or food efficiency ratios of rats exposed to oxygen. But it is clear that any possibly beneficial effect of ascorbate occurred at a specific concentration. Of the three levels used 250 mg/kg had no effect, 750 mg/kg produced a noticeable increase in gain and FER, and 1500 mg/kg actually decreased gain and FER in comparison with the diet lacking ascorbate, perhaps reflecting a net pro-oxidant effect of ascorbic acid at this concentration. Similarly the dietary ascorbic

acid had to be increased to 750 mg/kg before there was a significant rise in the blood ascorbic acid of the oxygen-exposed rats, but raising it to 1500 mg/kg caused no further significant increase.

The depression in blood ascorbic acid observed in the present experiments in exposed rats compared to their pair-fed air controls is in agreement with that observed previously.[5] It does not seem likely that the lower blood ascorbate is due to inhibition of microsomal ascorbic acid synthesis by lipid peroxidation as observed by Kitabchi et al.[9] since there is no significant difference between the ascorbic acid level of exposed rats fed diet -E-C and those fed E-C. Decreased synthesis due to starvation or carbohydrate deprivation as noted by Stirpe et al.[11,12] would be expected to occur in the pair-fed controls as well as in the oxygen-exposed rats, and thus could not account for the depression observed here. Further study of in vitro ascorbic acid synthesis by livers of exposed and pair-fed control rats in a later experiment[22] supports the conclusion that ascorbic acid synthesis is not limiting in animals exposed to oxygen at this pressure. It is yet possible that the decrease observed may be due to increased oxidative breakdown of the ascorbic acid. However, the method of ascorbic acid determination used in these experiments estimates the total concentration of ascorbic, dehydro-ascorbic, and diketogulonic acids, so the ascorbic acid must be actually broken down to oxalic and threonic acids, or CO_2 before it escapes detection. Finally, the depression in blood ascorbate may be due to stress in the oxygen-exposed animal. Brooksby et al.[20] found a significant difference in relative adrenal and thymus weights in rats exposed to oxygen at 600 mm Hg compared to air controls, indicating a stress response in the exposed animals. A decrease in plasma ascorbic acid

levels during periods of stress has been observed by others.[23] In any case the present experiments have shown that increasing the level of ascorbic acid in the diet can significantly increase blood ascorbate levels in exposed rats, but the level at which the ascorbate is supplied may determine whether the net effect is beneficial or detrimental.

As observed previously the vitamin A status of the rats is relatively unaffected by exposure to oxygen at 600 mm Hg.[5] Liver vitamin A of exposed rats showed no significant difference from either pair-fed or ad libitum fed control values. When the exposed rats were fed diets lacking ascorbic acid, plasma vitamin A was also relatively unaffected by oxygen exposure. However, on the diets containing ascorbic acid in the second experiment there was a noticeable decrease in plasma vitamin A in the exposed rats compared to air controls. It is possible that the decrease was caused by peroxidation of the plasma vitamin A catalyzed by ascorbic acid and traces of plasma iron or copper. However, the most significant decreases was noted in rats receiving the lowest amount of ascorbate (250 C) and was not observed in the groups fed diets -EC and EC in the first experiment. In general, there is no correlation between plasma vitamin A and vitamin A deficiency, since plasma A levels may be maintained after liver stores are depleted and plasma vitamin A levels may be altered by certain hormones.[24]

The particular lability of sulfhydryl groups to destruction by oxygen and by lipid peroxidation has been well documented.[25-29] Many workers have shown that both enzymes containing essential sulfhydryl groups and non-protein sulfhydryl compounds are oxidized and inhibited by oxygen at high pressure[25-28] and by peroxidizing lipid.[29] In many cases oxygen has been shown to cause a net decrease in total tissue

sulfhydryl groups.[14,30,31] However, in the present experiments no consistent difference was observed in liver non-protein or protein sulfhydryl groups between oxygen-exposed and pair-fed control rats. The apparently significant depressions seen in the first experiment could not be duplicated in the second experiment. We cannot say with certainty that there was no depression in the level of sulfhydryl groups in livers of the animals during exposure to oxygen at this pressure. In the present experiment the rats were returned to air before being sacrificed, and if a higher proportion of glutathione were present in the oxidized form under conditions of oxygen exposure, it could have been reduced at this time by glutathione reductase. In addition, preliminary experiments indicated that the error involved in estimates of -SH groups in duplicate samples of the same liver by the method described was about 5 percent. This error is probably reflected in the group standard deviations obtained and thus no smaller decrease in -SH group concentration than 10 percent is likely to be detected. The decrease in total -SH observed by Jamieson et al.[30] in lungs of rats exposed to OHP amounted to about 35 percent. However, if the rats were removed from oxygen before macroscopic lung damage had occurred, the decrease in -SH groups was only 10 percent. From the present experiments we may conclude that no permanent decrease in liver sulfhydryl groups greater than 10 percent is caused by chronic exposure to oxygen at 600 mm Hg, even when there is a relative deficiency of tocopherol in the diet. However, it is possible a selective oxidation may occur in essential enzyme or cofactor -SH groups which are particularly labile due to factors such as steric configuration or location in the cell.

Dietary ascorbic acid appeared to have some effect on liver non-protein sulfhydryl groups. The exposed rats fed either 750 or 1500 mg/kg

showed a significantly higher concentration of non-protein sulfhydryl groups in the liver than exposed rats fed diets -C or 250 C. The increased supply of non-protein sulfhydryl groups may represent an adaptive response since dehydroascorbic acid is reduced by glutathione *in vivo* and increased sulfhydryl compounds may also be required to inhibit peroxidative processes which may be catalyzed by pro-oxidant levels of ascorbic acid. The increase is also observed in pair-fed controls so that the effect is apparently not precipitated by oxygen exposure *per se*.

Although previous work suggested a possible increase in catalase levels during oxygen exposure, the present experiments indicate no consistent significant increase in liver catalase activity of rats exposed to oxygen compared to air controls. Others[2,3] have also been unable to find any consistent change in catalase activity in the red blood cell after exposure to OHP. Catalase is known to have a very high turnover number and is probably quite capable of decomposing any extra H_2O_2 produced by the cellular oxidases associated with it without extra enzyme being synthesized. Since catalase is largely latent within the peroxisome in the liver, any H_2O_2 produced in other parts of the cell must diffuse into the peroxisome before it can be decomposed by catalase. Because of the high concentration of catalase in the peroxisome and its first-order reaction rate, the rate of diffusion of H_2O_2 molecules becomes limiting, rather than the concentration of catalase in the cell.[32] Glutathione peroxidase has been shown to be more efficient than catalase in decomposing H_2O_2 produced in small, steady amounts[33] and is probably more important to the oxygen-exposed animal than catalase for the decomposition of H_2O_2 formed outside the peroxisome.

SUMMARY

Young male Sprague-Dawley rats fed defined diets containing various combinations of vitamins E and C were continuously exposed to oxygen at 600 mm Hg for 4 weeks. Gain and food efficiency ratios of exposed rats fed diets lacking either vitamin E or C were significantly lower than those of their pair-fed controls. The addition of 750 mg ascorbate/kg to the diet containing vitamin E tended to improve gain and FER of exposed rats, while 1500 mg/kg appeared detrimental. The addition of 750 or 1500 mg ascorbic acid/kg of diet increased the blood ascorbic acid in both exposed and control rats, and decreased the difference between the oxygen-exposed rats and their pair-fed controls. There was no consistent significant difference between exposed and pair-fed control rats in concentration of liver sulfhydryl groups or blood reduced glutathione. However, ascorbic acid at 750 or 1500 mg/kg diet increased the non-protein sulfhydryl groups in livers of both exposed rats and pair-fed controls. Liver vitamin A was not affected by oxygen exposure in any of the diet groups, but in one of the experiments plasma vitamin A was depressed in exposed rats fed diets containing ascorbic acid compared to their pair-fed air controls. No consistent difference in liver catalase activity was observed in exposed rats and pair-fed controls, suggesting that other means of H_2O_2 removal may be more important for the oxygen-exposed animal.

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TABLE 1

EXPERIMENTAL DIET GROUPS AND LENGTH OF EXPOSURE TO 100 PERCENT OXYGEN AT 600 mm Hg

Experiment	Group	Diet	Vitamin E mg/kg	Vitamin C mg/kg	Length of exposure days
I	I	-E-C	0	0	30
	II	-EC	0	250	31
	III	E-C	500	0	31
	IV	EC	500	250	32
II	I	-C	500	0	29
	II	250 C	500	250	29
	III	750 C	500	750	29
	IV	1500 C	500	1500	29

TABLE 2

RESULTS OF VARIOUS BIOCHEMICAL DETERMINATIONS ON BLOOD AND LIVERS OF OXYGEN-EXPOSED AND AIR CONTROL RATS FED COMBINATIONS OF VITAMINS E AND C

Experiment	Group	Diet	Glutathione blood mg/100 ml	Ascorbate blood mg/100 ml	Plasma vitamin A IU/100 ml	Liver vitamin A IU/liver	Liver catalase u/g
I	I O_2	-E-C	36.3±2.9	0.61±.07*	189±20	424±32	4290±411
	air		34.5±1.8	.86±.05	198±19	428±33	4210±352
	II O_2	-EC	36.8±2.6[a]	.67±.05[a]**	129±11[a]	469±48[a]	3940±261[a]
	air		36.9±2.9	.88±.03	111±19[a]	447±49	3760±306
	III O_2	E-C	35.4±2.9	.65±.05*	157±13[b]	576±72	4110±380
	air		37.3±2.9	.79±.03	149±9[b]	426±38	3460±243
	IV O_2	EC	34.9±2.8	.70±.02[a]**	150±9[a]	485±21	3920±376
	air		42.4±3.4[b]	1.04±.06[b]	125±16[b]	415±47[b]	3870±441[b]
	ad lib		44.0±1.4*	1.08±.04**	156±11	443±52	4120±73
II	I O_2	-C	34.0±3.1[a]	.64±.04[a]	130±6[b]	327±26[a]	3820±351[a]
	air		36.6±2.7	.71±.06	128±4[b]	294±18	4260±302
	II O_2	250 C	34.8±3.2	.72±.07	140±6**	343±23	3040±299
	air		36.6±1.1[a]	.85±.06[a]	178±6[a]	310±43[a]	2900±310[a]
	III O_2	750 C	29.8±2.3	.99±.09	122±11[a]	314±21	4400±234*
	air		34.8±2.0	1.03±.06	150±7[b]	323±28	3300±220
	IV O_2	1500 C	31.7±1.6	1.06±.05	153±12	293±22	3670±308
	air		30.0±1.8	1.15±.03	169±9	293±25	3520±284
	ad lib		41.2±3.6*	.97±.06	179±3	273±26	3840±281

*$p < 0.05$

**$p < 0.005$

[a] 5 rats

[b] 4 rats

All values are mean ± S. E. M. of 6 rats unless otherwise noted.

TABLE 3

LIVER SULFHYDRYL GROUPS IN OXYGEN-EXPOSED AND AIR CONTROL RATS FED VARIOUS COMBINATIONS OF VITAMINS E AND C

			Liver -SH, μmoles/100 mg protein			Liver protein
Experiment	Group	Diet	Non-protein	Protein	Total	mg/g
I	I O_2	-E-C	5.2±0.7	5.9±0.8	11.1±0.8	150±7**
	air		4.8±1.1[a]	6.1±0.4[a]	10.9±1.3[a]	184±8
	II O_2	-EC	4.5±0.3[a]	5.7±0.4[a]	10.2±0.3[a]	156±8[a]
	air		5.3±0.5[a]	5.0±0.4[a]	10.3±0.3[a]	164±5
	III O_2	E-C	5.0±0.2*	6.0±0.2	11.0±0.1*	145±4
	air		6.4±0.4	6.0±0.3	12.4±0.5	155±7
	IV O_2	EC	4.7±0.4[a]	5.1±0.5[a]	9.8±0.5[a]**	149±8
	air		6.0±0.8[b]	6.3±0.5[b]	12.4±0.3[b]	160±6[b]
	ad lib		5.2±0.6	4.5±0.3	9.7±0.4	165±9
II	I O_2	-C	5.0±0.7[a]	5.6±0.6[a]	10.5±0.7[a]	164±10[a]
	air		4.8±0.5	5.8±0.3	10.6±0.8	181±1
	II O_2	250 C	5.0±0.2	5.0±0.4	10.0±0.4	164±10
	air		5.4±0.2[a]	5.3±0.3[a]	10.7±0.2[a]	179±6[a]
	III O_2	750 C	6.8±0.4	5.7±0.2	12.5±0.6	163±4
	air		6.2±0.7	5.6±0.4	11.8±0.6	161±2
	IV O_2	1500 C	7.7±0.2	5.8±0.2	13.4±0.5	144±6
	air		7.7±0.6	5.8±0.7	13.6±0.3	155±4
	ad lib		5.9±0.6*	6.1±0.4	12.1±0.0*	147±7

[a] 5 rats

[b] 4 rats

*$p < 0.025$

**$p < 0.01$

All values are mean ± standard deviation of the mean of 6 rats unless otherwise noted.

FIGURE LEGENDS

Figure 1. Growth and daily food consumption of oxygen-exposed and air-control rats fed diet 1500 C. Each point represents the average weight or food consumption of 6 animals.

Figure 2. Average total weight gain, food consumption, and food efficiency ratios of oxygen-exposed and air-control rats fed various levels of vitamins E and C.

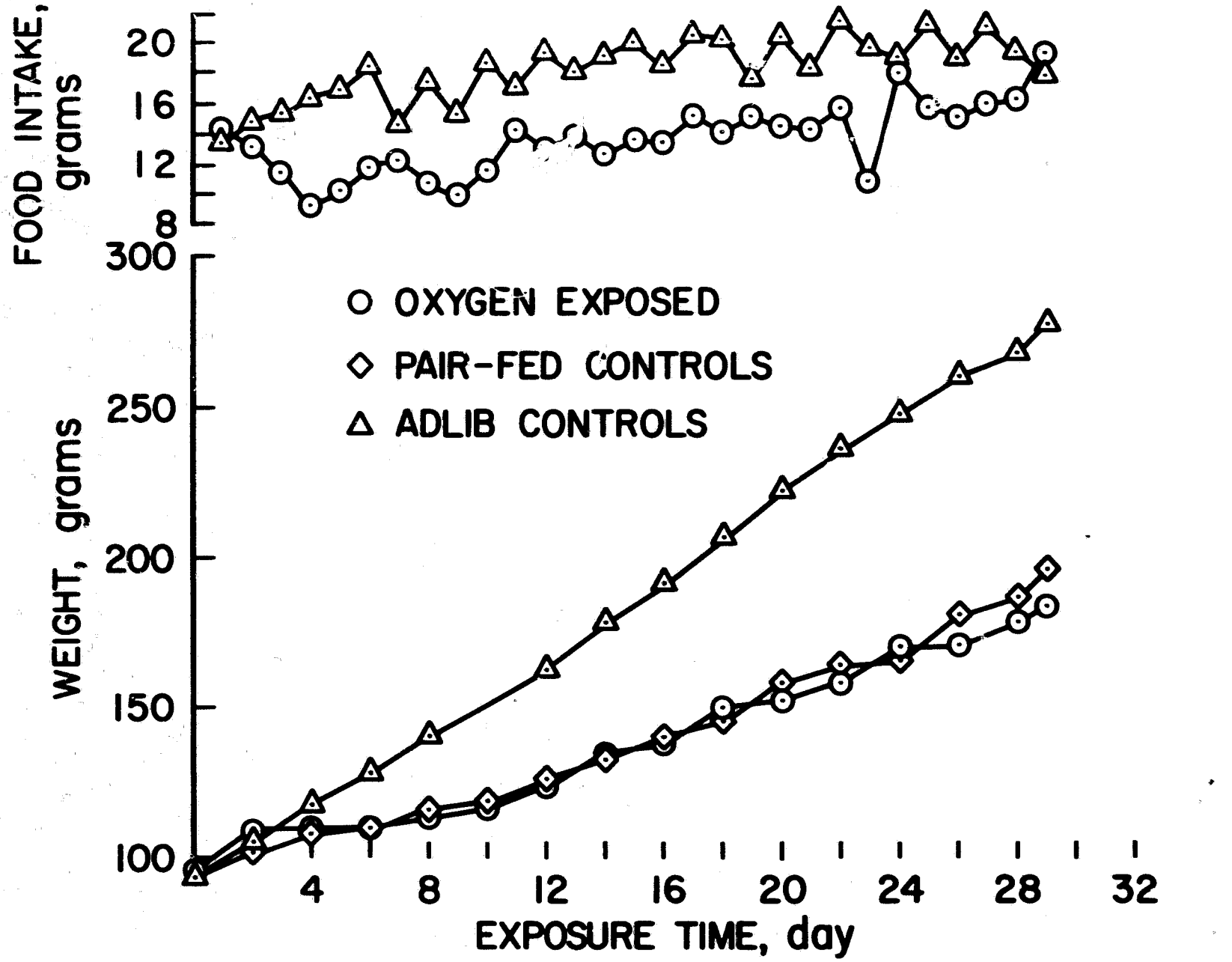

Figure 1.

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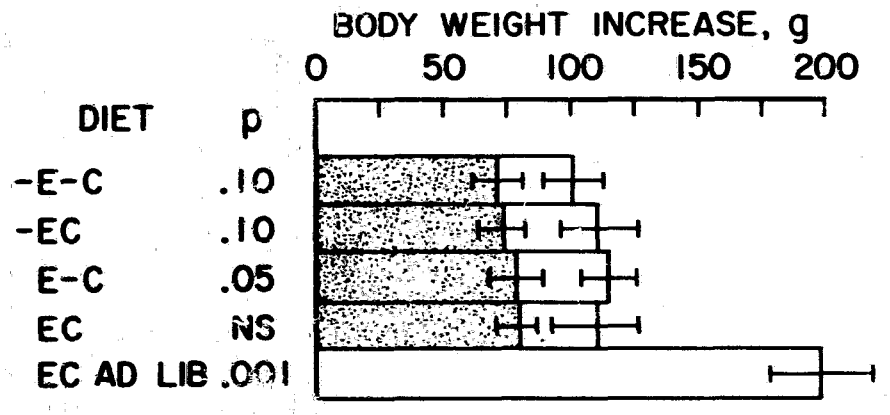

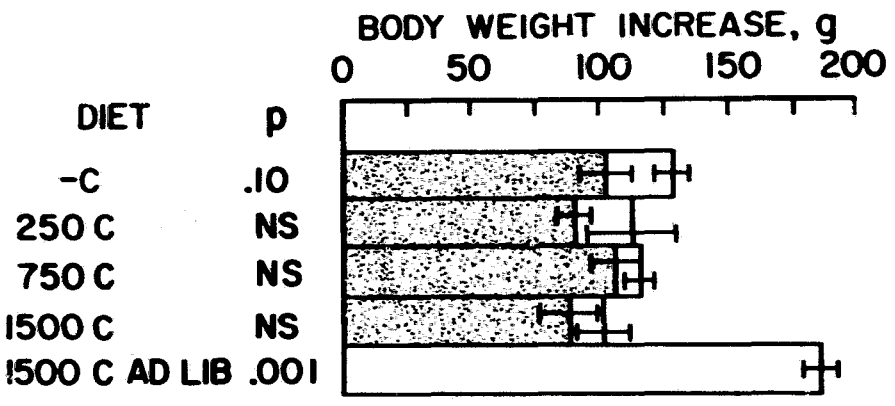

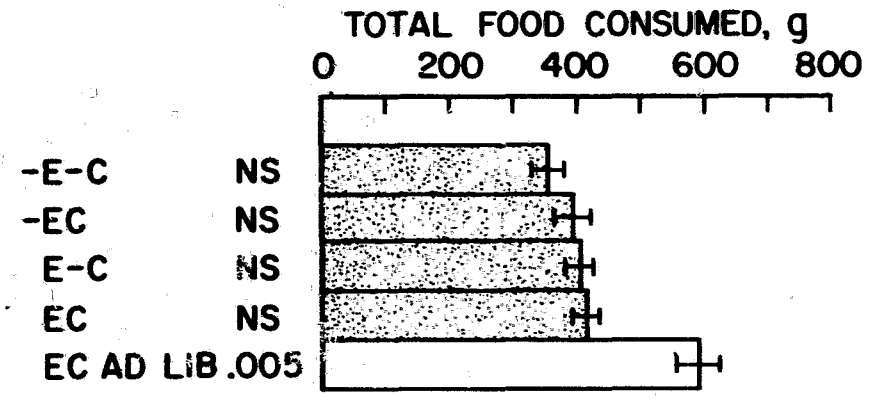

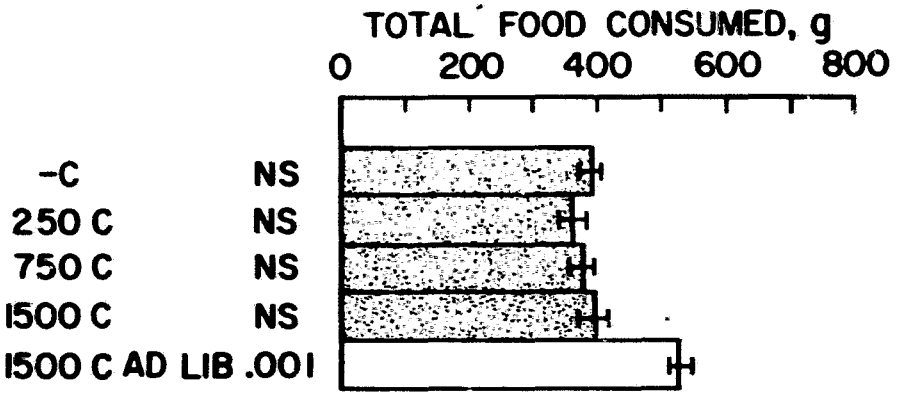

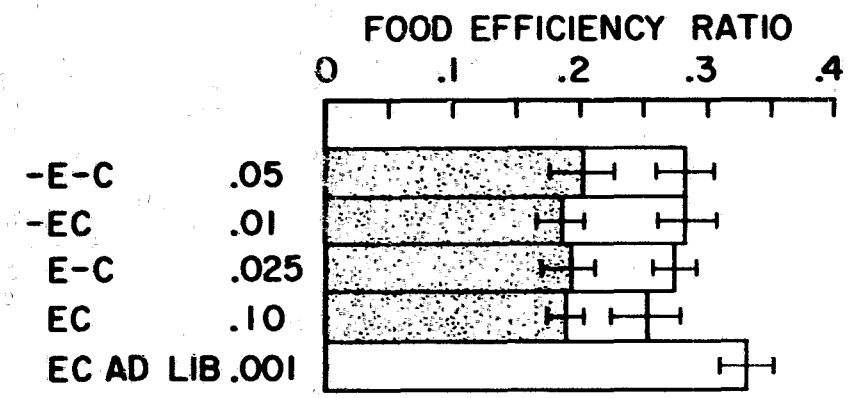

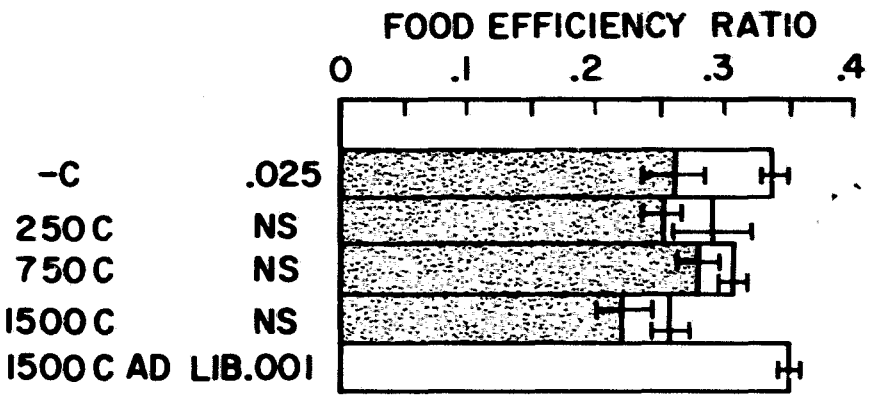

O_2 EXPOSED AIR CONTROL

EXPERIMENT I EXPERIMENT II

Figure 2.

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