THE EFFECT OF A CARROT DIET ON THE RESISTANCE OF THE RAT TO HYPOXIA

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By

CHARLES W. CRAVEN

Bachelor of Science

Oklahoma Agricultural and Mechanical College

Stillwater, Oklahoma

1947

Submitted to the Department of Chemistry

Oklahoma Agricultural and Mechanical College

In Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

1949

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AGRICULTURAL & MECHANICAL COLLEGE
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APPROVED BY:

Robert MacVicar
Chairman, Thesis Committee

O C Dermer
Member of the Thesis Committee

Otto M Smith
Head of the Department

D.C. McIntosh
Dean of the Graduate School

240283

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. Robert W. MacVicar, Associate Professor of Agricultural Chemistry Research and Chemistry, Oklahoma Agricultural and Mechanial College; and Dr. Herman I. Chinn, Chief of Department of Pharmacology and Biochemistry, School of Aviation Medicine, Randolph Field, Texas, for their very helpful assistance and suggestions throughout this investigation.

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INTRODUCTION

The failure of the tissues, for any reason, to receive an adequate supply of oxygen is called hypoxia. Hypoxia may result from defective oxygenation of the blood in the lungs, lowered oxygen capacity of the blood, or slowing of the movement of blood through the capillaries. Any condition which causes the oxygen tension to be lowered in the air that is breathed will bring about hypoxia. One such condition is encountered in ascents to high altitude.

As the pressure becomes reduced with increasing altitude the gases of the atmosphere and in the lungs must expand. As a result the quantity of oxygen and other gases of the atmosphere per unit volume becomes reduced proportionally. For this reason, at great altitudes (above 40,000 feet, inhalation of pure oxygen from a cylinder will not pevent hypoxia.(1, 3). A closed cabin containing air at a higher pressure is the only manner by which the problem of hypoxia in very high altitude flying can be solved at the present time.

With the advent of pressurized cabins for stratospheric flying in military aviation (especially in the case of jet-propelled aircraft), the problem of survival in the event of mechanical failure resulting in loss of cabin pressure has become an important problem. In a search for some means of increasing the survival time of animals subjected to lowered oxygen tension numerous substances have been tested. Most of these have, however, given no increase in survival time. This investigation has been made to further study factors which may increase the survival time of the rat when subjected to hypoxia.

REVIEW OF LITERATURE

Numerous factors have been shown to be concerned in the survival time of the rat when exposed to lowered oxygen tension. Notable among these are type of diet, temperature of the environment and loss of body weight (2).

Several years ago Campbell (5) showed that in many conditions in which tetany eventually occurs the oxygen tension of the tissues is very much decreased before the onset of tremors. Since muscular contraction causes a marked increase of oxygen tension in the tissues he suggested that this was the purpose of the muscular contraction in tremors and convulsions. He then began to investigate the influence of diet composition and the ingestion of various chemicals on the resistance to oxygen want in the rat. The following technique (6) was developed and used for his investigations: Weanling rats were fed a diet consisting of wheat, oats, peanuts, "puppy biscuits," potatoes, corn, bread, kitchen scraps, peas, beans and greenstuffs. When the rats reached about 80 grams in weight they were put into a decompression chamber in which the barometric pressure was gradually decreased to 240 - 250 mm Hg within a period of five minutes. The oxygen pressure at this stage was seven per cent of one atmosphere, corresponding to an altitude of about 30,000 feet. At temperatures of 20 to 25° C within the chamber rats fed a normal diet survived for at least thirty minutes; however, at 33° C the rats expired within ten minutes.

The first observed effect of dietary change tending to increase survival time of rats exposed to these latter conditions (low pressure and high temperature) was observed with animals which had been fed wholly on carrots for six days preceding exposure. The survival time was then increased up to thirty or sixty minutes. Other foods found to have a beneficial effect were beet root (boiled to remove some apparently toxic substance), apples and bananas.

In order to investigate further the resistance of the rat to oxygen-want a purified basal diet was used. The following substances were then added to this test diet without beneficial effect on resistance to hypoxia: vitamin A, carotene, nicotinic acid, riboflavin, cocaine, adrenosine, ammonium chloride, and sulphanilamide (6, 9). A full milk diet did not have any beneficial effect. A diet resembling the carrot in chemical composition (low-protein, high-fiber content) was decidedly beneficial.

Campbell (7) reported that the ten per cent loss in weight while on such a diet was not the responsible factor. This does not agree with the investigations of Le Blond, <u>et al</u> (18) who reported that starvation to a similar loss in weight increased the resistance of the rat to hypoxia. Hiestand (15), however, reported that inanition did not increase hypoxic resistance.

In a later communication Campbell (8) reported that if the protein of the diet consisted of casein or meat it was necessary to reduce it to five per cent before any beneficial effect could be detected. Gelatin, given in much larger amounts, and zein (11) in amounts as high as fifty per cent of the diet, on the contrary, actually increased the survival time of rats. Glutamine, tryptophane, tryosine and lysine were found to be somewhat beneficial. Charcoal or kaolin at the twenty-five per cent level, and agar at the ten per cent level of the diet were found to give protection equal to that afforded by a cellulose content of twenty-five per cent. Campbell (10) suggested that these may absorb some toxic substance produced by bacterial action in the intestine. He suggested that protein was involved rather than carbohydrate or fat since the amounts of these in the diet could be varied by twenty-five to thirty per cent without effect. He believed, however, that there was probably some other substance present in carrots which was helpful in raising the resistance of rats to oxygen want. The nature of this substance, if it exists, remains unknown.

Nelson, Goetzl, Robins and Ivy (19) have confirmed Campbell's observations concerning the protective effect afforded the rat by a carrot diet. They found that of 107 carrot-fed rats seventy-nine per cent survived the exposure to high altitude, whereas only twenty-one per cent of the control animals survived. Wetzig and D'Amour (22) were not able to observe any increase in "ear batting" reflex time of rats which had been fed a carrot diet, then exposed to anoxia.

Experience indicates (4, 16, 23) that in exposure to high altitude during flight a high carbohydrate diet is the most suitable for long-distance flights. It becomes of interest, therefore, to test whether a similar diet is also the most suitable for sudden exposure to high altitude. Campbell's animal experiments suggest that such may be the case. In his latest paper (8) he states that:

> suitable articles of diet for subjects exposed to the effects of oxygen-want are zein (but not whole corn), carrots, parsnips, beetroot, apples, bananas, starch, glucose, fat (lard), vitamins and salt. Gelatin and gluten (flour) might also be used in very moderate quantity and milk in small quantity.

The conclusion from this is that a diet for high altitude flight should be high in carbohydrate and low in proteins.

The experimental part of the present investigation was divided into two phases. Phase One (which was conducted at Oklahoma A & M College) was designed in the form of a preliminary investigation to confirm further previous reports (8, 19) on the relationship between a carrot diet and the survival time of the rat to hypoxia, and to obtain data which might indicate the responsible factor connected with a high carrot diet. In Phase Two of this investigation (which was conducted at the School of Aviation Medicine, Randolph Field, Texas) a more complete study of these factors was made.

EXPERIMENTAL

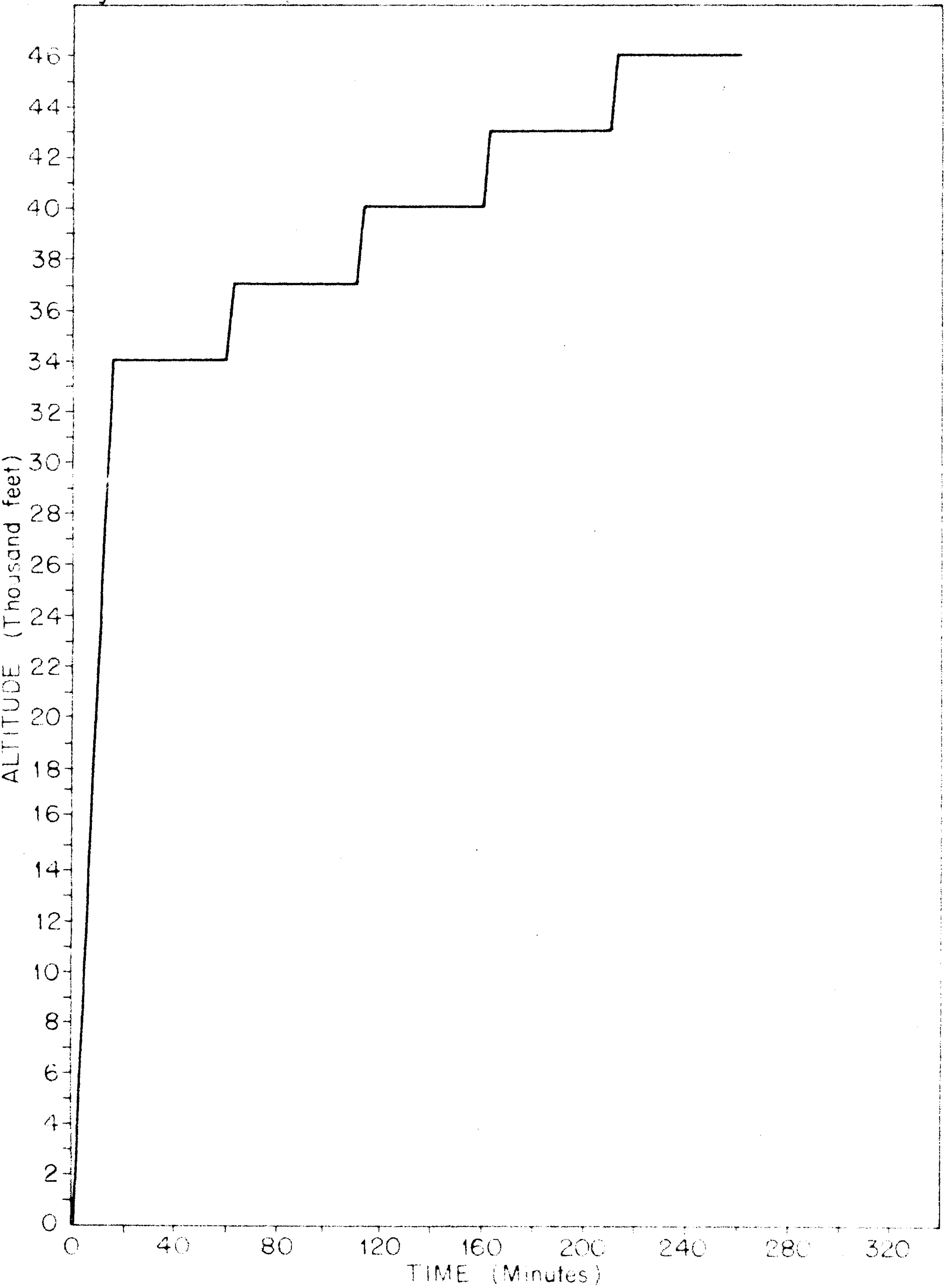
Phase I

Technique of Producing Hypoxia in Rats.

The method of investigation was to place the experimental animals under a large glass bell jar which rested on a glass-covered steel vacuum table (see plate 1), the seal between the bell jar and vacuum table being made with stopcock grease. The air pressure within this chamber was gradually reduced according to an exactly reproducible schedule (Figure I), the pressure being measured by a previously calibrated closed-end mercury manometer. The vacuum was produced by an oil vacuum pump, and the rate and degree of evacuation were controlled by leakage through two capillary orifices attached to the glass dome. Within the dome was placed a wire mesh cage with three compartments, each large enough to comfortably hold three 270-gram rats. This entire apparatus was then kept near 28° C. A reproducible rate of decompression was obtained by manipulation of pinch clamps on the capillary tubes (17).

The experimental animals were placed in this chamber and the pressure gradually reduced during 15 minutes to about 190 mm Hg (34,000 feet); rats survive best with slow decompression (12, 14, 15, 20). The pressure was held at this level for 45 minutes, then reduced to about 165 mm Hg (37,000 feet) in a period of one minute. The pressure was then lowered at 45-minute intervals through the following levels: 139 mm Hg (40,000 feet), 114 mm Hg (43,000 feet), then finally held at 88 mm Hg (46,000 feet) until any animals which had survived to this stage ceased breathing. The last few minutes of life were characterized by the onset of marked gasping which was termed the "respiratory crisis" (21). The survival time was measured from the time when 34,000 feet

Figure I PROCEDURE USED TO PRODUCE HYPOXIA IN PHASE I



was reached until death.

Dietary Studies

Animals used in this study were normal male albino rats (Wistar strain) weighing from 150 to 250 grams and were previously maintained on the laboratory stock ration which had been found suitable for normal rapid growth. This ration had the following composition:

Constituent	Per Cent
Yellow Corn Meal	58.00
Soybean Oil Meal	20.00
Dry Whole Milk Powder	20.00
Alfalfa Meal	2.00
Dry Brewers' Yeast	1.00
NaCl (Iodized)	0.50
$CaCO_3$	0.50
Cod-liver Oil	0.05

Determination of survival times under the previously described conditions of hypoxia were made for groups of rats which had been on one of the following diets or treatments:

Treatment No.	Description of Diet or Treatment
1	Basal Ration
2	Raw Carrots
3	Cooked carrots (by boiling 15 minutes)
4	Carrot juice (water extract) plus basal ration in the ratio of 1 to 1 by volume
5	Carrot pulp (water extract) plus basal ration, in the ratio of 1 to 1 by volume

6	Carrot juice plus carrot pulp (water extract) plus basal ration, in the ratio of 1 to 1 by volume
7	Carrot juice (alcohol extract) plus basal ration, in the ratio of 1 to 1 by volume
8	Carrot pulp (alcohol extract) plus basal ration, in the ratio of 1 to 1 by volume
9	Carrot juice plus carrot pulp (alcohol extract) plus basal ration, in the ratio of 1 to 1 to 1 by volume
10	Partial inanition

The carrots used throughout this experiment were fresh roots obtained on the local market and labeled as either California or Arizona grown.

The juice and pulp in both the water and alcohol extracts were obtained by grinding the carrots in an electric meat grinder, then adding liquid until a heavy slurry was obtained; this slurry was then macerated in a "Waring-type" blendor until a homogenate was obtained. The juice and pulp were then separated by squeezing the juice through muslin. The juice, in each case, was concentrated under reduced pressure at 60° C until a thin syrup was obtained. These preparations were then stored in a refrigerator at 5°C until used.

The average survival times and per cent loss in weight for each group are shown in Table I.

TABLE I

Relationship Between Treatment and Survival Times of Rats

Treatment	Duration of Treatment, Days	Number of Animals	Wt. loss per cent	Survival time minutes
1. Basal Ration	-	18	0.00	32.9

2.	Raw Carrots	10	12	6.8	133.9
3.	Cooked Carrots	10	18	21.9	72.0
4.	Juice, Water Extract	7	6	14.6	86.6
5.	Pulp, Water Extract	7	6	8.1	71.6
6.	Juice & Pulp, Water Ex.	7	6	8.0	36.3
7.	Juice, Alcohol Extract	6	9	6.2	69.3
8.	Pulp, Alcohol Extract	7	9	7.0	50.9
9.	Juice & Pulp, Alcohol Ex.	7	9	3.9	55.5
10.	Partial Inanition	8	6	11.7	63.0

Results

In this phase of this investigation it was found that rats fed only raw carrots and tap water ad libitum, showed a marked increase in survival time, as compared to control animals which had been fed the basal ration (Table I). Animals fed cooked carrots also showed an increase in survival time, but not to as high a degree as those fed the raw carrot diet. Of those rats which were fed preparations obtained by water extraction, those which had received the juice showed a greater survival time than those which had received the pulp.

In the case of those animals which were fed the alcohol preparations, the rats which received the juice portion again showed an increased survival time, as compared to those receiving the pulp. Partial inanition caused an increased survival time, but not to the degree afforded by the carrot diet.

These results are interesting in that an increase in survival time was noted in each case of carrot feeding (including the various fractions). Since a weight loss was apparent in all of these treatments, the question arises as to how partial starvation may be related to survival time.

These data, although interesting and useful as a guide for later investigations, are not easily analyzed statistically due to the rather complex test procedure. Further, due to the small number of animals used in these tests, these data are of little value other than to show a general trend.

In Phase Two the relationship between partial inanition and a full carrot diet as related to hypoxic sensitivity was further investigated.

Phase II

Dietary Treatment

Animals used in this phase of the investigation were normal albino rats (Wistar strain) weighing 150 to 300 grams. This investigation was made to determine the effect of partial inanition and carrot feeding on hypoxic sensitivity in both male and female rats. A paired-feeding technique was employed in all experiments of this phase.

Pairs of rats were selected, with particular attention being given to age, weight and sex. One animal from each pair received a diet of fresh raw carrots and tap water, ad libitum, while the other was given the basal ration, restricted in quantity so that the weight loss within pairs was nearly the same (see Tables II and III). The weight of each animal was recorded at the time a particular feeding trial was started; thereafter each animal was weighed daily. On the first day of feeding the rat receiving the restricted basal ration was given five grams of Purina dog chow (proximate analysis: Carbohydrate, 48.5; protein 26.2; fat, 5.4 per cent). On succeeding days body weight was controlled by either increasing or decreasing this quantity of feed in two gram increments.

Control animals were fed the basal ration (Purina dog chow) and tap water ad libitum, until the day preceding the test. In each case all water and food were taken away from the rats fourteen hours prior to the test so that they would be in a post-absorptive state during exposure.

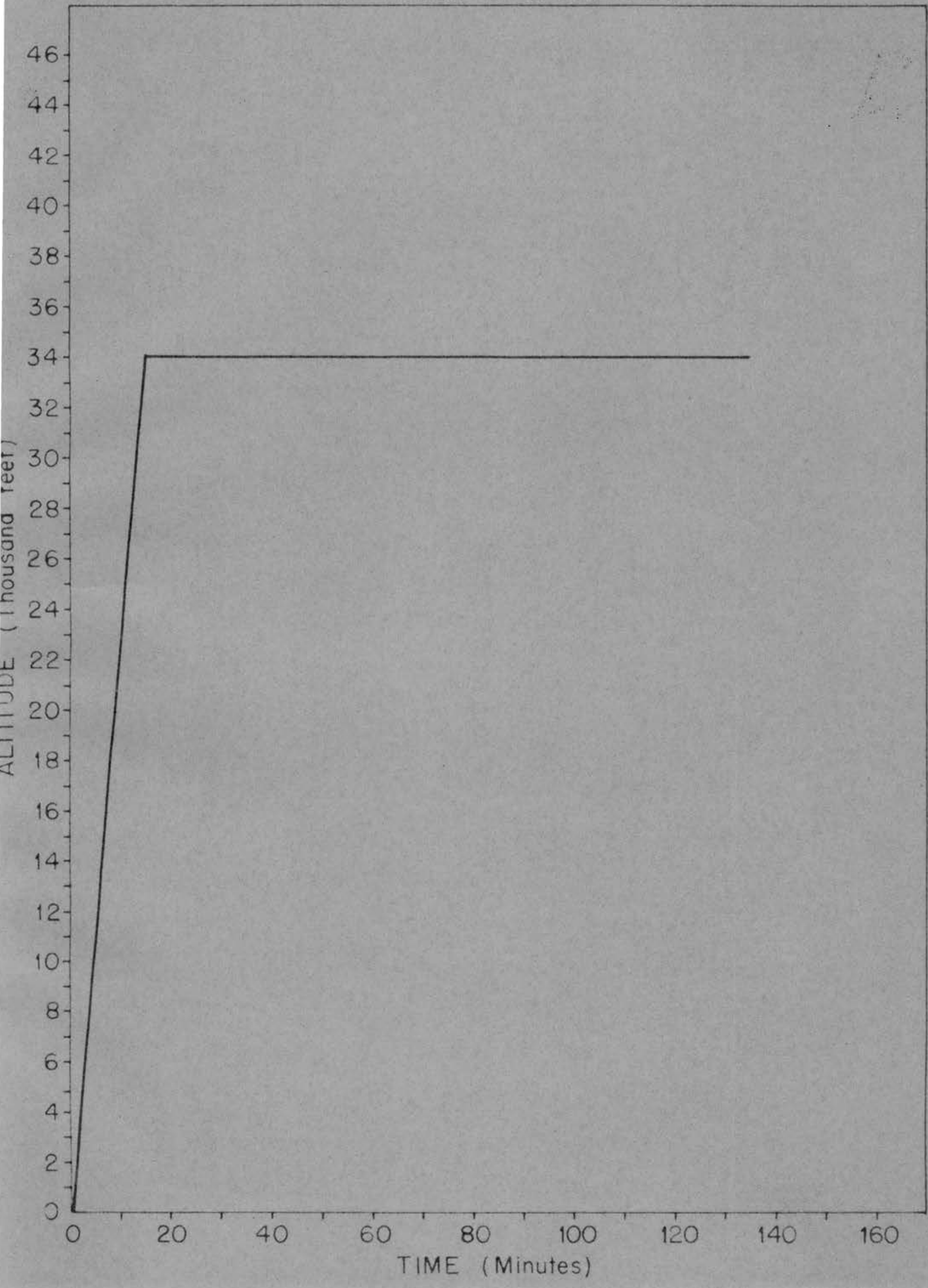
Technique of Producing Hypoxia in Rats

The method of producing a condition of hypoxia in this phase was to place the animals in one of two metal decompression chambers. One of these decompression chambers was designed for use in small animal experiments (see Plate 2); it was of sufficient size to accomodate eight rats and was equipped with a glass observation window. The rats were placed in a wire mesh cage which had eight separate compartments. The entire unit was maintained in an air-conditioned room at 28° C.

The other decompression chamber used in this phase was the large heavy steel type, which had been constructed for the Air Forces to be used in the oxygen indoctrination program for air crews (see plate 3). This chamber was of sufficient size to accomodate eighteen men, thus the observer was able, by breathing oxygen, to remain in the chamber during the tests. This unit was also maintained at 28° C.

The testing procedure in this phase was changed from that which had been used in Phase One. These changes were made to give a test which would yield data more easily analyzed statistically. The first testing procedure used in this phase was to lower the pressure at the rate of 2,000 feet per minute to a pressure altitude of 190 mm Hg. (34,000 feet). This altitude was then maintained for a period of two hours (see Figure II). This testing procedure was then used to test a series of female rats. Since this test was not found to be crucial for male rats it was again changed so that such a series could be tested. This last test for hypoxic tolerance was made as follows: the pressure was lowered at the rate of 2,000 feet per minute to a pressure altitude of 34,000 feet and maintained at this altitude for

Figure II PROCEDURE USED TO PRODUCE HYPOXIA IN PHASE II (FEMALES)



one hour, at which time it was further reduced to 155 mm Hg. (38,000 feet); it was held at this level for thirty minutes, then reduced to a pressure altitude of 128 mm Hg. (42,000 feet) and held at this level for thirty minutes (see Figure III). The survival times were recorded throughout the two-hour period, and those animals which survived the entire period were returned to ground level.

Results

Two series of rats, one male and the other female, were subjected to the effects of hypoxia. Each of these series contained three groups of rats with thirty rats in each group. The survival time of each rat was recorded to show the tolerance to hypoxia. These data, along with a description of treatment and weight losses, are shown in Tables IV and V. By using the number of rats surviving as the ordinate and the time in minutes as the abscissa, curves as shown in Figures IV and V were obtained.

Figure III PROCEDURE USED TO PRODUCE HYPOXIA IN PHASE II (MALES)

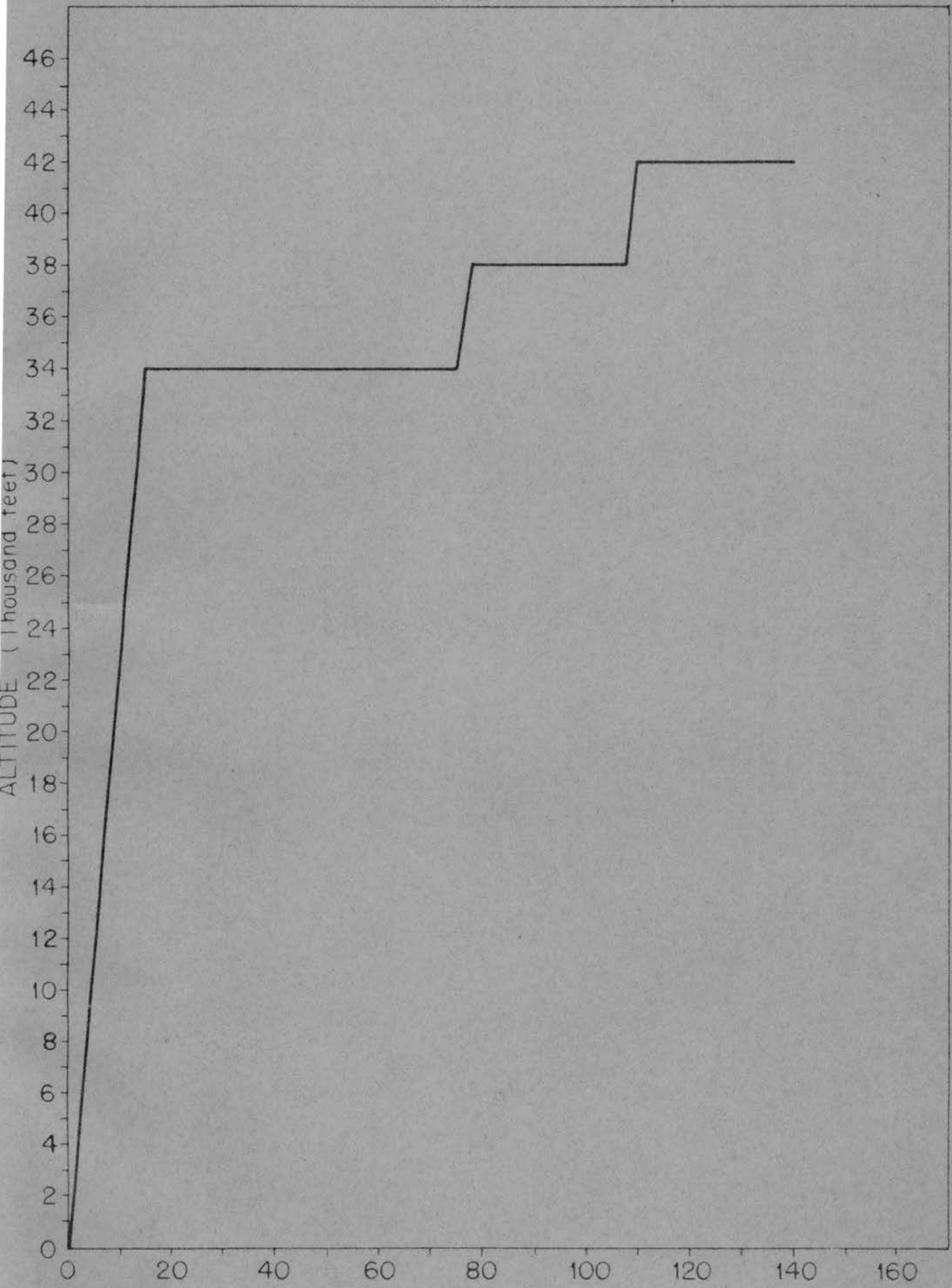


Figure IV COMPARISON OF GROUPS TREATED, SHOWING HYPOXIC TOLERANCE (FEMALES)

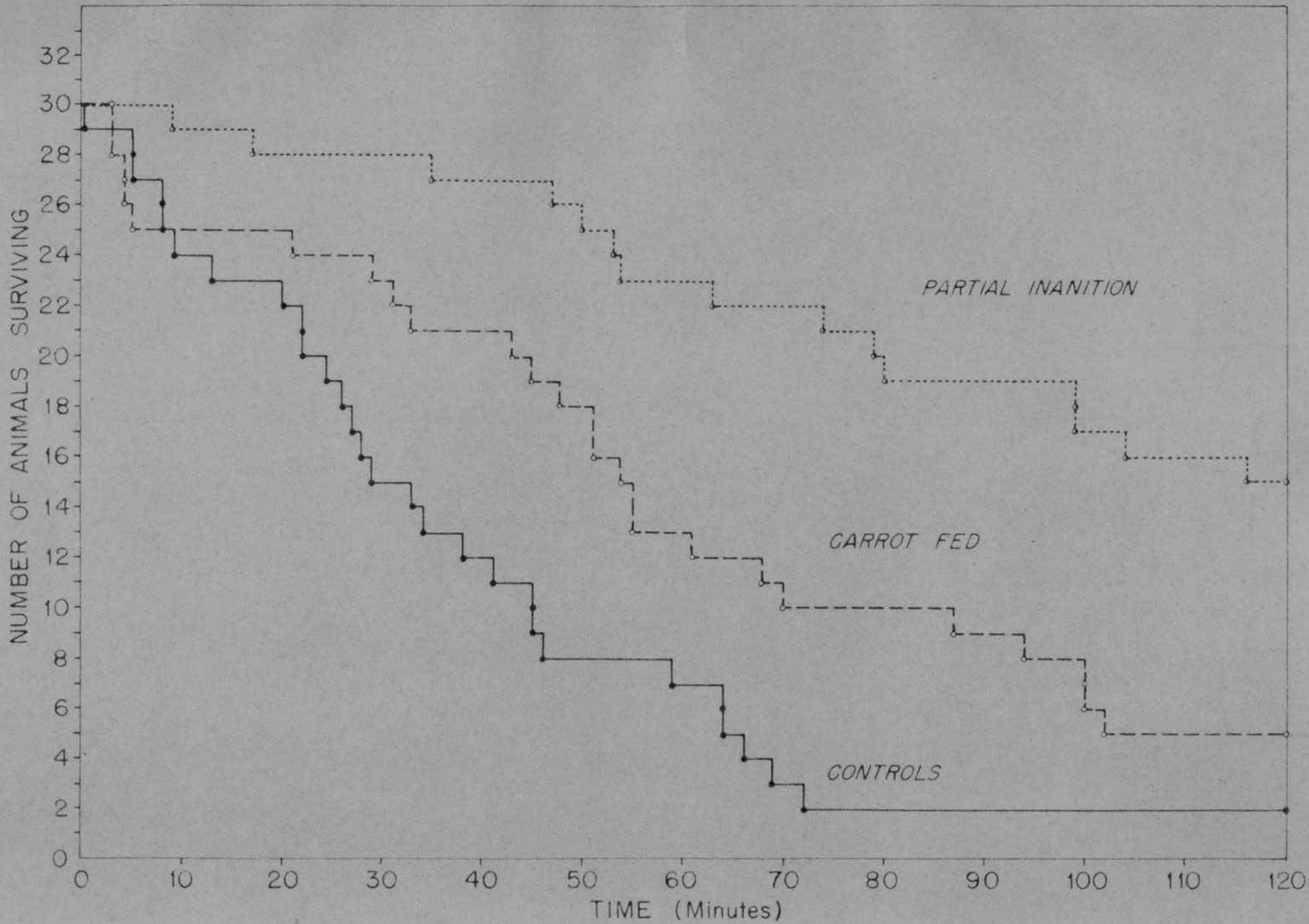


Figure V COMPARISON OF GROUPS TREATED, SHOWING HYPOXIC TOLERANCE (MALES)

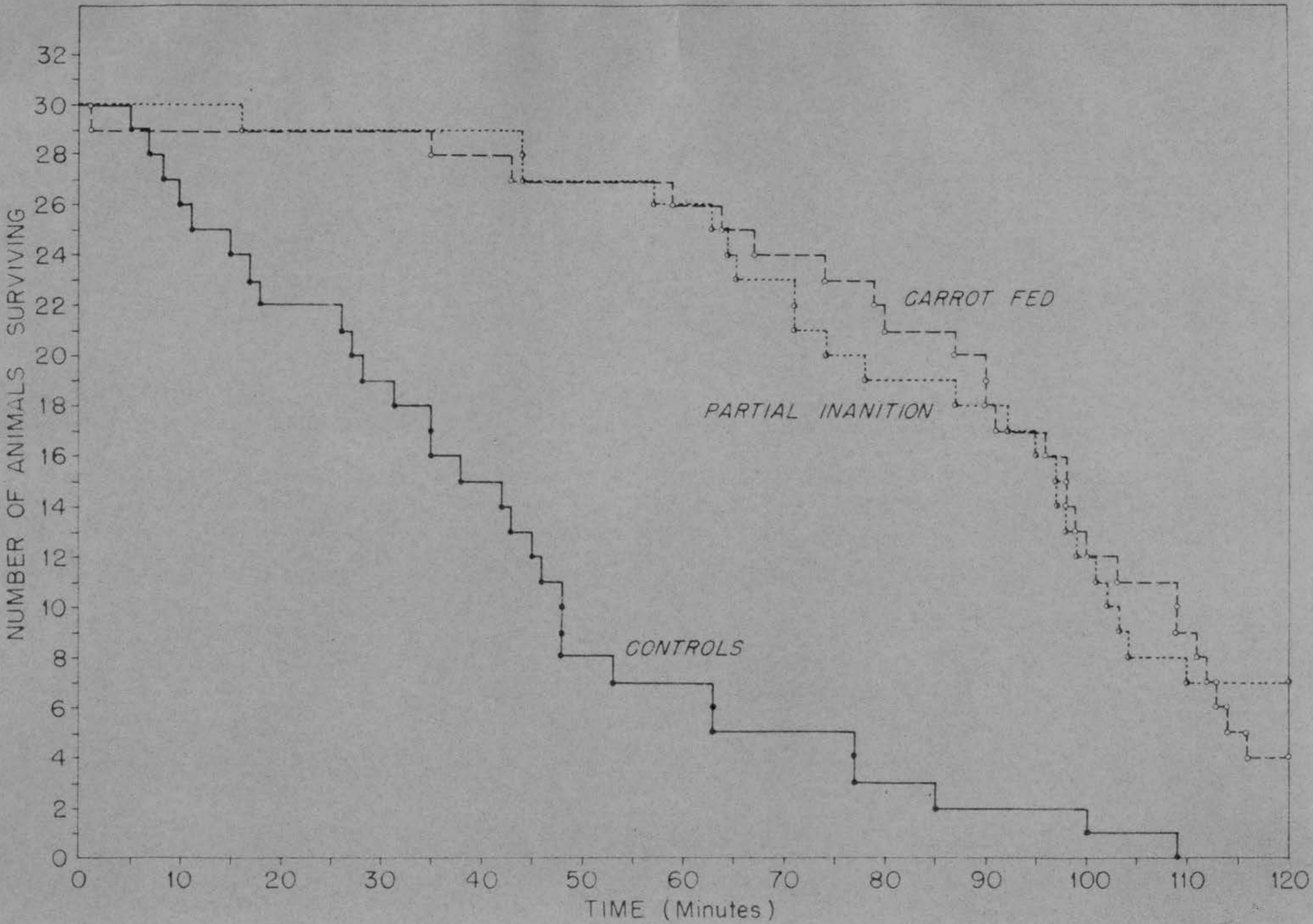


TABLE II. RESULTS OF PAIRED FEEDING, SHOWING INITIAL WEIGHT AND WEIGHT LOSS WITHIN PAIRS (FEMALES)

Pair Number	CARROT DIET Initial Wt. Grams	Wt. Loss Grams	Per Cent Lost	PARTIAL INANITION Initial Wt. Grams	Wt. Loss Grams	Per Cent Lost
1-11	140	20	14.3	140	16	11.4
1-12	148	28	18.9	148	28	18.9
1-13	132	8	6.1	132	6	4.5
1-14	156	12	7.7	156	14	9.0
1-15	160	22	13.7	160	24	15.0
2-1	154	30	19.5	154	32	20.1
2-2	172	38	22.1	172	34	19.8
2-3	172	34	19.8	172	34	19.8
2-4	190	44	17.9	190	34	20.0
2-5	220	38	17.3	220	36	16.4
3-11	188	36	19.1	188	36	19.1
3-12	210	48	22.8	210	50	23.8
3-13	162	30	18.5	162	26	16.0
3-14	192	36	18.7	192	40	20.8
3-15	140	18	12.9	140	18	12.9
5-1	180	28	15.6	180	22	12.2
5-2	180	22	12.2	180	32	17.8
5-3	170	22	12.9	170	20	11.8
5-4	188	32	17.0	188	30	16.7
5-5	176	30	17.0	176	28	15.9
5-6	166	20	12.0	166	20	12.0
5-7	170	22	12.9	170	18	10.6
5-8	184	28	15.2	184	28	15.2
5-9	166	10	6.0	166	10	6.0
5-10	162	22	13.6	162	18	11.1
5-11	162	22	13.6	162	18	11.1
5-12	152	14	9.2	152	18	11.8
5-13	176	30	17.0	176	34	19.3
5-14	190	24	12.6	190	24	26.7
5-15	184	16	8.7	184	14	7.6
AVERAGE	171.4	25.8	15.1	171.4	25.3	14.9

TABLE III. RESULTS OF PAIRED FEEDING, SHOWING INITIAL WEIGHT AND WEIGHT LOSS WITHIN PAIRS (MALES)

	CARROT DIET			PARTIAL INANITION		
Pair Number	Initial Wt. Grams	Wt. Loss Grams	Per Cent Lost	Initial Wt. Grams	Wt. Loss Grams	Per Cent Lost
8-1	292	68	23.3	292	58	19.9
8-2	292	52	17.8	292	50	17.1
8-3	292	46	15.8	292	34	11.6
8-4	300	38	12.7	300	38	12.7
8-5	300	52	17.3	300	48	16.0
8-6	300	52	17.3	300	30	10.0
8-7	282	44	15.6	282	40	14.2
8-8	282	36	12.8	282	28	9.9
8-9	248	42	16.9	248	42	16.9
8-10	262	46	17.6	262	42	16.0
8-11	262	46	17.6	262	48	18.3
8-12	256	52	20.3	256	48	18.8
8-13	240	48	20.0	240	44	18.3
8-14	270	46	17.0	270	34	12.6
8-15	256	40	15.6	256	40	15.6
9-1	246	32	13.0	246	42	17.1
9-2	246	16	6.5	246	22	8.9
9-3	224	18	8.0	224	14	6.3
9-4	320	22	6.9	320	18	5.6
9-5	210	12	5.7	210	8	3.8
9-6	210	20	9.5	210	24	11.4
9-7	216	30	13.9	216	18	8.3
9-8	220	22	10.0	220	6	2.7
9-9	218	26	11.9	218	24	11.0
9-10	230	44	19.1	230	36	15.7
9-11	264	30	11.4	264	18	6.8
9-12	280	32	11.4	280	28	10.0
9-13	200	18	9.0	200	14	7.0
9-14	290	28	9.7	290	24	8.3
9-15	288	52	18.1	288	56	19.4
AVERAGE	259.8	37.0	14.2	259.8	32.5	12.5

TABLE IV. RELATIONSHIP BETWEEN TREATMENT, FINAL WEIGHT, PER CENT WEIGHT LOSS AND HYPOXIC TOLERANCE (FEMALES)

CONTROLS		CARROT DIET				PARTIAL INANITION			
Final Wt.Grams	Survival Time, Min.	Pair Number	Final Weight	Per Cent Weight Loss	Survival Time, Min.	Pair Number	Final Weight	Per Cent Weight Loss	Survival Time, Min.
185	0	3-12	162	22.8	3	3-13	136	16.0	9
192	5	5-10	140	13.6	3	3-12	160	23.8	17
188	5	5-2	158	12.2	4	3-11	152	19.1	35
194	8	2-1	124	19.5	4	5-2	148	17.8	47
180	8	5-12	138	9.2	5	5-14	166	26.7	50
176	9	5-15	168	8.7	21	5-12	134	11.8	53
206	13	5-14	166	12.6	29	5-13	142	19.3	54
184	20	5-9	156	6.0	31	3-14	152	20.8	63
132	22	5-6	146	12.0	33	2-1	122	20.1	74
152	22	5-12	146	17.0	43	5-4	158	16.7	79
174	24	5-1	152	15.6	45	5-8	156	15.2	80
182	26	5-3	148	12.9	48	5-5	148	15.9	99
178	27	1-11	120	14.3	51	5-7	152	10.6	99
192	28	5-7	148	12.9	51	5-6	146	12.0	104
224	29	5-11	140	13.6	54	1-15	136	15.0	116
154	33	2-2	134	22.1	55	5-3	150	11.8	120
152	34	3-11	152	19.1	55	5-9	156	6.0	120
196	38	3-15	122	12.9	61	5-10	144	11.1	120
194	41	2-3	138	19.8	68	5-11	144	11.1	120
196	45	1-13	124	6.1	70	5-15	170	7.6	120
166	45	2-4	156	17.9	87	3-15	122	12.9	120
142	46	5-4	156	17.0	94	2-2	138	19.8	120
136	59	5-8	156	15.2	100	2-3	138	19.8	120
190	64	3-14	156	18.7	100	2-4	152	20.0	120
178	64	1-12	120	18.9	102	2-5	184	16.4	120
136	66	5-5	146	17.0	120	1-11	124	11.4	120
168	69	3-13	132	18.5	120	1-12	120	18.9	120
192	72	2-5	182	17.3	120	1-13	126	4.5	120
206	120	1-14	144	7.7	120	1-14	142	9.0	120
220	120	1-15	168	8.7	120	5-1	158	12.2	120
* 178.8			146.6	15.1			145.8	14.9	

*Average

TABLE V. RELATIONSHIP BETWEEN TREATMENT, FINAL WEIGHT, PER CENT WEIGHT LOSS AND HYPOXIC TOLERANCE (MALES)

CONTROLS		CARROT DIET				PARTIAL INANITION			
Final Wt. Grams	Survival Time, Min	Pair Number	Final Weight	Per Cent Weight Loss	Survival Time, Min	Pair Number	Final Weight	Per Cent Weight Loss	Survival Time, Min
212	5	9-8	214	23.3	1	8-3	246	19.9	16
204	7	8-5	252	17.8	35	9-11	234	17.1	44
300	8	9-4	302	15.8	43	9-8	198	11.6	44
250	10	9-11	246	12.7	59	9-14	262	12.7	57
288	11	9-12	256	17.3	64	9-13	182	16.0	63
250	15	9-7	198	17.3	67	8-9	206	10.0	64
284	17	8-9	206	15.6	74	9-7	186	14.2	65
236	18	9-3	210	12.8	79	8-8	246	9.9	71
200	26	9-2	224	16.9	80	8-7	238	16.9	71
224	27	9-6	186	17.6	87	9-2	230	16.0	74
222	28	8-6	270	17.6	90	9-3	206	18.3	78
260	31	8-15	216	20.3	90	8-4	262	18.8	87
220	35	8-4	262	20.0	91	8-5	248	18.3	92
230	35	9-1	204	17.0	96	9-12	248	12.6	95
222	38	9-5	202	15.6	98	8-13	192	15.6	97
224	42	8-13	196	13.0	98	8-11	216	17.1	97
280	43	9-15	232	6.5	99	8-6	248	8.9	98
312	45	9-13	186	8.0	100	8-12	204	6.3	99
206	46	8-8	254	6.9	103	8-10	216	5.6	101
202	48	9-9	194	5.7	109	9-6	190	3.8	102
306	48	8-3	248	9.5	109	9-1	214	11.4	103
280	48	8-2	242	13.9	111	9-4	298	8.3	104
260	58	8-10	220	10.0	112	9-5	198	2.7	110
288	63	8-11	214	11.9	113	9-15	236	11.0	120
248	63	9-10	194	19.1	114	9-9	192	15.7	120
244	77	8-12	208	11.4	116	9-10	186	6.8	120
242	77	9-14	266	11.4	120	8-2	242	10.0	120
250	85	8-14	236	9.0	120	8-1	224	7.0	120
242	100	8-7	242	9.7	120	8-15	216	8.3	120
276	109	8-1	234	18.1	120	8-14	224	19.4	120
* 248.7			227.1	14.2			222.9	12.5	

*Average

TABLE VI. FISHER'S "P" VALUES, SHOWING THE SIGNIFICANCE OF DIFFERENCES BETWEEN THE VARIOUS GROUPS

Groups Being Compared	Fisher's "P" Values*	
	Females**	Males
Control and Carrot Fed	0.066	0.00035
Control and Inanition	0.00055	0.00014
Carrot Fed and Inanition	0.14	1.0

*Low values for "P" indicate a high significant difference; for example, where "P" is 0.01 the difference is significant at the 1 per cent level.

**The $L.D._{50}$ for the controls in the female group was 29 min., while the $L.D._{50}$ for the control animals in the male group was 38 min.

DISCUSSION

During the exposure to hypoxia, the oxygen tension within the low pressure chambers was controlled by measuring the barometric pressure. However, the carbon dioxide tension was not controlled and it might well have influenced the survival times of the animals. To overcome such a limitation adequate ventilation was provided during each test.

The data from the experiments in Phase One indicated that a carrot diet and loss of body weight might well have increased hypoxic tolerance in the rat. These data were not conclusive, but did form a basis for further investigation.

By using a simplified test procedure, data was obtained in Phase Two which could be treated statistically. Using the dose of exposure that was lethal for fifty per cent of the animals (the $L.D._{50}$) as a basis of comparison between the groups in this phase the difference in hypoxic tolerance may be illustrated. In the female series the control group had an $L.D._{50}$ of 29 minutes, the carrot-fed group and $L.D._{50}$ of 54 minutes, and the inanition group an $L.D._{50}$ of 116 minutes. However, in the male series the control group had an $L.D._{50}$ of 38 minutes, the carrot-fed group an $L.D._{50}$ of 98 minutes, and the inanition group an $L.D._{50}$ of 97 minutes (see Tables IV and V).

By using a Fisher's Chi square method (13) the significance of these differences may be evaluated. The $L.D._{50}$ for the control animals may be used as the point to test the significance of these differences between groups within the series. By using this point there was found to be a significant difference in tolerance between the control animals and either the carrot-fed or inanition animals in each series. This difference was highly significant, except in the case of the control and carrot-fed rats in the female series. Here the difference is significant only to the 6.6 per cent level (see Table VI). The difference in tolerance between the carrot-fed and inanition groups was not found to be significant in either series; in fact, with the male series there was found to be no difference in tolerance to a highly significant level.

It should be pointed out that the tests for hypoxia with the male series were more carefully controlled than were those for the female series. This resulted from testing rats from each of the three groups (15 from each group) in the large pressure chamber simultaneously. Since, in these latter tests, the control, carrot-fed, and inanition animals were subjected to the exact same conditions, any source for error (such as high carbon dioxide or oxygen tension) was eliminated. Because of this the data from this male series are considered of more significance.

Since partial inanition gives the rat as great an increase in hypoxic tolerance as does a carrot diet it appears that this increase in tolerance is due to the loss in body weight rather than any substance present in the carrot. This increase in hypoxic tolerance, resulting from loss in body weight, may be the result of a lowered metabolic rate (20) which would lower the oxygen requirement of the tissues.

SUMMARY AND CONCLUSIONS

The investigation to test the survival time of rats subjected to hypoxia was made in two phases. In Phase One several groups of rats were fed various carrot diets and one group was subjected to partial inanition before being tested for hypoxic tolerance. In each of these groups an increase in tolerance was noted.

In Phase Two the question of influence of carrot diet and partial inanition was further investigated. Two groups of rats (both male and female) were "pair fed," tested for hypoxic tolerance, and compared to normal rats. One of these groups had been fed an exclusive carrot diet for ten days before the test while the other had been fed a restricted basal ration so that the weight loss between the two groups was similar. With each of these groups a significant increase in resistance to hypoxia was found. Data from the male series shows that either a carrot diet for ten days or a restricted basal diet, resulting in a similar weight loss, affords the rat a significant increase in hypoxic tolerance.

BIBLIOGRAPHY

1. Armstrong, H. S. Principles and Practices of Aviation Medicine. Baltimore: Williams and Wilkins, 1939.

2. Armstrong, H. W. and J. W. Hein. "Factors Influencing Altitude Tolerance During Short Exposure to Decreased Barometric Pressure." J. Aviation Med. 9, 45 (1938).

3. Best, C. H. and N. B. Taylor. The Physiological Basis of Medical Practice. Baltimore: Williams and Wilkins, 1945.

4. Butts, J. S., H. F. Mulholland and D. M. Green. "The Relationship of Anoxia Susceptibility to Diet." J. Aviation Med. 16, 311 (1945).

5. Campbell, J. A. "The Oxygen Deficiency Theory and Experimental Tetany." Lancet, 1, 72 (1926).

6. Campbell, J. A. "Increase of Resistance to Oxygen Want in Rats on a Carrot Diet." J. Physiol. 93, 31P (1938).

7. Campbell, J. A. "Increase of Resistance to Oxygen Want in Animals on Certain Diets." Quart. J. Exper. Physiol. 28, 231 (1938).

8. Campbell, J. A. "Diet and Resistance to Oxygen Want." Quart. J. Exper. Physiol. 29, 259 (1939).

9. Campbell, J. A. "Resistance to Oxygen Want." J. Physiol. 95, 1P (1939)

10. Campbell, J. A. "Protein and Resistance to Oxygen Want." J. Physiol. 95, 28P (1939).

11. Campbell, J. A. "Zein and Resistance to Oxygen Want." J. Physiol. 96, 33P (1939).

12. Emerson, G. A. and E. J. Van Liere. "Drug Prophylaxis Against Lethal Effects of Severe Anoxia." J. Lab. and Clin. Med. 28, 689 (1943).

13. Fisher, R. A. Statistical Methods for Research Workers, ed 7, Edinburgh: Boyd and Oliver, (1938).

14. Hailman, H. F. "Lowest Barometric Pressure Compatible with Life in an Atmosphere of 100 per cent Oxygen." Proc. Soc. Exper. Biol. and Med. 53, 221 (1943).

15. Hiestand, W. A. and H. R. Miller. "Further Observations on Factors Influencing Hypoxia Resistance of Mice." Am. J. Physiol. 142, 310 (1944).

16. King, C. S., H. F. Bickerman, C. J. Harper, J. R. Oyler and C. P. Seitz. "Aviation Nutrition Studies." J. Aviation Med. 16, 69 (1945).

17. Kolls, A. C. and A. S. Loevenhart. "Respiration Chamber for Small Animals." Am. J. Physiol. 39, 69 (1915).

18. LeBlond, C. P., J. Gross and H. Langier. "Effect of Fasting on Resistance to Anoxia." J. Aviation Med. 14, 262 (1943).

19. Nelson, D., S. Goetzl, S. Robins and A. C. Ivy. "Carrot Diet and Susceptibility to Acute Anoxia." Proc. Soc. Exper. Biol. and Med. 52, 1 (1943).

20. Quimby, F. H., N. E. Phillips and I. U. White. "Chronic Inanition, Recovery, and Metabolic Rate of Young Rats." Am. J. Physiol. 154, 188 (1948).

21. Stullken, D. E. and W. A. Hiestand. "Comparison of Survival to Decompression in Air and in Oxygen." Proc. Soc. Exper. Biol. and Med. 54, 260 (1943).

22. Van Liere, E. J. Anoxia, Its Effect on The Body. Chicago: The University of Chicago Press, 1942.

23. Wetzig, P. and F. E. D'Amour. The Effects of Polycythemia and of a Carrot Diet on Resistance to Anoxia." Am. J. Physiol. 140, 304 (1943).

24. Whittingham, C. "Diet, Oxygen Want, and High Flying." J. Roy. Naval Med. Service, 25, 317 (1939).

PLATE I. SMALL "BELL JAR" DECOMPRESSION CHAMBER

PLATE 2. SMALL ANIMAL DECOMPRESSION CHAMBER

PLATE 3. LARGE AIR FORCE DECOMPRESSION CHAMBER

Typist: Margaret Mary Collins