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PROGRESS REPORT

INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

1 July through 30 September 1966

R. G. Lindberg, Ph.D.
Principal Investigator

PREPARED UNDER CONTRACT NASw-812

for

OFFICE OF SPACE SCIENCES
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
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SUMMARY

Previous contract reports have verified the use of pocket mice as useful organisms for space biology research. A particularly appealing feature of these organisms are their relatively simple life support requirements. In a related contract (NASw-1191) prototype equipment was developed for execution of an experiment in space to study the effect of space residence on circadian rhythm. The prototype equipment emphasizes stability of the environment and maintenance of an oxygen/nitrogen atmosphere at 72°F and 50% RH. During development it was apparent that an extremely simple and compact piece of experiment hardware could be developed if more were known about the ability of pocket mice to survive environmental extremes, and further, if the effect of environmental extremes would perturb the biological end points being measured.

This report summarizes progress over the last quarter in which two kinds of studies were initiated. The first is concerned with regulation of body temperature in hypoxic atmospheres. It is believed that the temperature lability of Perognathus is a particularly useful feature for ellucidating mechanisms of thermoregulation in mammals. It is also believed that this study will establish the feasibility and practicality of maintaining pocket mice in a hibernating state for the possible purpose of transporting them long distances in space for experimentation at remote sites.

The second kind of studies is concerned with pilot experiments directed toward studying the response of pocket mice to environmental extremes. Responses to changes in ambient termperature have been reported under contract NASr-91. The studies when completed should contribute, both, to our knowledge of physiological ecology and to design concepts for simple experiment hardware for space research.

TEMPERATURE REGULATION IN HYPOXIC ATMOSPHERES

Page Hayden

The decrease in body temperature of animals, that are normally classified as homeotherms (including man), with decrease in ambient oxygen concentration has been known for many years (1). The observation of this phenomenon has been closely associated with studies on the protective effects of low ambient temperatures and anoxia. The lowering of body temperature in effect seems to put tissue respiration below the level at which damage might occur because of oxygen deprivation. Large animals are deprived of this "thermal-haven" unless the onset of hypoxic atmosphere is slow and moderate.

Hibernators and non-hibernators apparently are not radically different in their response to hypoxia. The exceptions being that hibernators are able to rewarm from temperatures at which non-hibernators must eventually perish.

The following portion of this report deals with preliminary results of one portion of a larger study concerning temperature regulation with hypoxia, oxygen affinity of blood, critical temperature of arousal and temporal effects in hypothermia in the genus Perognathus.

METHODS AND MATERIALS

The total experimental system consists of six major components: (1) animal chamber; (2) cooling unit; (3) oxygen monitor; (4) body temperature readout; (5) 2 pen analog recorder; (6) gas supply.

The animal chamber is made of lucite with a double wall water jacket construction for temperature control and is provided with heat exchange coils and humidifier for incurrent gas mixtures. An antenna system is fitted to a subfloor of the actual animal chamber to receive signals transmitted from a telemeter implanted abdominally in an animal (2). The signal is amplified, integrated and analog output fed to one pen of the recorder.

Various respiratory atmospheres can be made using two vernier valves and bottled gases under three stages of regulation. Pure breathing oxygen and 95% nitrogen with 5% carbon dioxide are mixed and supplied, at the rates of 100-200 ml/min., to the animal chamber. A small bypass shunt goes to the oxygen analyzer (Beckman F3) and monitors the mixture constantly. The O_2 analyzer output is fed to one channel of the recorder.

The animal chamber is maintained below ambient (8-9°C in most cases) by circulating water from a cooling source which can be regulated through adjustments of back-pressure in the refrigerating gas. Thermostatic adjustment devices could not be used since they caused perturbation in the temperature monitoring system.

In all experiments, animals were supplied with a piece of paper towel for urine absorbtion and sunflower seeds for food.

RESULTS AND DISCUSSION

To this date, 10 Perognathus longimembris have been experimentally treated and monitored (a total of 17 runs) for periods up to three days.

Analysis of temperature data was made by resolving all cooling and warming curves into simple linear segments for presentation of both graphic and digital data. Although inherent telemeter accuracy and reproducibility is within ±0.1°C, the data as detected, recorded and resolved is probably closer to ±0.5°C.

In Figure 1 are given representative temperature responses of Perognathus longimembris to different kinds, duration and onsets of hypoxic atmospheres. All of the plots in Figure 1 are from the same animal taken 1, 15, 47 days apart. Plot A was an initial attempt to determine the degree of hypoxia that would induce a change in body temperature (T_B) that could be reversed with application of normal air. Previous work (3) had been involved with immediate severe hypoxia. Plot B shows the T_B forced down to low temperature and not being reversed immediately by the application of normal atmosphere. External warming was applied in this case to decrease the overall time for return to normothermia. In the 12.3% oxygen regime the temperature drop was very linear and appeared to be the result

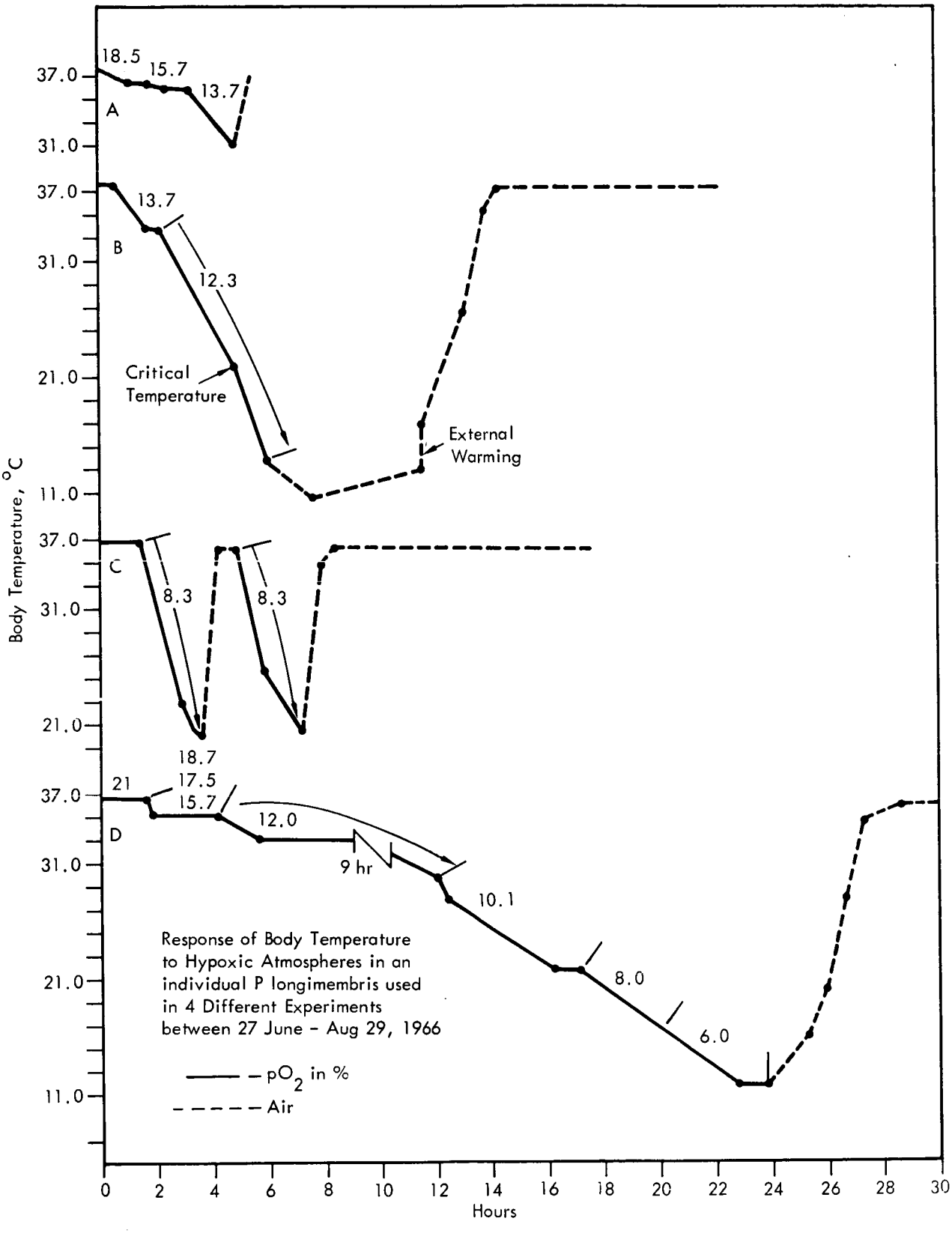

Fig. 1 Representative body temperature data from a single P. longimembris to various hypoxic mixtures of O_2 and N_2 + CO_2.

of an attempt to maintain a constant metabolism but the body temperature gradually slipped lower. At about $T_B = 22^{\circ}C$ there was an abrupt change with the "resistive metabolism" giving way to apparently passive cooling. This transition temperature has been noted in other P. longimembris under these same general circumstances. Although it varies in different individuals, (22-24°C) it is constant at a given value in any one animal. On two occasions it has been noted in induced torpor and in spontaneous torpor. In most spontaneous torpors it is undetected for the animal assumes passive cooling (follows Newton's law of cooling) from near normothermia.

Figure 1, plot C shows two successive temperature depressions with hypoxia. In the second depression, the cooling rate was faster than the first -.260° / min. vs -.180° / min., although total time to low temperature was the same. This might be interpreted as some kind of conditioning to the cardio-vascular system. The return to normal temperature required 35 mins. and a maximum warming rate of +.617° / min. in the first depression. While rewarming from the second, twice the time and the maximum rate was +.367° / min., a depletion, or slowing of mobilization, of energy stores is indicated by this response.

Figure 1, plot D is representative of an animal that resists torpor, as opposed to those that display a spontaneous torpor with mild hypoxia. Unfortunately in this case the oxygen concentration was decreased in the region of the critical temperature 21-22°C and could not be observed. The decrease to 8.0% apparently caused the animal to cool passively as a drop to 6.0% had no effect on cooling rate. The application of air caused the animal to arouse to normal temperature after a short lag period. This is in direct contrast to the state of "neural" hibernation of ground squirrels induced into torpor by hypoxia (4).

The application of air does not always act as a stimulus for the animal to resume normal metabolism. In several instances the application of air, post hypoxic exposure, caused a stabilization of temperature followed by a decline to a deeper torpor with subsequent arousal after a period of time. These cases occurred below the apparently transition temperature of 22-24°C. It would appear that the period of stabilization

of temperature upon application of air is some kind of checkout for systems that allow the animal to assume a passive metabolism at an even lower temperature. It seems the animal senses the change in the atmosphere and determines that a full torpor (depth dependent upon the ambient temperature) is possible and that arousal is compatible with the environment.

If air is applied to an animal that has exhibited a torpor with a minimal treatment with hypoxia, it usually does not respond immediately to the oxygen increase. There is a strong circadian component in these animals, in that arousal is not initiated until mid-afternoon (~ 1500 hrs.) which is in the range of time that spontaneous torpors normally terminate.

Table 1 is a comparison of induced torpor and arousal versus spontaneous torpor and arousal in the same animal. The entry into torpors are not directly comparable in time, in that one is passively cooling from the start; in the other, some resistive metabolism may be encountered when the body temperature has decreased sufficiently to be compatible with the ambient oxygen concentration. However, the end result may be essentially the same phenomenon. Initial cooling rates (Table 1) are very similar and may represent the maximum rate for this species.

The arousal from induced torpor and spontaneous torpor are very similar, if the initial warming trend of induced torpor is disregarded. The spontaneous arousal is more gradual and lacks the sharp increase at the terminus. It is interesting to note that there is a break in the curves at the transition temperature and the highest rates of warming occur above this point.

In Figure 2, body temperature, in relation to ambient oxygen concentration, have been plotted. Only those temperatures that remained constant for at least an hour have been used. This graph indicates that body temperature (by various amounts of metabolic effort) can be maintained down to about 20° C in 8% oxygen but by far, most are grouped above 30° C in 12% or greater oxygen concentration. It would seem that there

Table 1

Comparison of entry and arousal from spontaneous torpor and hypoxia induced torpor in Perognathus longimembris L-1650♂, 13-14 July 1966.

SPONTANEOUS Air			INDUCED (a) 7.25% O_2 & (b) 6.10% O_2		
ENTRY			ENTRY		
Temp Range °C	Rate of Change °C/min	Duration mins	Temp Range °C	Rate of Change °C/min	Duration mins
36.5-31.0	- 0.50	11	[a] 39.9-30.5	- 0.470	20
31.0-19.8	0.12	93	30.5-26.3	0.247	17
			26.3-21.8	0.112	40
19.8-10.7	0.08	109	21.8-17.3	0.067	67
			17.3-16.5	0.009	82
			[b] 16.5-11.9	0.147	31
			11.9-10.4	0.089	17
Total 36.5-10.7		113	39.9-10.4		274
AROUSAL - Air			AROUSAL - Air		
Temp Range °C	Rate of Change °C/min	Duration mins	Temp Range °C	Rate of Change °C/min	Duration mins
10.7-14.5	+ 0.047	80	10.4-11.2	+ 0.007	111
			11.2-14.1	0.026	110
14.5-17.3	0.053	52	14.1-17.7	0.064	56
17.3-21.5	0.182	23	17.7-21.0	0.110	30
21.5-32.3	0.272	40	21.0-25.2	0.210	20
32.3-35.6	0.143	23	25.2-35.2	0.400	25
Total 10.7-35.6		218	10.4-35.2		352

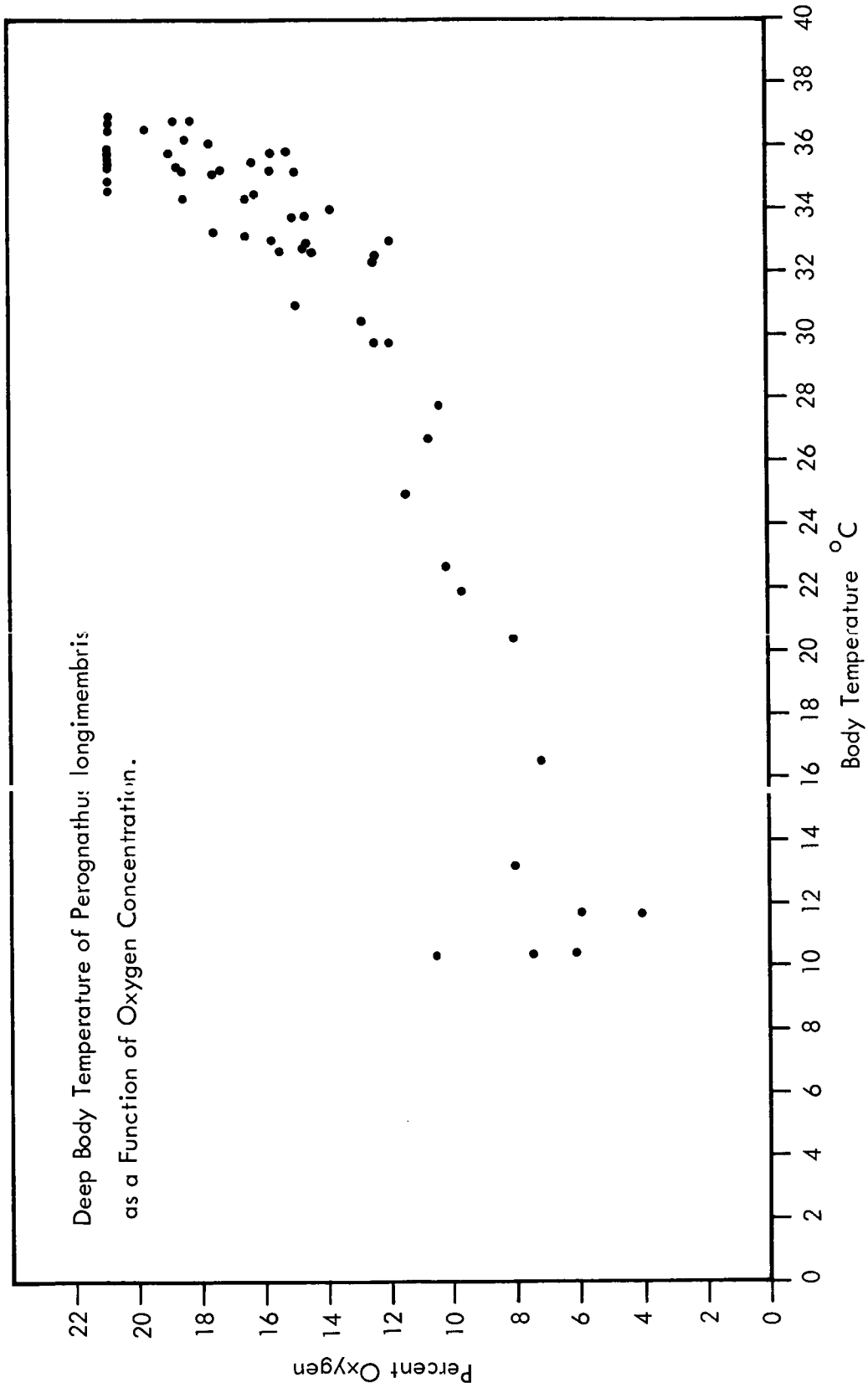

Fig. 2 Body temperature of P. longimembris maintained for at least an hour in various hypoxic atmospheres.

is a strong tendency to drop into a passive metabolism below this value and to go into torpor. Several attempts were made to monitor temperature changes with slight incremental decrease of oxygen concentration (in 1° steps from 21% - 15%). Although a step function in relation to oxygen can be noted, the most common are oscillatory fluctuations with some peaks in low concentration O_2 reaching higher temperatures than occurred in normal concentrations of oxygen.

The lowest body temperature that an animal has reached and rewarmed itself, under ambient conditions of the torpor, is 9.1° C at 6.8° C. This seems to put it well into the range occupied by hibernators, ground squirrel 11° C vs. rat 23° C (5). The conditions of this torpor were: air to 10% O_2 (180 mins.), to 7.2% O_2 (106 mins), to air (154 mins). Another animal was torpid for 14 hours at a body temperature of 7.5° C but was removed to room temperature to rewarm.

The temporal relationships between depth of torpor, ability to rewarm and survival have only been cursorily explored at this time, but several cases have been documented which are pertinent. Two animals were induced into torpor by stepwise reduction in oxygen concentration, one animal was maintained in the last stage of hypoxic mixture (10.6%) for 12 hours then switched to air, the other was subjected to 15.5% for 13 hours after induction into torpor with 12.5% then switched to air. The animal maintained in 10.6% before air, was alive after 44 hours but died sometime before 17° C when chamber was gradually warmed. The other animal was returned to room temperature after 36 hours where it regained normothermia. At this point it is not known if the experimental treatment was important or whether the time in induced hypothermia is in the marginal zone of life and death.

The maximum time that this species has been observed in natural torpor is 72 hours (6). This maximum duration is believed to have occurred under optimum conditions; time of year when natural prolonged torpor occurs, isolated from external noise and cold environment. It would seem logical that an induced torpor of 36 hours duration may represent a value near the limit compatible with survival.

It has been previously reported (3) that P. longimembris have been maintained in torpor for 140 hours. It is not known if this animal would have lived if rewarmed. It is quite possible that pocket mice exhibit the same kind of response to prolonged hypothermia as white rats which are able to live for 9-10 hours at $T_B = 15^{\circ}C$, but cannot be rewarmed after 5 hours (7), but pocket mice are on a prolonged time scale.

SUMMARY

1. P. longimembris can be induced by hypoxia into a state of deep torpor that seems to be very similar to natural torpor.
2. A critical body temperature of 22-24°C has been observed during cooling and warming, but has not yet been correlated with a specific physiological function.
3. The circadian rhythm of arousal does not seem to be affected by induction into torpor with hypoxic atmospheres.
4. Body temperatures as low as 9.1°C in an ambient of 6.8°C are compatible with natural rewarming processes.

RESPONSE OF POCKET MICE TO ENVIRONMENTAL EXTREMES

A. WATER BALANCE & FOOD CONSUMPTION IN TWO HUMIDITY REGIMES

Members of the genus Perognathus although adapted to desert life, probably are not subjected to such extreme dryness as we suspect. A major portion of the day is spent in a plugged burrow where the humidity is near 100% and they venture out in the evening when the temperature is relatively low and the humidity high. The sensible and the insensible water loss are directly related to the environmental humidity and to the body temperature of the mouse.

During some laboratory experimentation, animals may be placed under conditions that cause a negative water balance with resultant dehydration and weight loss. Questions have arisen on several occasions as to the transient nature of this weight loss and if a completely dry environment is incompatible with survival of this non-water drinking species.

MATERIALS AND METHODS

Two groups of 10 mice each were selected at random from the animal holding facility and brought into the laboratory. One group was placed on the shelf and exposed to conditions of 50-60% relative humidity. The other was fitted with lids so that dry air could be supplied near the bottom of the bottle, and waste air exhausted at the top. The air was dried by passing it through magnesium perchlorate and was metered to the individual bottles at about 200-250 ml min. from a manifold system. Humidity readings from a Serdex recording Hygrothermograph indicated essentially zero humidity at room temperature.

Each animal was given a weighed amount of sunflower seeds, wheat and rye seed.

All animals were weighed every 3-4 days and food consumption was determined twice during the 46 days of the study.

RESULTS & DISCUSSION

The weights of the two groups are plotted in Figure 3. Both groups lost weight rather rapidly at first but leveled out after a few days in their respective regimes. The animal holding facility has a higher humidity, in general, than in the laboratory and probably explains the weight loss of the group maintained on the shelf. The overall weight loss after 46 days of 7% and 11%, or a 4% greater weight loss of those maintained in dry air does not seem excessive. However, the possible method of maintaining this small difference is interesting. Table 2 is a summary of the kinds and amount of food eaten.

Table 2. Summary of kinds and amount of food eaten by two groups of pocket mice on different humidity regimes. (Values ± 1 SD)

Dry - <1% R.H

Total				
Wheat & Rye	Sunflower	Avg. Wt. gm	Wheat & Rye gm	Sunflower gm
22.4 ± 5.81	36.7 ± 3.96	8.59	2.61 ± .67	4.27 ± .46

Normal - 50-60% R.H

Wheat & Rye	Sunflower	Avg. Wt. gm	Wheat & Rye gm	Sunflower gm
30.3 ± 7.26	35.5 ± 3.30	9.38	3.23 ± .88	3.78 ± .35

It would appear that the animals in dry air preferred more sunflower seeds in their diet than did those in normal air. The total food consumed in each case was nearly equal (6.88 gms/gm mouse-dry vs. 7.01 gms/gm mouse-normal). This preference for sunflower seeds in the low humidity group may be a reflection of the greater demand for water. Sunflower seeds have a greater percentage oil content and would yield more water upon metabolism.

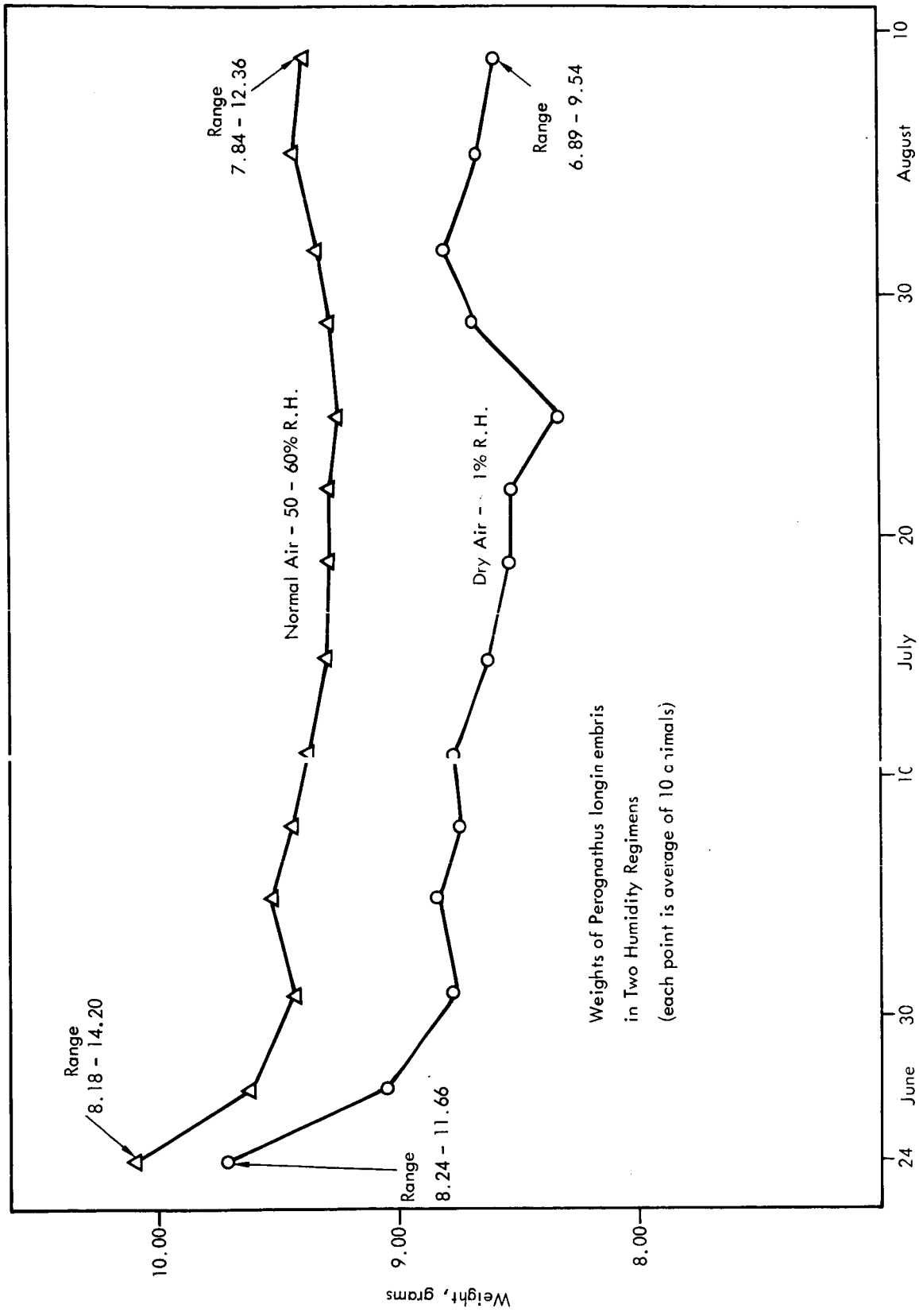

Fig. 3 Weights of two groups of pocket mice maintained in different humidity regimes with excess food for a period of 46 days.

SUMMARY

Pocket mice can live in an essentially dry environment with relatively little weight loss, if an adequate diet of high oil content seeds is provided.

RESPONSE OF POCKET MICE TO ENVIRONMENTAL EXTREMES

B. SURVIVAL AND WEIGHT LOSS IN 100% OXYGEN AT REDUCED PRESSURE

One solution to the problem of maintaining living beings in a viable atmosphere, in a closed system has been to use pure oxygen at reduced pressure to approximate the partial pressure of oxygen under normal conditions. This approach has certain engineering advantages. Except for minor difficulties, the absense of nitrogen and trace gases seem to have no short term adverse effects. The reduced total pressure, however, increases the rate of water loss and could completely off-set the balance in water metabolism of a non-drinking species of animal such as Perognathus sp. A preliminary experiment was run to gain some insight into possible problems.

METHODS AND MATERIALS

Two lucite chambers, 3" dia x 10" were devised and fitted with a pressure gauge, oxygen supply and gas removal connectors. Dry 100% breathing oxygen was metered into the chambers at about 150-200 ml/min. An absolute pressure of 1/3 atmosphere (10 inches of Hg) was maintained by a cartesian diver type regulator and a vacuum pump.

Four animals, Perognathus longimembris, were selected at random and placed in the divided chambers. Paper towels for grooming and urine absorbtion, and food (sunflower seed, wheat, millet & rye grain) were supplied to each chamber.

RESULTS AND DISCUSSION

Two mice were maintained under reduced pressure for 17 days and were returned to normal pressure four times for weight checks. On the 8th day two of the original four animals, with the least weight loss, were selected for the full run. Two were removed to provide more cage space. One of the animals removed at this time had been noted to

be excessively "jumpy" and weighed the least at the initiation of the experiment. By the 8th day this animal had lost 36% of its initial weight (as opposed to about 20% for other animals) and it died two days after removal.

The weights of the experimental animals are plotted in Figure 4. At the termination of the experiment, the animals were 7% and 14% less than their starting weight and it would appear that they would eventually regain all weight lost.

It has been demonstrated that genus Perognathus can be maintained under 100% oxygen reduced pressure for an extended period. The data also suggest that there may be a minimum weight animal that can be used. The heavier animals probably contain more fat that may be preferentially metabolized to liberate more metabolic water and help the animal over the adaptation period. A relatively light weight animal may have no "fat-buffer" for water and become dehydrated during the adaptation period to the point of non-recovery.

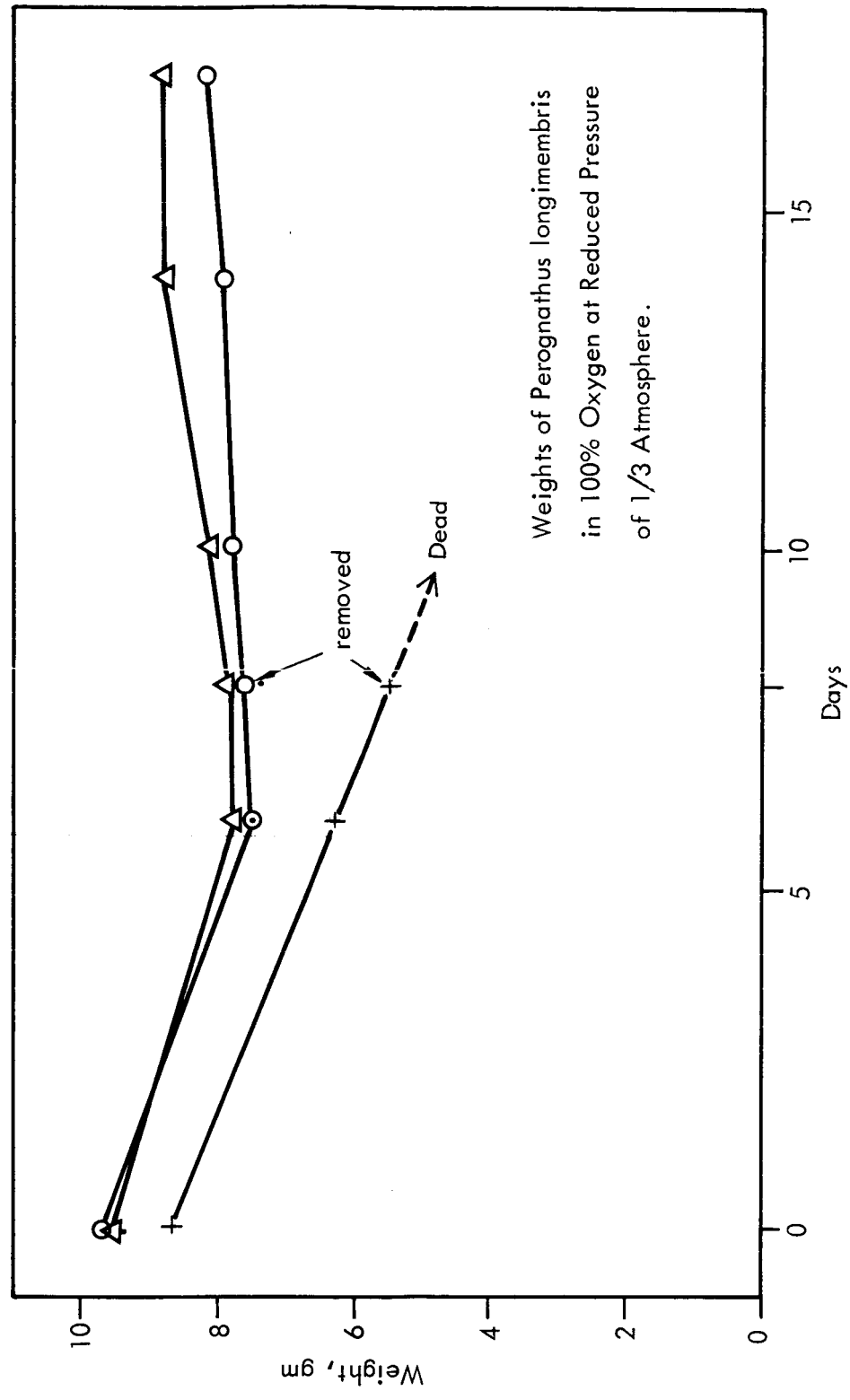

Fig. 4 Weiths of P. longimembris maintained in 100% oxygen, reduced pressure for 17 days. Two animals removed on 8th day, one of which was obviously distressed and died two days later.

C. PASSIVE LIFE SUPPORT SYSTEM USING POTASSIUM SUPEROXIDE

The use of a single chemical compound to provide oxygen and remove carbon dioxide and water from a sealed environment seems very applicable to small animal life support systems. Although some parameters for short term (24 hrs.) runs are known (8) the environmental stability of longer runs is subject to question. There is probably an over production of oxygen initially and a concomittant drop in water vapor with carbon dioxide being held at a relatively low value. These dynamic processes are expected to come to some kind of equilibrium as the KO_2 is used and a crust of potassium carbonate is formed.

The success of this kind of system may well be in the geometry of the active material and methods of passively regulating the reactants involved.

In order to obtain some preliminary empirical data on the total system-animal response with KO_2 as the atmospheric regulator, a mouse was sealed in a chamber for an extended period.

METHODS AND MATERIALS

The chamber, Bethlehem Corp. hyperbaric chamber, was fitted with a total pressure manometer, polarographic oxygen sensor and internal temperature sensor. The 4" x 4" x 10" plastic animal container rested on an antenna system so that deep body temperature data could be received from an abdominally implanted telemeter. The animal chamber had a sand substrate and sunflower, wheat and rye seeds were provided for food. A wire basket containing 50 grams of 2-4 mesh KO_2 was placed next to the animal container in the sealed chamber. The total system was passive, in that no fan was provided for internal circulation. Exchange of gases through the perforations in the animal chamber may have been aided by animal movements.

RESULTS AND DISCUSSION

In Figure 5 are plotted the results of the parameters measured in this experiment. After 18 days the experiment was terminated by choice. As suspected there was a heavy over production oxygen for about the first five days before some kind of stability occurred in the system. This area of dynamic stability lasted for about a week before oxygen consumption rates overtook oxygen productions rates and 0_2 concentration gradually fell to below normal atmospheric concentration. It is interesting to note that small peaks on the oxygen concentration curve, after the time of heavy 0_2 production, are directly correlated to periods of torpor in the animal. During torpor, the animal uses much less oxygen than when it is maintaining a normal mammalian type body temperature.

The periods of daily torpor in which the body temperature is not actively maintained and the animal cools to near ambient temperature, are given in the lower Figure 5. The times of entry into torpor and arousal from torpor are given in Table 3. As noted in other experiments, the arousal from torpor is a more clear cut marker to use if one is interested in determing the period length of this animal.

It is significant to note (in Table 3) that the duration or entry and arousal from torpor do not seem to be effected by concentration of oxygen or pressure within the system. It has been suggested (9) that barometeric pressure changes act as "zeitgebers" in the establishment of circadian periodicities. If this were true in this species, it would seem that this animal would have shown at least some confusion in its rhythm.

SUMMARY

1. A daily period of torpor was documented in a pocket mouse that was exposed to oxygen concentrations from 14-46%. The period length and duration were unaffected.

2. The use of potassium superoxide as an oxygen supply for long term small mammal experiments needs more investigation.

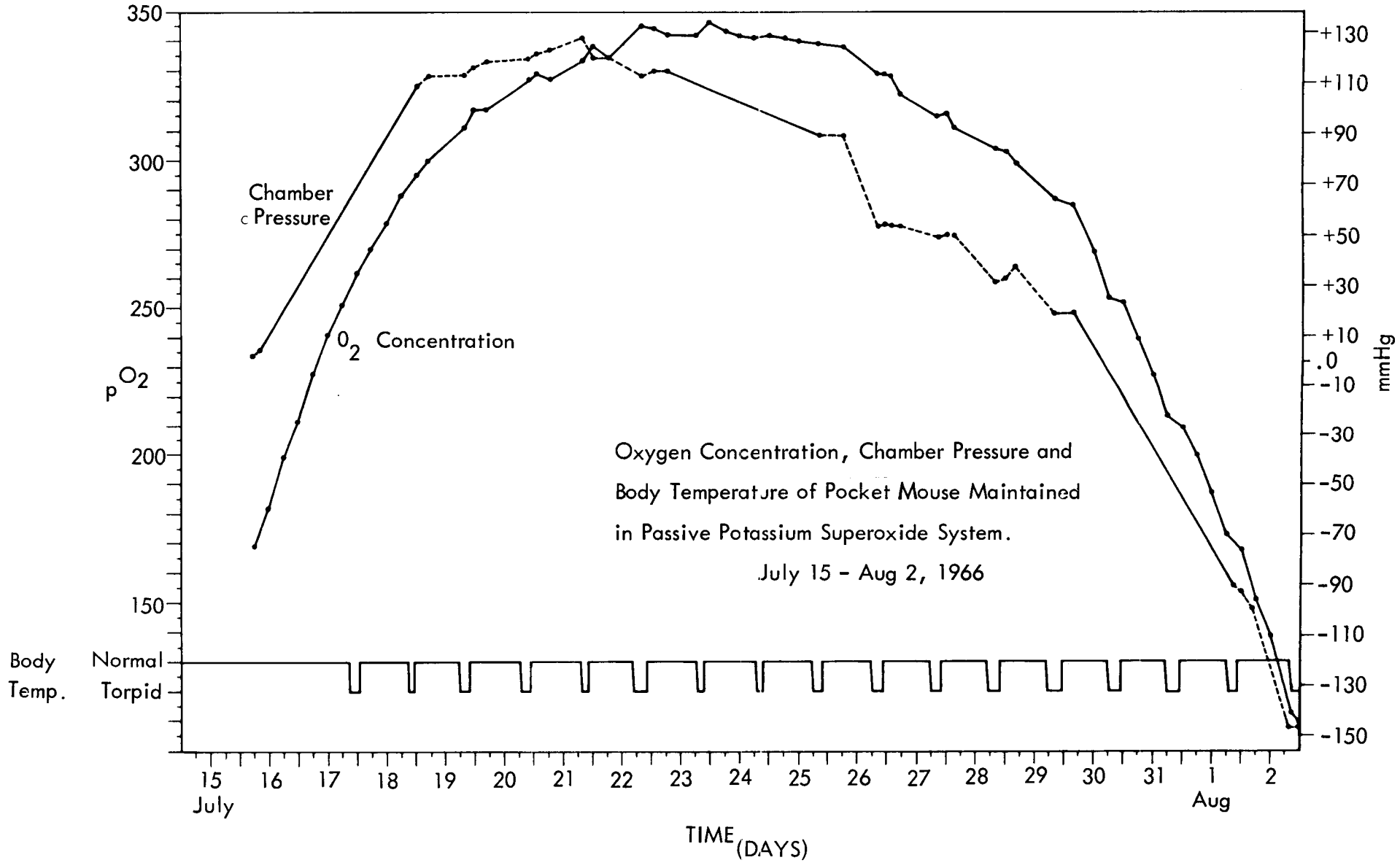

Fig. 5 Atmospheric measurements and body temperature of a P. longimembris sealed in a chamber for 18 days with potassium superoxide as oxygen supply, carbon dioxide and water vapor absorber.

Table 3

Daily entry and arousal from spontaneous torpor of Perognathus longimembris maintained in closed system with potassium superoxide as oxygen supply.

		Entry	Arousal	Duration Torpor	Oxygen Concentration
July	16	none	-	0	-
	17	0850	1300	250	20.9 - 41.8%
	18	0850	1030	100	-
	19	0530	1010	280	-
	20	0630	1040	250	-
	21	0640	1020	250	-
	22	0400	1000	360	-
	23	0610	0940	210	41.8 - 45.8%
	24	0640	0950	190	-
	25	0500	1005	305	-
	26	0520	1005	285	-
	27	0505	1015	310	-
	28	0435	1035	360	-
	29	0425	1115	410	-
	30	0540	1110	330	41.8 - 14.3%
	31	0500	1050	350	-
Aug.	1	0540	1000	260	-
	2	0700	1100	240	-

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