QUANTITATION OF WATER DEPRIVATION RESPONSES IN MERIONES UNGUICULATUS

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ABSTRACT

QUANTITATION OF WATER DEPRIVATION RESPONSES IN *MERIONES UNGUICULATUS*

By

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Studies were undertaken to determine the effect of prolonged water deprivation on the survival and metabolism of *Meriones unguiculatus*. Survival of this desert species on a dry diet varying in protein content followed the generalization that inability to conserve water when urea must be excreted limits longevity. On a diet containing 20% protein (Mouse Breeder Blox), mean survival was only 5 weeks. On a 9% protein (barley) diet, mean survival was in excess of 28 weeks yet the terminal whole body water content (64% of body weight) was the same for both groups. The conclusion was made that, although water deprived animals were in negative water balance, the proportion of water to protein remained essentially the same as the animals lost weight and was unaffected by dietary composition. The water content of ad libitum control animals (59.4%) under constant illumination was significantly different from that of "normal" controls.

The reasons are as yet, uncertain, but a possible endocrine effect has been suggested.

The effect of water deprivation on oxygen consumption has not heretofore been adequately investigated. Fertig and Edmonds (1969) demonstrated a fall in oxygen consumption for water deprived house mice, but these investigators failed to compare experimental and control patterns. In the present study, it has been shown that, aside from merely lowering oxygen consumption, water deprivation causes a "shift" in the system relating oxygen consumption (cc/day) to body weight (g). Quantitation of the response resulted in two significantly displaced regression lines representing control and water deprived animals. Simple prediction equations for oxygen consumption are, therefore, inadequate if the water status of the animal is not considered.

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Dedicated to my parents, Frank and Jessie
Norton, whose moral and financial
support made this research
possible.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The success of a species ability to survive in a desert environment depends upon its capacity to solve the problems of food, water, and body temperature maintenance. The factors which determine the availability of the first two commodities vary in different habitats and, as Chew (1951) has so aptly phrased it, can "influence the distribution and abundance of mammals" on earth. It is not a function of this dissertation to describe the ecological patterns of the world's deserts but only to examine some of the physiological mechanisms which arise from such an existance and which are necessary for survival.

The problem of food is one for which mammals have no physiological defense other than tolerance of starvation or semi-starvation. Food must be supplied by the environment. Survival depends upon it not only because food itself is needed for survival but also because it is a source of water; be it free or metabolic. The question which now arises is whether an animal can live in the absence of the second essential, water, if food is available. The answer will depend upon the animal's ability to balance water loss with oxidation or metabolic water and with that free water which may exist in the food.

Water is lost through the feces and urine, and by evaporation at the respiratory surface, while gain, in this case, can only be had via the food. Daily fluctuations in loss to gain or in gain to loss may exist but, in the long run, the animal must be in equilibrium if it is to survive. This investigation is meant to examine the tolerance to water deprivation of that group of desert mammals belonging to the order *Rodentia* and, in particular, a species of the genus *Meriones*, one of several in a large group of desert rodents commonly referred to as the desert gerbil.

Schmidt-Nielsen *et al*. (1948) have demonstrated the ability of the kangaroo rat (*Dipodomys merriami*) to exist on a diet of dry grain with no exogenous water available. Other investigators (Bartholemew and MacMillen, 1961; Kirmiz, 1962) have reported this independence of exogenous water in the kangaroo mouse, *Microdipodops pallidus*, and the jerboa, *Jaculus*. In contrast, Bartholemew and Hudson (1959) have shown that the antelope ground squirrel, *Citellus leucurus*, another desert rodent, has need of succulent plants in its diet. This dissertation attempts to classify the species *Meriones unguiculatus*, as either a "wet" or a "dry" desert rodent.

The third stress of the desert is that of heat and, as Schmidt-Nielsen (1964) points out, there are basically three ways in which an animal can meet it; evasion, tolerance, and thermoregulation. Evasion is generally accomplished by burrowing and by being nocturnal in nature. Misonne (1959) has reported that temperatures at a one meter depth below the surface usually vary within a range of 20-30 degrees centigrade; which is well under the surface temperature during the day. Consequently, burrowing animals do not meet with the severe stress of the desert heat that one might expect. Furthermore, most of the desert rodents are nocturnal, coming out at night to forage for food and remaining in burrows during the day. Hence they are further alleviated from the stresses imposed upon them by the severe desert heat such as the need to thermoregulate and the conservation of body water. To passively put up with the heat would involve an extremely high tolerance to increases in body temperature and, as yet, there are no indications that desert mammals are able to surpass the limits of heat tolerance found in most other mammals. The third mechanism is thermoregulation whereby the animal combats the heat by evaporation. This is the costliest method since it infringes upon the animal's precarious water balance and, in fact, poses a threat to survival. Desert species of the type studied here are not particularly tolerant of high temperatures, nor do they use water for heat regulation. It is the fact that most desert rodents are both fossorial and nocturnal that contributes to their capacity to withstand the aridity and temperature of the environment in which they live.

Gerbils are rodents of the family *Cricetidae*, subfamily *Gerbillinae*. They are referred to by a number of common names such as sand rats, desert rats, antelope rats, and jirds. Their natural diet consists of dry seeds and roots. They are distributed throughout Africa, Egypt, the Middle East, Southern Russia, Central and Eastern Asia, and India. The habitat of *Meriones unguiculatus* is described by Rich (1968) as extending from Mongolia to the northern sections of the Sinkiang, Shensi, Ordos, and Shansi provinces of China. Since there are twelve genera presently known, it is inadequate in reports such as this to ascribe only the word "gerbil" to the species under investigation. The name Mongolian gerbil, when presented in the literature, usually refers to *Meriones unguiculatus* and it is to this species that this report makes reference.

This research focuses on the environmental aspects of the animal's physiology with emphasis on water conservation and oxygen consumption. The problem of heat regulation in *Meriones* is not considered because, in their natural habitat, they exhibit the common behavioral characteristics described above as being typical of most desert rodents and, hence, are not subjected to the stresses of the desert heat. It only remains then to examine the problems imposed upon the animal by the desert dryness.

Of particular interest to this research was the ability to quantitate, or to describe in mathematical form, certain physiological responses as they relate to some independent variable.

The questions of interest were as follows:

1. Can ad libitum water consumption be successfully correlated to body weight in an interaspecific study such as this?

2. Is _Meriones_ _unguiculatus_ as adaptable to water deprivation as other desert species?

3. Does dietary composition have a significant effect on survival during water deprivation?

4. What effect does water deprivation have on mean oxygen consumption?

5. Can oxygen consumption be successfully quantitated in an intraspecific study in terms of cc/(g)(days)?

6. What effect does water deprivation have on percent body water and plasma osmolarity?

7. Are these six factors affected by an artificial environment such as constant illumination?

The particular parameters studied include food and water intake, body weight changes during water deprivation, oxygen consumption, body water, survival time as a function of dietary intake, and plasma changes during water deprivation.

REVIEW OF THE LITERATURE

The quantitation of a physiological process involves the establishment of a relation between a response, Y, and an independent variable, X, most notably surface areas, body weights, or survival times. The equations used for the expression of such relationships have been termed heterogonic (Adolph, 1949) since they reveal the degree of disproportionality existing between the two variables investigated. If, for example, the logarithms of a response Y are a linear function of the logarithms of X, then obviously the response must be proportional to a given power function of X. The equation is usually written in the form

$$Y = a X^{k} \tag{2.1}$$

in which Y is the dependent variable and X the independent variable. Taking the logarithms of both sides, the equation becomes

$$\log Y = \log a + k \log X \tag{2.2}$$

which has the same general form as the linear equation

$$Y = a + k X \tag{2.3}$$

Linear equation (2.3) represents a constant absolute increase in Y for a constant absolute increase in X, whereas logarithmic equation (2.2) represents a constant percentage increase for the two variables. Both equations are fitted to data by the method of least squares, but the procedure for equation (2.2) involves a logarithmic transformation of the measured data into terms of log Y and log X.

Brody (1964) has concluded that it is often more rational to assume that a given percentage deviation has about the same significance for a large as for a small animal than it is to assume that a given absolute deviation has approximately the same significance for a large as for a small animal. For example, if a 100 g animal looses 5 g of body weight it has lost only 5% of its initial weight, but the same absolute loss in a 10 g animal constitutes a 50% reduction in weight. As a result, the logarithmic equation (2.2) has a greater significance in such physiological quantitation than has the linear equation (2.3).

Controversy has arisen over the value of the exponent, "k," when body weight is the independent variable. Adolph (1949) suggested that the two-third power of body weight might be a common and reasonable coefficient of proportionality, whereas Klieber (1961) showed a greater proportionality between certain variables when the three-fourth power of body weight was used. Thus, studies on the interspecific comparison of metabolic rates in 26 groups of animals resulted in a regression coefficient of 0.756. Intraspecific comparisons, however, have usually resulted in higher exponential values. Lee (1939) reported a "k" value of 0.82 after metabolic studies on rabbits. Similarly, Benedict (1938), in his regression line for metabolic rates in mice, gave a "k" value of 0.89. Since animals are strikingly dissimilar in size and geometric shape, it seems illogical to equate "k" with a constant in dealing with intraspecific studies. It is more rational to take X^k as the reference base, the value of "k" being determined on the basis of actual data (Brody, 1964).

Studies in Ad Libitum Water Intake

The use of ad libitum water intake as an index of water requirements involves the assumption that it is an accurate index of the water intake to which the species is adapted. In support of this assumption, successful correlation between ad libitum water intake and habitat aridity has been shown for a number of rodents; mice originating in areas with more mesophytic vegetation drank more water than those from primarily xerophytic regions (Lindeborg, 1952). Schmidt-Nielsen (1964) has shown that those species of rodents most able to survive during water deprivation consume the least amount of ad libitum water and generally come from the most extreme desert habitats.

Lee (1963) found no correlation of water intake with habitat aridity in studies between coastal and desert *Neotoma lepida*. MacMillen and Lee (1967) noted that the Australian desert rodents, *Notomys alexis* and *Notomys cervinus*, which are generally independent of drinking water under moderate temperatures and a diet of carbohydrate-rich seeds, will drink water "greedily" in the laboratory. The jerboa, *Jaculus jaculus*, from the Sahara, has been observed to drink 4.3% of its body weight in water per day (Schmidt-Nielsen, 1964; see Table 1). It is apparent that the variables involved in ad libitum water consumption are numerous. Hudson (1962) found increased water consumption with rising ambient temperatures in studies on the desert ground squirrel, *Citellus leucurus*. Schmidt-Nielsen (1964) has reported that kangaroo rats, *Dipodomys merriami*, which ordinarily can survive water deprivation indefinitely, could not do so in relative humidities below 15%. Adolph (1943) showed that a decrease in water consumption occurs when food is restricted in studies on the laboratory rat. Williams (1959) has presented evidence that ad libitum water intake is affected by dietary composition. Alteration of dietary protein in studies on the deer mouse was directly related

Table 1. Ad Libitum Water Consumption in Some Rodents.

Species	Mean Weight (g)	N	Water Consumption % body wt./day	Reference
Sigmodon sp.	130.0	--	17.7	Spector, 1956
Citellus leucurus	98.8	12	13.4	Hudson, 1962
Neotoma fuscipes	186.7	10	24.5	Lee, 1963
Neotoma lepida	--	9	13.1	Lee, 1963
Peromyscus leucopus tornillo	31.1	20	5.5	Lindeborg, 1952
Dipodomys agilis	51.9	10	12.1	Hudson, 1962
Meriones unguiculatus	69.5	19	11.0	This study
Acomys cahirinus	--	--	11.38	Schmidt-Nielsen, 1964
Microtus p. pennsylvanicus	34.7	11	20.8	Lindeborg, 1952
Mesocricetus sp.	70.0	--	18.4	Spector, 1956
Acomys russatus	--	--	9.26	Schmidt-Nielsen, 1964
Jaculus jaculus	--	--	4.30	Schmidt-Nielsen, 1964
Meriones crassus	--	--	none	Schmidt-Nielsen, 1964
Gerbillus gerbillus	--	--	none	Schmidt-Nielsen, 1964
Dipodomys merriami	--	--	none	Schmidt-Nielsen, 1964

to the amount of water drunk. It is possible, therefore, that, although a general correlation between habitat aridity and water intake may sometimes exist, the measurements of ad libitum water consumption in the laboratory may not directly reflect the "normal" water consumption in nature. The natural environment itself is not usually "constant," unless behavioral adaptations carefully regulate the selection of the ambient environment.

The water requirements of *Meriones unguiculatus* appear to be intermediate between those of *Dipodomys merriami* and *Citellus leucurus*. Although both *M. unguiculatus* and *C. leucurus* require free water for maintenance, the former consume less water ad libitum (Winkelmann and Getz, 1962).

Adolph (1949) formulated an equation by which interspecific comparisons of ad libitum water intake could be made on the basis of body weight. His equation was heterogonic and expressed water intake as being proportional to the 0.88 power of body weight. Lindeborg (1952) found no meaningful correlation between water intake and body weight in mice from xeric and mesic habitats. Using a modification of Adolph's equation, Hudson (1962) compared the water consumption of 14 species of rodents with limited success. The trend relating water intake to differences in the habitat of the various species studied was also obscure. *Dipodomys merriami*, an animal from an extremely arid region, had a consumption equal to the predicted value, whereas the cotton rat, *Sigmodon sp.*, an animal from a moist habitat, had a water consumption higher than would be expected on the basis of body weight. *Microtus pennsylvanicus* and *Peromyscus leucopus tornillo* showed consumptions that correlated nicely with their habitats (Lindeborg, 1952), that is, mesic versus xeric, but did not follow the predicted values (Hudson, 1962). Lee (1963) has attributed this discrepancy to the heterogeneous conditions under which the data were collected. Hudson stated that comparisons of ad libitum water consumption on a relative weight basis are complicated by such variables as humidity, temperature, moisture content of the food and activity. These factors are often not explicitly acknowledged in the literature. Water intake for a number of species has been tabulated by Spector (1956).

Toleration of Water Deprivation in the Order Rodentia

"Dry" Rodents

The ability of rodents, particularly the desert species, to survive during periods of water deprivation has been extensively studied. Body weight changes, length of survival, and dietary intake are reviewed.

Desert rodents of the family *Heteromydae*: *Dipodomys sp*., *Dipodomys merriami* (kangaroo rats); and *Perognathus penicillatus pricei* (pocket mice) were able to maintain water balance on a diet of dry grain with no drinking water (Schmidt-Nielsen, *et al*., 1950). *Dipodomys merriami* survived for indefinite periods of time and were able to maintain their body weights on air dried food without access to drinking water when the diet consisted of pearled barley or rolled oats. Survival was limited to 16 days when a high protein diet of soybeans was given without water (Schmidt-Nielsen, 1964). Terminal weights in these animals was 60% of initial. Bartholemew and MacMillen (1961) have shown indefinite survival under water deprivation for a third genus, *Microdipodops pallidus*, the kangaroo mouse, when on a dry carbohydrate rich diet. Pocket mice, *Perognathus baileyi* and *Perognathus penicillatus pricei*, survived well without any moist food and appeared to be even more independent of moisture than the kangaroo rats (Schmidt-Nielsen, 1964).

A comparative study of the jerboa, *Dipus aegyptius* (family *Dipodidae*) and white rat, *Rattus sp*., was reported by Kirmiz (1962). On a dry grain diet (barley and wheat; 10% moisture) jerboas survived over a period of 1-3 years. Weight loss followed 10 months of water deprivation. In contrast, the white rats decreased food intake for the first three days of water deprivation after which they ceased to eat entirely. The finding for the rat was consistant with that of Adolph (1943). Laboratory rats refused to eat whenever some essential constituent of the diet (such as water) was lacking. As a result, the body weights of the rats diminished rapidly and survival was only one week (Kirmiz, 1962). Schmidt-Nielsen, *et al*. (1948) reported a 21 day survival for water deprived rats, *Rattus norvegicus*, accompanied by a 50% reduction in body weight. The capacity of jerboas to live on a dry diet was attributed to a reduced food consumption and metabolism, whereas the white rats ceased to eat after the third day on a dry diet. Total inanition as well as water fasting were considered the lethal factors (Kirmiz, 1962).

Data for the subfamily *Gerbillinae* has been reported by Burns (1956); Petter (1953); Schmidt-Nielsen (1964). *Gerbillus gerbillus*, from Egypt, *Meriones libycus* and *Meriones crassus*, both from the Sahara, lived well on dry food and survived indefinitely, often with an increase in weight. In a comparison of seven desert species, Schmidt-Nielsen (1964) showed *Gerbillus gerbillus* to be the most adaptive to water deprivation with *Jaculus jaculus* and *Acomys cahirinus* the least adaptive. The latter species suffered a 30-40% weight loss after three weeks of water deprivation and are reported to consume 11.38% of their body weight daily in ad libitum water.

Final mention of the "dry" rodents must include a recent study on the house mouse, *Mus musculus* (Fertig and Edmonds, 1969). Mice kept on a dry grain diet maintained themselves at full body weight for several months. When a high protein diet was introduced, the mice consumed less food, thereby subjecting themselves to a slow starvation. The mice tolerated a temporary loss of body weight of about 40%. High protein diets were lethal but urine concentrations often exceeded those of *Dipodomys.* The ability of *Mus musculus* to survive on limited water intake exceeded that of *Microtus* (Chew and Hindegardner, 1957). Independence of drinking water or succulent food is due to an extreme ability to reduce urine water loss by forming very hypertonic urine and by reducing evaporative water loss by a decrease in oxygen consumption (Chew, 1961).

"Wet" Rodents

Studies in water deprivation have been done on animals classified as "wet" rodents. Data on the North American pack rat, *Neotoma albigula*, has been compiled by the Schmidt-Nielsens, *et al*. (1948) and compared to the white rat, *Rattus norvegicus*. Like the kangaroo rat, the pack rats needed no source of drinking water but could not survive on air dried diets. Much of its moisture came from succulent vegetation. When given only air dried food, survival was only 4-9 days as compared to 15-21 days in the white rat, also a "wet" rodent. The rate of weight loss during water deprivation was similar for both animals but the pack rats died after only a 30% reduction in weight. White rats tolerated a 50% loss of weight. Adolph (1943) found a 46% reduction in weight and a 6-15 day survival time for water deprived rats. Emphasis has been placed on the seeming contradiction of a desert species being less tolerant to water deprivation than a relative of similar size with no special adaptation to a desert existance.

Findings for the sand rat, *Psammomys obesus* (Schmidt-Nielsen, 1964), and the carnivorous grasshopper mouse, *Onychomys torridus* (Schmidt-Nielsen and Haines, 1962), were similar to those of the pack rat.

Related species, *Neotoma lepida* and *Neotoma fuscipes*, have been studied by Lee (1963). Neither of these species are able to maintain initial body weight or to maintain a constant weight at a lower level when water is withheld. Survival of water deprived wood rats ranges from 2-16 days. *Neotoma lepida* experienced a 32.5% reduction in weight while *Neotoma fuscipes* lost a mean of 40.0% of their initial body weight. Animals which had experienced partial dehydration, rehydrated, and were then deprived of water, had survival times which were twice those of unacclimated animals. Similar results were found for Citellus leucurus (Hudson, 1962), but survival under water deprivation reached a maximum level for all "wet" rodents considered; 51 days.

Studies in Body Water

Khalil and Abdil-Messeih (1954) reported a lower water content in the tissues of desert animals than in other animals. Sokolov (1966) refuted these findings and found no such reduction in the tissues of desert rodents when compared to the dog, rat, and man. Schmidt-Nielsen et al. (1948) found the water content of kangaroo rats to be 66.5% after 7 weeks of water deprivation on a diet of pearled barley. Control animals averaged 67.2% after 54 days on fresh watermelon. Attempts at dehydration by feeding the rats a diet of dry soybeans resulted in an average body water content of 67.2% at the time of death. Weight loss in these animals was 66% of initial. Negative water balance was achieved but the proportion of water in the body remained the same as the animals lost weight. This implied that the animals were not really dessicated. Chew (1951) reported the same percent body water (66%) for other small rodents on water deprivation. Chew (1957) found a significant decrease in body water in water deprived mice, Mus musculus.

Oxygen Consumption in Relation to Body Size

The relation of metabolic rate to body size has been reported by a number of investigators (Adolph, 1949; Klieber, 1961; Brody, 1964). Interspecific comparisons have shown that metabolic rate is most nearly proportional to the three-fourth power of body weight or a regression coefficient (in the heterogonic equation) of 0.756 (Klieber, 1961). Brody's analysis was shown in his "mouse to elephant" curve.

Intraspecific comparisons of metabolic rate to body size have yielded slightly higher coefficients; 0.89 for mice (Benedict, 1938), 0.82 for rabbits (Lee, 1939), and 0.84 for dogs (Galvao, 1942). As a result, X^k has been suggested as the reference base; the value of "k" being determined on the basis of observed data (Brody, 1964).

Oxygen Consumption During Water Deprivation

The ability of desert rodents to reduce metabolic activity may be as important for water conservation as for energy conservation (Bartholemew and MacMillen, 1961). Schmidt-Nielsen (1964) has shown that a decrease in evaporative water loss accompanies a decrease in oxygen consumption. Since water conservation is essential to the water deprived animal, a reduction in oxygen consumption is therefore advantageous. Klieber (1961) reported an inverse relationship between oxygen consumption and days of starvation. Metabolism decreased with increased time on starvation in laboratory rats. Fertig and Edmonds (1969) have shown that water deprived house mice on a lethal diet (high protein) entered a state of torpor, as indicated by a reduced oxygen consumption, in order to conserve energy. The reduction in oxygen consumption was accompanied by a reduction in evaporative water loss. Adolph (1943) found a similar reduction in caloric output for water deprived rats.

A number of resting oxygen consumption values for certain rodents has been compiled in Table 2.

Table 2. Oxygen Consumption in the Order Rodentia.

Species	Mean Weight (g)	Oxygen Consumption ml/gm/hr (resting)	Ambient Temperature Degrees C	Reference
Dipodomys merriami	56.1	2.24	25	Schmidt-Nielsen et al., (1950)
Dipodomys merriami	56.1	1.47	28	MacMillen & Lee, (1967)
Dipodomys merriami	34.7	1.20	31	Dawson, (1955)
Dipodomys spectabilis	100.1	1.40	25	Schmidt-Nielsen, et al., (1950)
Dipodomys spectabilis	100.1	1.41	28	MacMillen & Lee, (1967)
Dipodomys panamintinus	56.9	1.20	33	Dawson, (1955)
Citellus leucurus	79.2	1.30	31	Dawson, (1955)
Citellus leucurus	96.1	1.03	30	Hudson, (1962)
Neotoma lepida (desert)	--	0.79	27	Lee, (1963)
Neotoma lepida (coastal)	--	0.72	27	Lee, (1963)
Neotoma fuscipes	--	0.79	25	Lee, (1963)
Mus musculus	33.0	2.92	25	Schmidt-Nielsen, et al., (1950)

Mus musculus (white)	29.2	3.61	25	Schmidt-Nielsen, *et al*., (1950)
Rattus norvegicus (white)	102.1	1.94	25	Schmidt-Nielsen, *et al*., (1950)
Microdipodops pallidus	15.2	1.30	35	Bartholemew & MacMillen, (1961)
Cricetus aureus	95.1	1.66	25	Schmidt-Nielsen, *et al*., (1950)
Peromyscus crinitus	23.0	2.98	25	Schmidt-Nielsen, *et al*., (1950)
Perognathus spp.	25.2	3.14	25	Schmidt-Nielsen, *et al*., (1950)
Reithrondontomys megalotis	9.0	2.50	33	Pearson, (1960)
Meriones unguiculatus	61-80	1.85	27	Robinson, (1959)
Gerbillus pyramidum	72-145	1.13	27	Robinson, (1961)
Notomys alexis	28.9	2.14	28	MacMillen & Lee, (1967)
Notomys cervinus	34.7	1.39	28	MacMillen & Lee, (1967)
Leggadina hermannsburgensis	12.6	3.43	28	MacMillen & Lee, (1967)

METHODS

Experimental Rationale

Meriones' response to prolonged water deprivation was observed over periods ranging from 20-40 days in several experimental trials. The designs of all trials were essentially the same although different parameters were investigated after each experimental run. The absolute values of the results obtained are of interest to this research only to the extent that they have led to a description or quantitation of a particular response. For example, the values for ad libitum water consumption are not as critical as the system which relates water consumption to body weight. The results formulate the system but the system alone describes the response.

With the exception of oxygen consumption, all parameters were observed directly. Oxygen consumption was found by calculation from the food intake and adjusted for body weight changes.

Experimental Design

Adult, male and female Mongolian gerbils, *Meriones unguiculatus*, were placed in individual cages containing a granulated corn cob litter and starved for 12 days in order to reduce body fat. A reduction in body fat was necessary to eliminate individual variations in percent body water inherent between animals. During the starvation period, water was available ad libitum. The animals were then transferred to clean cages, with no litter, and divided into two uniform groups of twenty animals each. One group was housed in a constant light laboratory while the other group was kept in the original laboratory under light-dark conditions (12 hours of light and 12 hours of darkness daily). The relative humidity in both laboratories ranged from 40-80%.

After the twelve days of starvation, each animal received approximately 50 g of Wayne mouse breeder blox (MBB) or barley (see Appendix I) and from then on food was added as needed. Food remaining at the end of the trial was measured and individual consumption was recorded as mg/(g mean body weight)(day). Ten animals from each group also received ad libitum water. The light-dark watered animals (LDW) were considered the normal controls. Body weights were measured every two days on a Mettler balance to the nearest 0.1 g beginning with the first day of the starvation period. Water bottles were also weighed at this time. Individual water consumption was measured using the techniques described by Bartholemew and Hudson (1959) using inverted water bottles fitted with L-shaped drinking tubes to reduce spillage. No correction was made for occasional spillage and evaporative water loss was negligible. Thus, the values for mean water consumption, and its error, may be slightly biased. Water consumed was expressed as g/(g of mean body weight)(day). Measurements made during the starvation period were separated from those made during the experimental period.

Determination of Body Water

After each animal died, its carcass was placed in a drying oven at 105 degrees centigrade and weighed periodically. A constant weight was recorded after three identical weighings. Body water was then recorded as a percent of the terminal wet body weight. Control animals were sacrificed after most of the water deprived animals had died.

Calculations and Assumptions in the Determination of Oxygen Consumption

Total food ingested was measured at the end of the experimental period and corrected for digestibility. Fat and starch were considered 90% digestible; protein was considered 80% digestible. The percent composition of the diets studied is given in Appendix I. Since these compositions are at 40% relative humidity, there exists a small percentage of water in each case. It was assumed that the oxidation of digestible foodstuffs led to the standard end products of carbon dioxide, water, and urea.

Table 3. Oxygen Consumption when Different Foodstuffs Are Oxidized.

Food Type	Gms. H_2O formed per gm. food	Liters of O_2 consumed per gm. food	Liters of O_2 used per gm. water formed
Starch	0.556	0.828	1.489
Fat	1.071	2.019	1.885
Protein	0.396	0.967	2.441

*From Schmidt-Nielsen, Desert Animals, 1964.

Oxygen consumption values were found using the values of Schmidt-Nielsen (Table 3) for calculating the amount of oxygen needed to oxidize one gram of starch, fat or protein. For example, the oxidation of one gram of starch requires the consumption of 0.828 liters of oxygen and from this gram of starch, 0.556 gms. of water are produced metabolically. To find the total amount of oxygen consumed, corrections had to be made for changes in body weight.

Estimation of Body Weight Changes

It was assumed that the composition of weight gain or of weight loss was probably a function of initial body weight. It was therefore necessary to estimate the initial and terminal body composition of each animal in order to determine what percentage of the weight change could be attributed to fat, protein, and water.

Terminal carcasses were ashed in a muffle furnace at a temperature of not less than 675 degrees centigrade for eight hours. The ash content of these carcasses was then expressed as a percent of the terminal wet body weight. The percent ash was considered constant during either weight gain or weight loss.

The animal with the highest percent body water was taken as the most "fat-free" animal. Its water content was associated with approximately 2.68% fat, a mean percentage calculated from the values of Pitts and Bullard (1968) on six small rodents trapped in the wild. These species were considered relatively "fat-free." The values for ash, body water, and estimated fat were subtracted from 100% to give this animal a protein percentage of 21.21% of its body weight.

J. T. Ried, _et al_. (1968) have shown, by their prediction equations for body composition in sheep, that the protein/water ratio during weight change remains essentially constant. It was assumed that this relationship was also true of rodents and, hence, knowing the ratio for one animal, it was possible to find the percentage of protein in the terminal carcass of every animal using the following identity:

$$\frac{69.57}{21.21} = \frac{\text{\% body water (measured in every animal)}}{\text{\% protein (unknown)}}$$

The percent fat in the terminal carcass of each animal was found by subtracting the values for water, protein, and ash from 100%.

Regression analysis of percent body fat versus terminal body weight showed that 40% of the variation in body weight could be attributed to percent body fat (Appendix II). Knowing the initial body weight, and using the regression line as the best predictor of percent body fat, the amount of fat present initially could then be estimated. The percent ash present initially was considered unchanged. The remaining percentage consisted of protein and water. The average protein/water ratio was 0.306156. Multiplying this value by the remaining percentage gave the percent protein present initially. Body water was found by multiplying the remaining percentage by 0.693844 or by subtracting the other components from 100%. The difference in composition between the initial and terminal weights constituted the body weight change. A detailed account of the procedure for estimating the composition of body weight change can be found in Appendix II.

The amount of fat and protein lost by water deprived animals was added to the total digestible food and the total amount of oxygen consumed was calculated. The amount of fat and protein gained by water ad libitum animals was subtracted from the food intake and again the total oxygen consumed was calculated.

Determination of Blood Osmolarity

Animals deprived of water over periods ranging from 20-46 days as well as ad libitum control animals were ether anesthetized and a 0.6cc. sample of blood obtained by direct heart puncture. Duplicate hematocrits were taken immediately. Blood samples were covered during the procedure to prevent evaporation and then centrifuged for 20 minutes. A 0.2cc. aliquot of plasma was withdrawn using a 0.2cc. diSPO Prothrombin Pipet (accuracy ± 2%; Scientific Products) fitted with an airtight gasket (Adams Suction Apparatus No. A-2473). In cases where blood samples were small, a 20μL pipet was fitted to the gasket. The 0.2cc. samples were diluted in 0.2cc. of ammonia-free distilled water; the 30μL samples were diluted in 0.3cc. of ammonia-free distilled water. All samples were placed in a Precision Systems Osmette calibrated to ± 3 mos. and the osmolarity measured in triplicate after three successful runs. A mean value was recorded for each sample. Comparisons were made between LD and LL (constant light) animals on high and low protein diets.

Statistical Considerations

Statistical significance was determined using either the Student's "t" test or the Analysis of Variance F-test for one-way classification (Sokal and Rohlf, 1969). Homogeneous within group variation was tested using the critical values of F_{max} (Sokal and Rohlf, 1969). When significance was revealed in the analysis of variance, the treatment sums of squares was partitioned into single-degree of freedom orthogonal contrasts for determination of the significant mean responses. Regression analysis of plasma osmolarity changes with time and percent body fat followed the procedures outlined by Sokal and Rohlf (1969). Quantitating equations were found using the method of least squares regression following a logarithmic transformation of the data points. Justification for the log-log scale in the survival response is best explained as "goodness of fit." A detailed account of the procedures can be found in the Appendix.

RESULTS

The rates of many and diverse physiological processes are proportional to some power function of body weight. It is possible, therefore, to express certain particular responses in such an equational form and thus to interrelate those parameters whose values are dependent upon the same variables. The purpose of this chapter is to present the results of a number of studies involving _Meriones_' response to water deprivation with significance levels for the treatment combinations (i.e., water deprived vs. controls; light-light vs. light-dark). A more detailed account of the statistical treatment is given in Appendix III. Whenever the data for a particular parameter are amenable to mathematical interpretation, the response is expressed in equational form. Predicted values can be obtained directly from the graphs or through a series of computations (see Appendix IV). Although several of the parameters already studied were quantified in this manner, the interrelationships of other closely allied responses were left for a future study.

Water and Food Consumption

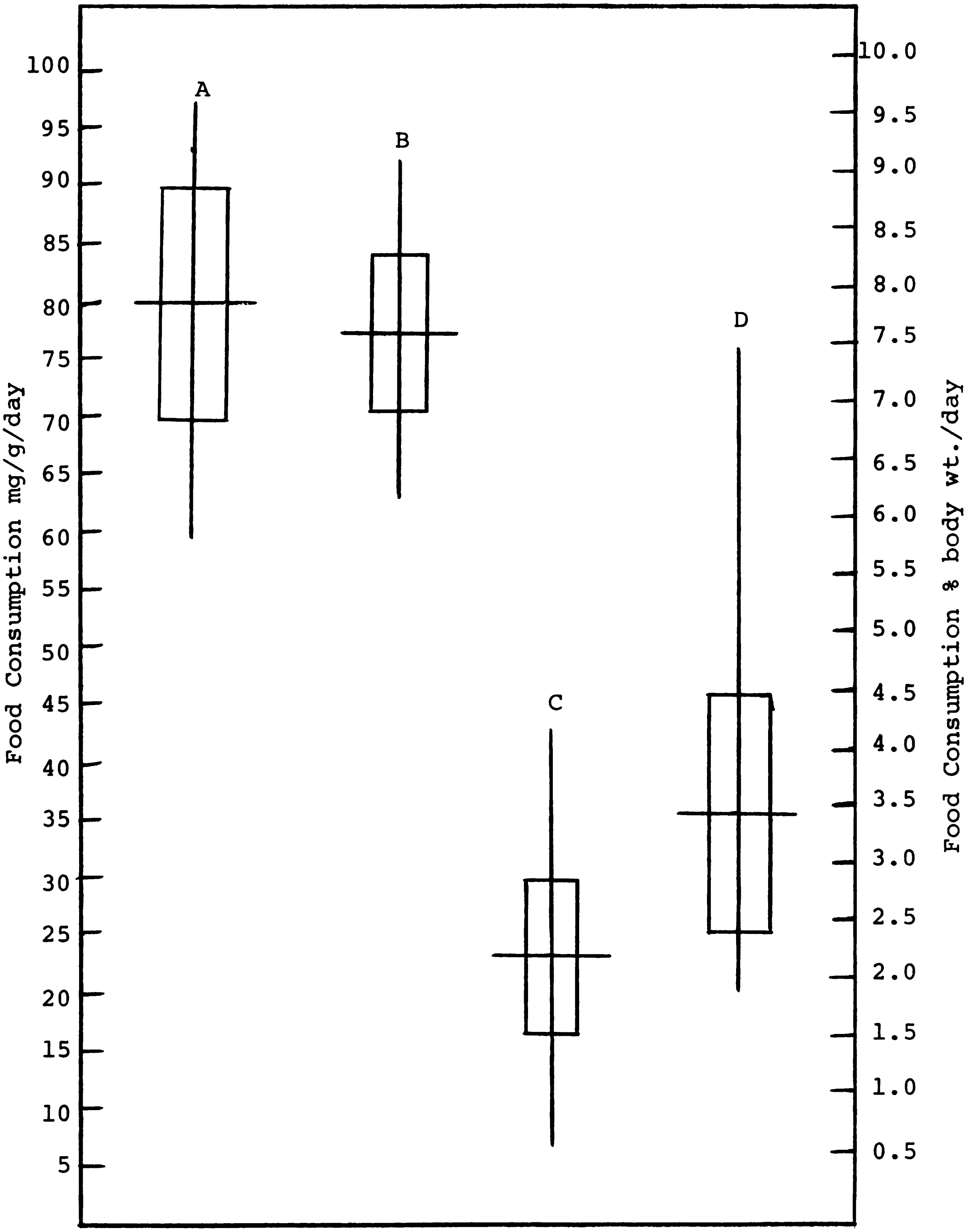
At room temperatures between 25-27 degrees centigrade, and with the relative humidity between 50-80%, the mean rates of ad libitum water consumption for light-dark (LD) and light-light(LL) animals (n = 20 animals not significantly different in body weight at time 0) were 0.113 g/(g mean body weight)(day) and 0.106 g/(g mean body weight)(day) when food was available. The difference in mean response was not significant ($p > 0.05$). When water consumption for these control groups was compared with water consumption during the initial starvation period, a significant difference was found. Mean water consumption during starvation was 0.046 g/(g mean body weight)(day) (n = 35) compared to 0.11 g/(g mean body weight)(day) (n = 19) when food was given ad libitum. The "t" value of 9.13 was highly significant at $p < 0.01$.

Food consumption during water deprivation was also measured (Figure 1). Ad libitum control animals under LD and LL conditions had an average food consumption of 80.59 mg/(g body weight)(day) and 77.69 mg/(g body weight)(day). Water deprived animals under LD and LL conditions had mean responses of 35.93mg/(g body weight)(day) and 24.95 mg/(g body weight)(day). A one-way analysis of variance was run to determine the significance of the four groups as a whole. The analysis resulted in an "F" value of 46.74 which was highly significant at $p < 0.01$.

Figure 1. Food consumption during water deprivation. Vertical lines represent ranges; horizontal lines represent the mean. Boxes imply ± 2 standard deviations from the mean.

A: LD Controls
B: LL Controls
C: LL Water Deprived
D: LD Water Deprived

(Width of vertical bars in Figure 1, and all similar figures, has no significance.)



Food Consumption mg/g/day
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
A
B
C
D
Food Consumption % body wt./day
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

Figure 1

An orthogonal breakdown of the treatment sums of squares resulted in the following null comparisons:

Ho_1: LDW animals = LLW animals (controls)

Ho_2: LDNW animals = LLNW animals (water deprived)

Ho_3: LDW:LLW animals = LDNW:LLNW animals (controls vs. water deprived)

The values of Q^2 for the first two nulls, testing the differences in mean response under light-dark and light-light conditions, were not significant ($p > 0.05$). It follows that constant light does not lower food consumption in animals under a similar water status.

The value of Q^2 for the third null hypothesis measured the significance between animals on a dissimilar water status. It was highly significant at $p < 0.001$.

From the above facts, it is evident that food deprivation causes a significant decrease in ad libitum water consumption and, likewise, that water deprivation significantly reduces food consumption. The absence of one dietary factor will lower the animal's response to the other. Light status apparently is not a significant factor.

Quantitation of Ad Libitum Water Consumption: The Heterogonic Equation

The time rate of water intake (cc/day) in relation to body weight (g) is shown in Figure 2. It is evident

Figure 2. Quantitation of ad libitum water intake; relation of water intake to mean body weight. $\log Y = 1.72 \log X - 2.309$

$r = 0.868$

$S_{y.x} = 0.0135$

Dotted lines represent 95% confidence on the line.

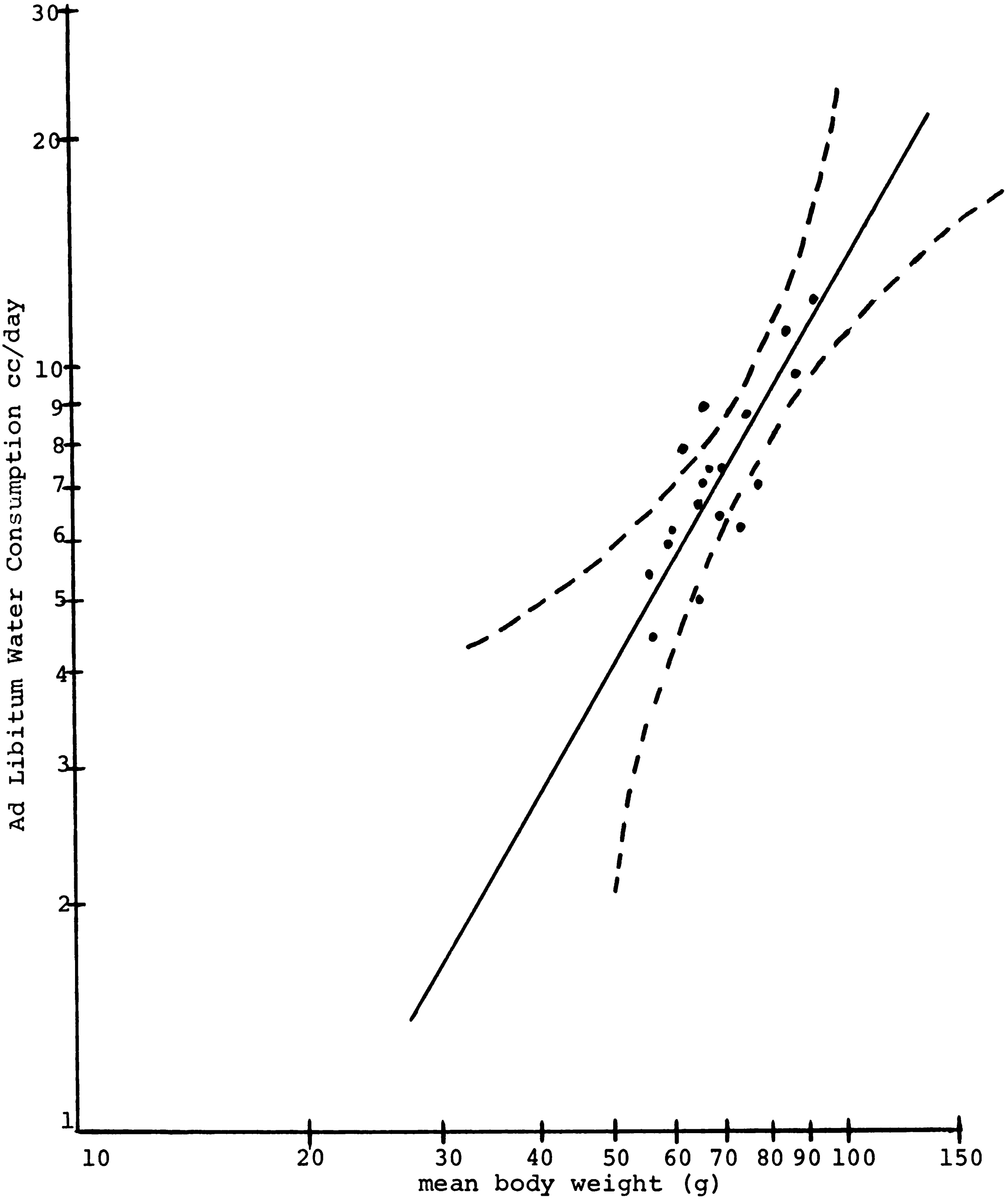

Ad Libitum Water Consumption cc/day
30
20
10
9
8
7
6
5
4
3
2
1
10
20
30
40
50
60
70
80
90
100
150
mean body weight (g)

Figure 2

that the logarithms of water intake are directly proportional to the logarithms of body weight. The data fit an equation of the form

$$\log Y = \log a + k \log X$$

where "k" represents the constant rate of change of the dependent variable function, log Y, for unit increments of log X, the independent variable ("a" is equal to $\overline{\log Y} - k\,\overline{\log X}$). The values of "a" and "k" were found and the line drawn using the method of least squares regression following a logarithmic transformation of the data points. The mean log of Y, for eighteen animals, was 0.8524, and the mean log of X was 1.8371. The slope of regression, "k," was 1.72. Expansion of the equation yielded

$$\begin{aligned}\log Y\ (\text{cc/day}) &= \overline{\log Y} - k\,\overline{\log X} + k \log X \\ &= 0.8524 - (1.72)(1.8371) + 1.72 \log X \\ &= 1.72 \log X - 2.309\end{aligned}$$

The mean drinking response, $\overline{Y}$, of eighteen animals averaging 69.5 g to ad libitum water was 7.39 cc/day.

The general form of this equation, $Y = a\,X^{k}$, expresses the degree of disproportionality between the physiological response, Y, and an independent variable, X.

The correlation coefficient, r, measuring the degree of interdependence between water consumption and body weight, was 0.868.

Effects of a Deficient Water Supply on Body Weight and Survival

Water deprived gerbils on a high protein diet of Wayne mouse breeder blox were unable to maintain their initial body weight or to maintain a constant weight at a lower level. The average weight loss experienced by constant light animals was 57.6% of their initial body weight. Animals under a light-dark cycle lost 58.2% of their initial weight. Upon the removal of drinking water, animals on a low protein diet of pearled barley experience an initial loss of weight followed by a plateau at about 66% of initial. At no time was a significant gain in weight observed for water deprived animals on either ration.

Survival of water deprived gerbils was found to be inversely related to protein intake (Table 4). Mean survival was slightly in excess of 22 and 35 days for LD and LL animals on a high protein diet. Barley fed animals had mean survival times of 92.3 days in LD and 199.9 days in LL. Statistical significance was found between the mean survival response of animals on the two diets as well as between animals on the same ration but under different environmental treatments (LD vs. LL). Animals under constant light survived for a significantly longer period than did animals on the same diet but under a light-dark cycle. Animals on a barley diet had

Table 4. Gerbil Survival Without Water.

mean survival (d)	protein intake*	diet	light	N
22.0	6.4	MBB	LD	11
35.0	6.1	MBB	LL	10
92.3	2.8	BARLEY	LD	8
104.3	2.3	SUNFLOWER	LL	7
199.9	2.4	BARLEY	LL	5

*mg protein/g mean body weight/day.

significantly longer survival times than those raised on a mouse breeder block ration ($p < 0.05$).

Tolerance to food deprivation might be an important physiological adaptation in desert species. In the absence of water, gerbil survival without food was only 21 days. A significantly longer survival period was observed in LD animals given ad libitum water (Table 5). The increase was also significant under LL but the mean response was only 30.4 days making it significant from water ad libitum animals under LD as well.

Table 5. Gerbil Survival Without Food.

	In LD: N	days ± S.E.	In LL: N	days ± S.E.
No water	18	21.0 ± 1.3	17	21.5 ± 1.7
Water ad lib.	16	41.2 ± 2.8	18	30.4 ± 1.7

Quantitation of the Survival Response To Water Deprivation

The effect of environmental treatment on the mortality rate of water deprived animals is shown in Figure 3. Least squares analysis resulted in two statistically different regression lines (Figure 3a; 3b). Figure 3a represents the mortality rate of LD animals and Figure 3b the mortality rate of LL animals. The logarithms of the percents of the population surviving (Y) was inversely related to the logarithms of survival days (X). The slopes of regression were $k_a = 1.58$ and $k_b = 0.41$. After a logarithmic transformation of the data points, the mean logs of Y for LD and LL animals were found to be 1.66 and 1.83 respectively. Likewise, the mean logs of X were 1.58 and 1.20. The heterogonic equations expressing the two responses are as follows:

LD Water Deprived Animals

$$\begin{aligned} \log Y &= \overline{\log Y} + k\,\overline{\log X} - k \log X \\ &= 1.66 + 1.58(1.32) - 1.58 \log X \\ &= 3.75 - 1.58 \log X \end{aligned}$$

LL Water Deprived Animals

$$\begin{aligned} \log Y &= \overline{\log Y} + k\,\overline{\log X} - k \log X \\ &= 1.83 + 0.41(1.20) - 0.41 \log X \\ &= 2.32 - 0.41 \log X \end{aligned}$$

Under constant light, 50% of the population were alive after 30 days of water deprivation whereas under a

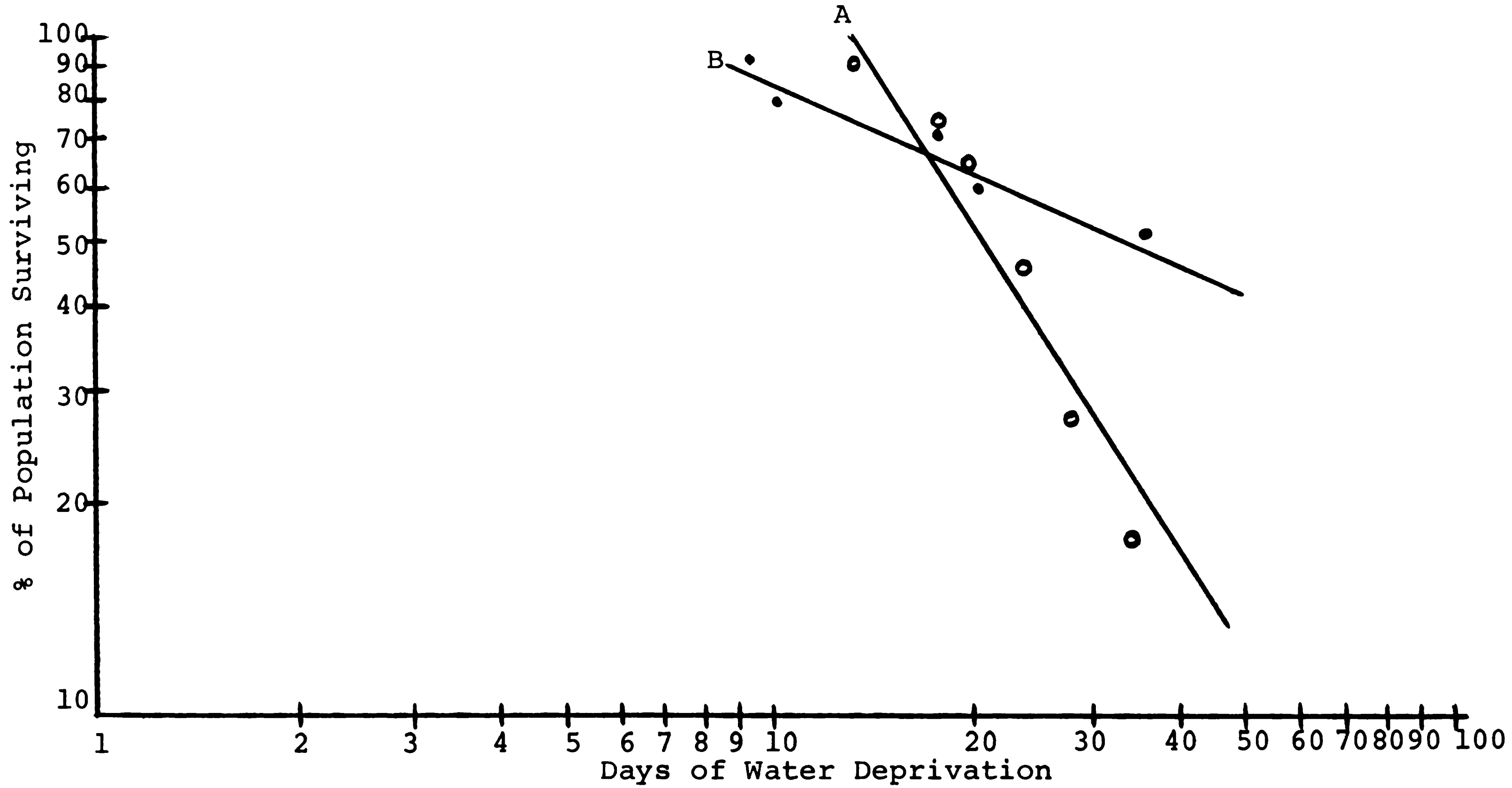


Figure 3. Mortality rates of LD (A) and LL (B) animals on water deprivation.
Figure 3A: $\log Y = 3.75 - 1.58 \log X$
Figure 3B: $\log Y = 2.32 - 0.41 \log X$

light-dark cycle 50% survived only 20 days. Since the function describing the survival response of LD animals appears sigmoid, the predicted values of the regression line may be slightly biased. Nevertheless, significance in survival was shown to exist between populations under a different light status.

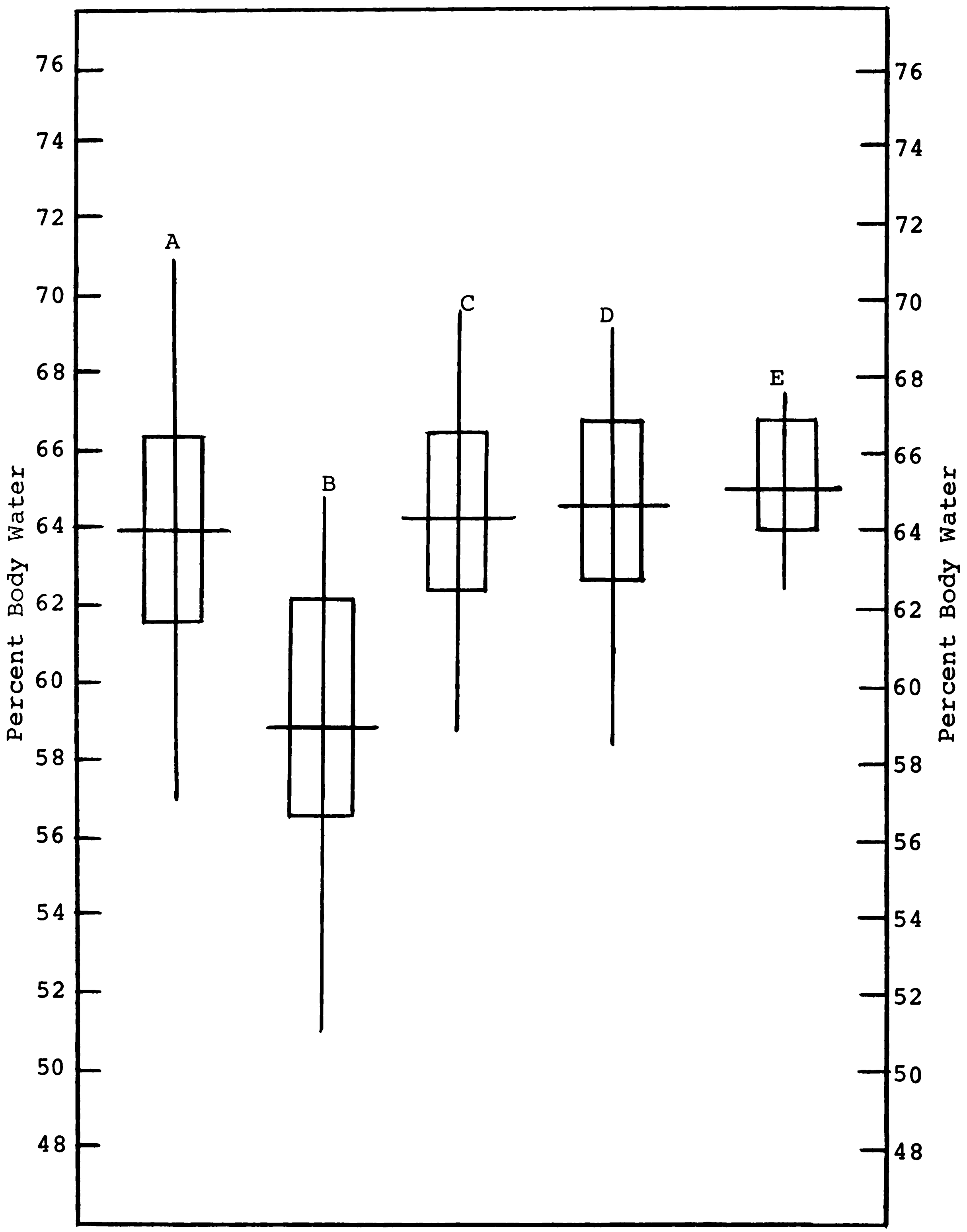
Body Water in the Water Deprived Animal in Light-Dark and Light-Light Environments

Since much of the variation in percent body water between individual animals can be attributed to percent body fat, animals were placed on a 12 day starvation period with ad libitum water. Afterwards, food was available ad libitum and 10 animals from each environmental treatment were water deprived. The starting weights of each group did not vary significantly. After 40 days of experimental treatment, body water was measured.

The results in Figure 4 represent percent water in the terminal carcass. LD and LL water deprived animals had an average percent body water of 64.49 and 64.51 respectively. Control animals averaged 64.06% under LD and 59.44% under LL. Water deprived animals on a diet of pearled barley and under constant light had an average percent body water of 65.8. A one-way analysis of variance determined the significance of environmental treatment. An "F" value of 5.052 was significant at $p < 0.01$.

Figure 4. Body water in the water deprived animal on high and low protein diets. Vertical lines represent ranges; horizontal lines represent the mean. Boxes imply ± 2 standard deviations.

A: (n=10) LD Controls MBB
B: (n=10) LL Controls MBB
C: (n=10) LL Water Deprived MBB
D: (n=11) LD Water Deprived MBB
E: (n= 6) LL Water Deprived Barley



Percent Body Water
76
74
72
70
68
66
64
62
60
58
56
54
52
50
48
A
B
C
D
E

Figure 4

Construction of orthogonal contrasts resulted in the following null hypotheses:

Ho_1: LDW = LLW

Ho_2: LDNW = LLNW

Ho_3: LDW:LLW = LDNW:LLNW

Comparison of barley animals with controls utilized the Student's "t."

The first and third null hypotheses were rejected at $p > 0.05$. No significance could be found between water deprived animals under LD and LL. Barley animals did not differ significantly from LD controls. The mean response of LL ad libitum animals was low enough to produce significance between water ad libitum and water deprived animals. When LD controls were compared to LD water deprived animals, no significance was found ($p > 0.05$). Thus, it was concluded that a significant decrease in percent body water resulted from a constant light environment when water was available. Since no significance was found between water deprived animals, it was concluded that the effect of constant light on body water is dependent upon the "water--no water" regime. Within group variation about the mean were homogeneous and hence not significant.

Body water after starvation was also measured (Table 6). No significance could be shown between LD and LL animals on a no water regime or between animals given water ad libitum. The populations were combined in column 3.

Table 6. Body Water After Starvation.

Treatment	In LD: n	% ± S.E.	In LL: n	% ± S.E.	Combined: n	% ± S.E.
No water	18	67.17 ± 0.32	17	66.72 ± 0.33	35	66.95 ± 0.23
Water ad lib.	16	68.47 ± 0.31	18	68.62 ± 0.44	34	68.55 ± 0.27
	p	0.004	p	0.002	p	0.002

A significant difference in percent body water was observed between water ad libitum and water deprived animals during starvation. Environmental light status apparently has no effect during starvation.

Effect of Water Deprivation on Mean Oxygen Consumption

Comparisons of mean oxygen consumption values between water deprived animals and controls are shown in Figure 5. The results are expressed in cc O_2/(g mean body weight)(day) on four treatment groups. LD controls had a mean oxygen response of 56.72 cc/(g mean body weight) (day) compared to 52.21 cc/(g mean body weight)(day) in LL ad libitum animals. No significance could be shown ($p > 0.05$) with the Student's "t." LD water deprived animals had a mean oxygen consumption value of 41.27 cc/(g mean body weight)(day) while LL water deprived animals consumed an average of 41.62 cc/(g mean body weight)(day). As with the control groups, no significance could be shown.

A one-way analysis of variance was run to determine significance among the four groups as a whole. The treatment sums of squares gave an "F" value of 5.3 which was highly significant at $p < 0.01$. An orthogonal breakdown of the treatment sums of squares into single degree of freedom contrasts was used to determine the significance of water availability (water status) since no

Figure 5. Comparison of mean oxygen consumption during water deprivation. Vertical lines represent the ranges: horizontal lines represent the means. Boxes imply ± 2 standard deviations.

A: LD Controls
B: LL Controls
C: LL Water Deprived
D: LD Water Deprived

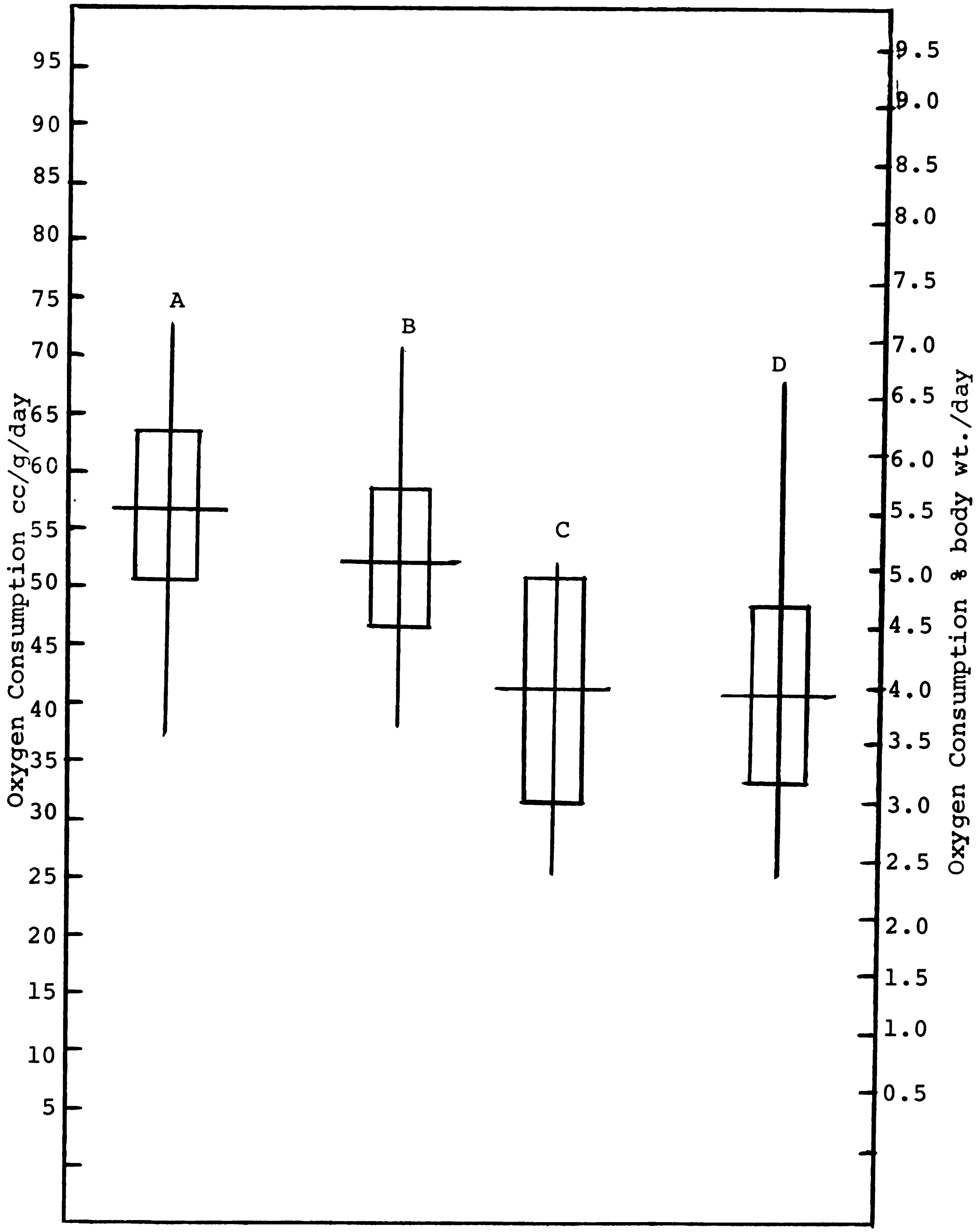

A
B
C
D
Oxygen Consumption cc/g/day
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
Oxygen Consumption % body wt./day
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

Figure 5

significance was found between environmental treatments (light status). The breakdown resulted in the following null hypotheses:

Ho_1: LLW = LLNW

Ho_2: LDW = LDNW

Ho_3: LDW:LDNW = LLW:LLNW

The values of Q_1^2 and Q_2^2 representing the first two nulls were highly significant at $p < 0.01$ and $p < 0.002$ respectively. Q_3^2 for the third null was not significant ($p > 0.25$), as could be expected from the results of the Student's "t." Water deprivation significantly lowered mean oxygen consumption in those animals so treated as compared with control animals on ad libitum water. No significance could be attributed to the two conditions of environmental light.

Effect of Water Deprivation on the Quantitation of the Oxygen Consumption Response

Graphic interpretation of oxygen consumption (cc/day) and mean body weight (g) resulted in two statistically significant regression lines. Figure 6a and Figure 6b represent data from ad libitum and water deprived animals respectively. As evidenced by the graph, the logarithms of oxygen consumption are proportional to the logarithms of mean body weight. The equations for the regression lines are again heterogonic. Following a

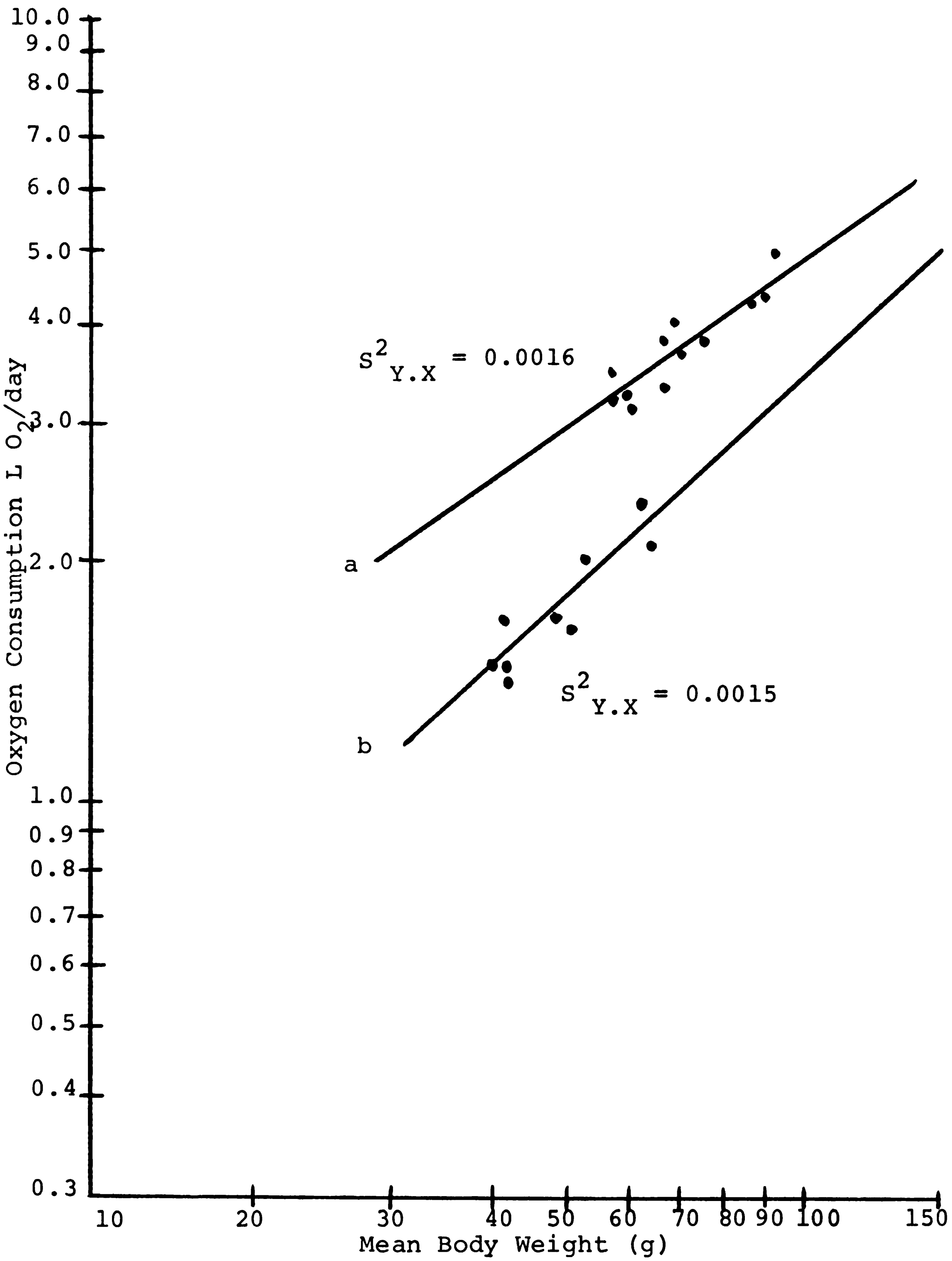


Figure 6. Effect of water deprivation on the quantitation of oxygen consumption. Figure 6a - controls; Figure 6B - water deprived animals. S^2 = standard error of the line.

logarithmic transformation of the data, the slopes of regression were found to be 0.736 for controls and 0.938 for water deprived animals. The slopes of the two systems are not significantly different. The degree of disproportionality existing between oxygen consumption and mean body weight is less than that found for water consumption and mean body weight. The mean logs of Y were 3.58 and 3.24 for ad libitum and water deprived animals respectively; the mean logs of X were 1.84 and 1.68. The prediction equations for ad libitum and water deprived animals are as follows:

Ad Libitum Controls (Y = cc/day; X = g)

$$\begin{aligned} \log Y &= \overline{\log Y} - k\,\overline{\log X} + k \log X \\ &= 3.58 - 0.736(1.84) + 0.736 \log X \\ &= 2.23 + 0.736 \log X \end{aligned}$$

Water Deprived (Y = cc/day; X = g)

$$\begin{aligned} \log Y &= \overline{\log Y} - k\,\overline{\log X} + k \log X \\ &= 3.24 - 0.938(1.68) + 0.938 \log X \\ &= 1.67 + 0.938 \log X \end{aligned}$$

It was concluded that water deprivation caused a significant "shift" in the oxygen consumption to body weight relationship.

The correlation coefficients, r, measuring the degree of interdependence between oxygen consumption and body weight were $r_a = 0.828$ and $r_b = 0.888$ for control and water deprived animals respectively.

Plasma Osmolarity Changes During Water Deprivation in Animals on High and Low Protein Diets

Blood osmolarity changes in animals on a water deficient diet varying in protein content were measured over a period of days (Table 7). Each animal was used once and therefore variations between individual animals were not accounted for. They appeared to be insignificant (see standard errors). Regression analysis of osmolarity (mOs/L) versus days yielded insignificant slopes ($k = 0$) for all groups. Comparisons of mean responses utilized the Student's "t" test. Animals under similar environmental treatments (LD and LL) were compared to each other to determine the effect of the diet on plasma osmolarity changes. Likewise, comparisons were made between animals on the same diet but under different environmental treatments. No significance could be attributed to light status ($p > 0.10$). Animals raised on a barley diet were not significantly different from controls. The difference between LL controls and LL animals raised on mouse breeder blox bordered on significance. Only one animal survived under LD and mouse breeder blox and hence, no meaningful comparison could be made. Since the slope of regression for LL animals on mouse breeder blox did not differ significantly from zero after 20 days of water deprivation,

Table 7. Plasma Osmolarity During Water Deprivation.

(N)	Sex	Water Status	Range of Days	Hct. %	Osmolarity mOsm/kg*	Diet	Light
3	M	ad lib.	33-46	40.95	305.10 ±9.56	MBB	LD
1	M	none	33	40.80	367.40	MBB	LD
6	M	none	33-46	37.95	321.66 ±10.90	Barley	LD
6	M	ad lib.	20-46	41.60	300.33 ±15.13	MBB	LL
5	M	none	20-33	40.16	341.92 ±7.35	MBB	LL
5	M	none	33-46	40.90	302.36 ±9.61	Barley	LL

* ± Standard Error

it was concluded that plasma osmolarity rose during the initial phase of water deprivation (although probably not to a significant level) and then leveled off during the remaining days of the trial.

DISCUSSION

Among the species of rodents listed in Table 1, the ad libitum water intake of Meriones unguiculatus (11% of their body weights/day) is between that of Dipodomys agilis and Acomys cahirinus. In contrast to the findings of Lindeborg (1952) or of Lee (1963), successful correlation of ad libitum water intake (cc/day) to body weight (g) was found for this species. The correlation coefficient of 0.868 is well above those of Lindeborg (which ranged from 0.032 in Noveboracensis bairdi to 0.536 in Tornillo blandus). The reason for such a contrast might be explained on the basis of individual methods.

In this study, prior to the measurement of ad libitum water intake, all animals were starved in an attempt to reduce individual variation in percent body water due to the presence of fat. Fat deposition acts as a "sink" in the physiological system and removes this tissue from the lean body mass. Fat adds to the weight of the animal but in no significant way does it reflect the requirements of the active cell mass. As a result, the highest correlation of a physiological process to body weight would be expected to occur when the body weight of an animal is a close approximation of the lean body mass (i.e., an essentially "fat-free" animal). Evidence for the assumption that ad libitum control animals were relatively "fat-free" during the experimental period is supplied by the fact that the percent body water in these animals did not differ significantly from that of water deprived animals. Water deprived animals (which continue to loose weight during water deprivation) were almost certainly low in body fat since the highest percent body water recorded was for this group. It is assumed, therefore, that the body weights of control animals more accurately reflected the lean body masses of the animals than did the body weights of the animals used by Lindeborg and Lee, and are the reason for the high correlation of water intake to body weight seen in this study.

Quantitation of ad libitum water consumption (cc/day) versus mean body weight (g) resulted in a regression equation of the form

$$Y\ (\text{cc/day}) = 0.0045\ X^{1.72}$$

On a weight relative basis (cc/g/day), the equation becomes

$$Y\ (\text{cc/g/day}) = 0.0045\ X^{0.72}$$

revealing that, relative to weight, water intake is proportional, not to body weight, but to the 0.72 power of body weight. The exponent of X, 0.72, is a close approximation of Klieber's (1961) use of body weight to the three-fourth power as the reference base for physiological quantitation. It must be said, then, that water intake is relatively size dependent.

Water deprivation is an extreme procedure and probably unnatural, but survival time without water may reflect the extent to which a species is adapted to limited amounts of water. In addition, this study has shown, that dietary intake is an important factor to the survival of water deprived animals in that survival time was found to be inversely related to protein intake (Table 4). Increasing the amount of dietary protein significantly reduced the time at which 50% of the population of water deprived animals survived. A significant increase in the survival of constant light animals was also observed but this was probably the result of a slightly increased protein intake in these animals since no significance could be shown in either the starting weights or in the oxygen consumption of these animals as compared to LD controls.

Aside from the small amount of metabolic water formed from the oxidation of protein, the formation of urea as a degredative product of protein metabolism necessitates an increase in the obligatory water needed for urine production. The combined effects of water deprivation and a high protein diet are lethal to *Meriones*. Animals fed a diet of pearled barley, however, had a significantly longer survival time which is attributed to a reduction in the need for urea processing.

It has been assumed (Howell and Gersh, 1935) that certain desert species like the kangaroo rat, are able to withstand a greater degree of dessication than other mammals. The present study does not concur with this assumption. Water deprived gerbils contined to lose weight on a high protein diet but, after 40 days of water deprivation, the body water percentage was not significantly different from the normal LD controls given ad libitum water (Figure 4). The difference between normal controls and barley-fed animals was also insignificant. On a starvation regime, however, water deprived animals had a significant reduction in percent body water when compared to animals given ad libitum water. Apparently, enough free and metabolic water can be obtained from the food intake to handle urine and evaporative water loss. The conclusion must be made that, although water deprived animals were in negative water balance, the proportion of water in the body remained essentially the same as they gradually lost weight. Even though a considerable amount of water had been lost by these animals their bodies were not really dessicated.

The average percent body water of 10 animals under constant light was significantly lower than that for normal LD controls. The reasons for this finding are as yet uncertain but a possible hormonal effect has been suggested. Piacsek and Meites (1967), working with underfed rats, found a decrease in reproductive function which they attributed to a deficiency of follicle stimulating hormone (FSH) and luteinizing hormone (LH) release from the pituitary. Reactivation of gonadotropin release (FSH: LH) in the starved rats was achieved under constant illumination. Estrogen secretion was indicated by an increase in uterine weight, enhanced mammary duct development, and by increased pituitary weight. Although the animals in the current study were randomly assigned to treatment groups, six of the 10 animals under constant light were females. It is suggested that estrogen secretion in these water ad libitum animals resulted in a synthesis and deposition of fat which lowered the mean percent body water of the group as a whole. Since no estrogen levels were measured, the problem, however interesting, needs further research.

Plasma osmolarity changes in the water deprived gerbil appear to be insignificant under both environmental treatments and type of diet. The plasma osmolarities of animals on a high protein diet were higher but not significantly higher than control or barley fed animals. Apparently, plasma osmolarity rises slightly during the initial phases of water deprivation after which the animal retains relative homeostasis. The initial stages of water deprivation are probably transient phases during which time the kidney is adjusting to plasma osmolarity changes. Homeostasis is regained but at a level slightly higher than normal.

It is known that a reduction in food intake occurs during periods of water deprivation in rodents (Klieber, 1961; Fertig and Edmonds, 1969). Moreover, during periods of relative starvation, there is a decrease in thyroid stimulating hormone (TSH) by the pituitary resulting in a depression of thyroid function. This decrease in thyroid activity causes an concommittant decrease in oxygen consumption by the tissues. In the present study, water deprived animals reduced their food intake and showed a 24% reduction in oxygen consumption below that of control animals (Figure 5), supporting the work of these investigators.

Robinson (1959) has reported a resting oxygen consumption value of 2.14 cc/(g)(hr) for *M. unguiculatus* at 25 degrees centigrade. In the current study, the mean value for oxygen consumption in control animals was found to be 2.27 cc/(g)(hr), which approximates Robinson's figure. When gerbils are housed individually rather than in groups, activity is very often at a minimum. It is assumed, therefore, that the values obtained for control animals approximated the normal resting condition of the animal, being only 6% above Robinson's figure.

The physiological significance of a reduced metabolic rate during water deprivation is two-fold. A reduction in energy expenditure, similar to that seen in aestivating mammals, would prolong survival by extending the period during which the energy reserves of the organism can last. Moreover, Fertig and Edmonds (1969), using water deprived mice, have reported a decrease in evaporative water loss following a reduction in oxygen consumption. It is, therefore, apparent that a decrease in oxygen consumption during water deprivation is as important for water economy as it is for energy conservation.

The system relating oxygen consumption (L/day) to mean body weight (g) is shown in Figure 6. Regression analysis of the data points resulted in two significantly displaced regression lines representing control Figure 6a and water deprived Figure 6b animals respectively; whereas the slopes of each system were not significantly different. Three significant physiological features can be elucidated from this regression analysis. First, water deprived animals show a significant reduction in oxygen consumption below control animals but the data points for this group do not constitute the lower end of regression line a, instead, the entire system correlating oxygen consumption to body weight is shifted downward so that animals of the same weight will have different oxygen consumption values depending upon their water status. The use of a prediction equation for oxygen consumption must, therefore, acknowledge the water status of the animal since two different systems relating oxygen consumption to body weight can be shown to occur.

Secondly, there exists a high degree of correlation between oxygen consumption (L/day) and body weight (g). The correlation coefficient (r_b) for water deprived animals, 0.888, is slightly but not significantly higher than that for controls ($r_a = 0.828$). Since oxygen consumption is a function of the active cell mass of an animal, the high correlations suggest that the body weights of the animals were a close approximation of the lean body mass; the starvation period significantly reduced the variation inherent between individuals due to the presence of fat.

The third significant feature of Figure 6 involves the use of the heterogonic equations which describe the response. The equations for control and water deprived animals were as follows:

Control Animals:

$$Y \text{ (cc/day)} = 54.95\ X^{0.736}$$

Water Deprived Animals:

$$Y \text{ (cc/day)} = 36.31\ X^{0.938}$$

On a weight relative basis, the equations become

Control Animals:

$$Y\ (cc/g/day) = 54.95\ X^{-0.264}$$

Water Deprived Animals:

$$Y\ (cc/g/day) = 36.31\ X^{-0.162}$$

the slopes of which are not significantly different from zero. This implies that on a weight relative basis (i.e., cc/g/day) oxygen consumption is virtually size independent in intraspecific studies. It is possible, however, that the range of body weights within this species is not significant enough to show the inverse relationship of O_2 (cc/g/day) to body weight (g) that is seen between species. It seems logical to assume that, given the conditions of this study (i.e., relatively "fat-free" animals) this relationship of oxygen consumption to body size probably would not exist in intraspecific studies.

SUMMARY AND CONCLUSIONS

1. Physiological processes which reflect the requirements of the active cell mass of an animal can be successfully correlated to the body weight of the animal. The highest correlations between the responses and the independent variable (body weight) will occur when the body weight is a close approximation of the lean body mass.

2. Quantitation of ad libitum water intake showed that water intake was proportional to the 0.72 power of body weight.

3. *Meriones unguiculatus* appear to be less adaptable to water deprivation than the kangaroo rat, *Dipodomys merriami*. On a dry barley diet, *Meriones*' survival is indefinite but does not reach the limits seen for the kangaroo rat.

4. Survival under water deprivation is inversely related to protein intake. Increasing the amount of portein in the diet necessitates an increase in the obligatory water needed for urea excretion and infringes upon the animal's water balance.

5. The percent body water of water deprived animals does not vary significantly from that of control

animals and is not influenced by dietary composition.

6. A significant reduction in oxygen consumption is observed during periods of water deprivation. The reduction is an important mechanism for energy conservation and water economy.

7. Water deprivation causes a significant "shift" in the system relating oxygen consumption (cc/day) to body weight (g). Thus, animals of the same body weight will have different oxygen consumption values depending upon their water status.

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APPENDICES

APPENDIX I

Percent Composition of Air-Dry Diets

	Mouse Breeder Blox	Sunflower Seeds	Pearled Barley
Water*	13.3	4.8	11.1
Ash	4.7	4.0	0.9
Starch	55.1	19.9	78.8
Fat	9.1	47.3	1.0
Protein	17.8	24.0	8.2
kcal/100gm	356	560	349

*water content variable with humidity; values are for equilibration with 40% R.H.

Metabolic Parameters for (a) Diets as Fed (40% R.H.) and (b) Fat Free Weight Loss

	Mouse Breeder Blox	Sunflower Seeds	Pearled Barley	Weight Loss
Caloric Density (kcal/100gm)	356	560	349	130
Protein/Energy Ratio (g/kcal)	0.0500	0.0429	0.0235	0.2419
Urea/Oxygen (mg/ml)	0.0685	0.0555	0.0341	0.3548
Urea/Total Water* (mosmols/liter)	1527	1644	721	2222
Total Water*/Oxygen (mg/ml)	0.748	0.562	0.788	2.664

*Total Water = free water in diet plus water formed by oxidation.

APPENDIX II

Regression Analysis of Body Fat Least Squares Prediction from Body Weight

Regression ANOVA

Source of Variation	d.f.	SS	MSS	F
Regression	1	481.274	481.274	25.21
Residual	39	744.662	19.094	
Total	40	1225.935		

$P(F = 25.21) < 0.001$

Least Squares Regression Line

$$Y = a + bX$$

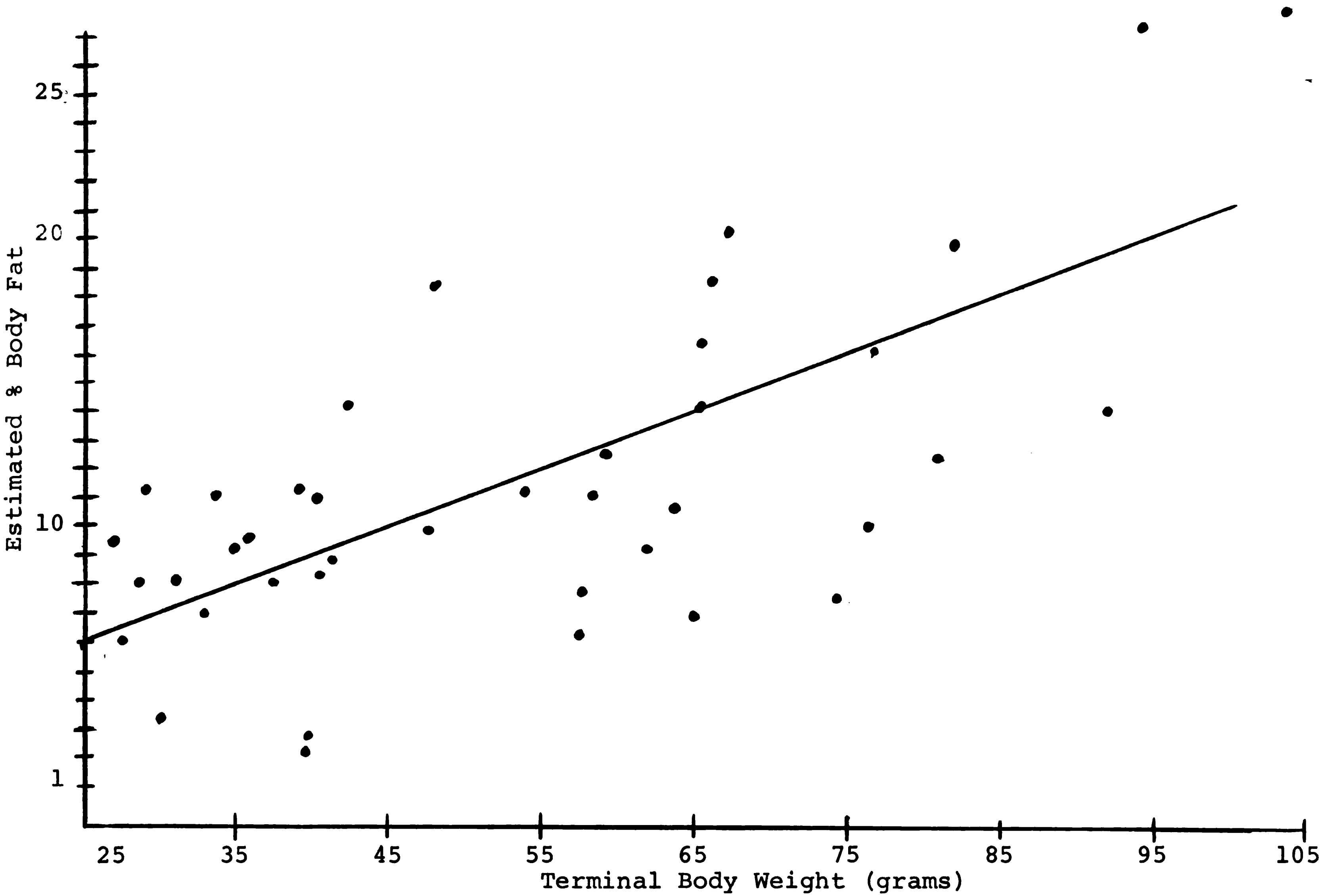
$b = 0.17 \quad a = \overline{Y} - b\overline{X} = 2.01$

$\overline{Y}$ = estimated mean % fat $\quad \overline{X}$ = mean body weight

$$Y' = 2.01 + 0.17X$$

39.25% of variation in body weight is due to fat content

$r = 0.625$



Regression analysis of body fat; least squares prediction from body weight. Correlation coefficient = 0.625. Y' = 2.01 + 0.17X

APPENDIX III

Statistical Treatment of Parameters Analysis of Variance and Orthogonal Linear Contrasts

N = number of observations
n = sample size
a = number of treatment groups
$E\underline{y}$ = grand sum
y = sample mean
Ey^2 = sum of squared observations
Ey_i = sum of sample n_i
CT = grand total squared and divided by N

The Sum of Squares:

SS (Total) = $Ey^2 - CT$

SS (Treatments) = $(Ey_i)^2/n_i - CT$

SS (Error) = $SS_T - SS_t$

The anova table is constructed as follows:

Source of variation	d.f.	SS	MSS	F
Among groups	a-1	SS_t	$SS_t/a-1$	MS_t/MS_e
Within groups	N-a	SS_e	$SS_e/N-a$	
Total	N-1	SS_T		

When significance is found with the F test, the among group sums of squares can be partitioned into single-degree of freedom contracts which are orthogonal and hence ask independent questions about the treatment combinations.

The Orthogonal Breakdown

There are as many orthogonal contrasts in a statistical analysis as there are degrees of freedom in the treatment sums of squares. An orthogonal set consists of scalars which sum to zero when any combination of "vectors" is cross multiplied. The test statistic is Q^2.

$$Q^2 = (\overset{\Sigma}{\mathrm{E}}\mathrm{MT})^2/\mathrm{N}\overset{\Sigma}{\mathrm{E}}\mathrm{M}^2$$

M = scalar value = 1,0,-1

T = treatment total

Q^2 = SS + sum of squares

The anova table is identical to that of the analysis of variance F test. The mean sums of squares are equal to the sums of squares since each contrast has but one degree of freedom.

Variance Homogeneity

$$F(\max) = S^2\max/S^2\min$$

$$p = 0.05$$

Method of Least Squares Regression (log transformation)

X = log X   Y = log Y

Ex = sum of independent variable

Ey = sum of dependent variable

Ex^2, Ey^2 = sum of squared variable

Exy = sum of cross products (SP)

b = slope of the line = SP/SS_x

a = ordinate intercept = $\overline{\log Y} - b\,\overline{\log X}$

$$Y = a + bX$$

Coefficient of Correlation "r"

$$r^2 = \mathrm{SSregression}/\mathrm{SStotal}$$

Student's "t"

$$t = \overline{Y}_1 - \overline{Y}_2/SE$$

t = test statistic

$\overline{Y}_1,\overline{Y}_2$ = mean response

SE = standard error = $(SE_1)^2 + (SE_2)^2$

"t" is significant when p is less than or equal to 0.05.

APPENDIX IV

Log Computation of the Heterogonic Equation

$$Y = aX^k$$

$$\log Y = \log a + k \log X$$

$$a = \overline{\log Y} - k\ \overline{\log X}$$

$$\log Y = \overline{\log Y} - k\ \overline{\log X} + k \log X$$

The value of any one Y can be found by taking the antilog. The least squares regression line uses only the logarithmic values of the data points.