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The role of the adrenal cortex in fat metabolism of Meriones unguiculatus, the Mongolian gerbil

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THE ROLE OF THE ADRENAL CORTEX IN FAT METABOLISM OF *MERIONES UNGUICULATUS*, THE MONGOLIAN GERBIL

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ABSTRACT

The effects of adrenalectomy and corticoid replacement therapies on survival, weight loss, depot fat and blood free fatty acid were studied in Meriones unguiculatus, the Mongolian gerbil. Adrenalectomized gerbils that received cortisol and aldosterone lived significantly longer and lost significantly more weight than the controls (adrenalectomized gerbils not receiving any treatment) and the sham-operated groups. Cortisol and aldosterone treatment were also effective in mobilizing significantly greater amounts of depot fat into free fatty acid. Cortisol and aldosterone treated groups had significantly less blood free fatty acid than the control and the sham-operated groups.

ACKNOWLEDGEMENT

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Finally, I would like to express my sincere gratitude to my parents for their constant support and encouragement during the course of my studies.

INTRODUCTION

Meriones unguiculatus, the Mongolian gerbil, is a small desert rodent with a distribution that includes the desert and semi-arid steppe areas of northern China and Mongolia (Rich, 1968). It was introduced in the United States in 1949 and since has gained interest among researchers primarily because of its great adrenal dependence for survival and unusual osmoregulatory capabilities.

Many investigators have pointed out that the gerbil has unusually large adrenal glands, its adrenal to body weight ratio being 3 to 4 times that of the white rat (Gorden et al., 1961; Roscoe and Fahrenbach, 1962; and Cullen and Scarborough, 1971). Roscoe and Fahrenbach (1962) suggested that the size of the adrenal glands may relate to the rate of steroid hormone synthesis in the gerbil, which is 10 times that of the white rat. It has been shown that the gerbil is highly dependent on the adrenal glands as adrenalectomy (ADX) causes death within 4 to 5 days in contrast to white rats which typically show survival of 16 to 17 days following ADX (Cowie, 1949).

A number of studies have indicated that M. unguiculatus shows an extraordinary capacity to conserve water and electrolytes, particularly sodium, and can withstand extreme osmotic stress without obvious physiological strain. Winklemann et al. (1962) and Arrington and Ammermann (1969) showed that compared to non-desert rodents the Mongolian gerbil has low water requirement.

Its daily water intake averages between 3.9-4.7 ml/100g body weight, approximately one-half the daily intake of white rats (Baker et al., 1979) and white mice (Green, 1966). Other researchers have indicated that gerbils can survive indefinitely on dry grains and seeds, ingesting no water per se and excreting minute amounts of highly concentrated urine (Rich, 1968). Hagood (1982) gave gerbils highly concentrated NaCl solution (6 %) as drinking water and found that urine output was reduced when compared to urine output of gerbils on low and sodium-free water. Moreover, under extreme hypo-osmotic (0 % NaCl) and hyperosmotic (6 % NaCl) conditions gerbils are able to maintain their body weight for a 9 week period (Freeman and Leftwich, 1981).

In mammals osmotic regulation is partly under the control of adrenal mineralocorticoids and removal of the adrenal glands causes death due to the loss of sodium retention ability. The results of several experiments suggests that unlike other mammals, the Mongolian gerbil may not be dependent on its adrenal glands for sodium regulation. Cullen and Scarborough (1970) and Kozub and Leftwich (1982) showed that adrenalectomized gerbils do not compensate for the loss of its mineralocorticoids by drinking salt (NaCl) solution and die within 4 to 5 days. In earlier comparable study, Richter (1936) using a two-bottle preference test showed that ADX rats voluntarily drink sodium chloride water instead of tap water. Furthermore, adrenal corticoid hormonal replacement therapy did not significantly prolong survival in ADX gerbils. In experiments by Cullen and

Scarborough (1970) and Kozub and Leftwich (1982) ADX gerbils given corticoid replacement therapy survived 17 days and 6 days respectively. In a comparable study ADX white rats survived indefinitely with hormonal replacement therapy (Pincus and Thimann, 1950 and Wolf, 1965). Histological changes in the adrenal glands of gerbils placed on 0 % and 6 % NaCl solution did not show glomerulosa hypertrophy or atrophy according to Freeman and Leftwich (1981). However, in the same study they observed increased mitotic figures in the *zona fasciculata* region of the adrenal cortex. This suggests that under osmotic stress gerbils may produce greater quantities of glucocorticoids. It is well documented that glucocorticoids secreted by the fasciculata region promote fat mobilization and elevate blood glucose levels (Turner and Bagnara, 1976). Experiments have shown that when rats are placed under adrenal stress an increased secretion of glucocorticoid is followed by an increase in mobilized free fatty acid (Exton et al., 1972 and Gasquet et al., (1975).

The purpose of the present experiment was to confirm the findings that adrenalectomy in the Mongolian gerbil causes rapid death and that adrenal corticoid replacement therapy only partially increases survival. Further it was desirable to determine if partial increase in survival is due to the animal's ability to maintain its body weight and to observe any co-relation between maintenance of body weight and fat mobilization in the adrenalectomized Mongolian gerbil receiving hormone treatment.

MATERIALS AND METHODS

Sixty-four, 13-18 weeks old male Meriones unguiculatus were obtained from Tumblebrook Farms, Mass.. The animals were individually housed in standard 8" X 8" X 10" wired cages and kept under a 12 hour light, 12 hour dark photoperiod in a secluded room with an ambient temperature of 24 - 26°C. Each animal was provided tap water and Rodent Laboratory Chow (purchased from Ralston Purina Company) ad libitum. The animals were acclimated for two weeks prior to the experiment and weighed 60-80 g just prior to surgery.

The gerbils were divided into groups of 16 animals each. Groups 1, 2, and 3 were bilaterally adrenalectomized (ADX) and treated with cortisol, aldosterone and propylene glycol respectively. Group 4 was sham operated and received propylene glycol (Table 1). From previous work, (Gaunt et al., 1971) it was shown that 1 mg/day of cortisol and 50 ug/day of aldosterone given to golden hamsters maintained 100 % survival during therapy (21 days). The same dosages were used in the present experiment and the appropriate amount was dissolved in 0.1 ml of propylene glycol. All injections were administered subcutaneously. Hormone treatment was started immediately after the surgery. All animals were weighed daily and their survival time recorded. Shams were killed on the eighth day after all adrenalectomized animals had died. The four groups were subdivided so that eight animals from each group were tested daily for blood free fatty

acid (BFFA). Blood was milked from the tail into 50ul heparinized micropipettes, and analyzed for free fatty acid by a colorimetric procedure described by Koichi and Michio (1965) with some modifications (Appendix). Values of free fatty acid were calculated as microequivalents (ueq) per ml of blood.

When dead, the animals were necropsied and visible body fat from the body cavity below the diaphragm was removed. This depot fat was then analyzed for total lipid content by a separation procedure using chloroform and methanol. (Johnson, A. R. 1971) (Appendix).

Data were subjected to Bartlett test. If significant F-values were found, Dunnett's Multirange test was used to determine statistically significant difference between groups. Duncan's test was applied to determine statistically significant groups for each parameter (α=.05).

RESULTS

Adrenalectomized (ADX) gerbils that received cortisol ($\bar{x}$= 5.9 days) and aldosterone ($\bar{x}$= 6.8 days) lived significantly longer than untreated ADX animals ($\bar{x}$= 4.1 days). The type of corticoid (cortisol and aldosterone) made no difference in survival time (Fig. 1).

All groups except the sham-operated group lost weight from the time of surgery until death. There was a significantly greater loss in weight of ADX animals that received cortisol ($\bar{x}$= 27.2 g) and aldosterone ($\bar{x}$= 24.8 g) than the untreated ADX group ($\bar{x}$= 13.3 g) and the shams ($\bar{x}$= 10 g) (Fig. 2). No other differences in weight loss were significant between groups. The pattern of weight loss differed between the groups (Fig. 3). Untreated ADX gerbils and ADX gerbils that received cortisol lost weight at approximately the same rate (avg. 17 g) for the first three post-operative days. The untreated ADX animals were all dead by day four and the average weight loss by the third day was 20 g. The ADX groups that received aldosterone and the sham-operated group lost weight at about the same rate (11 g) for the first three days following surgery. No further decrease in sham weight occurred whereas the aldosterone treated ADX group continued to lose weight until death.

ADX animals that received cortisol ($\bar{x}$= 0.20 g) and ADX group that received aldosterone ($\bar{x}$= 0.26 g) contained significantly less depot fat than did the sham ($\bar{x}$= 0.58 g) and the

untreated ADX groups ($\bar{x}$= 0.57 g). There were no other statistically significant differences in depot fat between groups.

Blood free fatty acid (BFFA) of ADX untreated group ($\bar{x}$= 1.4 ueq/ml) one day after surgery was significantly greater than cortisol ($\bar{x}$= 0.5 ueq/ml), aldosterone ($\bar{x}$= 0.76 ueq/ml) and sham-operated groups ($\bar{x}$= 0.48 ueq/ml). Values of BFFA for the second post-operative day showed a similar trend. The untreated group ($\bar{x}$= 1.3 ueq/ml) had a significantly higher BFFA than did the group receiving cortisol ($\bar{x}$= 0.34 ueq/ml), the group treated with aldosterone ($\bar{x}$= 0.46 ueq/ml) and the sham ($\bar{x}$= 0.58 ueq/ml) (Fig. 5).

DISCUSSION

In the present experiment ADX gerbils that received no treatment survived 4 days. This result is in close agreement with the findings of Cullen and Scarborough (1970), 5 days, Kozub and Leftwich (1982), 4 days and Jefferson (1983), 4 days. Furthermore, results of the present experiment also confirms the findings of Kozub and Leftwich (1982) that glucocorticoid or mineralocorticoid are both effective in partially increasing survival of ADX gerbils. Glucocorticoid (cortisol in the present study and dexamethasone in Kozub and Leftwich's experiment) increased survival by 2 days and aldosterone by 3 days. However, in an experiment by Cullen and Scarborough (1970) ADX gerbils receiving cortisol (1mg/day) survived almost 5 days longer than the non-treated group. This is slightly longer than survival time of cortisol treated group in the present experiment. Such a difference could be due to an age and sex of the animals used in the two experiments. Cullen and Scarborough (1970) used both sexes whereas males were used in the present experiment. Progesterone produced by the ovaries is known to act as an intermediate to a number of steroid hormones, including adrenal corticoid hormones (Turner and Bagnara, 1976). Experiments by Swingle and Remington (1944) and Cowie (1949) have shown that female white rats outlived males after adrenalectomy.

The age of the ADX animals at the time of surgery also

affects survival time. The survival period of white rats (both) sexes) increases with age probably reaching a maximum at about the fourth month (Cowie, 1949). Mongolian gerbils in the present experiment were 15-20 weeks old at the time of surgery and weighed 60-80 g. Cullen and Scarborough (1970) did not indicate the age of their gerbils; they did however, indicate that the weight of their gerbils ranged from 71-108 g at the time of surgery. This would suggest that their animals were older than those in the present experiment. However, the authors found no statistically significant differences between the sexes.

Mesocricetes auratus, the golden hamster a desert mammal like the Mongolian gerbil shows a relatively short survival period after adrenalectomy. Unlike the Mongolian gerbil, however, adrenal cortical replacement therapy does increase the hamster's survival considerably (21 days) (Snyder and Wyman, 1951; Gaunt et al., 1971; Nickerson and Molteni, 1971; and Salber and Zucker, 1974). Non-desert mammals, e.g. house cat, domestic dog and white rat are not as highly adrenal dependent. Upon replacement therapy with adrenal mineralocorticoid and/or NaCl solution as drinking water they live normal lives. (Pincus and Thiamann, 1950).

In the present experiment the untreated group progressively lost weight until death. The percent weight loss in gerbils receiving cortisol is similar to losses in Gerbillus gerbillus, the jerboas, as reported by Burns (1956). McManus (1972), in his experiment on water relations and food consumptions

of the Mongolian gerbil subjected animals to increasing concentrations of salt (NaCl) solution as drinking water. Considering weight loss to be an index of the degree to which NaCl solutions are tolerated he observed that these animals lost almost 50 % of their weight before death. This is higher than in the present experiment. Lethal weight loss due to desiccation (water deprivation) in the Mongolian gerbils was shown by McManus (1972) to be approximately 48 % at death. A closely related species Meriones crassus showed 36 % weight loss due to desiccation (Missone, 1959; cited by Chew, 1965). McManus showed that intact gerbils after being exposed to high concentrations of salt (NaCl) solution, and then absolutely deprived of water continued to lose weight. He attributed weight loss to desiccation.

Figure 3 shows daily percent change in body weight. All groups including the sham showed initial weight loss immediately after surgery. This is most likely due to surgical trauma. From day 1 to day 3 after surgery aldosterone treatment was effective in maintaining body weight of ADX gerbils close to that of the sham. If weight loss during this period were due to water loss, aldosterone as a mineralocorticoid was affective in preventing this in the Mongolian gerbil. The cortisol treated group on the other hand showed progressive weight loss as did the untreated ADX group. It is interesting to note that weight loss after the third post-operative day in the aldosterone treated group was less compared to the cortisol treated group.

Moreover, the aldosterone treated group showed a slight weight recruitment on day 6 just before the animals died. These findings suggest that adrenal mineralocorticoid initially play a significant role in the ADX gerbils. However, these results also indicate that although replacement with cortisol or aldosterone increased survival in ADX gerbils, such an increase was not due to the animals ability to maintain their body weight. Hence, these findings lead to the question of the cause of progressive weight loss. Losses in body weight in animals under stress and/or starvation could be attributed to three factors: water loss; reduction in body fat mass due to lipolysis and wasting of musculature because of gluconeogenic activity which involves break down of muscle protein. Part of the present experiment was designed to determine if progressive weight loss in cortisol or aldosterone treated groups was due to lipolysis in depot fat. Depot fat in the present experiment were analyzed at death of the animals. It is therefore not possible to statistically compare the differences in the four groups. However, as the shams maintained a steady weight from day 6 on and cortisol treated gerbils died on the 6th day depot fat of these two groups are compared. The ADX group that received cortisol had significantly less depot fat than the shams, indicating lipolytic activity of glucocorticoid. Although there is no evidence in the literature that aldosterone has any lipolytic actions, results of the present

experiment suggest that it may have some in the Mongolian gerbil. Aldosterone treatment was as affective in reducing fat mass presumably due to lipolysis, as there was no significant difference in depot fat between this group and the cortisol treated group. These findings support the hypothesis that progressive weight loss in ADX Mongolian gerbils was partly due to loss in fat mass. Lipolytic actions of glucocorticoids have been shown in other animals. Exton et al. (1972) and Gasquet et al. (1975) pointed out that in bilaterally ADX white rats glucocorticoid replacement therapy was affective in increasing adipose tissue lipoprotein lipase activity.

BFFA (blood free fatty acid) in the gerbil was measured to see if lipid mobilized by corticoid hormones was reflected as increased FFA (free fatty acid) in blood. The results were contrary to expectation. Both cortisol and aldosterone were effective in keeping BFFA level close to that of the sham. It is therefore suggested that maintenance of normal BFFA may be an early affect of the hormones and that this may ultimately be a controlling mechanism for the ADX gerbil's partial increase in survival when given adrenal corticoid hormone treatment.

In conclusion, based on the results of the present study and other studies it seems unlikely that cortisol or any other adrenal hormone alone could completely prevent adrenal insufficiency in the Mongolian gerbil. Indeed, even with replacement therapy with adrenal hormones, survival increased by only 3-4 days. It has been shown that M. unguiculatus is

unusual in producing significant amounts of 19-hydroxy-11-deoxycortisol. Oliver and Peron (1964) demonstrated that the gerbil produces equal quantities of this hormone and cortisol. Studies on white rats have shown a 19-nor-deoxycorticosterone to be a potent mineralocorticoid (Gomez-Sanchez et al., 1979 and Perrone et al., (1980). It is possible that the 19-hydroxy-11-deoxycortisol or other 19-hydroxy steroid may play a major role in the Mongolian gerbil's adrenal dependency, or the gerbil may depend on an untried combination of mineralocorticoids and glucocorticoids.

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Table 1. Treatment of (Meriones urguiculatus) Mongolian Gerbils

GROUP	SURGERY	TREATMENT
1	ADX*	Cortisol
2	ADX	Aldosterone
3	ADX	Propylene Glycol
4	SHAM	Propylene Glycol

* Adrenalectomized.

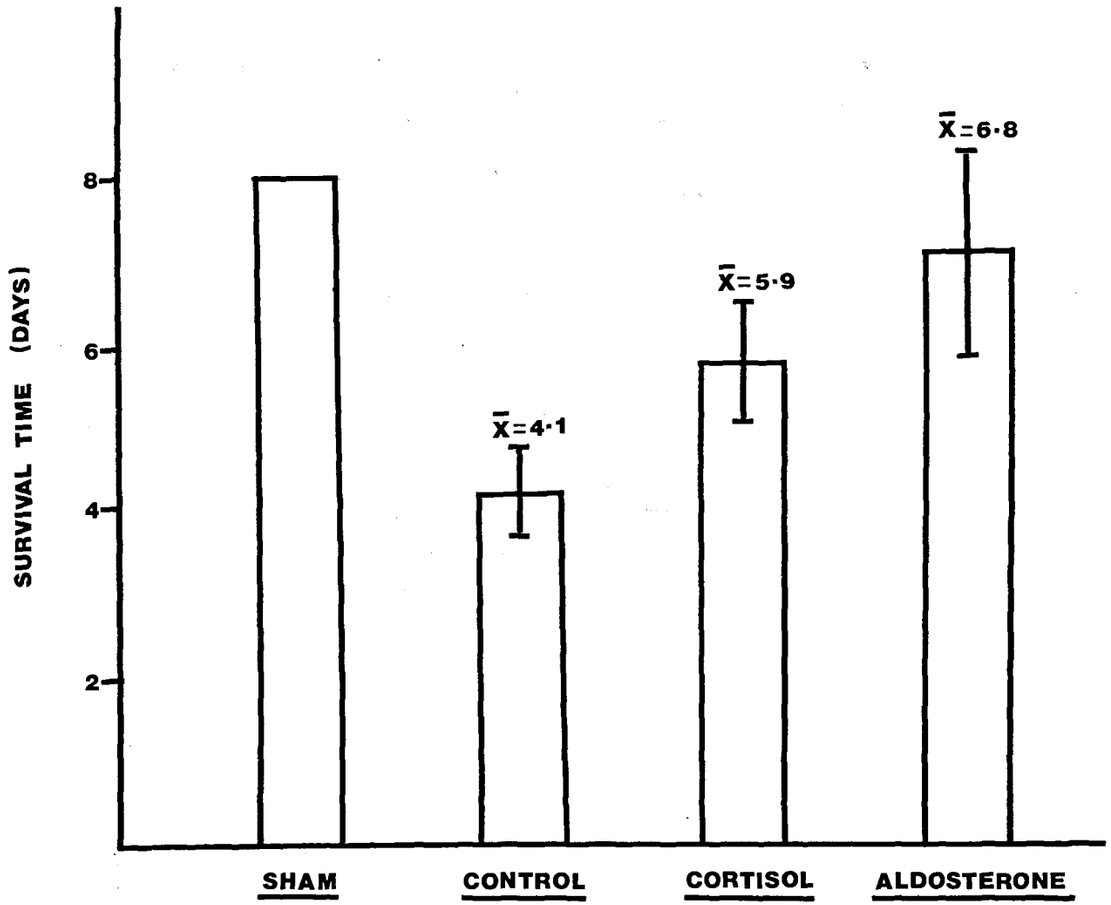
Figure 1. The effects of cortisol and aldosterone on survival of adrenalectomized Meriones unguiculatus, the Mongolian gerbil± Standard Error (N= 16).

Means underscored by the same line do not differ significantly at p= .05 (Duncan's test).

$\bar{x}$= mean survival (days)

Shams were killed on Day 8.

Control = ADX, untreated group.



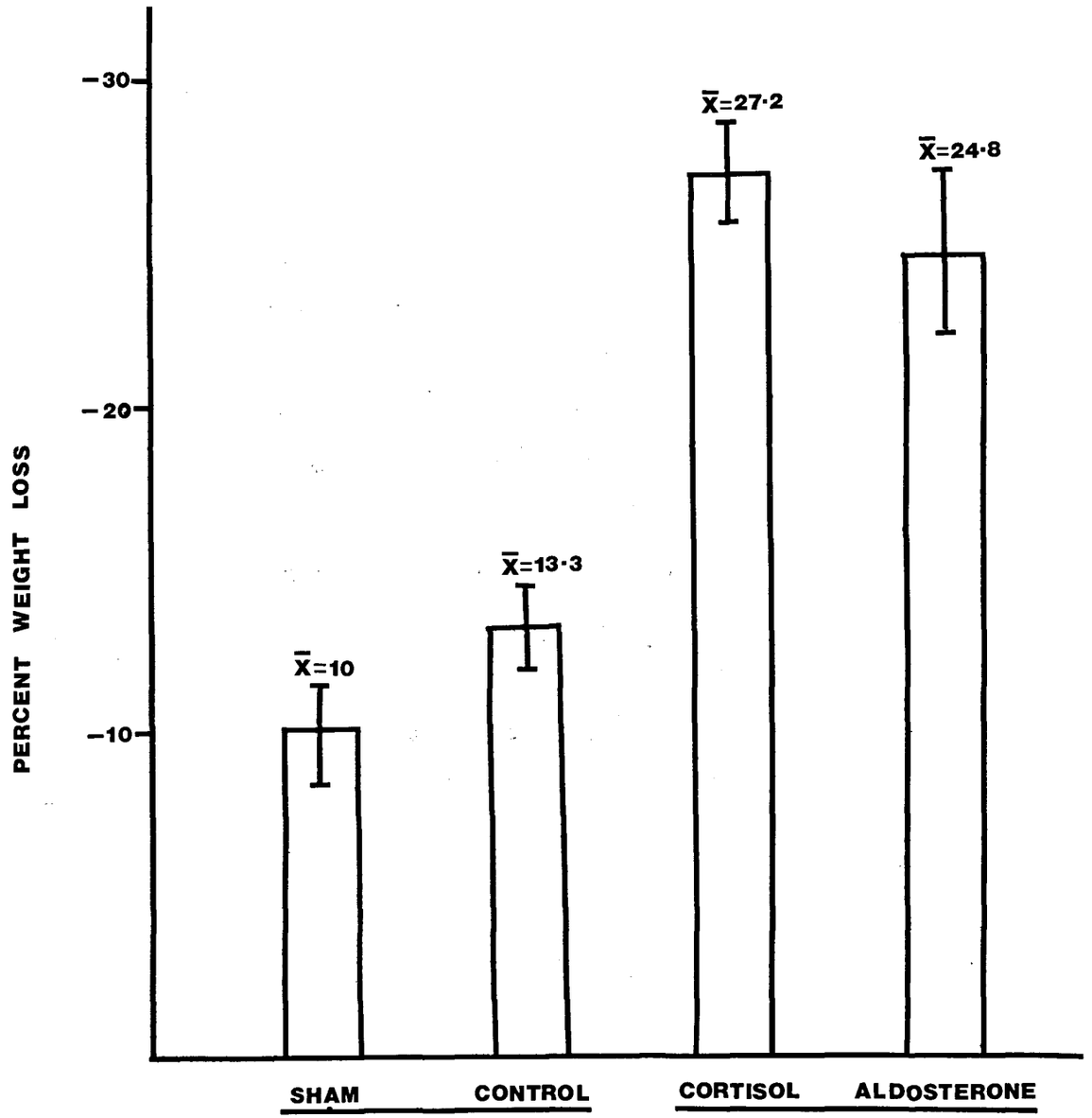


Figure 2. The effects of cortisol and aldosterone on weight loss at death of Meriones unguiculatus, the Mongolian gerbil, expressed as percent body weight ± Standard Error (N= 16).

Means underscored by the same line do not differ significantly at p= .05 (Duncan's test).

$\bar{x}$= mean percent weight loss

Control = ADX, untreated group.

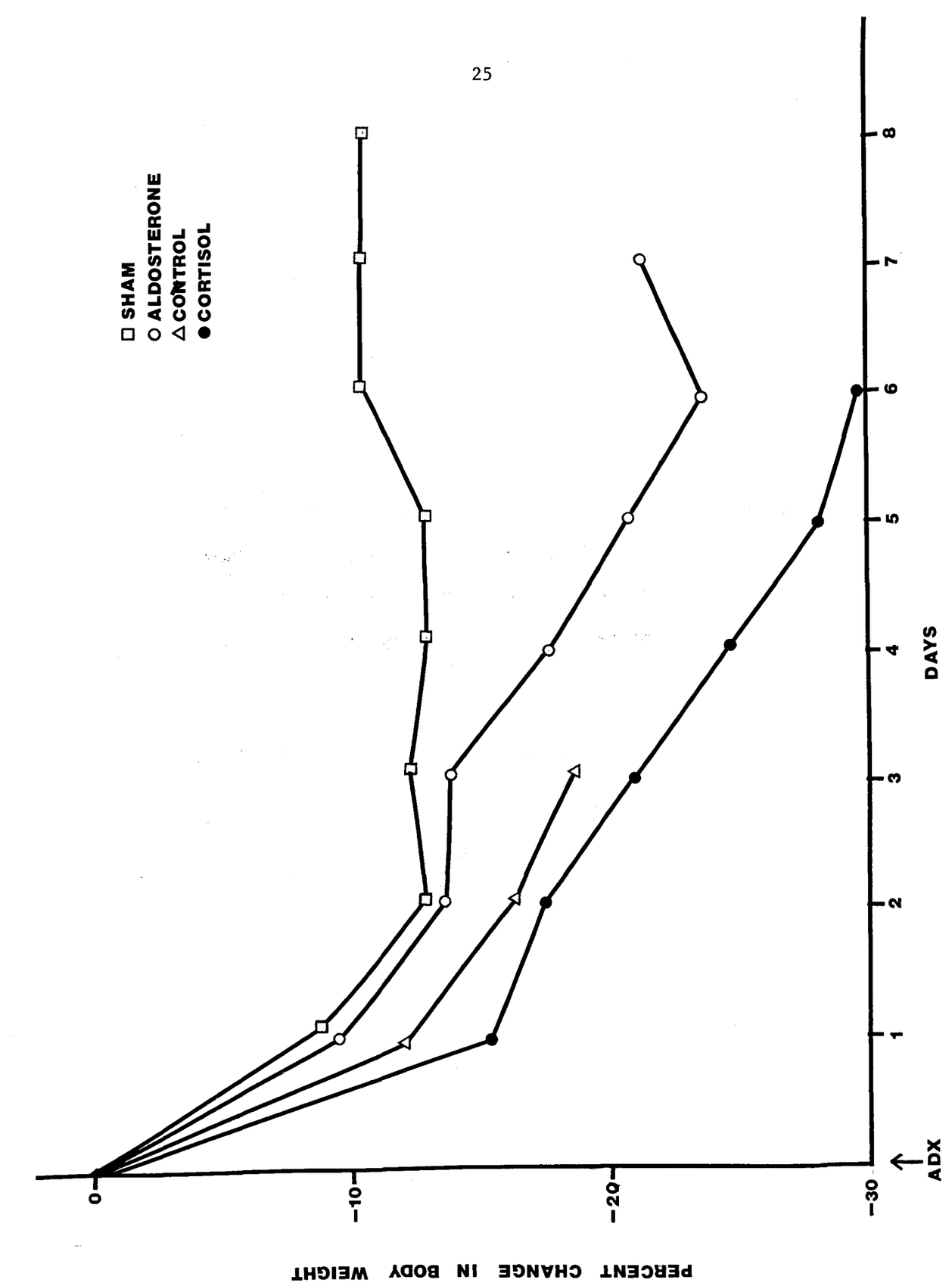


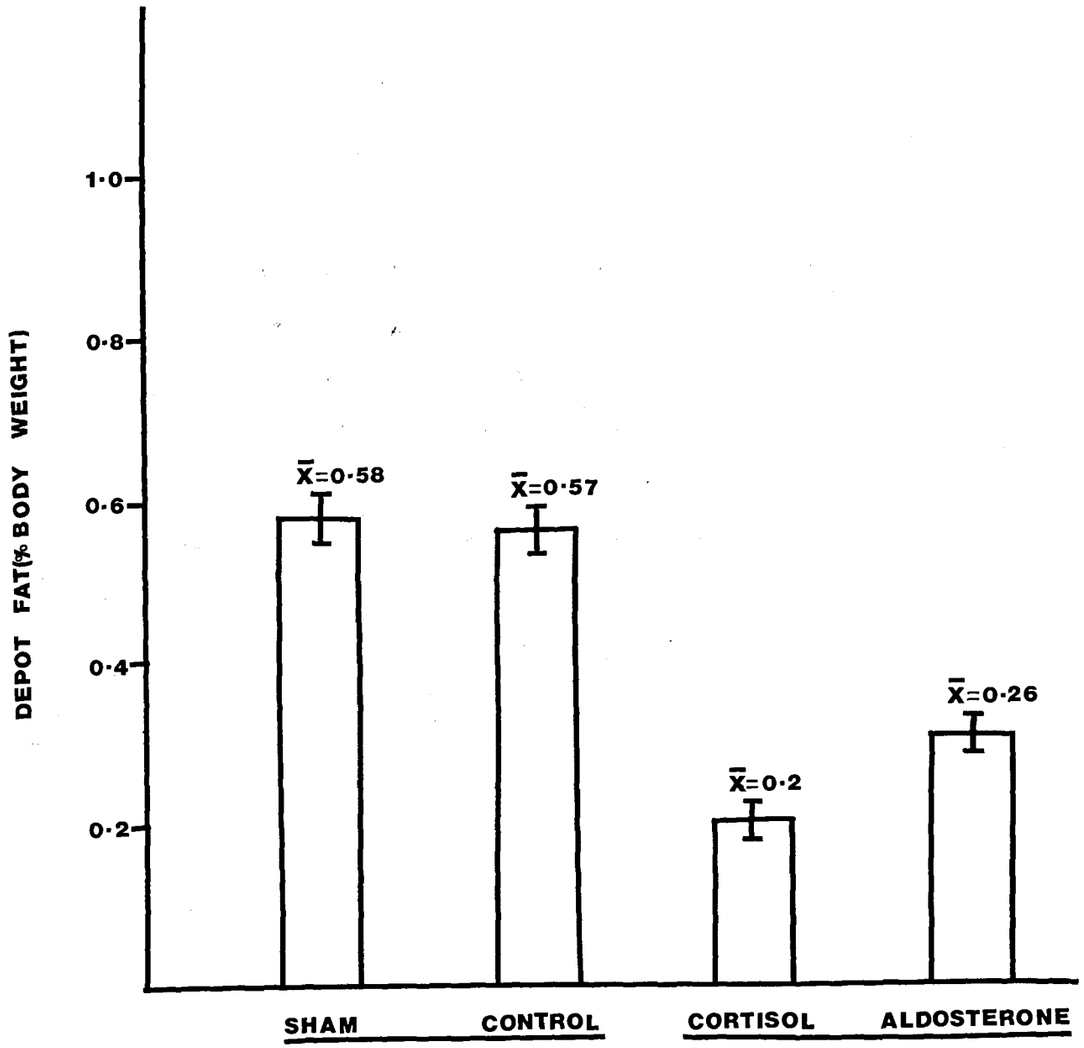
Figure 3. The effects of cortisol and aldosterone on daily weight of Meriones unguiculatus, the Mongolian gerbil, expressed as mean percent weight change. (N= 16)
Control = ADX, untreated group

Figure 4. The effects of cortisol and aldosterone on depot fat of Meriones unguiculatus, the Mongolian gerbil at necropsy, expressed as percent body weight ± Standard Error (N= 16).

Means underscored by the same line do not differ significantly at p= .05 (Duncan's test).

$\bar{x}$= mean depot fat (% body fat).

Control = ADX, untreated group.



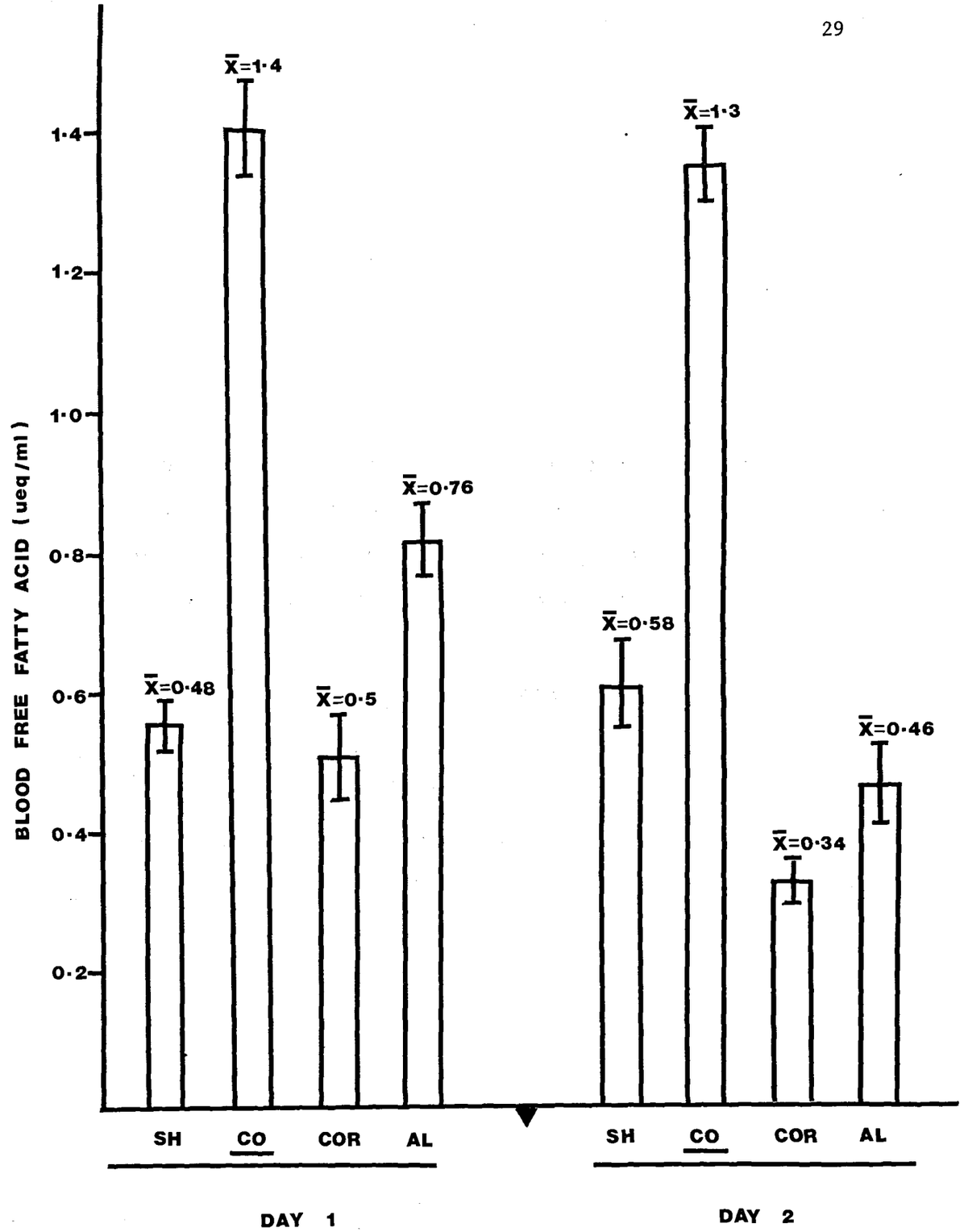


Figure 5. The effects of cortisol and aldosterone on blood free fatty acid of Meriones unguiculatus, the Mongolian gerbil. Values are expressed as ueq/ml. ± Standard Error (N= 16).

Means underscored by the same line do not differ significantly at p= .05 (Duncan's test).

$\bar{x}$= mean blood free fatty acid.

Sh = sham, Co = control, (ADX, untreated group), Cor = cortisol,

Al = aldosterone.

APPENDIX

MEASUREMENT OF BLOOD FREE FATTY ACID

(a procedure described by Koichi, I. and Michio, Ui (1965) with modifications)

Standards:

1) 0.0051 g of palmitic acid (P. A.) (Mol. wt. 256.4) was dissolved in 1000 ml of chloroform to give a concentration of 0.1 ueq/ml. Subsequent dilutions of 0.1 ueq/ml P. A. solution were made to give the following concentrations: 0.016, 0.012, 0.008 and 0.004 ueq/ml.
2) All glass tubes used in this procedure were thoroughly cleaned with soap, rinsed with tap water and distilled water and let dry. To ensure complete dryness and cleanliness before use, glass tubes were rinsed with methanol and finally with chloroform.
3) 6 ml. of standard and 3 ml. of Cu-triethanolamine solution (1M-triethanolamine, 1N-Acetic acid, 4.6 % - $CuCl_2 \cdot 2H_2O$, 9:1:10) were placed in a glass stoppered centrifuged tube, shaken 60 times and let stand for 30 minutes.
4) The upper layer was gently aspirated and the lower layer filtered into a cuvette, using a small glass funnel lined with filter paper.
5) Two drops of 0.1 % sodium diethyldithiocarbamate was added to the solution in the cuvette and the

absorbance read immediately at 440 nm.

Free Fatty Acid In Blood:

1) 6ml of chloroform, 1 ml of phosphate buffer (pH 6-7), and 0.2 ml of blood were placed in glass stoppered centrifuge tubes. The tubes were shaken for 90 secs. and let stand for 15 min.
2) The upper layer was aspirated and the lower layer decanted into a second glass stoppered centrifuge tube (making sure no blood is present in the decanted layer, as this interferes with the readings).
3) To the decanted layer, 3 ml of Cu-triethanolamine solution were added. The tube was shaken for 60 seconds and let stand for 30 minutes.
4) Follow steps 4 and 5 of the standard. Care must be taken that the blue Cu-triethanolamine solution does not filter through into the cuvette. Also, at all times contact of skin with the solution must be avoided.

LIPID EXTRACTION PROCEDURE

(as described by Johnson, A.R. and Davenport, J.B. (1971))

1) As different tissues have different water content, tissue water content must be corrected to 80 %. Water content of adipose tissue was determined as follows: a previously weighed sample of adipose tissue was completely dried in an oven. Water content was calculated by subtracting dry weight from wet weight. Adipose tissue water content was found to be approximately 60 %.
2) The tissue was homogenized with 3 vol. of chloroform/methanol (C/M) (1:2).
3) The homogenate was centrifuged. (If sample size is large, more than 1 g. filteration using Buchner funnel and suction works better).
4) The supernatant from step 3 was set aside and the residue rehomogenized with 3.8 vol. of C/M/Water (1:2:0.8).
5) The supernatant from steps 3 and 4 were combined and to this 2 vol. of chloroform and 2 vol. of water were added. (If volumes are measured correctly throughout, this mixture will be biphasic).
6) The top layer was aspirated (using separatory funnel if sample size is large, more than 1 g).
7) Dry the remaining chloroform/lipid layer in a water bath at 60°C (app. 24-48 hours).

VITA

Safia Baggia was born on June 30, 1953 in Rangoon, Burma. She received her primary education at St. Johns Old Convent, in Rangoon. She graduated from Walthamstow Senior High School, in London, England in 1975. She then attended Bridgewater College, Bridgewater, Virginia where she held a foreign student scholarship. At Bridgewater she received her Bachelor of Arts degree in general science in 1981. At the University of Richmond, she completed her graduate work in Biology and obtained a Master of Science degree in 1983. While there, she was initiated into Beta Beta Beta Honorary Biological Society and received a graduate assistantship. She will enter the doctoral program in cellular endocrinology at the Eastern Virginia Medical School in July, 1983.