METABOLIC RESPONSES OF FEMALE RATS TO THERMAL STRESS

by

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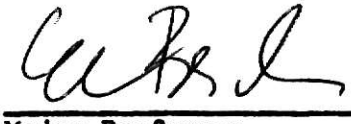

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INTRODUCTION

Maintenance of a relative constancy of bodily functions--homeostasis--prevents significant deviations from the normal state. Thus, when an animal is exposed to one or more stressful or potentially stressful stimuli (i.e., stressors), metabolic, neuroendocrine and motor responses act together to maintain "near-constant" body conditions (11). All of these changes tend to favor survival of an animal in a new environment. Under some climatic conditions, like in the tropics, elevated temperature and relative humidity, in addition to irregular and scanty precipitation, may result in sparce vegetation. This creates an environment that is generally unfavorable to most animals (28), and may result in two major physiological problems--obtaining sufficient water for the needs of the body and maintaining body temperature within physiological limits.

Previous reports show that in all homeotherms, food intake is negatively correlated and water intake positively correlated (8, 9, 22, 29) with environmental temperature. In the thermoneutral state, food and water consumptions appear to be interdependent (3), but such a relationship does not appear to exist during thermal stress. In the latter, physiological demand for water, primarily for evaporative cooling (19), is out of proportion to the amount of food consumed (22).

On the other hand, in the female rat, the influence of estrous cycle on food and water intake is as predictable as that of temperature (33). As the animal enters estrus, food and water intake and body weight decrease; but significantly ($P < 0.01$) increase during diestrus

(10, 21). These variations suggest a regulation that takes precedence over maintenance of energy balance (21).

In all mammalian species studied, the hypothalamus subserves an important function in the control of reproduction (15), the regulation of food and water intake (3), and of body temperature (16). Since the centers controlling these functions are anatomically closely related, they may show interactions, in addition to their various vegetative functions. However, the physiological mechanisms that enable some mammals to survive under harsh environmental conditions (e.g., increased heat and decreased water), intolerable to most animals, is not well understood. Thus, the study reported here describes some physiological responses of the cycling female rat to thermal stress.

Materials and Methods

Animals

Twelve adult cycling female rats (Holtzman), each weighing 220 to 240 grams, were used in each experiment. The animals were fed rat chow mash (Purina) from feed cups with perforated inserts to minimize spillage. Watering containers were calibrated 100 cc graduated cylinders to which a specially devised "L" glass tube had been attached. Food and water were available ad libitum. Duplicate feed cups and water tubes allowed for rapid interchange with minimal disturbance to the animals. The rats were preconditioned to chamber conditions for 10 days preceding each treatment (24.5 C, 29.2 C, 34.0 C). From day 11 of each treatment group, various measurements were recorded for each rat for a duration of at least 5 estrous cycles (about 25 days)--about 20% of the rats exhibited four-day estrous cycles.

Chamber

The animals were housed individually in metabolic cages in a controlled environment room (Scherer-Gillet, CER 810), under control (24.5 C), followed by two experimental temperatures (29.2 C and 34.0 C). A relative humidity of 50% ± 5%, (mean ± S.E.), and a 12L:12D photoperiod (L = 0600 to 1800 Hours), were employed in all tests.

Measurements

Food intake was measured by the net difference in the weight of the feed cups between the beginning and the end of a particular sample period. Water intake was easily measured by taking the difference in the level at the time of observation from the premeasured 100 cc level. Food and water intakes were calculated on a relative basis (4). Daily vaginal smears were made from each animal to determine the stages of estrous cycle. All of the above measurements were performed at the same relative time each day.

Body water turnover was studied using tritiated water (^3H_2O), by a modification of the technique previously described by Chapman and Black (12). Blood samples--50 µl each--obtained from each rat via the tail vein, were digested with 0.5 ml Protosol (NCS*), decolorized with 0.15 ml of a freshly prepared solution of 20% Benzyol peroxide in toluene, and counted for radioactivity, using toluene cocktail.

Packed cell volume was determined by the microcapillary hematocrit method and plasma protein concentrations with a hand protometer. Plasma corticosteroid levels were determined according to the method described by Mattingly (24).

Results

It is apparent from the data of Table 1 that the experimental temperatures used in this study constituted stressful environments for the rats as evidenced by significantly ($P < 0.01$) elevated corticosteroid levels for both experimental groups. There also was significant ($P < 0.05$) relative lymphopenia and neutrophilia, both of which have been reported as stress indicators (6). No significant changes were noted in the total numbers of white blood cells.

The influence of temperature on relative food intake on different days of the estrous cycle is illustrated in Figure 1. The cyclic estrual variation of food intake at the temperatures employed agrees with similar data reported previously (10, 33). Food intake is elevated during diestrus (days 3 and 4), but becomes significantly reduced on the day of estrus (day 1). This cyclic variation did not appear to be affected by the temperatures employed; however elevated temperatures caused significant ($P < 0.01$) decreases in the average relative food intake of the rats (Table 2). As temperature was increased above control value, food intake was reduced; the lowest value was at the highest temperature (34.0 C). It is of interest to note that although 29.2 C is midpoint between 24.5 and 34.0 C, the food intake curve was not; furthermore, the rats still gained weight at 29.2 C and 34.0 C despite the significant reductions in food intake.

Relative water intake at the three temperatures, as illustrated in Figure 2, appears to be altered in a manner opposite to that of food intake. The influence of estrous cycle is not abolished by thermal stress, but the volume of water consumed by the rat is significantly

Table 1. Total white blood cells, (WBC), relative lymphocyte and neutrophil values and corticosteroid levels for female rats. All values are from blood samples obtained on day 25 of each treatment.

Treatment	n[1/]	Total WBC (1000's)	Lymphocytes (%)	Neutrophils (%)	Cortico-steroids (µg/100 ml plasma)
CONTROL (24.5 C)	12	11.0 ± 0.8[2/]	78.2^{a} ± 1.0	14.9^{d} ± 10	21.5^{g} ± 3.0
EXPERIMENTAL #1 (29.2 C)	12	10.4 ± 0.6	68.3^{b} ± 1.7	22.6^{e} ± 1.5	71.9^{h} ± 5.7
EXPERIMENTAL #2 (34.0 C)	12	10.2 ± 1.0	60.9^{c} ± 1.4	29.3^{f} ± 1.4	84.8^{i} ± 6.4
REFERENCES[3/]					
Albriton, E. C.(2)		14.0 5.0 to 25.0	73.0 65.0 to 84.0	22.0 9.0 to 34.0	-
McCarthy, J. L. et al. (25)		-	-	-	23.0

1/ n is the number of animals

2/ Mean ± Standard Error

3/ numbers in parenthesis correspond to reference page listing

Values with different superscripts are significantly different ($P < 0.05$) as determined by analysis of variance.

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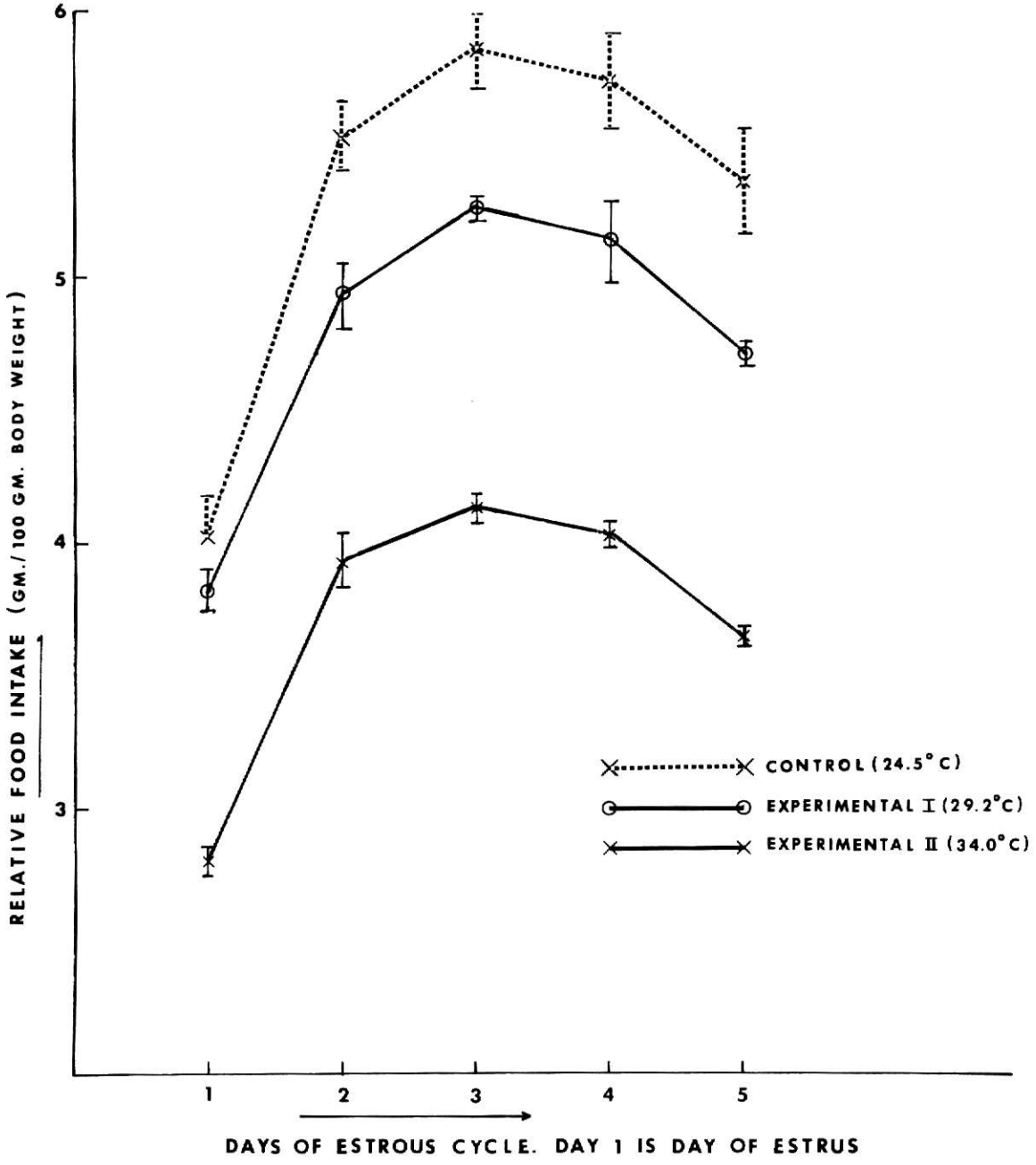

FIGURE 1. DAILY FOOD INTAKES OF RATS AT THREE DIFFERENT TEMPERATURES. EACH REPRESENTS THE MEAN OVER FIVE ESTROUS CYCLES STANDARD ERRORS ARE SHOWN VERTICALLY.

Table 2. Mean body weight gain, food intake and water intake for control and experimental animals for 25 days.

Treatment	n[1/]	Body weight gain (gm/day)	Relative Intake Food (gm/100 gm body weight)	Relative Intake Water (ml/100 gm body weight)
CONTROL (24.5 C)	600	$0.92^{a} \pm 0.08$[2/]	$5.9^{c} \pm 0.4$	$12.8^{f} \pm 0.6$
EXPERIMENTAL #1 (29.2 C)	300	$0.54^{b} \pm 0.04$	$4.5^{d} \pm 0.4$	$14.2^{g} \pm 0.8$
EXPERIMENTAL #2 (34.0 C)	300	$0.51^{b} \pm 0.05$	$3.9^{e} \pm 0.3$	$18.7^{h} \pm 0.8$

[1/] n is the number of rat days (number of animals per group x 25 days)

[2/] Mean ± Standard Error

Values with different superscripts are significantly different ($P < 0.01$), as determined by analysis of variance.

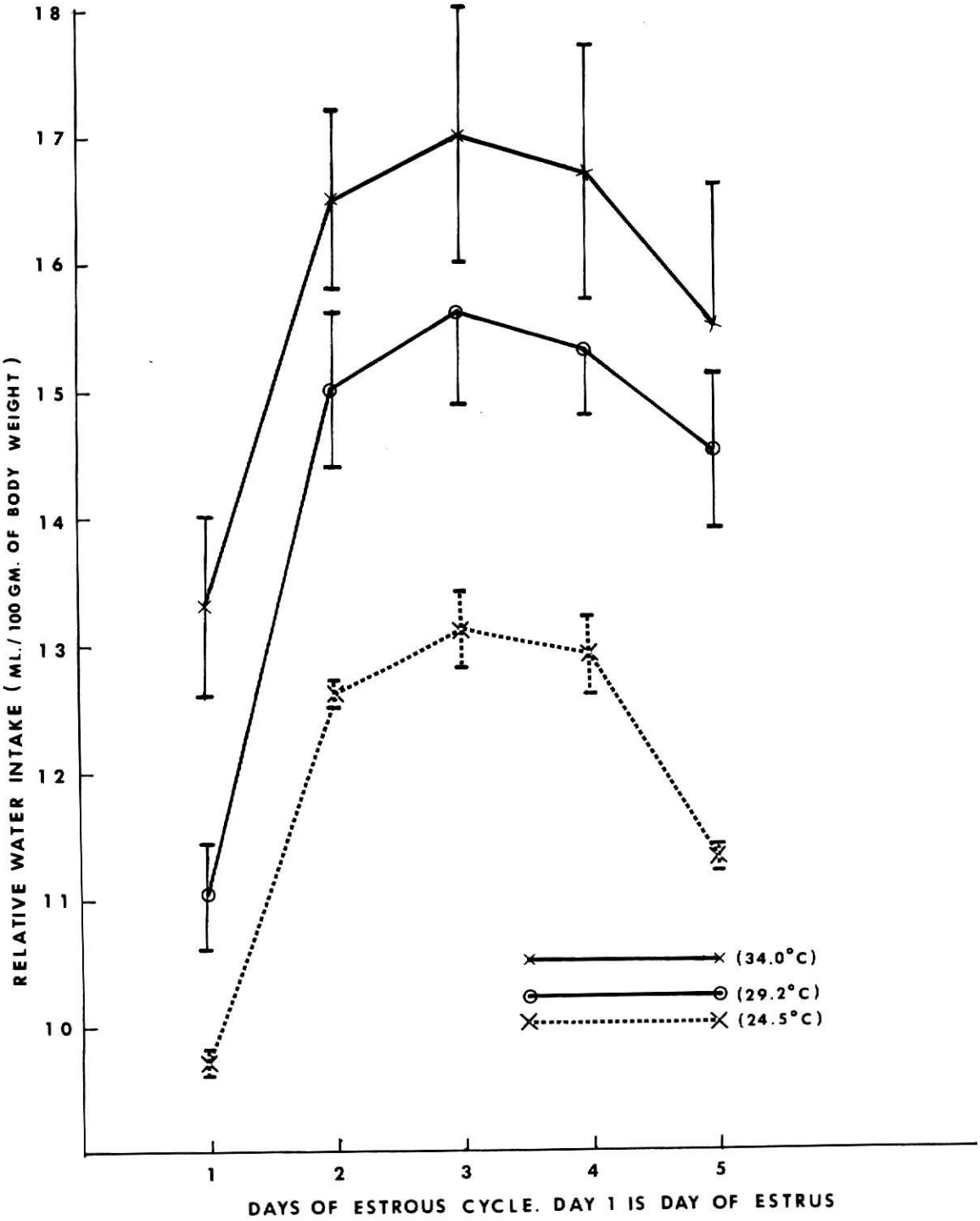

FIGURE 2. DAILY WATER INTAKES OF RATS AT THREE TEMPERATURES. EACH POINT REPRESENTS THE MEAN OVER FIVE ESTROUS CYCLES. STANDARD ERRORS ARE SHOWN VERTICALLY.

($P < 0.01$) increased under conditions of elevated temperature (Table 2). The ratios of the amount of water to that of food consumed during each day of the estrous cycle, at 34.0 C, also were significantly ($P < 0.01$) increased over control values (Figure 3).

Water turnover data are shown in Table 3. The results indicate that, compared to control conditions, thermal stress resulted in a slight but nonsignificant decrease in percent body water, in significantly ($P < 0.01$) increased body water flux, and a significantly ($P < 0.01$) shortened biological half-life of $^{3}H_2O$. The rate of water pool turnover also is significantly ($P < 0.01$) increased in all cases.

The contributions of preformed water to the daily water turnover--as per cent of daily flux--are shown in Table 4. Significant increases ($P < 0.05$) were observed in the amounts contributed by drinking water (i.e., free water ingested) and by total preformed water at 34.0 C compared to control values. In addition, per cent contributions to daily flux by food water, (i.e., water contained in the food), are significantly decreased ($P < 0.05$) at the two experimental temperatures.

Packed cell volumes (PCV) and plasma protein concentrations showed slight but nonsignificant elevations ($P > 0.05$) under both experimental conditions (Table 5).

Discussion

A stress reaction (32) in an animal may be produced by any condition or agent (stressor) for which the animal is unaccustomed or unadapted. All stressors have in common the ability to evoke the release of ACTH (32) and all require physiological adjustments on the part of the animal (5). An animal, for example, a food animal

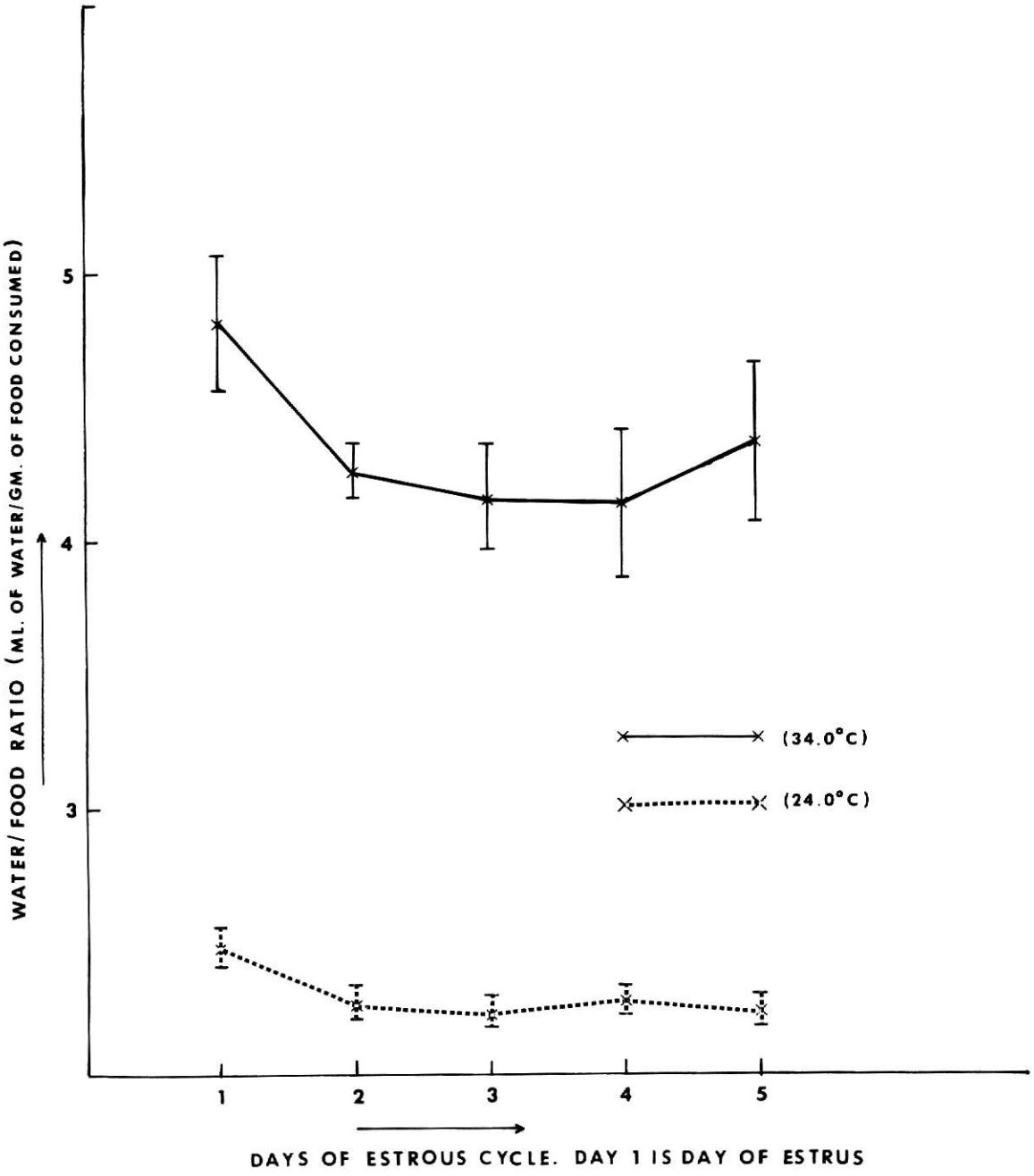

FIGURE 3. RATIO OF WATER INTAKE PER GRAM OF FOOD CONSUMED. EACH POINT REPRESENTS THE MEAN OVER FIVE ESTROUS CYCLES. STANDARD ERRORS ARE SHOWN VERTICALLY.

Table 3. Characteristics of body water turnover and pool size of female rats. Determinations were made after 10 days exposure to each of three temperature conditions.

Treatment	n[1/]	Body water (% body weight)	Kinetic parameters of water pool: Flux (ml/100 gm body wt/ day)	Half-life (days)	Pool turnover (fraction/ day)
CONTROL (24.5 C)	12	$70.0^{a} \pm 1.5$[2/]	$15.6^{b} \pm 0.5$	$3.2^{e} \pm 0.1$	$0.22^{h} \pm 0.01$
EXPERIMENTAL #1 (29.2 C)	12	$68.5^{a} \pm 1.7$	$18.6^{c} \pm 0.8$	$2.3^{f} \pm 0.1$	$0.30^{i} \pm 0.01$
EXPERIMENTAL #2 (34.0 C)	12	$66.8^{a} \pm 1.9$	$20.4^{d} \pm 0.6$	$1.9^{g} \pm 0.2$	$0.36^{j} \pm 0.03$

1/ n is the number of animals

2/ Mean ± Standard Error

Values with different superscripts are significantly different ($P < 0.01$) as determined by analysis of variance.

Table 4. Contributions of drinking water and food water to daily water turnover--as per cent of daily flux. Average values were calculated for the first 10 days of each 25-day period in each treatment group (see flux values in Table 3). All calculations were corrected for evaporative water loss from drinking tubes.

Treatment	n[1/]	Drinking Water (%)	Food Water (%)	Total Preformed Water (%)
CONTROL (24.5 C)	120	$81.1^{a} \pm 2.1$[2/]	$2.15^{c} \pm 0.02$	$83.3^{f} \pm 2.5$
EXPERIMENTAL #1 (29.2 C)	120	$85.8^{ab} \pm 2.4$	$1.34^{d} \pm 0.03$	$87.1^{fg} \pm 2.0$
EXPERIMENTAL #2 (34.0 C)	120	$89.7^{b} \pm 3.0$	$1.01^{e} \pm 0.01$	$90.7^{g} \pm 3.0$

1/ n is the number of rat days (number of animals per group x 10 days)

2/ Mean ± Standard Error

Values with different superscripts are significantly different ($P < 0.05$) as determined by analysis of variance.

Table 5. Packed cell volume (PCV) and plasma protein concentrations for control and experimental animals. Values are for blood samples obtained on day 25 of each treatment.

Treatment	n[1]	Packed Cell Volume (%)	P	Plasma Proteins (gm/100 ml plasma)	P
CONTROL (24.5 C)	12	43.6 ± 0.4[2]	> 0.05[3]	9.4 ± 0.8	> 0.05
EXPERIMENTAL #1 (29.2 C)	12	44.3 ± 0.3	> 0.05	9.8 ± 0.1	> 0.05
EXPERIMENTAL #2 (34.0 C)	12	45.3 ± 0.3		10.1 ± 0.9	

1/ n is the number of animals

2/ Mean ± Standard Error

3/ Statistical probability as determined by Students t-test

exposed to the stress of extreme environmental conditions (e.g., elevated ambient temperature), may have altered physiological characteristics that may not be optimum for production. Thus tropical breeds of livestock do not produce meat or milk as much as their temperate counterparts.

The decreased food intake associated with rising temperatures agrees closely with previously reported data (8, 9, 14, 20, 29). Under stress--in this case thermal stress--the major responses of the body are directed towards maintenance of a "constancy" of body functions and a reduction in the physiological impact of the stress. For example, at temperatures above the zone of thermoneutrality--upper critical temperature--food intake is reduced, presumably because of the difficulty of heat dissipation, since any extra heat obtained from the food may embarass the system, resulting in hyperthermia (9). The effect of temperature on food intake may be primarily central (3) and appears to have a homeokinetic significance (9).

The gain in body weight despite a significantly reduced food intake at 29.2 C and 34.0 C may be due to a number of reasons. Firstly, efficiency of food conversion is increased at high temperatures (26) and although the rats may be decreasing their food intake, they may be utilizing it more efficiently, thereby displaying weight gain. Secondly, at high ambient temperatures, less food is required for maintenance of body temperature (26) and weight gain may be observed in spite of drastically reduced food intake. This could also explain why domestic animals in arid and tropical areas of the world grow and gain weight despite reduced availability of feed and elevated ambient temperatures.

That the food intake curve at 29.2 C does not lie at the exact midpoint of 24.5 and 34.0 C, probably implies that the response of food intake to temperature is not linear. Furthermore, from the data reported here it is evident that the influence of estrous cycle on food intake in the rat may not be temperature-labile.

Control values for water turnover are similar to those previously reported (Richmond et al., 1962, Foy et al., 1960). Changes in these parameters and in the quantity of water intake under thermal stress, reflect the physiological cost to the rat of maintaining homeokinesis with respect to water balance. Water is the main source of cooling the body when ambient temperatures rise beyond the critical point (19), and mechanisms are initiated which operate to replace water lost by evaporative cooling (19). Thus water intake becomes significantly elevated, and is turned over at a faster rate and in greater volume, accounting for the shortening of the biological half-life of injected $^{3}H_{2}O$.

Decreases in the per cent contributions by food water to daily water flux (Table 4) is probably due to the significant decreases in the amounts of food consumed at the two experimental temperatures (Table 2; Fig. 1). On the other hand, increases in the contributions of drinking water to flux (Table 4) may be probably due to increased water intake (Table 2; Fig. 2). Since the total water available to an animal is from three sources (free water, water contained in the food and metabolic water), the contributions of metabolic water to total flux may become reduced at these experimental temperatures.

Inspite of compensatory adjustments to high temperatures, the rat may not be able to consume enough water to replace the loss. This may result in the slight but nonsignificant dehydration observed, in spite of water intake in amounts greater than control values. Alterations in the ratios of water to food consumed indicate the relative degrees to which these parameters are affected by thermal stress.

Although an attempt was made to control relative humidity in this study, it was observed that the effective water vapor pressure at a constant relative humidity is a function of temperature. Thus, in this and other studies, where relative humidity is a variable, it would perhaps be more accurate to control water vapor pressure rather than relative humidity.

Data obtained for water turnover at elevated temperatures in these studies, appear to have other biological significance. For example, in a tropical environment where the supply of water may be severely restricted, and where high ambient temperatures may impose a strain on the water economy of domestic animals (1, 31), increased turnover of available body water without concomitant replenishment, could further aggravate the problem of water needs (7, 27, 31). Under such circumstances, water may become a limiting factor in animal production (18).

Conclusion

Data reported here indicate that when an animal, like the rat, is subjected to the stress of elevated ambient temperature, it adjusts its food and water metabolism in such a manner as to reduce the physiological impact of the stress. In addition, thermal stress, of

the nature employed here, does not abolish the cyclic variation of food and water intakes nor their relationship to the estrous cycle. However, the physiological mechanisms that underline the responses observed here are largely unknown, and need to be investigated.

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APPENDIX A

Pattern of daily food intake through one typical estrous cycle

CONTROL (24.5 C)

Rat No.	ABSOLUTE FOOD INTAKE (gm) Days of Estrous Cycle 1	2	3	4	5	Grand Mean	RELATIVE FOOD INTAKE (gm/100 gm body weight) Days of Estrous Cycle 1	2	3	4	5	Grand Mean
1	8.4	14.4	12.9	13.4	14.2		3.56	6.08	5.42	5.63	5.94	
2	11.5	15.4	16.4	15.1	12.5		4.60	6.14	6.51	5.97	4.92	
3	11.1	14.7	16.6	16.0	13.8		4.66	6.13	6.83	6.20	5.57	
4	13.1	15.4	15.9	15.8	-		5.24	6.11	6.29	6.05	-	
5	10.9	17.0	18.1	15.9	13.2		4.18	6.49	6.91	6.01	5.02	
6	10.7	16.8	18.6	16.0	14.9		4.08	6.36	6.99	5.94	5.58	
7	8.4	14.1	13.8	14.9	15.3		3.42	5.69	5.54	6.21	6.07	
8	9.5	11.4	13.0	14.6	11.8		4.15	4.94	5.58	6.08	5.00	
9	11.6	13.6	16.4	15.1	12.8		4.72	5.51	6.61	6.90	5.12	
10	11.0	16.7	17.8	17.8	-		4.35	6.55	6.93	7.08	-	
11	12.3	17.7	17.7	18.4	-		4.84	6.91	6.86	6.53	-	
12	10.9	15.1	15.0	16.0	15.4		4.56	6.27	6.17	6.50	6.21	
Mean	10.8	15.20	16.0	15.8	13.8	14.3	4.4	6.10	6.4	6.3	5.5	5.7
± S.E.	0.6	0.8	0.9	0.8	0.6	0.7	0.3	0.4	0.4	0.4	0.3	0.4

EXPERIMENTAL (29.2 C)

Rat No.	ABSOLUTE FOOD INTAKE (gm) Days of Estrous Cycle 1	2	3	4	5	Grand Mean	RELATIVE FOOD INTAKE (gm/100 gm body weight) Days of Estrous Cycle 1	2	3	4	5	Grand Mean
1	12.2	14.1	13.5	16.9	13.9		4.33	4.97	4.77	5.89	4.90	
2	11.0	13.1	13.3	14.2	13.8		4.02	4.82	4.83	5.17	5.05	
3	10.5	14.0	14.0	14.3	-		4.07	5.33	5.34	5.46	-	
4	12.0	15.6	14.7	15.5	-		4.37	5.55	5.23	5.22	-	
5	12.5	14.3	16.8	15.7	14.6		4.33	4.87	5.69	5.37	5.03	
6	11.7	12.0	14.8	16.4	16.0		4.11	4.25	5.17	5.60	5.50	
7	8.6	13.5	15.4	14.6	11.4		3.14	4.85	5.50	5.23	4.13	
8	8.7	14.0	14.6	15.5	-		3.33	5.26	5.47	5.79	-	
9	10.3	14.1	16.2	15.0	-		4.09	4.61	5.21	4.87	-	
10	10.0	14.2	17.0	13.1	13.3		3.77	5.27	6.15	4.79	4.72	
11	10.6	14.9	17.1	16.9	15.3		3.81	5.19	5.91	5.82	5.34	
Mean	9.8	12.8	14.0	14.0	14.0	12.9	3.6	4.6	5.0	4.5	5.0	4.5
± S.E.	0.5	0.8	0.9	0.9	0.6	0.6	0.2	0.3	0.3	0.4	0.4	0.4

EXPERIMENTAL (34.0 C)

Rat No.	ABSOLUTE FOOD INTAKE (gm) Days of Estrous Cycle 1	2	3	4	5	Grand Mean	RELATIVE FOOD INTAKE (gm/100 gm body weight) Days of Estrous Cycle 1	2	3	4	5	Grand Mean
1	8.4	9.85	11.20	12.21	10.05		2.97	4.55	4.72	4.42	3.60	
2	9.4	12.4	14.1	12.4	10.40		3.47	4.55	5.15	4.47	3.77	
3	9.2	11.8	12.0	11.6	10.3		3.42	4.33	4.38	4.26	3.77	
4	10.6	13.3	12.6	11.8	11.3		3.93	4.92	4.62	4.34	4.17	
5	7.7	8.6	8.9	7.5	6.4		2.99	3.38	3.49	2.93	2.54	
6	5.7	12.2	12.9	11.8	10.1		2.05	4.48	4.64	4.19	3.64	
7	8.0	12.1	12.2	10.5	10.7		2.94	4.43	4.42	3.77	3.84	
8	7.8	11.7	11.2	10.4	10.2		3.02	4.54	4.28	3.92	3.90	
9	7.7	10.4	10.2	10.4	8.5		2.99	4.04	3.89	3.98	3.28	
10	7.7	10.8	10.9	11.3	8.5		2.85	4.02	4.03	4.12	3.15	
11	9.3	11.8	11.0	13.3	10.1		3.35	4.27	3.93	4.81	3.59	
12	10.1	11.6	11.9	12.3	11.7		3.62	4.23	4.29	4.48	4.19	
Mean	9.1	11.4	11.6	11.3	9.9	10.7	3.1	4.3	4.3	4.2	3.6	3.9
± S.E.	0.6	0.5	0.7	0.4	0.5	0.5	0.2	0.4	0.4	0.5	0.2	0.30

APPENDIX B

Pattern of daily water intake through one typical estrous cycle

CONTROL (24.5 C)

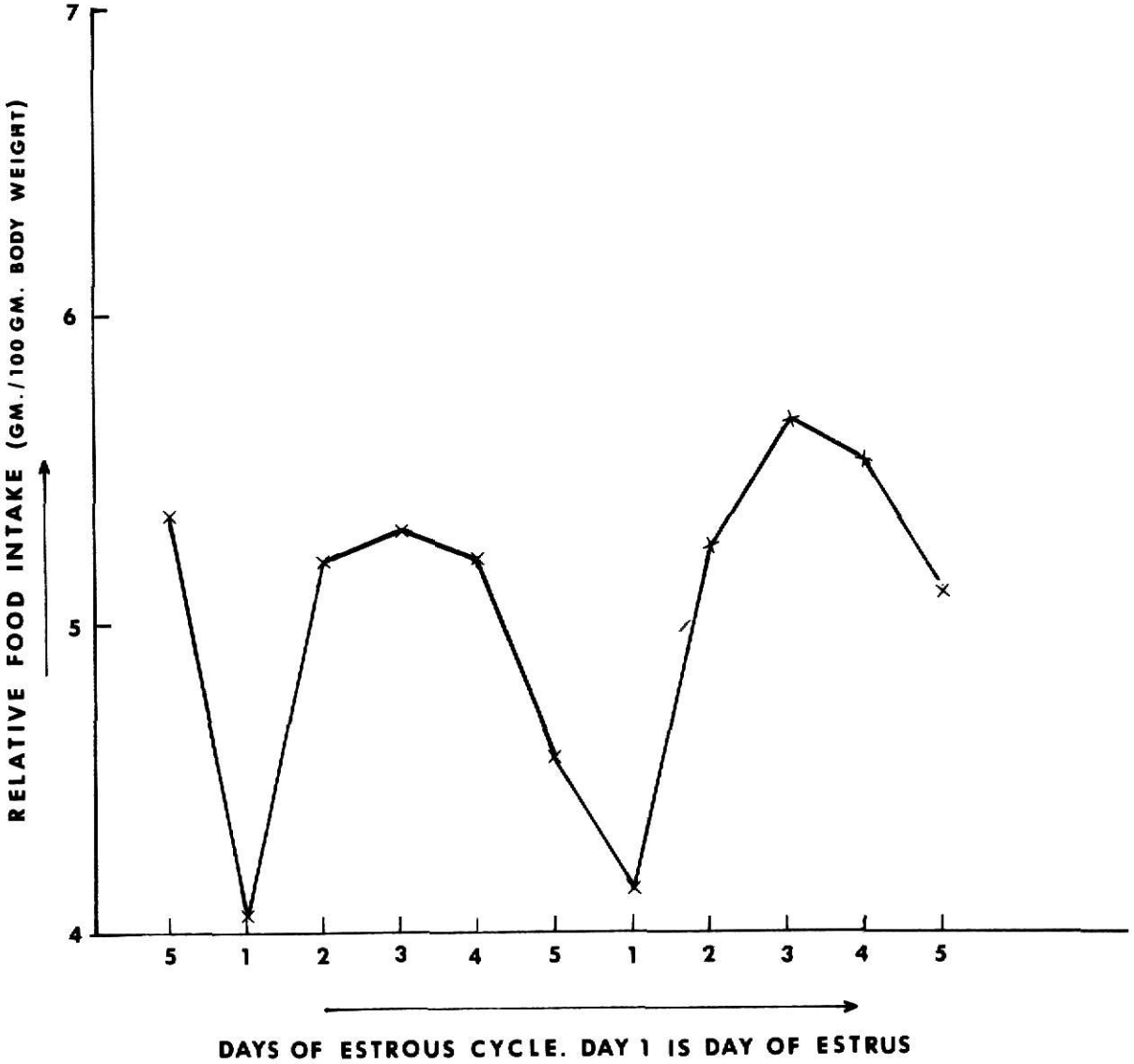
	ABSOLUTE WATER INTAKE (ml)						RELATIVE WATER INTAKE (ml/100 gm body weight)					
Rat No.	Days of Estrous Cycle 1	2	3	4	5	Grand Mean	Days of Estrous Cycle 1	2	3	4	5	Grand Mean
1	24	29	29	30	37		10.17	12.24	12.19	12.61	15.48	
2	29	33	36	34	29		11.60	13.15	14.29	13.44	11.42	
3	26	34	37	35	32		10.92	14.17	15.23	14.29	12.90	
4	27	28	31	30	-		10.80	11.11	12.25	11.77	-	
5	21	33	38	32	28		8.05	12.60	14.50	12.17	10.65	
6	28	35	38	38	36		10.69	13.26	14.34	14.29	13.48	
7	20	27	26	30	27		8.13	10.89	10.44	11.95	10.71	
8	18	21	25	28	22		7.86	9.09	10.73	11.91	9.32	
9	30	40	40	39	33		12.20	16.19	16.13	15.66	13.20	
10	25	39	43	42	-		9.88	15.29	16.73	16.28	-	
11	21	38	34	37	-		8.27	14.84	13.18	14.23	-	
12	19	27	27	32	25		7.95	11.20	11.11	13.01	10.08	
Mean	24	29.2	33.7	33.9	29.9	30.1	9.7	12.8	13.4	13.5	11.9	12.26
± S.E.	2.4	2.5	1.5	1.2	1.8	2.1	0.6	0.8	0.9	0.8	0.6	0.8

EXPERIMENTAL (29.2 C)

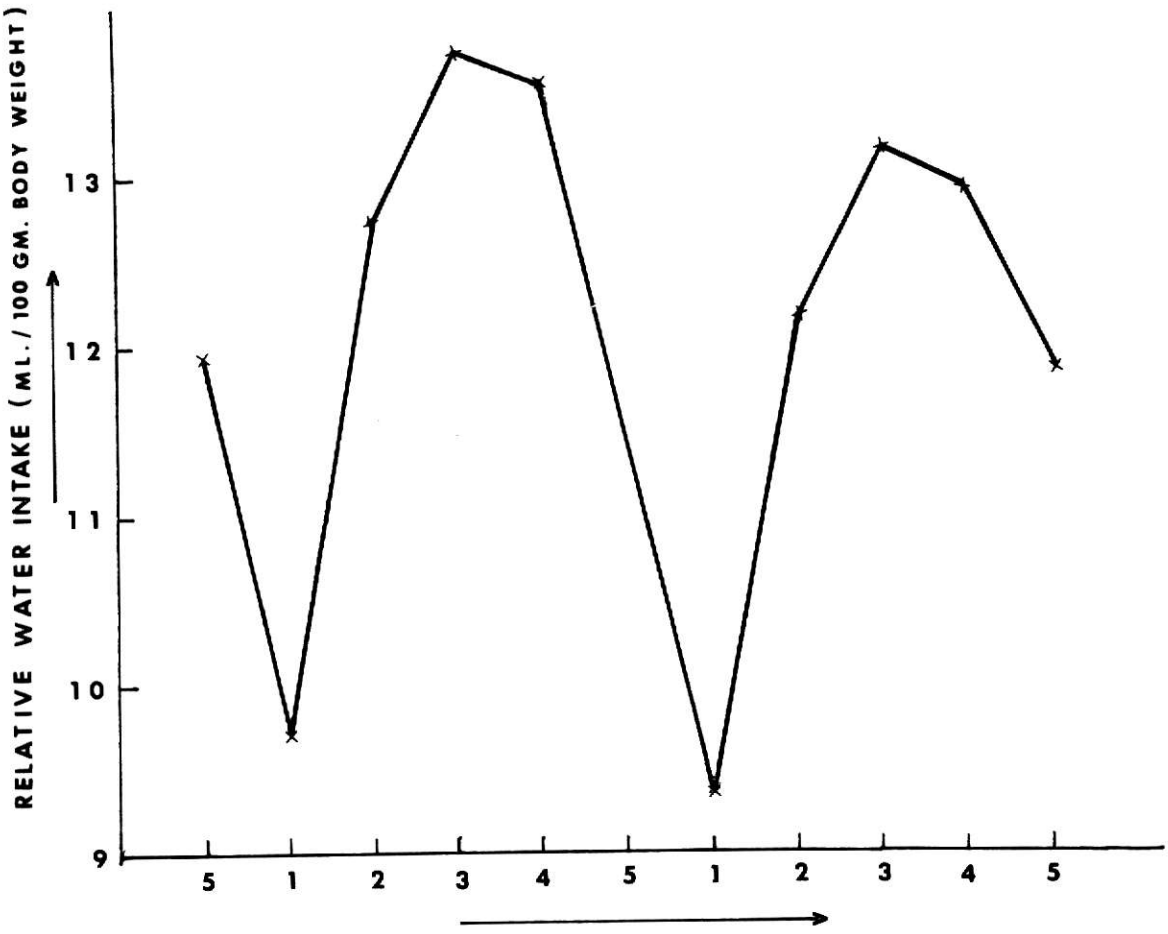
Rat No.	ABSOLUTE WATER INTAKE (ml) Days of Estrous Cycle 1	2	3	4	5	Grand Mean	RELATIVE WATER INTAKE (ml/100 gm body weight) Days of Estrous Cycle 1	2	3	4	5	Grand Mean
1	31	36	44	47	46		11.42	13.02	15.90	18.01	16.90	
2	27	32	33	38	36		9.87	11.60	11.96	13.80	13.10	
3	33	42	50	50	41		13.13	16.32	20.05	20.55	19.00	
4	34	38	44	47	40		12.16	13.49	15.60	16.63	14.29	
5	23	30	30	25	20		9.18	14.65	11.63	9.82	7.94	
6	29	35	41	45	41		10.34	12.27	14.30	15.68	14.43	
7	26	32	45	48	36		9.61	14.75	16.08	17.03	13.12	
8	30	33	48	50	45		11.27	12.34	17.75	18.35	16.62	
9	31	40	43	45	40		12.02	16.07	16.43	17.01	15.39	
Mean	29.3	35.3	42.0	43.9	38.3	37.8	11.0	13.8	15.5	11.3	14.5	14.2
± S.E.	2.3	1.2	2.0	2.0	2.1	1.8	0.4	0.6	0.5	0.9	0.8	0.8

EXPERIMENTAL (34.0 C)

Rat No.	ABSOLUTE WATER INTAKE (ml) Days of Estrous Cycle 1	2	3	4	5	Grand Mean	RELATIVE WATER INTAKE (ml/100 gm body weight) Days of Estrous Cycle 1	2	3	4	5	Grand Mean
1	34	45	43	43	43		12.70	17.80	16.95	16.15	15.56	
2	34	49	47	59	67		12.54	16.52	16.42	15.51	15.57	
3	44	50	52	45	48		12.63	17.98	17.16	21.68	24.53	
4	42	48	52	46	49		16.30	18.48	19.08	16.53	17.71	
5	40	44	47	49	49		15.55	17.27	18.42	19.13	19.45	
6	58	80	88	91	68		20.83	29.35	31.68	32.33	24.48	
7	41	63	63	54	52		15.06	23.07	22.81	19.37	19.37	
8	23	29	32	28	29		8.90	11.25	12.22	10.92	11.09	
9	55	71	104	88	107		21.32	27.57	39.70	33.65	41.28	
10	35	54	51	60	41		12.94	20.07	18.85	21.88	15.19	
11	34	42	40	47	38		12.26	15.18	14.30	16.99	13.50	
12	36	41	42	50	39		12.92	14.96	15.13	18.20	13.95	
Mean	39.7	51.3	55.1	55.0	52.5	50.7	14.5	19.1	20.2	20.2	19.3	18.7
± S.E.	1.2	2.0	2.1	1.8	2.3	3.3	0.6	0.7	1.5	2.0	0.3	0.8



APPENDIX C. VARIATION OF FOOD INTAKE WITH THE ESTROUS CYCLE IN THE RAT.



APPENDIX D. VARIATION OF WATER INTAKE WITH ESTROUS CYCLE IN THE RAT.

METABOLIC RESPONSES OF FEMALE RATS TO THERMAL STRESS

by

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AN ABSTRACT OF A MASTER'S THESIS

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The effects of thermal stress and estrous cycle on food and water intake and on the kinetics of water metabolism were studied in Holtzmann rats -- 12 adult females per group. These animals were housed individually in metabolic cages, in series, in a controlled environment room (Sherer-Gillet, CER 810) at control (24.5C) followed by two experimental (29.2C and 34.0C) temperatures. In all cases the relative humidity was 50% and the photoperiod 12L:12D (L=0600-1800 hours). Food and water were available ad libitum and the intake of each was measured and recorded daily for at least five consecutive estrous cycles (about 25 days total time). Water turnover was measured using tritiated water. The results indicate that, compared to control, the experimental conditions constituted stressful environments for the rats as evidenced by elevated ($P < 0.01$) corticosterone levels and significant ($P < 0.01$) relative lymphopenia and neutrophilia. In addition, experimental conditions resulted in reduced ($P < 0.01$) food intake and increased ($P < 0.01$) water intake. Body water turnover was increased ($P < 0.01$) while body water pool size and the biological half-life for ^3H_2O were reduced ($P < 0.01$). However, the cyclic variation of food and water intake and its relationship to the estrous cycle was unchanged. These data suggest that changes in metabolic responses of a rat exposed to thermal stress tend to favor survival of the animal.