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THE EFFECTS OF THE FEMALE SEX HORMONES ON FOOD AND WATER INTAKE AND ON BODY WEIGHT AFTER HYPERTONIC STRESS IN THE FEMALE RAT

BY

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APPROVED:

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VITA

John L. Kibler, III was born on August 1, 1944, in Richmond, Virginia. After living in five small Virginia towns, he moved with his family to Richmond where he was graduated from George Wythe High School in 1962. He then attended Randolph-Macon College where he was invited into the membership of Lambda Chi Alpha social fraternity in 1963. With a major in psychology, he was graduated from Randolph-Macon in 1966. After working as a recreational instructor until December of that year, he enlisted in the United States Air Force and received a commission in April 1967. He left the Air Force in December, 1970, having attained the rank of Captain and served in Denver, Omaha, and Saigon, Vietnam. Upon resignation of his commission, he entered the Graduate School, Department of Psychology, at the University of Richmond. After receiving his Master of Arts degree, he will attend Texas Christian University in order to earn a Ph.D. in physiological psychology.
It was hypothesized that replacing female sex hormones in ovariectomized rats would enable them to maintain their normal food intake and body weight after hypertonic NaCl loads as opposed to the decrease in both measures typically observed in male and ovariectomized rats. A group of 6 rats received sham operations and sesame oil injections; 4 groups of 6 rats received ovariectomies with a group each receiving oil, progesterone, estrogen, or progesterone-estrogen injections. No difference was found among the groups 24 hours after a 3% body weight stomach load of 10% NaCl solution in either food intake, water intake, or body weight. The data failed to support the hypothesis. Extreme variability in the dependent measures, which correlated with temperature and humidity fluctuations in the colony room, masked the results.

A great deal of research has been conducted investigating the nature of the regulatory mechanisms governing fluid balance in mammals. Yet, due to the complexity of the systems involved and the difficulties inherent in the assay of the components, there are still large gaps existing in the understanding of many aspects of those mechanisms. A recent discovery added another facet to the investigation and offers a novel approach to the problem (Kosub, in press). Working with adult rats, Kosub discovered that females were able to tolerate induced hypertonic stress more effectively than males. The study is intended
to investigate the causes of the differential response between sexes in the rat when exposed to hypertonic stress. It will be necessary, however, to first discuss in detail several aspects of the regulatory mechanisms to establish the background on which this study is based.

Mammalian fluid regulation is a multidimensional, dynamic process. It is multidimensional because there are two separate elements which must be maintained within critical limits—volume and tonicity. It is dynamic because the mammal's environment places a constant stress on the organism's state of fluid balance. Due to such factors as respiration, perspiration, and elimination of wastes, the mammal constantly loses water from its body. In addition to the constant water loss, gradients of tonicity are in a continual state of flux and force the continuous adjustment of the regulatory mechanisms.

The body water can be conceptualized as existing in three compartments. These divisions include the extracellular fluid (ECF), the intracellular fluid, and the transcellular fluid (Pitts, 1968). The ECF comprises approximately 20-25% of body weight and consists of the blood plasma, interstitial fluid, and lymph. The rapidly flowing, vascularized blood plasma is separated from the pool of interstitial fluid only by the walls of the capillaries through which the compartments exchange nutrients and waste products. The interstitial fluid provides the environment for the cells and exists, as the name implies, in the interstices between the cell bodies. The intracellular fluid, accounting for slightly more than half of all body water, is not a single entity, but it is rather the conglomerate of the fluid content of the cells of the body. It is functionally defined as the
total of all body water excluding the ECF and transcellular fluid. The latter totals less than 3% of the body water and differs from ECF in that it is separated from blood plasma by a single continuous layer of epithelial tissue in addition to the capillary membrane.

Intracellular fluid and the ECF are separated by the semi-permeable membrane of the cell walls which are relatively permeable to water but only selectively permeable to most solutes except urea. Fluid transport into and from the cells is largely determined by the gradient of osmotic pressure. Effective osmotic pressure is defined as that hydrostatic pressure which must be applied to a solution to prevent its being diluted by a less concentrated solution when the two solutions are separated by a semipermeable membrane (Pitts, 1959). In general, the greater the number of solute particles per unit volume of solution, the higher the osmotic pressure. Two solutes, potassium and particularly sodium, are the prime contributors to the effective osmotic pressure across the mammalian cell membrane (Stevenson, 1965). Changes in ECF tonicity, through osmotic pressure, produce changes in both the volume and tonicity of the intracellular fluid. Yet, there are narrow, critical limits within which each dimension must be maintained for effective cellular function.

The primary vehicle of the water regulatory function is the kidney. It plays the key role in the retention and excretion of both water and sodium. Regulation of excretion and filtering functions by the kidney is controlled by the hypothalamic-pituitary-kidney-adrenal system. The role of this system in the control of fluid volume will be addressed first.
Volume increases occur naturally through an organism's water intake. A blood volume increase without change in tonicity may be artificially produced by the administration of isotonic (0.9% weight by volume) NaCl solution (Stricker, 1966). Injections of isotonic saline have also been found to increase the volume of blood plasma without a corresponding change in ECF tonicity (Corbit, 1967; Hatton & Thornton, 1968). Darrow and Yannet (1935) illustrated that ECF volume increases with isotonic NaCl loads, but intracellular fluid undergoes no change in volume or tonicity, since there is no change in the effective osmotic gradient. Accordingly, Corbit (1967) noted no increase in drinking accompanying an isotonic volume increase in the ECF.

Volume decreases, on the other hand, occur in nature through water deprivation and hemorrhage. The former results in a concomitant tonicity increase, while the latter does not (Fitzsimons, 1961). While water deprivation is a strong stimulus for drinking (Gilman, 1937; Novin, 1962; Stricker, 1966; Stricker & Wolf, 1966), hypovolemia alone apparently is not a sufficient stimulus for drinking amounts of water that would restore the volume deficit (Schnieden, 1962). It should be clear that volume deficit alone would have no effect on the intracellular fluid volume or tonicity.

That urination or drinking are the responses available to an organism to alleviate volemic stress is an observation made obvious by experience. Less obvious are the physiological adjustments made by the organism to correct for volume imbalance. As in most regulatory systems, the detection and correction of volemic stress appear to
be under multiple control. Several sites have been proposed as detectors of changes in ECF volume. Bartter and Gann (1960) propose pressure receptor cells at the junction of the thyroid and carotid arteries; Gauer, Henry, and Sieker (1961) suggest the left atrium of the heart. Each hypothesizes that the respective sites respond to changes in pressure corresponding with changes in blood volume to initiate the hypothalamic-pituitary responses which begin monitoring the restoration of the fluid balance. Davis (1961) proposes that the renal arterioles respond similarly to volemic changes to regulate the kidney-adrenal axis.

Volume control involves, through retention or excretion, regulation over both water and sodium. Water retention is facilitated by intake precipitated by thirst and by anti-diuresis. Excretion of excess water volume is facilitated by water diuresis. Sodium adjustments are thought to be made by hemodynamic changes in the renal tubules, changes in the nephron distribution of filtrate, and varying degrees of sodium reabsorption at the renal tubules by aldosterone (Pitts, 1968).

When volume is increased, the receptors signal the posterior pituitary to inhibit the flow of anti-diuretic hormone (ADH). The reduced flow of ADH promotes water diuresis and the resultant return to an efficient volume level in the ECF. The elimination of water, though, is limited by the increasing level of ECF tonicity that also results from the diuresis (Pitts, 1968).

During volume decreases, the dual mechanisms of the hypothalamic-pituitary-kidney-adrenal axis are more evident since the components
play an active role rather than one of inhibition as in volume increases. Bartter and Gann (1960) have suggested that the volume receptors at the thyroid-carotid artery junction stimulate the hypothalamus via the vagus nerve. In turn, the hypothalamus stimulates the posterior pituitary to increase its production of ADH. The increased amounts of ADH in the system serves several functions in water conservation. It acts directly on the kidney to concentrate the urine by causing the distal tubules and collecting ducts to become more permeable to water (Pitts, 1968). ADH also stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) which acts upon the adrenal cortex and stimulates the production of aldosterone (Sawyer et al., 1960). In addition, ADH directly stimulates aldosterone production by the adrenal cortex. Aldosterone, the critical agent in the kidney-adrenal loop of the regulatory axis, serves to promote the reabsorption of sodium at the renal tubules of the kidney. The process of transporting the sodium back into the vascular system requires water. Thus sodium retention enhances water retention. Eilers and Peterson (1964) have shown that aldosterone production in the rat increases markedly with decreases in blood volume and further substantiate the role of aldosterone in water retention.

The hypophyseal hormones are probably, however, not the primary stimulating agents for the production of aldosterone. Davis, Higgins, and Urquhart (1964) report a series of ablation experiments during which the aldosterone stimulating hormone was sought. It was discovered that even decapitation of dogs (and thereby the removal of the source of ADH and ACTH) did not markedly reduce aldosterone secretion
in response to acute hemorrhage. Removal of the kidneys, however, did significantly lower the level of aldosterone produced (Davis et al., 1961). Through fractionation, renin was isolated as a precursor to angiotensin II which was found to induce the secretion of aldosterone by the adrenal cortex (Davis et al., 1964). It is apparent, therefore, that the components of the regulatory systems are under multiple control in a manner similar to the volume detectors.

In summary, there are two major systems contributing to water retention under hypovolemic conditions. The hypothalamic-pituitary system produces ADH with its various effects; the kidney-adrenal loop produces aldosterone. Both elements play a role in water conservation.

As previously mentioned, volume is not the sole dimension of water regulated in the mammalian body. The tonicity of the bodily fluids must also be maintained within strict limits. Hypertonicity of the ECF occurs in nature primarily during water deprivation (Hatton, 1971), but it has also been shown to accompany stress in rats (Kakolewski & Deaux, 1970). Still another possible natural source of hypertonic ECF stress is the intake of dry food (Hatton & Alali, 1969; Novin, 1962). In the laboratory, the administration of concentrated NaCl is typically employed to induce a high level of ECF tonicity (Gilman, 1937; Novin, 1962; Stricker, 1966; Stricker & Wolf, 1966). According to the model of Darrow and Yannet (1935), an increase in ECF tonicity produces an equal rise in the intracellular fluid tonicity but a decrease in intracellular volume.

The detection of ECF tonicity changes may well be under multiple control as is volume detection. Verney (1954), however, has shown that
there is a response mechanism within the internal carotid loop and suggests the receptors are located in the supraoptic nuclei of the hypothalamus. He injected hypertonic NaCl into the carotid loop and found that increasing osmotic pressure by as little as 2% produced an increase in ADH production by the posterior pituitary. An identical injection administered to the peripheral vascular system or the injection of urea, which has virtually no osmotic effect, caused no change in ADH production. Thus, when the osmometers of the supraoptic nucleus detect an increase in serum tonicity, the increased ADH flow causes the kidney to excrete hypertonic urine as described earlier. Conversely, a decrease in tonicity stems the flow of ADH permitting large amounts of dilute urine to be excreted. It was subsequently demonstrated by Corbit (1969), that virtually any measurable change from isotonicity is sufficient to initiate sodium retention or elimination responses.

When hypertonic NaCl is administered in sufficiently large doses, however, the water required to excrete even the concentrated urine may create a critical reduction in fluid volume (Blass, 1968). The resulting secondary hypovolemia initiates the renin-angiotensin-aldosterone loop. The aldosterone, since it serves to retain water through sodium reabsorption, is thereby competing with the ADH influence. Although the ADH system predominates and hypertonic urine is excreted (Stricker, 1969), clearly efficiency in sodium elimination is reduced by the action of aldosterone. Logic further dictates that were any process to interfere with aldosterone's efficiency in reabsorbing sodium, it would greatly enhance an organism to relieve itself of the cause of its hypertonic stress.
Since, as previously noted, hypertonic NaCl administration and dry food intake both cause an increase in ECF tonicity, it is not surprising that several investigators have found rats to decrease their food intake and lose body weight following concentrated NaCl loads (Adolph, 1947; Gutman & Krausz, 1969). Prior to the report by Kozub (in press), however, researchers either used male rats for stomach-loads of concentrated NaCl, or they failed to report the sex of the Ss. Kozub found the expected loss of body weight and decrease in food intake among his male rats. The females, however, differed significantly from the males for both dependent variables, but did not differ significantly from their own baseline levels of food intake and body weight. He further reported that ovariectomized rats behaved not like the intact females, but they actually lost weight and decreased their food intake as did the males. The data was reported for the period 24 hours after the administration of the NaCl load. It seems clear that the intact female has at her disposal the means to recover from the level of stress imposed by Kozub within a day; the male and ovariectomized female lack the same feature.

In trying to hypothesize what enables the intact female rat to tolerate more efficiently hypertonic ECF stress, attention focuses on two subjects. The kidney, since it is the primary organ involved in electrolyte excretion, is very likely a factor. The sex hormones, which differ between the sexes and which the ovariectomized female essentially lacks, are the focus of most animal sex difference studies. There is evidence also that the female sex hormones play a role in regulating the effects of aldosterone, which has been shown to have an
influence on sodium excretion. The hypothesis follows that the presence of the female hormones enables the female rat to tolerate hypertonic ECF stress more effectively than the male which essentially lacks the hormones.

Progesterone, a female sex hormone, is known to inhibit the action of aldosterone reabsorption of sodium into the vascular system (Sharp & Leaf, 1966; Uete & Venning, 1963). Further, progesterone and estrogen, the other major female hormone, have both been shown to inhibit the production of aldosterone by the adrenal cortex (McKerns & Bell, 1960). On the other hand, testosterone, the male hormone, has little effect on aldosterone secretion (McKerns & Bell, 1960), nor does it influence the clearance of electrolytes by the kidney (Thorn & Engel, 1938).

If progesterone and estrogen are the primary contributors to the female rat's ability to tolerate hypertonic ECF stress, a direct demonstration should be possible. When females are effectively deprived of sex hormones through ovarieotomy, they are not able to maintain their diet level and lose weight when administered hypertonic NaCl solution (Kozub, in press). If the sex hormones were replaced, a return to normalcy should occur if the proposed hypothesis is correct. The purpose of this study was to determine whether the replacement of progesterone and estrogen, individually and in combination, would enable ovarieotomized rats to tolerate the stress of hypertonic NaCl loads as do their intact counterparts. It was hypothesized that progesterone and/or estrogen would enable the ovarieotomized rats to retain their normal body weight and consume their normal intake of food in the
24 hour period after receiving a stomach load of concentrated NaCl solution. Rats which are ovariectomized and do not receive hormone replacement, it was predicted, would lose body weight and decrease their food intake for the corresponding period.

Method

Subjects. Thirty female albino rats of the Sherman-Wistar strain were procured from a professional breeding service at approximately 60 days of age. Ss were individually housed in metal wire cages throughout the experiment. Purina Laboratory Chow was available ad lib in pellet form prior to the onset of data collection and in powdered form during experimental operations. The powdered food was provided in metal cups with no-spill rings inserted and wire mesh screens attached to the bottom of the cups to prevent spillage. Pans under the cages were inspected daily to detect spillage and none was found. Ad lib water was available at all times in polyethylene graduated cylinders.

Ss were maintained in constant light in an air conditioned colony room except for two nights when a power failure occurred. Temperature range in the colony room was recorded daily, and except for the day following the first session of stomach loads when it reached 79°F, was maintained at 74°F ± 3°F. Humidity was recorded twice daily at 1:00 p.m. and at 7:00 p.m. when data collection was conducted. The relative humidity ranged from 65-84%.

Each S was used only once in the study. Five Ss were discarded from the experiment. Ss #2 and 25 died following the operation prior to data collection. S #29 failed to show a weight gain.
following the ovariectomy and was discarded as an operative failure (Hervey & Hervey, 1966). S #24 demonstrated erratic body weight changes in excess of pre-experimental criteria for establishing baseline. Finally, S #27 died 2 days after the stomach load and was eliminated from the study because of possible internal damage caused by the load.

**Design.** A five-level single-factor completely randomized design was employed for each of three dependent variables. A control group (C) received sham ovariectomies and sham injections prior to the stomach load. The 4 experimental groups all received ovariectomies with one group each receiving progesterone (P), estrogen (E), progesterone and estrogen (PE), and sham (S) injections. Each condition included 5 Ss.

The dependent variables were percentage changes in food intake, water intake, and body weight as measured 24 hours after the stomach load in relation to pre-load baseline values. Body weight and food intake were measured to the nearest 1 gram; water was measured to the nearest 1 cc of intake. Baseline was computed for food and water intake by taking the mean of the four 24-hour readings prior to loading. Baseline for body weight consisted of the S's body weight immediately prior to loading.

**Procedure.** Each of the 30 Ss was randomly selected to participate in one of the 5 experimental conditions. Once assigned to a condition, the S received the appropriate operation and was allowed to recover for 8-10 days before the onset of data collection.

Data collection times were assigned to each of the 30 Ss (including the 5 discarded Ss) at exactly 24-hour intervals. Each S was allotted an 8-minute period between 7:00 and 11:00 p.m. for data
collection and experimental operations. Data collection was staggered with measurements taken on 10 Ss the first night and on 10 additional Ss on each of the two succeeding nights. Staggering the collection periods allowed F to perform the necessary administrations while maintaining strict 24-hour measurement periods and was intended to keep minor environmental fluctuations from creating a unidirectional bias.

For each S, body weight was measured and initial recordings of food and water were taken beginning 8-10 days following the operation. On the succeeding 4 days, food and water intake and body weight were measured to establish a baseline, and the series of injections was conducted. On the sixth experimental day, stomach loads were administered after the continuation of data collection and injections. For 3 days following the stomach load, data collection was continued in the absence of injections to establish the dependent variable measures and observe whether any S had suffered internal damage as a result of the stomach load (see page 1, appendix).

Operations. All operations were performed under ether anesthesia according to the general procedure outlined by Zarrow, Yochim, and McCarthy (1964). A longitudinal incision approximately 1½ inches long was made through the skin and fascia in the area near the spinal column in the lower back. A similar incision through the muscle walls of the back was made on one side. The ovary was then exposed and removed. After the muscle wall was sutured with silk thread, the process was repeated on the remaining side. Finally, the skin was sutured with silk thread. Ss were observed in recovery cages until they regained sufficient strength to be returned to the home cage. Body weight was
taken 4 days after surgery to ensure that the increase in body weight
typical of ovariectomized rats occurred (Hervey & Hervey, 1966). One
ovariectomized S failed to show the expected weight gain and was dis­
carded; two sham operates showed large weight gains but were retained
(see page ii, appendix).

Sham operations were conducted in a similar manner, but the
ovaries were replaced intact after they had been exposed through the
muscle wall.

**Injections.** All injections were given subcutaneously into the
lower back. Progesterone recipients were injected for each of 5 days
prior to the load; Ss receiving estrogen received 2 injections with
one 5 days prior to load and the other immediately prior to the load.
Sham injected Ss received the full 5 day injection schedule.

Progesterone injections consisted of 8 mg/kg of the S's body
weight. The progesterone was dissolved in a sesame oil base with an
8 mg/cc solute to solvent ratio. Estradiol benzoate was administered
with Ss receiving 100 mg/kg body weight. The estrogen solution was
prepared in a 100 mg/cc solute/solvent ratio. Sham injections con­
sisted of amounts of the vehicle oil equivalent to the volume of pro­
gesterone injected.

**Loads.** All stomach loads were performed under ether anesthesia.
Although the use of ether has been criticized for distorting food and
water intake values, earlier studies have concentrated on short term
effects (less than 4 hours) or have used ether in large doses (Czech
et al., 1967; Wayner et al., 1967). The technique, however, was
found not to interfere with intake data for rats 24 hours after the
administration of a hypertonic NaCl load (Kibler & Wornoa, 1972). The use of anesthesia was an attempt to reduce stress, a factor that has been shown to alter intake (Kakolewski & Deaux, 1970), and to complete accurate loads without injuring the Ss.

Each S was placed in a covered bell jar containing cotton balls soaked in ether. S was removed from the jar 5 seconds after collapse. The load, consisting of 3% body weight of 10% NaCl solution, was administered by passing an 8 French catheter through the mouth of the S and into the stomach. The S was allowed to revive before being returned to the home cage, and, in every case, was alert within 2 minutes after the load was accomplished.

Results

The mean percentage changes from baseline 24 hours after the administration of hypertonic NaCl loads are presented for body weight, food intake, and water intake in Table 1.

Cochran's C statistic was computed to test for homogeneity of error variance (Myers, 1969). Body weight percentage changes were found to contain heterogeneous error variances between groups, \( C(4,5) = .6073, p < .05 \). In addition, body weight measures were found to correlate significantly with the high extremes in temperature and relative humidity on the critical day following the stomach loads. Each predictor yielded an \( r(23) = .45, p < .05 \). A square root transformation, therefore, was applied to the raw data to produce an acceptable level of error variance homogeneity, \( C(4,5) = .5128, p > .05 \), and an analysis
Table 1
Mean Per Cent Change in Weight, Food, and Water Intake From Baseline in Ovariectomized (OV) and Sham-Operated (SOV) Female Rats 24 Hours after Stomach Loading with 10.0% NaCl

<table>
<thead>
<tr>
<th>Measure</th>
<th>SOV oil injection</th>
<th>OV oil injection</th>
<th>OV progesterone injection</th>
<th>OV estrogen injection</th>
<th>OV progesterone-estrogen injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight change</td>
<td>+0.3</td>
<td>-1.0</td>
<td>+0.4</td>
<td>-0.9</td>
<td>-0.2</td>
</tr>
<tr>
<td>Change in food intake</td>
<td>-19.5</td>
<td>-19.5</td>
<td>-6.4</td>
<td>-4.0</td>
<td>-8.9</td>
</tr>
<tr>
<td>Change in water intake</td>
<td>+78.4</td>
<td>+71.0</td>
<td>+51.4</td>
<td>+88.0</td>
<td>+57.8</td>
</tr>
</tbody>
</table>
of covariance was performed on the transformed data using humidity extremes as covariates (Winer, 1962). No significant differences between groups were found, $F(4,19) < 1$. The mean body weight data for all groups is displayed in Figure 1.

The percentage change in water intake between groups also showed heterogeneity of error variance, $C(4,5) = .5713, p < .05$. The square root data transformation was applied and again produced homogeneity, $C(4,5) = .4503, p > .05$. Since the water intake data did not correlate significantly with humidity or temperature, $r(23) = .31, p > .05$, an analysis of variance was performed on the transformed data. No significant difference was detected, $F(4,20) = 1.11, p > .05$. Water intake means by groups are graphically presented in Figure 2.

Neither the correlation with humidity, $r(23) = -.30, p > .05$, nor the heterogeneity of error variance, $C(4,5) = .4349, p > .05$, was significant for the percentage food intake data. An analysis of variance, therefore, was performed on the data adjusted by a constant and no significant differences between groups were discovered, $F(4,20) < 1$. The mean food data for the entire study is depicted by group in Figure 3.
Changes in Body Weight in Ovariectomized and Sham-Operated Rats
Following Hormone Injections and Concentrated NaCl Loads
Changes in Water Intake in Ovariectomized and Sham-Operated Rats
Following Hormone Injections and Concentrated NaCl Loads
**Figure 3**

Changes in Food Intake in Ovariectomized and Sham-Operated Rats Following Hormone Injections and Concentrated NaCl Loads
Discussion

The failure to find significant differences among the groups for any of the dependent variables was especially disappointing since there were overt indications that the dosage levels of the hormonal injections were physiologically active. Dosage levels have varied greatly among previous researchers (Pfaff, 1970; Rodier, 1971; Wade & Zucker, 1970; Zucker, 1969), and there appears to be little agreement in the appropriate amounts of progesterone and estrogen necessary to restore a natural hormonal balance in ovariectomized rats. It had been previously demonstrated that rats when ovariectomized gain weight faster than their intact counterparts (Hervey & Hervey, 1966; Kakolewski, et al., 1968), when Zucker (1969) reported that replacement of estrogen attenuated the weight gain and decreased food intake.

Reference to Figures 1 and 3 indicates similar results during the baseline period of the present study. Group E maintained body weight for one day following the initial injection and subsequently failed to attain the pre-injection weight level. Group PE follows a similar pattern although there is a return to the pre-injection level 3 days after the stomach-load. Zucker (1969) found progesterone alone to have no effect on body weight or food intake, but that, when combined with estrogen, it had a slight reversal of the estrogen effect. In contrast to the E and PE groups, the S and P groups gained weight continuously except following the stomach load when the S group, as expected, lost weight.
The food intake data are less conspicuously in accord with Zucker's data although the S and P groups consumed at least as much as on the pre-injection day on 3 of the 5 days following the initial injection (excluding the post-load day). The PE group reached the injection day level of consumption only once; the E group twice.

Since the PE and E groups were losing body weight and had decreased their food intake prior to load, the effect of estrogen on these dependent variables was to establish a trend, independent of stomach load, which was the opposite of the effect desired for estrogen-treated Ss following the stomach load. Kozub (in press), however, established differences in body weight loss and food intake decreases between intact females, which have a supply of estrogen, and ovariectomized females. Since the differences in availability of estrogen are at least approximately the same between intact and ovariectomized rats as between estrogen-treated and ovariectomized rats, it appears unlikely that the availability of estrogen alone should be sufficient to confound the data. Nor, in fact, should estrogen replacement cause the extremely heterogeneous error variance that was found. The significant correlation of the body weight change data with temperature levels, however, suggests an alternative factor which may well have erased the treatment effect and have caused the heterogenous error variance.

Extremes in temperature are known to influence food intake. Hamilton (1963) has demonstrated that with small increases in environmental temperature (+5.4°F) the albino rat decreases its food intake dramatically. The reverse phenomenon was reported by
Weiss (1958) who observed significant increases in food intake following very brief exposure to cold. While the temperature decreases by Weiss were rather extreme, from 18-54°F, exposure time as little as 20 minutes per day was sufficient to effect the observed change. It is apparent, then, that the rat's food consumption is extremely sensitive to environmental temperature changes.

A related phenomenon involves an increase in the rat's activity level as environmental temperature declines (Weiss, 1958). Further, it was demonstrated that food deprived rats live significantly longer under warm conditions (see Kleiber, 1961). In essence, then, as the temperature increases, the level of food consumption required to maintain the rat's body weight decreases.

During the course of the present experiment, there were power failures which prevented adequate maintenance of environmental controls, especially temperature increases, following the stomach loads of the first and third groups of rats. Figure 4 depicts the relation of body weight and food intake to the extreme temperature experienced as a result of the first power failure.

With the large temperature increase, a sharp drop in food intake occurred which would be expected from the results of Hamilton (1963). In addition, the rate of body weight gain increased, the significance of which will be discussed below.

The data plotted in Figure 4 are based on Ss from all experimental groups and on whom baseline was being established. The remaining Ss,
Contrast in Food Intake and Body Weight Changes During Baseline Period in Comparison with Daily High Temperature Extremes
also representing all experimental conditions, had been stomach loaded, and the measurements required to test the hypothesis were collected under unfavorable temperature conditions. Clearly, from the effects that accompanied the extreme temperature increase for the non-treated Ss, it is probable that the group loaded represented a contaminated population sample.

In addition, since a lesser power failure occurred on the third day of loading, essentially there existed 3 separate load conditions. Since Ss were randomly scheduled for load times, differing numbers of Ss from each group were loaded under each environmental condition. The heterogeneity of error variance could have been expected to occur under such conditions, and the data support this contention.

The fact that Ss increased body weight gain although they decreased sharply their food intake presents yet another difficulty. The thesis hypothesis depends upon the well established observation that when rats decrease their food intake, their weights also decrease. From the conclusions of Kleiber (see, 1961), the exception occurs when temperature increases. An explanation follows logically from the work of Weiss (1958). Since the rat's activity level is inversely related to environmental temperature, the decrease in activity could explain body weight maintenance or gain even though food intake requirements diminish. Since activity levels were not recorded throughout the study, no data can be presented to support the explanation. However, it seems likely that the temperature changes were accompanied by large differences in within-group variation and by effects either negatively biasing the treatment groups (e.g. a uniform decrease
in food intake for Ss loaded on a day of power failure) or at least confounding the results (e.g. divergence of food intake and body weight curves on the day of extreme temperature).
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APPENDICES
Appendix A

Temporal Scheme of Experimental Manipulations by Group

Group 1

Group 2

Group 3

June 5 10 15 20 25

Key: O - Ovariectomy  I - Injection and data collection
D - Data collection only  L - Load, injection, and data collection
Appendix B

Mean Percentage Increase in Post-Operative Body Weight

Four Days after Surgery and at Initial Injection

<table>
<thead>
<tr>
<th>Group</th>
<th>Surgery</th>
<th>4-Day</th>
<th>Initial Injection</th>
</tr>
</thead>
<tbody>
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**Appendix C**

### Analysis of Covariance: Body Weight

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### Analysis of Variance: Water Intake

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### Analysis of Variance: Food Intake

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