GOTSHALL, Robert William, 1945-
WATER AND ELECTROLYTE METABOLISM IN ADRENAL-
ECTOMIZED-NEPHRECTOMIZED RATS AND THE EFFECTS
OF ADRENALEORTICAL HORMONE REPLACEMENT.

The Ohio State University, Ph.D., 1971
Physiology

University Microfilms, A XEROX Company, Ann Arbor, Michigan
WATER AND ELECTROLYTE METABOLISM IN ADRENALECTOMIZED-
NEPHRECTOMIZED RATS AND THE EFFECTS OF
ADRENALCORTICAL HORMONE REPLACEMENT

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Robert William Gotshall, B.S., M.S.

The Ohio State University
1971

Approved

Stephen J. LeBrie
Adviser
Department of Physiology
PLEASE NOTE:

Some Pages have indistinct print. Filmed as received.

UNIVERSITY MICROFILMS
ACKNOWLEDGMENTS

I particularly wish to express my sincere appreciation to my adviser, Professor Stephen J. LeBrie, for his advice and assistance. I am also grateful to the Department of Physiology, The Ohio State University for facilities and opportunities provided.
VITA

Date of Birth: April 28, 1945
Place of Birth: Carrollton, Ohio

Educational Preparation:

B.S. Mount Union College, Alliance, Ohio, 1967
M.S. The Ohio State University, Columbus, Ohio, 1969

Professional and Academic Experience:

The Ohio State University, Columbus, Ohio
September 1967 through August 1971
Teaching Assistant, Research Assistant and
University Fellow in the Department of Physiology
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>PURPOSE</td>
<td>8</td>
</tr>
<tr>
<td>METHODS</td>
<td>10</td>
</tr>
<tr>
<td>CALCULATIONS</td>
<td>17</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>20</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>31</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>35</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>49</td>
</tr>
</tbody>
</table>
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mean Data of the Five Groups of the Non-hydrated Series</td>
<td>36</td>
</tr>
<tr>
<td>2.</td>
<td>Mean Data of the Four Groups of the Hydrated Series</td>
<td>38</td>
</tr>
<tr>
<td>3.</td>
<td>Plasma Volumes (PV) and Interstitial Fluid Volumes (ISFV) as a Per Cent of Body Weight, and the Ratio PV/ISFV</td>
<td>40</td>
</tr>
<tr>
<td>4.</td>
<td>Ratios of Sodium and Urea to Osmotic Pressure</td>
<td>41</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.</td>
<td>ECFV as a Per Cent of Body Weight and the Hematocrit</td>
<td>42</td>
</tr>
<tr>
<td>2.</td>
<td>The Per Cent Change in Body Weight from Day One to Day Two</td>
<td>43</td>
</tr>
<tr>
<td>3.</td>
<td>Plasma Concentration of Sodium and Total Extracellular Sodium</td>
<td>44</td>
</tr>
<tr>
<td>4.</td>
<td>Plasma Concentration of Chloride and Total Extracellular Chloride</td>
<td>45</td>
</tr>
<tr>
<td>5.</td>
<td>Plasma Concentration of Potassium and Total Extracellular Potassium</td>
<td>46</td>
</tr>
<tr>
<td>6.</td>
<td>Plasma Concentration of Urea and Total Extracellular Urea</td>
<td>47</td>
</tr>
<tr>
<td>7.</td>
<td>Plasma Osmotic Pressure</td>
<td>48</td>
</tr>
</tbody>
</table>
INTRODUCTION

Development of Concepts in Adrenal Cortical Physiology

In 1849, Addison first described a disease state which he attributed to a deficiency in suprarenal (adrenal) function. Very little was known concerning the physiology of the adrenals at that time, and it was almost eighty years before the disease was fully characterized and attributed specifically to adrenal malfunction. It was Marine and Bauman, in 1927, who demonstrated that there was a decrease in blood sodium concentration following adrenalectomy in the cat (38). Loeb, in 1932, described a decrease in sodium concentration in the blood of three Addisonian patients (36). He was able to demonstrate that adrenal insufficiency was characterized by a renal wastage of sodium salts (37). In the same year, 1933, Harrop et al. demonstrated this fact in adrenalectomized dogs (24, 25). It was the contention of Loeb and Harrop that the fundamental physiological change in adrenal insufficiency was the loss of sodium from the blood via the kidney. Therefore, emphasis was placed on the renal actions of the cortical hormones on electrolyte metabolism, to which changes in body water was a secondary osmotic consequence.

If this were all that was involved, cortical hormone administration would result in the retention of water as a result of sodium reabsorption, and the absence of cortical hormones would result in the loss of body fluids as a result of renal sodium loss. Dehydration was commonly described as a symptom of adrenal insufficiency (45). But, unfortunately for this theory, a study of
total body water revealed no significant decrease in the percentage of water determined on a fat-free basis (23). Reductions in blood volume (24, 48, 27) and of the extracellular fluid volume (24, 25) were observed and attributed to the loss of fluid through the kidneys (24, 25). However, it was shown that the losses of extracellular fluid and sodium were greater than could be accounted for on the basis of renal excretion (49, 50, 10).

Thus, two concepts emerged from the early work on adrenal insufficiency. The first was the view that the sole effect of the adrenal cortex upon electrolyte metabolism was through its action on the kidney, and changes in water metabolism were due to this action (37, 24, 25). The other view was that the cortical hormones not only exerted a renal action but also significantly influenced the distribution of electrolyte, and consequently water, between extracellular and intracellular compartments (49, 50).

Various investigators have studied the problem in many ways. In 1936, Dwight Ingle hypothesized that if the main action of the adrenal cortex were on the kidney, nephrectomy should benefit an adrenal insufficient animal by preventing loss of electrolytes. Adrenal cortical extract should not benefit such an animal. His studies showed that adrenalectomized-nephrectomized rats lived a shorter period of time than did nephrectomized rats. Also, the injection of adrenal cortical extract into the adrenalectomized-nephrectomized rats increased their survival time to that of nephrectomized animals (30). Gaudino and Levitt demonstrated (18) that normal dogs treated with desoxycorticosterone showed a decrease in intracellular water and an increase in extracellular water, with no change in total body water. Administration of adrenal cortical extract in the same animals caused a similar change without a change in renal function. Flanagan et al. (10) in 1950, used adrenalectomized dogs and showed that the
extracellular sodium and chloride losses in acute adrenal insufficiency were not due totally to renal excretion or fecal loss. Also, recovery from acute insufficiency with deoxycorticosterone resulted in increases in extracellular sodium and chloride greater than intake and renal retention, so they had to come from endogenous sources.

Woodbury (63), in 1953, studied the effects of deoxycorticosterone and adrenal cortical extract in nephrectomized rats. In a detailed study of plasma and tissue electrolytes he concluded that the adrenal cortical hormones influenced electrolyte metabolism in all tissues of the body and that this influence was exerted independently from the effects of these hormones on the kidneys. In the same year, Hills et al. (29) studied the distribution of water and electrolytes in patients with adrenal insufficiency. Patients in acute insufficiency showed a significant decrease in their inulin space (used as a measure of extracellular fluid volume) and the calculated losses of sodium, chloride, potassium and water from the inulin space could not be accounted for by external losses. They concluded that internal transfers of fluid and electrolytes had occurred. Desoxycorticosterone, hydrocortisone and cortisone were able to reverse these changes.

More recently, Swingle has added more evidence in support of his original hypotheses that the adrenal hormones are important in water and electrolyte distribution. His basic experimental design was to use adrenalectomized dogs which had been maintained in health with hormone replacement, usually desoxycorticosterone. These animals were taken off treatment and allowed to develop adrenal insufficiency. They were fasted and dehydrated for forty-eight hours prior to the experiment and were allowed no food or water during the time course of the experiment. Using this design, he and his coworkers published a series of papers from 1957 through 1959 (54, 55, 56, 57, 58). In these papers, it was demonstrated that glucocorticoids
and their analogs were able to restore these animals to health and to re-establish the blood volume and electrolytes. Since these animals had no exogenous sources of water and electrolytes, there had to be an internal redistribution.

It was also demonstrated that desoxycortisone and aldosterone were not able to restore these animals to health nor re-establish the distorted water and electrolyte pattern. This finding indicated to Swingle that a distinction existed between the major sites of action of glucocorticoids and mineralocorticoids on water and electrolyte metabolism; glucocorticoids acting extra-renal and mineralocorticoids acting mainly on the kidney. It was noted that the failure of the mineralocorticoids to reverse the adrenal insufficiency was in fasted animals. Mineralocorticoids did prevent symptoms of adrenal insufficiency if the animal was allowed free access to food and water as had long been known.

Two studies by Friedman et al. in 1958 (11, 12) involved rats which had been adrenalectomized and nephrectomized for twenty-four hours. Extracellular fluid volumes, plasma sodium and potassium concentrations as well as total extracellular sodium and potassium were measured. Their data suggested that adrenalectomy resulted in a decrease in sodium concentration and an increase in potassium concentration. These changes occurred with a decrease in total extracellular sodium and an increase in potassium. There were no changes in extracellular fluid volumes when compared to nephrectomy alone. However, their conclusions were drawn from data which in some cases was not significantly different from nephrectomy alone and showed only a trend. There was no information in their experiments as to how the animals were handled or whether or not in all cases food and water were withheld. Therefore, their results did not lend themselves to definitive conclusions.
Related to the role of the adrenal gland in affecting body water and electrolyte distribution is the increased susceptibility to water intoxication seen in adrenal insufficiency. Rowntree and Snell (40) in 1931, were the first to observe that water was not excreted normally when given to a patient with adrenal insufficiency. Various experiments demonstrated that there was a marked susceptibility to water intoxication after adrenalectomy (19, 50). Symptoms of water intoxication appeared rapidly after the administration of a water load and were attributed to cellular over-hydration. This sensitivity to excess water was demonstrated not to be wholly due to deficient water excretion (51, 20, 28). This fact was further demonstrated by Birnie, Eversole and Gaunt in 1948 (2) when they showed that desoxycortico-sterone greatly increased the resistance of adrenalectomized-nephrectomized rats to water loads.

The mechanism of impaired water excretion in adrenal insufficiency has been investigated by various investigators, the more recent work having been done by Kleeman et al. (32, 33, 34). Briefly, what has been demonstrated is that the glucocorticoids and not the mineralocorticoids are most efficient in correcting the impaired water excretion. The mechanism of action of the glucocorticoids in correcting this impairment is not dependent on altered renal hemodynamics, sodium reabsorption, changes in glomerular filtration rate or on the release, metabolism or action of antidiuretic hormone. The suggestion is that the major effect of the glucocorticoids in correcting the water excretion is to directly effect the permeability of the kidney tubule.

**History of Isolation and Purification of Adrenal Cortical Hormones**

In 1930, Swingle and Pfiffner (47) and Hartman and Brownell (26) prepared the first adrenal extracts that could serve as substitution therapy, for indefinite periods, in the absence of endogenous
adrenal function. Reichstein (9) in 1937, described nine compounds extracted from the adrenal cortex which he labeled A through I. Compound H was the only one found to have the biological activity of adrenal cortical hormone. He called this hormone "corticosteron". Kendall and his co-workers (31) soon after, in 1937, isolated an active compound which they called "B". This compound "B" was found to be identical with the corticosterone of Reichstein. The first synthesis of corticosterone acetate was done in 1944 and was accomplished by von Euiv, Lardon and Reichstein (9).

Steiger and Reichstein (9), in 1937, synthesized a crystalline substance from inactive materials which possessed marked adrenal cortical activity. This compound differed from corticosterone only by the fourth oxygen and was called desoxycorticosterone. Two years later the synthetic production of desoxycorticosterone acetate was accomplished by Serni, Logemann and Hildebrand (9).

In 1934 and 1935 Wintersteiner, Vars and Pfiffner (61, 39) found a non-crystalline residue after removing the various crystalline substances from adrenal extracts. This residue had a very potent life-maintaining activity in the adrenalectomized animal. This residue, termed the amorphous fraction, was much more potent than any of the separated crystalline components. This fraction contained about one-half of the life-sustaining activity of whole adrenal extract and had powerful sodium-retaining activity. It was not until 1953 that the steroid hormone active in the amorphous fraction was isolated by Simpson, Tail, et al. (42). These workers called it electrocortin because of its potent effect on the excretion of certain electrolytes. After further investigation they renamed it aldosterone (43) in 1954.
Gross and Gyesl (22) and Swingle, et al. (53, 52) found that minute doses of aldosterone maintained life and electrolyte balance indefinitely in adrenalectomized dogs. Spiers, et al. (46) showed that aldosterone had one hundred and twenty times the potency of desoxycorticosterone in decreasing the ratio of sodium\(^{24}\) to potassium\(^{42}\) excretion in adrenalectomized rats. Thus, it was quickly recognized that aldosterone is the most potent mineralocorticoid isolated from adrenal extracts.
Evidence for direct adrenal control over internal water and electrolyte distribution has been presented. To date, the approach to this problem has been mainly indirect with the result that there has been a lack of definitive data. Attempts at more direct studies have resulted in non-significant data with conclusions drawn from trends in the data. Thus, the question of adrenal cortical control over the internal distribution of water and electrolytes is still unanswered.

There is a need to study the problem directly using an experimental model which will most likely yield significant and conclusive results. The purpose of this dissertation is to study the role of the adrenal cortex in water and electrolyte distribution using an experimental model which will enable a direct study of the problem.

Since we were interested in the non-renal actions of the adrenal gland, the nephrectomized rat was used as a model for this study. Nephrectomy eliminates any involvement of the kidneys. Measuring the extracellular fluid volume and electrolytes in the adrenalectomized-nephrectomized animal and comparing these measurements to the same parameters in the nephrectomized animal should demonstrate the effect of removing the adrenal gland. Then, replacing adrenal cortical hormones in the adrenalectomized-nephrectomized rats and comparing these animals to the nephrectomized and untreated adrenalectomized-nephrectomized groups should enable one to demonstrate any extra-renal actions of the hormones. Any parameter which was initially altered by removal of the adrenals should be returned to the
nephrectomized value, if the hormone used had any extra-renal action on water and electrolyte metabolism.

It would seem that the nature of the role of the adrenal cortex in water intoxication is related to its role in determining the distribution of body water and electrolytes. Based on this assumption, it was decided to give each of the above groups of animals a small water load and observe how the water was distributed within the body. If a water volume were injected directly into the extracellular fluid of a nephrectomized rat, that volume should be distributed among the body compartments in a manner characteristic of this group. On the other hand, in an adrenalectomized-nephrectomized animal, one should expect a different distribution assuming that the adrenal gland is involved in controlling the distribution. By then replacing adrenal cortical hormone in the adrenalectomized-nephrectomized animal, the distribution of the water load should approximate that of the animals which were only nephrectomized. This would be expected if the particular hormone used was capable of controlling body water and electrolyte distribution.

Therefore, two series of animal groups were investigated. The first series constitutes the non-hydrated groups of animals and the second series, the hydrated animals. The non-hydrated animals showed some changes in water and electrolyte distribution which demonstrated adrenal cortical involvement in this regulation. A deficit in a regulatory mechanism is usually more detectable when the system is stressed, thus, the hydrated animals yielded more definitive results indicating that the adrenal cortex was involved in regulating the distribution of water and electrolytes. This action was separate from any adrenal action on the kidney.
METHODS

Female Sprague-Dawley (Madison, Wisconsin) rats weighing approximately 160 to 180 grams were used in this investigation. Five rats were grouped together in one cage. The temperature of the cage was maintained at 26° C ± .05°C throughout the experimental period.

In our early studies the mortality rate after nephrectomy or adrenalectomy-nephrectomy was high and the animals exhibited blood potassium levels higher than ten milliequivalents per liter within 24 hours after surgery. Others have shown that the survival time of nephrectomized and adrenalectomized-nephrectomized rats can be increased by placing the animals on a diet deficient in potassium prior to surgery (6, 3, 35). Therefore, twenty-four hours prior to surgery, a feeding bottle containing 40 grams of Controlyte (D.M. Doyle Pharmaceutical Co.) dissolved in 125 milliliters of distilled water was placed on each cage. This diet is a low electrolyte, protein-free diet with carbohydrate and fat contributing the necessary calories. Its constituents are 72 per cent carbohydrate, 24 per cent fat, zero per cent protein, .01 per cent sodium and .004 per cent potassium. The diet was presented ad libitum, although the theoretical amount was 8 grams of Controlyte and 25 milliliters of water per rat in order to supply the daily caloric requirement as indicated by the Biological Handbook (1). Since instituting both this diet and the regulation of the cage temperature, both nephrectomized and adrenalectomized-nephrectomized animals are in good condition for 24 hours post-operatively. Survival studies indicated that these animals generally
lived at least 72 hours postoperatively, if they were allowed water and
diet at 48 hours. This fact strongly indicated that the animals at 24
hours postoperative were not moribund.

Each cage of five rats was permitted the above diet ad libi-
tum for 24 hours. At this time, the feeding bottle was removed and
replaced with a bottle of distilled water. The animals were allowed
this water ad libitum for approximately four hours, after which time
period, they underwent nephrectomy or adrenalectomy-nephrectomy.
Surgery was accomplished under light ether anesthesia. A single in-
cision was made in the skin of the back where upon first the left, then
the right adrenals and/or kidneys were removed through lateral in-
cisions in the muscle. All surgical instruments were maintained
aseptic by keeping them in a solution of zephiran chloride. After the
surgery was completed and the incisions closed by suture, the rat was
weighed. This weight was recorded as the "day one" weight. Each
group of five rats so operated were placed back in a litter-free cage
and returned to the temperature regulated area. They were left there
for 24 hours without food or water.

Twenty-four hours after the time of surgery, the rats were
weighed ("day two" weight) and placed under ether anesthesia. A
femoral vein was exposed and a known amount of inulin was injected
via a 27 gauge 3/8 inch needle. One hour after the injection of inulin
a blood sample was obtained by heart puncture. Sampling was accom-
plished through a 26 gauge 3/8 inch needle. Samples were collected
consecutively in sterile, disposable, one milliliter tuberculin syringes.
Total volume of the samples never exceeded 3.5 milliliters. Samples
were transferred immediately to Spinco micro test tubes into which
heparin had been placed and allowed to dry (sodium heparin was utili-
zed in tubes slated for urea analysis and ammonia heparin in tubes
slated for sodium and potassium analysis). The concentration of heparin used was 10,000 units per liter of blood (5 units per 500 lambda of blood). The samples were immediately mixed and centrifuged. The plasma was transferred to corresponding tubes and frozen until analysis. A small amount of the fresh plasma was placed in tared beakers, weighed, placed in the oven at 100°C and dried to constant weight. At the time of the sampling a small amount of the fresh blood sample was collected in heparinized capillary tubes for micro hemocrit and pH determinations. The hematocrit was determined immediately; the pH capillary tubes were sealed and were read within one hour.

Inulin was used for the measurement of extracellular fluid volume (ECFV). A period of one hour was chosen for equilibration of the injected inulin with the ECFV. This period was chosen because equilibration occurs in two phases, a rapid and a slow phase. A period of one hour has been shown to extend into the slow phase with periods up to two hours showing undetectable differences (5). Experiments in our laboratory comparing ECFV as calculated at inulin equilibration times of 30 minutes, 60 minutes and 120 minutes showed essentially no differences in the last two time periods. Any longer period of time led to overestimation of the ECFV due to metabolism of inulin and sequestering of inulin within cells (60). Our values for ECFV and standard errors indicated a low variability and were quite compatible with similar experiments where the equilibration time was two hours (11). Thus, our techniques of measuring extracellular fluid volumes give values which are compatible with the literature.

The ECFV is calculated from the amount of inulin injected divided by the concentration of inulin in the blood sample taken after one hour, therefore, it was necessary to determine both of these
values. The amount injected was determined by injecting a known volume of a known concentration of inulin. A one milliliter syringe was weighed with volumes of 0.25 milliliters of water as indicated by the syringe markings. The syringes were weighed on a Mettler H-16 balance and read to the fifth decimal place. The average of the weighings was the actual volume in the syringe at that marking. A 10 per cent inulin solution was used. By filling the syringe to the 0.25 milliliter mark with the 10 per cent solution, the actual amount of inulin in the syringe was determined in milligrams. After injecting the contents of the syringe into the rat, the amount of inulin left behind in the syringe was determined by rinsing the syringe into a 10 milliliter volumetric flask which was then filled to the 10 milliliter mark. This solution was analyzed for inulin and the amount present was calculated. The amount left in the syringe subtracted from the amount initially in the syringe yields the amount of inulin injected.

The concentration of inulin in the blood sample was determined by the method of Galli (17). The plasma samples were compared to standard inulin solutions obtained by diluting a ten per cent inulin solution with water to a concentration within the range of the plasma samples. Inulin methods usually read plasma glucose. Since plasma samples from rats not injected with inulin read an appreciable amount with this method (2-5 mg %), it was necessary to run plasma blanks. However, it appeared undesirable to affect the experimental animals by taking a blood sample previous to inulin injection. Therefore, the possibility of using one rat from each group of five rats as the blank for the other four was tested. This one animal was treated exactly like the others except that no inulin was injected. Several experimental groups were run through this protocol except for inulin injection. The plasma obtained from these animals was then analyzed for inulin.
The results demonstrated that there was ($<.3 \text{ mg} \%$) essentially no difference between animals of a group. Thus, in subsequent experiments, one animal was used to represent the others within the group. The blank plasma sample obtained from this animal was treated exactly like the other plasma samples taken from that group and all samples were analyzed simultaneously. The blank was then subtracted from the samples containing inulin.

Plasma sodium and potassium were analyzed using a flame photometer (I. L. model 143) and were obtained from plasma samples which had been heparinized with ammonium heparin. Urea was analyzed by the method of Conway (4), and was obtained from plasma samples heparinized with sodium heparin. Chloride was determined by the use of the chloride titrator (Aminco-Cotlove); osmotic pressure was measured using the Aminco freezing-point depression apparatus; hematocrit was obtained by microhematocrit tubes; and pH was determined using the Radiometer/Copenhagen, model 27, pH meter.

Nine different experimental groups were employed in this study. These nine groups were divided into a non-hydrated series consisting of five groups and a hydrated series consisting of four groups.

The groups of the non-hydrated series were as follows:

1. **Control**: These animals were fed Controlyte for 24 hours, then given distilled water for 3-4 hours before undergoing surgery for removal of the kidneys. At this time, they were injected with inulin and, after one hour, the blood samples were obtained.

2. **Nephrectomized animals (NEPHX)**: These animals were fed Controlyte for 24 hours, then given distilled water for 3-4 hours before undergoing bilateral nephrectomy. They were placed back in the
cage in the temperature unit for 24 hours. Twenty-four hours after surgery, inulin was administered. One hour later, the blood samples were obtained.

(3) Adrenalectomized-nephrectomized animals (ADX-NEPHX): These animals were treated in the same way as in group 2 except that the adrenals were removed together with the kidneys.

(4) Adrenalectomized, nephrectomized, and aldosterone treated animals (ADX-NEPHX-ALDO): The procedure was the same as in group 3 except that d-aldosterone (Ciba) was administered subcutaneously. The aldosterone was dissolved in alcohol with the injection solution being no more concentrated than a 10 per cent solution. The dose given was 2.5 micrograms and the volume of the injection was 0.25 milliliters. This dosage was administered three times beginning immediately after surgery. The second dose was given eight hours after surgery and the final dose four hours before the start of the inulin space determination. The total dose, 7.5 micrograms per day, is physiological for the rat, based on the work of Eilers and Peters (7) and Friedman et al. (14).

(5) Adrenalectomized, nephrectomized, and corticosterone treated animals (ADX-NEPHX-"B"): The protocol for this group was the same as in group 4 except that corticosterone (Sigma) was administered. The corticosterone was dissolved in alcohol with the injection solution being less than a 10 per cent alcohol solution. The corticosterone was given three times in a manner identical with the aldosterone administration as in group 4. The dose was 375 micrograms per day (125 micrograms per injection). This dosage is physiological based on the work of Eilers and Peters (7) and Friedman et al. (15).
The four groups composing the hydrated series were identical to groups 2 through 5 of the non-hydrated series except for the following differences: After nephrectomy or adrenalectomy-nephrectomy, a 0.25 per cent solution of sodium chloride was infused into the femoral vein. The total volume infused was one per cent of the body weight determined immediately after surgery. The infusion rate was 0.2 milliliters per minute using a constant rate infusion pump. There was no noticeable hemolysis or any other difficulty observed in these animals during the infusion.

For the analysis of variance, the simple classification as described by Snedecor (44) was used in this investigation. The difference between sample means was tested at the five per cent level using Hartley's modification of Tukey's test (44).
CALCULATIONS

(1) Calculation of extracellular fluid volume (ECFV).

\[
ECFV \ (ml) = \frac{\text{mg. of inulin injected}}{\text{conc. of plasma inulin (mg/ml)}}
\]

(2) Calculation of extracellular volume as per cent of body weight.

\[\frac{ECFV}{\text{day two wt. (gm)}} \times 100\% = ECFV \ (\% \ body \ wt.)\]

(3) Per cent change in body weight (\% change body wt.).

\[\frac{\text{day 1 wt. (gm)} - \text{day 2 wt. (gm)}}{\text{day 1 wt. (gm)}} \times 100\% = \% \ change \ in \ body \ wt.\]

(4) Per cent of plasma which is water (\% plasma H_2O).

\[\% \ plasma \ H_2O = \frac{\text{wet wt. beaker, plasma} - \text{dry wt. beaker, plasma}}{\text{wet wt. beaker, plasma} - \text{wt. beaker, empty}} \times 100\%\]

(5) Amount of substance in ECFV per 100 grams body wt. (extracellular \(x/100 \ gm. \ body \ wt.\)).

\[\frac{ECFV \ (1.) \times \text{conc. of } X \ (mEq/l)}{\text{day 2 body wt. in 100 gms}} = \frac{mEq}{100 \ gm \ body \ wt.}\]

(6) Ratio of the concentration of sodium or urea to osmotic pressure.

\[\frac{\text{Na (mEq/l)}}{\text{osmotic pressure (mOsm/kg H}_2\text{O)}} = \text{ratio}\]

(7) Calculation of plasma volume and interstitial volume.
The ECFV is compartmentalized into the plasma volume (PV) and the interstitial fluid volume (ISFV). The blood volume is divided into the red blood cell volume and the plasma volume. The hematocrit is the percentage of the total blood volume which is occupied by the red blood cells. One minus the hematocrit gives the per cent of the total blood volume which is contributed by the plasma volume. Assuming that the total volume of red blood cells in any one group of animals is not affected by the experiment and remains unchanged over the 24 hour experimental period, differences in hematocrit observed must be due to differences in plasma volume. Using the fact that the normal plasma volume is approximately five per cent of the body weight, the plasma volumes of the other groups can then be calculated by comparing the hematocrits and plasma volumes in the following formula (59):

$$\frac{PV_2}{PV_1} = \frac{Hct_1}{Hct_2}$$

$PV_1$ is the normal plasma volume (5%), $Hct_1$ is the normal hematocrit (42.4% in these experiments). $Hct_2$ is the hematocrit of the experimental group and $PV_2$ is the plasma volume of the experimental group. This formula can only be used if there is no osmotic shrinking or swelling of the red blood cells from group to group. Although in our experiments there are changes in osmotic pressure, these changes are due to changes in urea concentration. Since urea is readily diffusible across cell membranes the osmotic pressure is quickly equilibrated on both sides of the membrane and no change in cell volume occurs.

The calculated plasma volume can be subtracted from the value measured for the ECFV. The result is the ISFV as a per cent of body weight. In the normal animal with an ECFV of 21% of body weight, the PV is 5% and the ISFV is 16%.
The ratio of the plasma volume to the interstitial fluid volume (PV/ISFV) can also be obtained. This ratio presents information on the respective roles of the PV and ISFV in changes in the ECFV. Obviously, if the change in ECFV involves a proportionate change in PV and ISFV, the ratio will not change from normal. However, if there is a greater change in either the PV or ISFV, the ratio will change accordingly. Therefore, with these calculations it can be determined which compartment (PV or ISFV) is primarily involved in the ECFV changes.
RESULTS AND DISCUSSION

Table 1 and 2 contain the mean values and standard errors of all the parameters studied in the non-hydrated and hydrated series respectively. Figures 1 through 7 illustrate corresponding data with the non-hydrated series on the left and the hydrated series on the right. Significance at the five per cent level is represented by an asterisk below the bar graphs. An asterisk in a box indicates a significant difference between the row and column represented by that box.

In the nephrectomized animals, changes in the extracellular fluid volume may be attributed to two factors, changes in total body water or a redistribution of fluid into or out of the ECFV. An increase in total body water may be caused by either increasing exogenous water intake or increasing the water formed by oxidation. In these experiments there was no intake of exogenous H₂O, therefore only water resulting from oxidation was available. Rats have no sweat glands and do not pant, therefore, water loss in the nephrectomized rat is caused primarily by respiration. The volume of water due to oxidation is dependent upon the kind of foodstuff being metabolized. The oxidation of 1.0 gram of fat results in the formation of 1.07 grams of water. The oxidation of 1.0 gram of carbohydrate yields .60 grams of water. From these values it would appear that sufficient amounts of water could be formed in 24 hours to cause a significant increase in total body water. However, incidental to the oxygen required for the oxidation of a particular foodstuff, there is an adjustment in respiration with a resultant change in evaporative water lost. Although there may be a considerable amount of water formed, there is an obligatory loss
in excess of gain. For fat, 1.6 times as much water is lost as is formed. For carbohydrate, 1.3 times as much is lost as is formed (41). Combining these facts with the fact that all our animals lost weight in the 24 hours of the experiment would indicate that there is no increase in total body water.

Extracellular fluid volume as a per cent of body weight is presented in Tables 1 and 2 and Figure 1. Adrenalectomy at the time of nephrectomy (Figure 1) resulted in a significant decrease in ECFV. The decrease ranged from 22.3 ± .44 per cent to 20.2 ± .33 per cent in the non-hydrated series and from 22.9 ± .20 per cent to 19.0 ± .21 per cent in the hydrated series. Due to the nature of the experiment, this loss of fluid had to be due to an internal shift of fluid out of the extracellular space. This finding demonstrated that the adrenal gland controlled the distribution of body water, an effect which was separate from its action on the kidney.

To determine the adrenalcortical hormones which were responsible for this control, the experiments with aldosterone and corticosterone were performed. In the non-hydrated series, the ECFV of the ADX-NEPHX-ALDO group was not significantly different from that of the ADX-NEPHX group (Figure 1). This indicated that aldosterone was not effective in preventing the decrease in ECFV which occurred with adrenalectomy. In the hydrated ADX-NEPHX-ALDO group, the ECFV was significantly higher than in the ADX-NEPHX group but significantly lower than in the NEPHX group. It appears that, in this series of hydrated animals, aldosterone was effective in preventing, to a small extent, the change in ECFV occurring with adrenalectomy.

On the other hand, corticosterone was capable of preventing the loss of extracellular fluid. This is demonstrated in the non-hydrated series (Figure 1) by the fact that the ADX-NEPHX-"B"
group (21.9 ± 0.32 per cent) was not significantly different from the NEPHX group (22.9 ± 0.20 per cent). In the hydrated series, the ADX-NEPHX-"B" group (25.0 ± 0.53 per cent) is actually larger than the NEPHX group (22.9 ± 0.20 per cent). Apparently, corticosterone not only prevented the loss of extracellular fluid but shifted fluid into the ECFV.

From these data it is apparent that aldosterone is not very effective in preventing the loss of ECFV associated with adrenalectomy. Corticosterone, however, is most effective. This finding indicates that corticosterone is the more important factor in the adrenal's direct control over the distribution of body water.

As indicated in Tables 1 and 2 and Figure 2, all animals lost weight from day one to day two. There was a difference among the groups as to how much weight was lost. There was a significant weight difference between the NEPHX group (-4.9 ± 0.29 %) and the ADX-NEPHX-ALDO group (-4.0 ± 0.13 %). In the hydrated series, the ADX-NEPHX-ALDO group (-4.3 ± 0.49 %) lost significantly less weight than both the NEPHX groups (-5.6 ± 0.18 %) and the ADX-NEPHX group (-5.1 ± 0.30 %). The ADX-NEPHX-"B" group (-4.7 ± 0.17 %) also lost significantly less weight than the NEPH group. In both series, the NEPHX group lost the most weight in the 24 hours of the experiment and the ADX-NEPHX-ALDO group lost the least.

If we assume that a large amount of the weight loss in these groups was due to evaporative water loss, the effect of this loss on the other measured parameters, especially the ECFV, will have to be considered. It is immediately apparent that there was no effect on the ECFV. The NEPHX group which lost the largest amount of weight, had the greater ECFV. The ADX-NEPHX group lost as much weight but had the greatest decrease in ECFV. Both groups then experienced
the same change in weight, but an opposite change in ECFV. The ADX-NEPHX-ALDO group lost the least amount of weight, but had a decrease in ECFV similar to the ADX-NEPHX group. Thus, the ADX-NEPHX-ALDO and ADX-NEPHX groups experienced a similar change in ECFV but an opposite change in weight. It is evident from these observations that factors other than evaporative water loss must have controlled ECFV. In fact, regardless of what the cause for the weight loss may have been, the factors governing the changes in ECFV were obviously different.

Hematocrit changes reflected the changes in ECFV (Figure 1). The normal (Control) hematocrit (42.4 ± .56 %) decreased subsequent to nephrectomy (37.0 ± .58 %). This decrease was most probably due to an increase in plasma volume as indicated by the increase in ECFV. A decrease in the number of red blood cells was not a likely cause of this change in hematocrit since the depression in red cell production associated with renal failure or nephrectomy has a latent period of several days. The large increase in hematocrit which occurred with adrenalectomy in both series reflected the decrease in ECFV. The changes seen in the ADX-NEPHX-ALDO and ADX-NEPHX-"B" groups were also in the direction expected with an increasing ECFV. The changes in hematocrit, although in the direction of the changes in ECFV, were not as large as would be expected if the changes in ECFV involved proportionate changes in interstitial fluid volume and plasma volume. The obvious example of this is the ADX-NEPHX-"B" groups of both series. Here the hematocrit remained significantly higher than in the NEPHX group. Thus, even though corticosterone was able to prevent ECFV changes, it was not able to completely prevent the changes in hematocrit due to adrenalectomy. This finding suggests that the changes in ECFV which occurred in the ADX-NEPHX-"B" groups primarily involved the interstitial fluid volume.
Table 3 contains the PV, ISFV and PV/ISFV values calculated as described in the methods section. With these values, the statements made in the above paragraph can be elucidated. It is seen that the NEPHX group of both series showed an increase over control in both the PV and ISFV. The ratio PV/ISFV also increased. This increase was significant at the five per cent level and indicated that the PV increased proportionately more than the ISFV. Adrenalectomy in both series resulted in a lowering of the PV/ISFV ratio. This finding indicated that there was a proportionately greater loss of PV than ISFV. Aldosterone did not change this distribution. However, with corticosterone, the changes in PV and ISFV were most interesting. In the non-hydrated series, the ECFV's of both the NEPHX group and the ADX-NEPHX-"B" group were the same. However, this ECFV was distributed differently in the ADX-NEPHX-"B" group, since the PV/ISFV ratio was significantly lower (.296 ± .006 as compared to .351 ± .006). In the hydrated series, where the ECFV of the ADX-NEPHX-"B" group was significantly larger than in the NEPHX group, this difference in distribution of the ECFV between the PV and the ISFV was even more prominent. The smaller plasma volume and large interstitial fluid volume of the ADX-NEPHX-"B" group resulted in a PV/ISFV ratio which was lower than in any other group. It is evident that, in this group treated with corticosterone, the interstitial fluid volume was contributing the most to the ECFV.

The data of Table 3 shows that nephrectomy resulted in a larger increase in PV than in ISFV. Adrenalectomy caused a greater decrease in PV than ISFV. The addition of aldosterone did not alter this distribution. Corticosterone prevented the loss of ISFV at the expense of some of the PV in both series. The additional ECFV observed in the hydrated ADX-NEPHX-"B" group was mostly contained in the ISFV.
Plasma sodium concentration did not change from group to group (Figure 2). Since sodium concentration was unchanged while fluid volumes changed significantly from group to group, sodium had to move with the fluid. This fact is demonstrated in Figure 3 by the changes in the total amount of sodium present in the ECFV. In both series, extracellular sodium decreased with adrenalectomy. Since in the ADX-NEPHX group the ECFV decreased while the plasma concentration of sodium remained the same, sodium must have moved out of the ECFV along with the water. The decrease in ECFV in this group then, was not due to the movement of water alone, but to the movement of an isosmotic sodium solution. As with the ECFV, the addition of aldosterone did not prevent the loss of sodium from the extracellular space while corticosterone prevented this loss. In fact, in the hydrated series, corticosterone actually increased the extracellular sodium to levels significantly higher than in the NEPHX group. The increase in ECFV in this group was caused by the movement of an isosmotic sodium solution into the extracellular space.

These data indicate that the adrenal gland was involved in the control of the distribution of sodium. Loss of the adrenal gland resulted in a loss of this electrolyte from the extracellular space. Aldosterone was not effective in preventing this loss, whereas, corticosterone was effective, indicating that, as in the case of the ECFV, it was the more important hormone in the adrenal control over this parameter.

Plasma chloride concentrations and total extracellular chloride are present in Figure 4. In both instances, the basic pattern of changes that occurred among groups in the non-hydrated series was repeated in the hydrated series. These data indicate that adrenalectomy resulted in a decrease in extracellular chloride. This decrease
was prevented by aldosterone as well as corticosterone. In the case of corticosterone, there was an increase in chloride to a value greater than that observed after nephrectomy alone.

The general pattern of changes in extracellular chloride among groups followed the pattern of changes in extracellular sodium. This was especially noted in the hydrated ADX-NEPHX-"B" group. It would appear that the movements of extracellular chloride followed the movements of extracellular sodium.

Nephrectomy resulted in an increase in plasma potassium concentration (from 3.9 ± .14 mEq/l to 6.2 ± .19 mEq/l). This rise was due to the increase in total extracellular potassium in the nephrectomized animal (Figure 5). Adrenalectomy at the time of nephrectomy resulted in a further increase in plasma potassium concentration to 7.6 ± .18 mEq/l in the non-hydrated series and from 6.6 ± .22 mEq/l to 7.7 ± .36 mEq/l in the hydrated series. It appears that this increase in plasma potassium concentration was caused by the decrease in ECFV. This conclusion is based on the fact that there was no change in total extracellular potassium from the NEPHX group to the ADX-NEPHX group of either series (Figure 5). Similar experiments by Friedman et al., in adrenalectomized-nephrectomized rats (11, 12) showed a significant increase in extracellular potassium in these animals when compared to nephrectomized animals. Our data indicate a similar trend, although, the small increases in extracellular potassium were not significant. It is possible that large changes in extracellular potassium were not seen because our animals were kept on a low potassium diet whereas Friedman did not use such a diet. The purpose of the diet in these experiments was to lower the potassium level. It has been shown that this diet depletes both cellular and extracellular potassium (6). This depletion may have prevented significant
potassium changes. The addition of aldosterone did not change the
extracellular potassium level from that of the NEPHX group. Only
the ADX-NEPHX-"B" group of the non-hydrated series showed a sig-
nificant change in extracellular potassium (Figure 5). This increase
in potassium also occurred in the hydrated series but was not signifi-
cant. It is not felt that corticosterone is resulting in the movement
of potassium into the ECFV, however, further evidence is needed to
clarify this problem.

Normal plasma urea concentration was 7.73 ± 1.16 mEq/l
(Table 1). This concentration increased to 46.94 ± 1.23 mEq/l by
nephrectomy. This rise was due to the large increase in total extra-
cellular urea occurring in the NEPHX group (Figure 6). This finding
is not surprising since protein metabolism and urea formation continue
in the nephrectomized animal. Since the kidney is the excretory organ
for urea, removal of the kidney must result in accumulation of urea.
In both series, adrenalectomy at the time of nephrectomy resulted
in a significant decrease in plasma urea concentration and also in
total extracellular urea (Figure 6). This decrease was not altered
by aldosterone or corticosterone. These data would suggest that in the
nephrectomized animal the intact adrenal gland may have a protein
catabolic effect which is involved in the increase in urea seen in the
NEPHX group. Adrenalectomy causes a depression in protein turn-
over resulting in reduced urea accumulation. This explanation has
been suggested by the results of survival studies on nephrectomized
and adrenalectomized-nephrectomized rats which were kept on a low
potassium diet (3).

Plasma osmotic pressure (Figure 7) was significantly de-
creased by adrenalectomy in the non-hydrated series. This decrease
was not prevented by aldosterone nor by corticosterone. The same
trend was observed in the hydrated series where only the ADX-NEPHX-"B" group showed a significantly decreased osmotic pressure. By calculating the ratios of the sodium or the urea concentration to the osmotic pressure (Table 4), the contribution of each to the osmotic pressure was investigated. In the normal animal the ratio of sodium concentration to osmotic pressure was .47 ± .007 (Table 4). This ratio decreased with nephrectomy to .41 ± .004 in the non-hydrated series and to .42 ± .003 in the hydrated series. The ratio decreased in both cases because sodium concentration was unchanged (Figure 3), while osmotic pressure increased (Figure 7). However, in the non-hydrated series, the ratio increased significantly to .44 ± .005 with adrenalectomy. Again the sodium concentration remained unchanged but the osmotic pressure decreased resulting in an increase in the ratio of the two. This ratio remained elevated for the ADX-NEPHX-ALDO and ADX-NEPHX-"B" groups. In the hydrated series, the ratio of sodium concentration to osmotic pressure was significantly changed only in the ADX-NEPHX-"B" group, reflecting a decrease in osmotic pressure while the sodium concentration remained unchanged. These data indicate that the change in osmotic pressure was not due to plasma sodium changes.

The normal ratio of urea concentration to osmotic pressure was .03 ± .004. This ratio increased significantly to .14 ± .004 in the NEPHX group of the non-hydrated series and to .13 ± .003 in the NEPHX group of the hydrated series. Both the urea concentration (Figure 6) and the osmotic pressure (Figure 7) increased over control in these groups. However, the increase in the ratio of the two indicated that the increase in urea concentration was greater than the increase in osmotic pressure. From this data it was concluded that this increase in urea was mainly responsible for the increase in osmotic pressure. In the non-hydrated series, the urea concentration
decreased significantly with adrenalectomy (Figure 6), as did also the osmotic pressure. However, at the same time the ratio of the two became significantly smaller (Table 4) indicating a proportionately greater decrease in urea. This ratio was also the same for the ADX-NEPHX-ALDO and ADX-NEPHX-'B' groups. In the hydrated series, the ratio of urea concentration to osmotic pressure was significantly decreased in the ADX-NEPHX-'B' group only. This group also showed the greatest decrease in urea and osmotic pressure. These data suggest that the decrease in osmotic pressure which occurred with adrenalectomy was primarily caused by the decrease in urea concentration.

These data indicate that urea was primarily involved in the changes in osmotic pressure. However, urea is nearly as diffusible across cell membranes as water, and it equilibrates rapidly across these membranes. Therefore, changes in urea concentration resulted in virtually no movement of water. For this reason there occurred no change in effective osmotic pressure. The changes in ECFV seen in this investigation were not the result of changes in urea concentration with the resultant changes in osmotic pressure.

The values for blood pH are given in Tables 1 and 2. The normal blood pH was 7.4 ± 0.029. Nephrectomy resulted in a decrease in pH to 7.23 ± 0.01. This is a significant decrease in pH. This acidosis was not altered by either adrenalectomy or the replacement of aldosterone or corticosterone in both series. Tables 1 and 2 present values for the plasma water content (% plasma H₂O). These values were not significantly different throughout. None of the experimental procedures appeared to have affected plasma water content. Thus, changes in blood pH and in plasma water content could not have attributed to the changes in other parameters measured.
The data presented demonstrate that the adrenal gland controlled the movement of water and electrolyte into and out of the extracellular compartment. This control was significant and independent of the adrenal's effect on the kidney. Removal of the adrenal in the nephrectomized animal resulted in a significant decrease in the ECFV. The electrolyte changes occurring with adrenalectomy indicated that sodium moved in the same direction as the fluid and did so isosmotically. Chloride tended to follow the sodium. Changes in potassium concentration were due to the decrease in fluid volume, although there was a trend toward an increase in total extracellular potassium. Physiological levels of aldosterone were not effective in preventing these changes. Physiological levels of corticosterone, however, were most effective in preventing these changes. In this adrenal control over water and electrolyte metabolism, the glucocorticoid was evidently the most important agent.
CONCLUSION

This investigation demonstrated that adrenalectomy resulted in the movement of water and sodium out of the extracellular fluid space. There was no direct evidence as to how or where this volume was relocated. In the more direct studies where both the tissue water and electrolytes were measured, there occurred a movement of water into the cells of adrenalectomized animals (13, 63). However, these animals had intact kidney function. It was not possible to definitively separate the effect of adrenalectomy on distribution of water and electrolytes in these experiments. Swingle et al. (54, 55, 56, 57, 58), in experiments on fasted adrenalectomized dogs, revived these dogs from insufficiency by injection of adrenalcortical hormone. They demonstrated that these hormones were capable of mobilizing water and electrolytes from cells, bone or some other internal sources. Our own experiments indicated that absence of adrenal hormone resulted in the loss of extracellular fluid to some location yet undisclosed. Possibly the water and sodium lost from the extracellular fluid of the adrenalectomized-nephrectomized rat entered the cells. The adrenal may have acted at the cell membrane by either affecting permeability or active transport mechanisms. Both of these possible effects would control the movement of water and electrolytes between extra- and intracellular compartments. The greater susceptibility of the adrenalectomized animal to water intoxication lends support to this theory. Since water intoxication is attributed to cellular over-hydration, the adrenalectomized animal may be less capable of keeping water out of the cells. Alterations in cell permeability or active transport of sodium could account for this over-hydration.
It is becoming increasingly evident that the connective tissue, specifically hyaluronic acid, are involved in maintaining the composition of the extracellular fluid. This ground substance, as it is called, is capable of alterations in the physicochemical state in response to such stimuli as pH, environmental changes, internal secretions, growth and injury (8, 62). It reacts with water, salts and other charged molecules and in this way changes the volume and composition of the extracellular fluid (8). These reactions are of particular interest if adrenalectomy resulted in alterations of the ground substance in our experiments. Such changes could involve the removal of water and sodium from the extracellular fluid with the resultant change in ECFV we have reported. Particularly the lack of corticosterone may have been the factor which effected the physicochemical properties of the ground substance, since replacement of this hormone prevented changes in water and sodium. It has not been determined as yet, to what extent changes in the ground substance may affect the extracellular fluid. Under some conditions, such as extremes of pH, changes in the ground substance have significant effects on the volume and composition of the extracellular fluid (62). It is conceivable, therefore, that changes in the ground substance accounted for the changes which were observed in our experiments.

The experimental model which is presented in this dissertation can be used to clarify the movements of water and sodium. Studies of tissue water and electrolytes in the adrenalectomized-nephrectomized rat should shed some light on the relocation of the ECFV. Also, if total body water as well as the ECFV were measured, movements of water could be identified. Should the total body water be found unchanged from nephrectomized to adrenalectomized-nephrectomized rats while the ECFV decreased, this would indicate a movement of
fluid into the cells. However, any method used in measuring total body water needs to be first examined to determine whether it also measures water bound to the ground substance. If it measures this water also, the method would not distinguish between changes in the ground substance and movement of water into the cells.

Swingle et al. (57, 58) concluded that the primary site of action of mineralocorticoids and glucocorticoids are different. Mineralocorticoids acted mainly via their effect on the kidney while glucocorticoids were primarily responsible for the internal redistribution of water and electrolytes. The results of our own experiments substantiate these conclusions in as much as corticosterone and not aldosterone was effective in preventing the changes associated with adrenalectomy. Thus, it appears that the adrenal gland possesses two independent control mechanisms for water and electrolyte metabolism; one through mineralocorticoids and the kidneys and the other through glucocorticoids and their extrarenal effects.

One facet of any investigation such as this should be pointed out. By performing adrenalectomy we have removed a gland which has multiple secretions. In these experiments we replaced two of these secretions separately. However, hormones may have entirely different effects when given in combination. When one considers the general permissive effect of glucocorticoids on functions and actions of other hormones, this point becomes important. Recent studies by Friedman et al. (15, 16) on neurohypophysectomized-adrenalectomized rats demonstrates this fact. In these studies effects of aldosterone, vasopressin and corticosterone on salt and water distribution were compared when each of these hormones was administered separately. It was shown that vasopressin and corticosterone showed similar effects on the measured parameters which were opposite to those of aldosterone.
However, when these hormones were injected in various combinations, the effects of corticosterone and vasopressin were no longer similar. This example demonstrates that caution must be applied in interpreting the physiological effects of a hormone when it is removed from its natural hormonal environment. In future experiments, these hormones should perhaps be administered in combination and in varying ratios. Such an approach would enable one to arrive at more conclusive statements concerning the actions and interactions of the adrenal cortical hormones.

It should also be recognized that both the medulla and the cortex are excised in adrenalectomy. Although epinephrine is not generally associated with water and electrolyte control, studies on a possible involvement of epinephrine in this regulation have not been conclusive. Most of the epinephrine effects were related to the kidney and the production of a diuresis or antidiuresis (21). It appears from a search of the literature that possible effects of the adrenal medulla on nonrenal aspects of water and electrolyte control have not been studied. Nevertheless, the adrenal medulla may be involved in this area and further studies are needed to resolve this problem.

It is now evident that the adrenal glands are involved in determining the volume and electrolyte composition of the extracellular fluid. This control is exerted not only through its renal effects but also through its non-renal actions. Apparently, the adrenals have direct control over the internal distribution of body water and electrolytes. However, the actual mechanisms of this control are still unknown. It appears as though the glucocorticoids are most effective in the non-renal regulation while the mineralocorticoids are most important in the renal regulation. However, this difference in functional significance needs further clarification.
Table 1
Mean Data of the Five Groups of the Non-hydrated Series
<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>NEPHX</th>
<th>ADX-NEPHX</th>
<th>ADX-NEPHX-ALDO.</th>
<th>ADX-NEPHX-ALDO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gm)</td>
<td>180.2±6</td>
<td>163.5±7</td>
<td>174.9±7</td>
<td>169.2±8</td>
<td>163.8±8</td>
</tr>
<tr>
<td>% Change in body weight</td>
<td>1.8±7</td>
<td>2.1±7</td>
<td>2.2±7</td>
<td>2.5±7</td>
<td>2.6±7</td>
</tr>
<tr>
<td>ECFV (%)</td>
<td>20.2±1</td>
<td>20.8±1</td>
<td>20.8±1</td>
<td>21.9±2</td>
<td>21.9±2</td>
</tr>
<tr>
<td>% of body weight</td>
<td>42.4±7</td>
<td>47.9±7</td>
<td>45.9±7</td>
<td>44.2±8</td>
<td>44.2±8</td>
</tr>
<tr>
<td>Na (mEq./L)</td>
<td>138.1±7</td>
<td>141.1±7</td>
<td>141.8±7</td>
<td>139.2±8</td>
<td>140.0±7</td>
</tr>
<tr>
<td>Extracellular Na (mEq./100 gm)</td>
<td>2.9±7</td>
<td>3.1±7</td>
<td>2.8±7</td>
<td>2.9±7</td>
<td>3.0±7</td>
</tr>
<tr>
<td>K+ (mEq/L)</td>
<td>3.9±7</td>
<td>6.2±7</td>
<td>7.6±7</td>
<td>7.0±7</td>
<td>7.3±7</td>
</tr>
<tr>
<td>Extracellular K (mEq./100 gm)</td>
<td>0.8±7</td>
<td>1.3±7</td>
<td>1.5±7</td>
<td>1.4±7</td>
<td>1.3±7</td>
</tr>
<tr>
<td>urea (mEq/L)</td>
<td>7.7±7</td>
<td>3.9±7</td>
<td>3.9±7</td>
<td>3.8±7</td>
<td>3.7±7</td>
</tr>
<tr>
<td>Extracellular urea (mEq./100 gm)</td>
<td>0.1±7</td>
<td>0.3±7</td>
<td>0.2±7</td>
<td>0.2±7</td>
<td>0.2±7</td>
</tr>
<tr>
<td>Osmotic pressure (mosmoles/Kg H2O)</td>
<td>295.3±7</td>
<td>341.3±7</td>
<td>323.9±7</td>
<td>326.1±8</td>
<td>328.6±8</td>
</tr>
<tr>
<td>pH</td>
<td>7.4±7</td>
<td>7.2±7</td>
<td>7.2±7</td>
<td>7.2±7</td>
<td>7.2±7</td>
</tr>
<tr>
<td>% Plasma H2O</td>
<td>91.6±7</td>
<td>93.4±7</td>
<td>92.4±7</td>
<td>92.6±7</td>
<td>92.8±7</td>
</tr>
</tbody>
</table>

a = mean  
b = Standard Error  
c = Number of animals
Table 2
Mean Data of the Four Groups of the Hydrated Series
<table>
<thead>
<tr>
<th></th>
<th>NEPHX</th>
<th>ADX-NEPHX</th>
<th>ADX-NEPHX-ALDO</th>
<th>ADX-NEPHX-(\text{&quot;B})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight</strong></td>
<td>156.6±</td>
<td>162.4±</td>
<td>176.3±</td>
<td>171.1±</td>
</tr>
<tr>
<td>(grams)</td>
<td>4.06(\text{a})</td>
<td>2.74±</td>
<td>2.52±</td>
<td>2.92±</td>
</tr>
<tr>
<td>(11)(\text{c})</td>
<td>(9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td><strong>% Change in</strong></td>
<td>-5.6±</td>
<td>-5.1</td>
<td>-4.3±</td>
<td>-4.7±</td>
</tr>
<tr>
<td><strong>Body Weight</strong></td>
<td>±1.8±</td>
<td>±1.30±</td>
<td>±1.15±</td>
<td>±1.17±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ECFV</strong></td>
<td>22.9±</td>
<td>19.9±</td>
<td>21.1±</td>
<td>25.0±</td>
</tr>
<tr>
<td>% of body weight</td>
<td>±.20±</td>
<td>±.21</td>
<td>±.29±</td>
<td>±.55±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hematocrit</strong></td>
<td>36.2±</td>
<td>47.1±</td>
<td>45.1±</td>
<td>43.0±</td>
</tr>
<tr>
<td>(%)</td>
<td>±1.57±</td>
<td>±1.67±</td>
<td>±1.55±</td>
<td>±1.49±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(12)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma Na</strong></td>
<td>142.1±</td>
<td>141.5±</td>
<td>140.7±</td>
<td>140.2±</td>
</tr>
<tr>
<td>mEq./L.</td>
<td>±1.67±</td>
<td>±1.63±</td>
<td>±1.87±</td>
<td>±1.72±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Extracellular Na</strong></td>
<td>3.27±</td>
<td>2.84±</td>
<td>3.00±</td>
<td>3.55±</td>
</tr>
<tr>
<td>mEq./100 gm.</td>
<td>±.03±</td>
<td>±.04±</td>
<td>±.04±</td>
<td>±.08±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma Cl</strong></td>
<td>92.61±</td>
<td>95.38±</td>
<td>97.11±</td>
<td>91.96±</td>
</tr>
<tr>
<td>mEq./L.</td>
<td>±1.50±</td>
<td>±1.95±</td>
<td>±1.24±</td>
<td>±1.14±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Extracellular Cl</strong></td>
<td>2.13±</td>
<td>1.91±</td>
<td>2.07±</td>
<td>2.38±</td>
</tr>
<tr>
<td>mEq./100 gm.</td>
<td>±.03±</td>
<td>±.03±</td>
<td>±.05±</td>
<td>±.08±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma K</strong></td>
<td>6.6±</td>
<td>7.7±</td>
<td>7.1±</td>
<td>6.8±</td>
</tr>
<tr>
<td>mEq./L.</td>
<td>±1.22±</td>
<td>±1.36±</td>
<td>±1.25±</td>
<td>±1.19±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Extracellular K</strong></td>
<td>1.152±</td>
<td>1.155±</td>
<td>1.152±</td>
<td>1.172±</td>
</tr>
<tr>
<td>mEq./100 gm.</td>
<td>±.005±</td>
<td>±.006±</td>
<td>±.006±</td>
<td>±.006±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma Urea</strong></td>
<td>44.06±</td>
<td>38.85±</td>
<td>38.80±</td>
<td>34.72±</td>
</tr>
<tr>
<td>mEq./L.</td>
<td>±1.10±</td>
<td>±1.14±</td>
<td>±1.36±</td>
<td>±1.50±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Extracellular Urea</strong></td>
<td>1.01 ±</td>
<td>1.779±</td>
<td>.828±</td>
<td>.872±</td>
</tr>
<tr>
<td>mEq./100 gm.</td>
<td>±.027±</td>
<td>±.024±</td>
<td>±.030±</td>
<td>±.026±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma Osmotic</strong></td>
<td>338.7±</td>
<td>322.34±</td>
<td>328.15±</td>
<td>313.69±</td>
</tr>
<tr>
<td>Pressure, mosmoles/Kg H_2O</td>
<td>±2.92±</td>
<td>±5.60±</td>
<td>±5.53±</td>
<td>±4.54±</td>
</tr>
<tr>
<td>(11) (8)</td>
<td>(13)</td>
<td>(11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>7.24±</td>
<td>7.25±</td>
<td>7.24±</td>
<td>7.22±</td>
</tr>
<tr>
<td>pH</td>
<td>±.01±</td>
<td>±.01±</td>
<td>±.01±</td>
<td>±.03±</td>
</tr>
<tr>
<td>(11) (8)</td>
<td>(9)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>% Plasma H_2O</strong></td>
<td>91.6±</td>
<td>92.9±</td>
<td>93.3±</td>
<td>93.6±</td>
</tr>
<tr>
<td>(11) (9)</td>
<td>(10)</td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\text{a = mean}\)
\(\text{b = Standard error of mean}\)
\(\text{c = Number of animals}\)
### Table 3

Plasma Volumes (PV) and Interstitial Fluid Volumes (ISFV) as a per cent of body weight, and the ratio PV/ISFV

<table>
<thead>
<tr>
<th>PV per cent body wt.</th>
<th>ISFV per cent body wt.</th>
<th>PV/ISFV</th>
<th>PV/ISFV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-Hydrated</td>
<td>Hydrated</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td>5.0%±.063b</td>
<td>16.0%±.291</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10)</td>
<td>(10)</td>
</tr>
<tr>
<td><strong>NEPHX</strong></td>
<td></td>
<td>5.7%±.091</td>
<td>16.6%±.321</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td><strong>ADX-NEPHX</strong></td>
<td></td>
<td>1.4%±.053</td>
<td>15.8%±.311</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16)</td>
<td>(16)</td>
</tr>
<tr>
<td><strong>ADX-NEPHX-ALDO</strong></td>
<td></td>
<td>4.6%±.073</td>
<td>16.2%±.403</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11)</td>
<td>(15)</td>
</tr>
<tr>
<td><strong>ADX-NEPHX-&quot;B&quot;</strong></td>
<td></td>
<td>4.8%±.093</td>
<td>16.5%±.309</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(15)</td>
<td>(15)</td>
</tr>
</tbody>
</table>

a = mean  

b = ± Standard error  
c = Number of animals
Table 4

Ratios of Sodium and Urea to Osmotic Pressure

<table>
<thead>
<tr>
<th></th>
<th>Na mEq/l osmotic pressure</th>
<th>Urea/l mEq osmotic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-hydrated</td>
<td>hydrated</td>
</tr>
<tr>
<td>CONTROL</td>
<td>±.007 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>(10) &lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>NEPHX</td>
<td>±.004 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>±.003</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>(s&lt;sub&gt;c&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td>ADX-NEPHX</td>
<td>±.005</td>
<td>±.008</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td>(s&lt;sub&gt;c&lt;/sub&gt;, s&lt;sub&gt;n&lt;/sub&gt;)</td>
<td>(s&lt;sub&gt;c&lt;/sub&gt;, s&lt;sub&gt;n&lt;/sub&gt;)</td>
</tr>
<tr>
<td>ADX-NEPHX-ALDO</td>
<td>±.008</td>
<td>±.007</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(13)</td>
</tr>
<tr>
<td></td>
<td>(s&lt;sub&gt;c&lt;/sub&gt;)</td>
<td>(s&lt;sub&gt;c&lt;/sub&gt;)</td>
</tr>
<tr>
<td>ADX-NEPHX-&quot;B&quot;</td>
<td>±.004</td>
<td>±.008</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>(s&lt;sub&gt;c&lt;/sub&gt;)</td>
<td>(s&lt;sub&gt;n&lt;/sub&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup> = mean  
<sup>b</sup> = ± standard error  
<sup>c</sup> = number of animals  
(s<sub>c</sub>) = significantly different from control  
(s<sub>c</sub>, s<sub>n</sub>) = significantly different from NEPHX
Figure 1

ECFV as a Per Cent of Body Weight
and the Hematocrit
Figure 2

Percent Change in Body Weight

From Day One to Day Two

The percent change in body weight
Figure 3
Plasma Concentration of Sodium and Total Extracellular Sodium
Figure 4

Plasma Concentration of Chloride and Total Extracellular Chloride
Figure 5
Plasma Concentration of Potassium and Total Extracellular Potassium
Figure 6

Plasma Concentration of Urea and Total Extracellular Urea
Figure 7

Plasma Osmotic Pressure

Osmotic Pressure
Milliosmoles/Kg H2O

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>ADX-NEPHX</th>
<th>ADX-NEPHX-ALDO</th>
<th>ADX-NEPHX</th>
<th>ADX-NEPHX-ALDO</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>325</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>340</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Non-Hydrated

Hydrated

* indicates significant difference

---

84
BIBLIOGRAPHY


