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THE RICE INSTITUTE

FLUID AND ELECTROLYTE SHIFTS IN THE
HYDRATED ADRENALECTOMIZED RAT

by

ROBERT C. FROST

A THESIS

SUBMITTED TO THE FACULTY
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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Prof. Talmage

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FOREWORD

The author wishes to express his appreciation for the cooperation and valuable assistance of Dr. Roy V. Talmage of the Rice Institute Biology Department which has made this study possible. He is also indebted to his wife, Anna Ruth, who cheerfully undertook the burden of arranging, typing, and proofreading the manuscript.

R. C. F.

Houston, Texas

April, 1952

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INTRODUCTION

Although the adrenal gland was first described in 1563 by Eustachius, it was not until three centuries later that significant studies concerning its functions were begun. Then in the year 1885 Thomas Addison described the disease which now bears his name and attributed a life-maintaining function to the adrenal cortex. His observations stimulated investigation concerning the vital nature of the gland in animals and by the turn of the century it was generally agreed that the adrenals were essential for life. Biedl has recorded the history of the subject up to the year 1913.

Progress continued slowly and the next two decades were spent largely in an endeavor to determine whether it was the adrenal cortex or medulla that was the essential component for life. The isolation of epinephrine in 1901 did much to divert attention away from the adrenal cortex. Consequently, it was not until after Zwemer's papers in 1927 that the cortex was generally accepted as the life-maintaining portion of the gland.

There had also been many reports describing the characteristic symptoms of adrenal insufficiency, but the absence of some of the key facts prevented any convincing interpretation of these observations at that time.

The announcement of the first good adrenal cortical extract

preparation by Swingle and Pfiffner, (1929, 1930), and Hartman, Brownell et al., (1930), marked the opening of a new era in adrenal cortical physiology, and the endocrine nature of cortical function was soon established.

The use of cortical extracts also permitted the chemists to begin their identification of the active cortical principles. Kendall, Wintersteiner and Pfiffner in the United States and Reichstein and associates in Switzerland were the early pioneers in the field of adrenal chemistry during the years 1934 and 1935 (Pfiffner, 1942; Reichstein, 1943; Kuizenga, 1944). In a few years close to thirty steroids had been isolated from the adrenal cortex. Of these, only six were physiologically active and as yet it is not known how many hormones the adrenal cortex actually secretes, perhaps only a few or possibly only one.

Recently, however, Zaffaroni and his collaborators, using new micro analytical methods for the isolation and identification of adrenocortical steroids, have analyzed the effluent blood of both *invivo* and perfused isolated glands and have suggested that 17-hydroxycorticosterone (compound F) and corticosterone (compound B) are the main secretory products of the adrenal cortex. They have also made some interesting observations in regard to the biosynthesis of the adrenal steroids. Since there is a pronounced fall in adrenal cholesterol following ACTH

injection, cholesterol has been considered a precursor of the adrenal steroids. Also, cholesterol has been shown to arise from acetate condensation in adrenal slices. Therefore, their reports, on the basis of perfusion and radioisotope tracer experiments, that both acetate and cholesterol can be transformed into adrenocortical steroids by the isolated adrenal, support current theory concerning adrenal cortical hormone synthesis (Zaffaroni, 1951). It may be concluded then, that many of the steroids which have been isolated are probably merely intermediate products in the biosynthesis of one or more of the true cortical hormones.

Following the chemical isolation and subsequent synthesis of many of the adrenal steroids, both clinical and experimental investigation concerning the role of the cortex in regulating the various aspects of organic and electrolyte metabolism has been extremely rapid. Also, an added stimulus to adrenal cortex research was prompted by the importance which Selye has ascribed to the gland in endowing an organism with resistance to stress (Selye, 1946, 1950). This current interest in adrenal cortical physiology is well reflected by the numerous conference and review articles which have been concerned with the various aspects of this subject (Kendall, 1948; Gaunt and Eversole, 1949; Gaunt et al., 1949; Hartman and Brownell, 1949; Ralli, 1949; Christman, 1950; Gordon, 1950; Pincus and Thimann, 1950;

Sayers, 1950; Selye and Stone, 1950; Mote, 1951; Pincus, 1951; White, 1951).

Some Historical Aspects of the Adrenal Cortex in Water and Electrolyte Metabolism

The role which the adrenal cortex plays in water and electrolyte metabolism was one of the first important functions to be recognized early in the developmental history of the gland. Nearly two decades ago it was demonstrated that adrenal insufficiency was associated with a marked degree of hemoconcentration (Banting and Gairns, 1926; Lucas, 1926). Later it was demonstrated by Swingle et al. (1933) that this hemoconcentration was due to a declining plasma volume.

Emphasis, however, was placed on electrolyte metabolism after Loeb et al. (1933), and subsequently, Harrop et al. (1933 a,b) noted in adrenal insufficiency a marked decrease in plasma sodium due to excessive renal excretion. This suggested that the hemoconcentration previously observed was due to extracellular dehydration. These observations became all the more significant in the light of Loeb's (1933) demonstration that sodium chloride alone would maintain an Addisonian patient.

It was found in subsequent investigation that while salt was helpful in maintaining adrenalectomized animals, they could not be maintained indefinitely unless their diet was also low

in potassium (Allers and Kendall, 1937). This observation was in agreement with the earlier work of Baumann and Kurland (1927) and Hastings and Compere (1931) in which they demonstrated that potassium might rise to toxic levels during adrenal insufficiency. The accumulation of this ion in both plasma and tissues has been subsequently well confirmed (Swingle and Remington, 1944; Kendall, 1948; Hartman and Brownell, 1949). The loss of sodium and retention of potassium which occurs after adrenalectomy has been shown to be due to a defect in renal tubule function (Harrison and Darrow, 1939; Swingle and Remington, 1944; Rommelt et al., 1949).

There are indications, however, that neither the loss of sodium nor the retention of potassium are necessarily essential for the development of adrenal insufficiency (Hartman and Brownell, 1949). Subsequent investigation has demonstrated that there are many additional factors which are responsible for the complex insufficiency syndrome. (For further information on this aspect of the subject, one is referred to the review articles on adrenal cortical physiology listed above).

In the mid-thirties most investigators felt that the primary function of the adrenal cortex was to regulate the reabsorption of potassium and sodium from the renal tubules. Any observed action on water metabolism was considered to

be merely a secondary osmotic consequence. During the intervening years, however, sufficient information has accumulated to indicate how incomplete this simple explanation was. It is now known that the cortex can not only directly effect the reabsorption of water from the renal tubules, but may play an important part in regulating its distribution in the fluid compartments of the body.

Furthermore, in addition to the corticoids it has been demonstrated that many of the sex steroids have some influence, at least pharmacologically, on salt and water metabolism. Physiologically, the anterior and posterior pituitary glands have likewise been shown to play very important roles in the regulation of electrolyte and water metabolism.

It will be desirable, therefore, to examine the actions of some of the endocrines and their respective hormones in regard to the phenomena to be discussed. The complexity of this subject is well reflected in the many recent review articles which have been concerned with the hormonal regulation of electrolyte and water metabolism (Swingle and Remington, 1944; Verney, 1947; Kendall, 1948; Gaunt et al., 1949; Gaunt and Eversole, 1949; Hartman and Brownell, 1949; Newton, 1949; Stehle, 1949 a,b; Christman, 1950; Elkington, 1950; Gaunt, 1950; Noble, 1950; Overman, 1950; Selye, 1950; Waring and Landgrebe, 1950; Wolff, 1950; Bradley, 1951; Danowski and

Elkington, 1951; Gaunt, 1951; Gaunt and Birnie, 1951; Smith, 1951).

Influence of the Steroids on Water and Electrolyte Metabolism

Ever since crystalline cortical steroids became available, desoxycorticosterone, which is relatively inexpensive, has been one of the most widely used adrenal compounds. It is the most potent of the corticoids in causing the retention of sodium and excretion of potassium and has been considered by many the prototype of the adrenal "salt and water" hormones. However, since it has been found in only very small amounts in adrenal tissue, there is some question as to whether it is an adrenal hormone of physiological importance. For this reason caution is necessary in interpreting physiological experiments based on its use.

All of the cortical steroids which have been tried (desoxycorticosterone, 17-hydroxy-11-dehydro-corticosterone, the amorphous fraction and whole adrenal extracts) have been shown to enhance urine excretion. Likewise, most of the cortical and sex steroids cause some sodium retention with the exception of the 17-ketosteroids which may cause a transient sodium loss.

The estrogens and androgens share with many other steroids the property of reducing the excretion of sodium and

causing water retention. Like desoxycorticosterone they also reduce serum potassium. After chronic administration, progesterone has been shown to enhance daily water exchange in both normal and hypophysectomized rats and to increase the rate of excretion in hydrated animals. Progesterone is also the only sex steroid which is capable of prolonging life during adrenal insufficiency. Both estrogen and testosterone differ from progesterone in that they do not provoke diuresis under any conditions yet observed. It is thought, however, that all the steroid hormones affect salt and water metabolism in some way (Gaunt and Eversole, 1949; Gaunt et al., 1949).

The Anterior Pituitary and Water Metabolism

That the anterior pituitary may play an important part in water metabolism is suggested by the fact that the maintenance of diabetes insipidus, which results when the neurohypophyseal mechanism is inactivated, is dependent upon the intact anterior lobe. This maintenance of a diuresis in the absence of the antidiuretic hormone of the posterior lobe may be dependent in part upon the trophic action of ACTH on the adrenal cortex; some of the cortical hormones are not only themselves natural diuretic agents, but are also essential to the action of other natural diuretics such as epinephrine and thyroxine.

Epinephrine, while not considered to play an important role in the regulation of water metabolism, is one of the few non-steroid agents which specifically exerts a diuretic effect in adrenalectomized animals (Stein and Wertheimer, 1944). It has also been shown, when given in large amounts, to enhance both diuresis and chloride excretion in normal, medullectomized and adrenalectomized rats, all of which had been hydrated (Caunt et al., 1945).

Likewise, thyroxine has been shown to exert some influence on water and salt metabolism. The hyperthyroid animal is able to excrete large amounts of water with a low chloride content. Similar results are obtained with normal animals under thyroxine therapy.

Nevertheless, it should again be emphasized that the adrenals must be intact for the full expression of these two natural diuretics.

Even the corticoids, however, do not completely restore to normal the impaired diuresis in hydrated hypophysectomized animals. Boss and Osborn (1950) have shown that while cortical hormone therapy does decrease renal tubule reabsorption in hypophysectomized rats, the filtration rate is not simultaneously increased sufficiently to yield a normal diuresis. Therefore, there must be some anterior pituitary influence other than ACTH which is necessary for repairing this type of

renal deficiency.

In this regard, White et al., (1949) have found various renal deficiencies in the hypophysectomized dog that cannot be repaired with cortical hormones, ACTH, thyrotrophin or gonadotrophin but which will respond to some growth hormone preparations. Earle et al., (1951) have also reported that continued administration of purified growth hormone significantly improves the hypophysectomized dog's impaired response to water load by increasing the renal plasma flow and the glomerular filtration rate. Therefore, it is very suggestive that the growth hormone is also a factor which is concerned to some extent with the maintenance of normal water metabolism.

One must also remember that the animal which does not have a functional posterior pituitary is unable to concentrate his urine, so actually any of the hormones of the anterior pituitary which would cause directly or indirectly an increase in the excretion of salts or metabolites, automatically produce a simultaneous increase in the excretion of water. Normally, then, there are probably several factors in the anterior lobe which could produce a diuresis if unchecked by the antidiuretic hormone of the posterior pituitary.

The Posterior Pituitary in Water Metabolism and Possible
Interrelationships with the Adrenal Cortex

The role which the posterior pituitary plays in regulating water and electrolyte balance must also be considered before alterations which occur during adrenal insufficiency can be fully appreciated. It has been shown that the action of the antidiuretic hormone (ADH) of the posterior pituitary in regulating renal excretion is in some ways antagonistic to that of the cortical hormones. As a rule ADH increases the renal tubule reabsorption of water and decreases the reabsorption of sodium. The adrenal cortical hormones, on the other hand, act in just the opposite manner. These hormones are not antagonistic in every respect, however, for they both enhance potassium excretion by inhibiting its reabsorption by the renal tubules.

It should be remembered that the actions of these hormones may at times be masked by the interrelationships of sodium and water metabolism. For instance, ADH given to a dehydrated animal may actually cause an augmented urine flow which is obligated by the increased elimination of solids. Likewise, the cortical hormones under certain conditions may produce a retention of salt which is of such a magnitude as to hide any diuretic action which would otherwise be observed.

The antagonistic effects which have been demonstrated for the posterior pituitary and adrenal cortical hormones suggest the possibility of a functional relationship. That is, perhaps

a normal electrolyte and water balance is dependant upon the ratio between the amounts of circulating ADH and cortical hormone. The question would then arise as to whether a slight excess of one hormone would cause a compensatory increase in the secretion of the other in order to maintain this normal balance. Recent investigations by Nagareda and Gaunt (1951) and Lloyd (unpublished observations) suggest that while the administration of an excess of either adrenal or posterior pituitary hormones may cause a hypersecretion of the other, there is no evidence that mild fluctuations in what might be considered the usual physiological range will do so.

The normal diuresis which follows excessive hydration, however, has been considered to be due to the decreased plasma osmotic pressure which inhibits the release of the antidiuretic hormone from the posterior pituitary (Chambers et al., 1945; Verney, 1946, 1947). One then wonders whether there may not also be a simultaneous increase in the secretion of cortical hormone to facilitate the excretion of this water. In agreement with their considerations described above, Nagareda and Gaunt (1951) demonstrated that only large water loads would elicit an excess corticoid secretion as measured by the depletion in ascorbic acid of the adrenal cortex. Experiments in which animals were loaded with salt water suggest that this adrenal cortical response is due to alterations in

osmotic pressure and not to the fluid load per se. They consider it probable, therefore, that mild fluctuations in water exchange are the result of a sensitively variable rate of ADH secretion, but that any major increase in water load elicits an excess corticoid secretion which contributes significantly to the observed renal response.

Water Metabolism in the Adrenalectomized Rat

In the adrenalectomized rat the normal diuresis which follows the ingestion of large amounts of water is impaired. This altered response is due in part, of course, to the absence of the cortical hormones which are necessary for a high rate of excretion. It is also due to an increased sensitivity of the renal tubules to an antidiuretic substance (ADS) in the blood which accumulates after adrenalectomy (Birnie et al., 1950). Various tests have indicated that this ADS is very likely either the posterior pituitary antidiuretic hormone or causes its production. The reason for ADS accumulation after adrenalectomy may be due to either an increased production or a decreased inactivation of the substance. Investigation of the latter possibility by Eversole et al. (1949) and Birnie (1950) has yielded suggestive results. It was found that both Pitressin and the ADS of serum are inactivated apparently by an enzyme system present in various tissues but notably in

the liver. Furthermore in the adrenalectomized animal the effect of this inactivating system is enhanced, indicating that the cortical hormones are apparently necessary for its normal expression. Thus, it is highly probable that the accumulation of ADS in the adrenalectomized animal is due largely at any rate to a decreased destruction rather than an overproduction.

In summary then, the absence of the adrenal cortical hormones during adrenal insufficiency plus the increased sensitivity of the renal tubules to an accumulating amount of an antidiuretic substance in the serum, provides the primary reason for water retention in the hydrated adrenalectomized rat.

Introduction and Description of the Research Problem

Gaunt (1944) has demonstrated that a deficiency in the intestinal reabsorption of water and sodium salts may play an extrarenal role in regard to water retention in hydrated adrenalectomized rats. He also reported a shift of salt from the extracellular fluid to the unabsorbed water in the gut of such animals. This decrease in the electrolyte concentration of the extracellular space would result in a shift of water to the cells and a consequent increase in the intracellular space.

Shipley (1945), however, was unable to show a marked delay in the intestinal reabsorption of water in hydrated adrenalectomized rats. He also concluded, on the basis of thiocyanate volume and total body water determinations, that the greatest portion of the absorbed but unexcreted water was retained almost entirely in the extracellular fluid compartment.

Recent investigation has shown, however, that a large fraction of an injected quantity of thiocyanate becomes bound to the serum albumin (Schienberg et al., 1950). Since there may be an increased capillary permeability to serum proteins during adrenal insufficiency, the thiocyanate space would then not be a consistent index of the extracellular fluid volume. Furthermore, it has also been reported that due to interfering chromogens, urinary thiocyanates are difficult to determine accurately (Doxiadis and Gairdner, 1948).

The present study was partly conducted, therefore, in order to satisfactorily determine the sites of the retained fluid in hydrated adrenalectomized rats and to examine what role shifts in electrolytes might play in this regard.

In order to avoid the pitfalls inherent in the determination of thiocyanate space, it seemed advisable to use the sodium space as measured with radiosodium 22 as an index of extracellular fluid volume. All fluid compartment determinations were made on both the intact and gutless rats in order to

eliminate any error in the determination of sodium space which would arise if the sodium concentration in the gut were higher than the serum concentration. This also afforded a means of determining in which compartments absorbed but unexcreted water was located. Serum electrolytes and fluid compartment determinations were made on both non-hydrated and hydrated normal and adrenalectomized rats.

It is also conceivable that some of these fluid and electrolyte shifts which occur during adrenalectomy may be due to changes in extrarenal phenomena. This concept has been suggested by the older work of Swingle et al. (1944) and more recently by that of Flanagan et al. (1950) and Gaudino and Levitt (1949), and others. They all agree that the alterations of body water and electrolyte metabolism in adrenal insufficiency cannot be explained entirely by an altered excretion of these substances. For instance, adrenalectomized animals show a sensitivity to excess water which is not wholly due to a deficient water excretion (Swingle et al., 1944; Hays and Mathieson, 1945; Gaunt, 1944). Furthermore, DCA has been shown to enhance the resistance of adrenalectomized animals to water intoxication even after nephrectomy (Birnie et al., 1948).

It has been suggested by Hays and Mathieson (1945) and Birnie et al., (1948) that the extrarenal protection which

adrenal steroids afford to adrenalectomized rats which have received toxic doses of water is very likely due to their effect on the distribution of water between extracellular and intracellular compartments, to prevent excessive intracellular hydration.

In order to study these extrarenal phenomena more directly the experiments which were described above for intact, nonhydrated and hydrated normal and adrenalectomized rats was also conducted on nephrectomized animals. It was also considered desirable to investigate the extrarenal effect which corticoid therapy (DCA) might have on the electrolyte and water metabolism of animals treated in this manner. Thus, perhaps some insight might be obtained concerning the possible extrarenal role which the adrenal cortical hormones may play in maintaining a normal electrolyte and water balance.

MATERIALS AND METHODS

The outline of the experimental procedure was as follows:

I Non-nephrectomized Rats

A. Non-hydrated

1. controls
2. two-day adrenalectomized

B. Hydrated

1. controls
2. two-day adrenalectomized

II Nephrectomized Rats

A. Non-hydrated

1. controls
2. two-day adrenalectomized

B. Hydrated

1. controls
2. two-day adrenalectomized
3. DCA-treated, two-day adrenalectomized

Animals in the non-nephrectomized group (Group I) weighed between 140-160 grams; those of the nephrectomized group (Group II) were between 130-180 grams. The adrenalectomized animals in Group I had both adrenals removed two days before the experiment and no extra salt or hormone

therapy was administered. The nephrectomized animals underwent a two-stage operation; the left kidney was removed five days before the experiment and the right one at least two hours before the experiment. The adrenalectomized rats of Group II also underwent a two-stage operation; the left adrenal was removed at the same time as the left kidney, and the right adrenal removed two days before the experiment. The controls were also etherized two days before the experiment in order to standardize the procedure. A dummy operation, however, was not performed. In Group II the DCA (desoxycorticosterone acetate)-treated two-day adrenalectomized rats received 2 milligrams of the hormone subcutaneously and 1 milligram intramuscularly when the last kidney was removed. Two milligrams were again injected intramuscularly at the beginning of the actual experiment. Equivalent amounts of sesame oil were simultaneously injected into untreated control and adrenalectomized rats.

At the beginning of an experiment, the animals, which previously had been starved for about 22 hours, were weighed, injected intraperitoneally with approximately 3 microcuries of Na^{22} as sodium chloride, and placed in metabolism cages for a period of 2 hours. In addition, the rats to be hydrated were also given two doses of water (5 cc. per 100 gm. of weight) by stomach tube, an hour apart. At the end of the

two-hour period, the animals were removed, any urine excretion measured, and the metabolism cages washed down with distilled water. The urine, then diluted to a known volume, was collected in order to determine the amount of radiosodium excreted. The rats were weighed again and a known quantity of blood was removed by heart puncture and centrifuged for serum electrolyte and radiosodium determinations. The animals' stomachs and intestines were removed and weighed and the carcasses were dried to a constant weight at approximately 110 C. The calculated dry weight of the removed blood was then added to the dried carcass weight. The gut was also dried to a constant weight after its radiosodium activity was determined. The radiosodium activities of the serum, urine, and gut were measured in respect to standards of the injection solution by means of a cup-type (Toroidal) gamma counter. All samples were counted twice and for a sufficient period of time to give one per cent accuracy.

Serum sodium and potassium values were determined by means of a Beckman Flame Spectrophotometer according to the method described by Mosher et al. (1949). Serum chlorides were measured by the method of Sendroy as modified by Van Slyke and Hiller (Hawk, Oser, and Summerson, 1947).

Total body water values were obtained by dividing the water content of the intact or gutless animal by its

corresponding nonhydrated weight after the two-hour run. In the hydrated animals this latter weight value was obtained by subtracting from their pre-run weight, the average weight loss during this two-hour period of the non-hydrated animals. In this way, changes in total body water could be read directly as cc. per 100 gms.

The two-hour sodium space which serves as an index of the extracellular fluid volume was determined from the following formula:

$$\frac{\text{counts/second injected} - \text{total counts/second excreted in urine}}{\text{serum activity in counts/second/cc.}}$$

X 100 ÷ nonhydrated body weight at the end of the run in grams.

In calculating the two-hour sodium space of the ratless rat the activity of the rat was subtracted from the total activity injected. Again changes in the sodium space could be read as cc./100 gms.

The extra-sodium space (the difference between the total body water and sodium space) was used as an index of intracellular space. The total sodium in the two-hour sodium space was obtained by multiplying the sodium space by the serum sodium concentration. Use of the Gibbs-Donnan equilibrium ratio was not considered necessary for this study.

Intestinal absorption was obtained by comparing the rat weights of starved hydrated rats with those which had received

no water. In all cases mentioned above, control and adrenal-ectomized rats were run simultaneously.

EXPERIMENTAL WORK

I Non-nephrectomized Rats

A. Non-hydrated Animals

The total body water of non-hydrated adrenalectomized rats was not found to be significantly different from their controls (Table 1 and 2). However, the sodium space, which is an index of the extracellular fluid volume, decreased during this period of adrenal insufficiency. Therefore, there was implied a shift of fluid to the cells which was indicated by an increase in the extra-sodium space.

Table 3 shows the typical electrolyte alterations which occur in the adrenalectomized animal. There was a marked decrease in the serum sodium and chloride and a concurrent rise in potassium. There was also a consequent reduction in the total sodium in the two-hour sodium space. A slight increase in the radiosodium activity of the gut was shown to be significant at the five per cent level according to Fisher's "t" test. This suggests a possible impairment in the absorption of diffused radiosodium from the gut. An increase of this magnitude, however, would not be significantly reflected in the sodium space.

As would be expected, fluid and electrolyte values for

TABLE I

The Distribution of Water in the Intact Rat

	No.	Total body water cc./100 gm. ± S.E.	Expected total body water cc./100 gm.	Sodium space cc./100 gm. ± S.E.	Water retained in sodium space cc./100 gm.	Extra- sodium space cc./100 gm. ± S.E.	Water retained in extra- sodium space cc./100 gm.
Normal rats	No water	24	71.7 ± 0.3	34.6 ± 0.3	-	37.1 ± 0.2	-
	Water	18	76.0 ± 0.5	35.6 ± 0.4	1.0	40.4 ± 0.4	3.3
Adrenal- ectomized rats	No water	28	72.0 ± 0.2	32.6 ± 0.3	-	39.5 ± 0.2	-
	Water	17	81.8 ± 0.4	35.7 ± 0.4	3.1	46.2 ± 0.6	6.7

TABLE 2

The Distribution of Absorbed Water in the Gutless Rat

	No.	Total body water cc./100 gm. ± S.E.	Expected total body water cc./100 gm.	Sodium space cc./100 gm. ± S.E.	Water retained in sodium space cc./100 gm.	Extra- sodium space cc./100 gm. ± S.E.	Water retained in extra- sodium space cc./100 gm.
Normal rats	No water	24	70.8 ± 0.3	-	33.4 ± 0.3	37.4 ± 0.2	-
	Water	18	74.8 ± 0.4	74.8	34.0 ± 0.4	40.8 ± 0.3	3.4
Adrenal- ectomized rats	No water	28	71.2 ± 0.2	-	31.1 ± 0.3	40.0 ± 0.2	-
	Water	17	77.7 ± 0.3	77.6	31.7 ± 0.4	46.0 ± 0.5	6.0

TABLE 3

Electrolyte Changes in Nonhydrated and Hydrated

Normal and Adrenalectomized Rats

	No.	Serum Na m. eq./liter $\pm$ S.E.	Serum Cl m. eq./liter $\pm$ S.E.	Serum K m. eq./liter $\pm$ S. E.	Total sodium in 2-hour sodium space in m. eq./100 gm. $\pm$ S.E. Intact Gutless	Radiosodium activity of gut in counts/second/gm. divided by counts/second/cc. of serum $\pm$ S.E.
Normal rats	No water	142.3 $\pm$ 0.7	111.0 $\pm$ 0.5	4.09 $\pm$ 0.17	4.93 $\pm$ 0.05	48.5 $\pm$ 0.8
	Water	136.8 $\pm$ 1.1	109.9 $\pm$ 0.9	3.75 $\pm$ 0.20	4.87 $\pm$ 0.05	49.6 $\pm$ 0.6
Adrenal- ectomized rats	No water	135.5 $\pm$ 0.8	107.7 $\pm$ 0.9	5.58 $\pm$ 0.16	4.41 $\pm$ 0.04	50.9 $\pm$ 0.7
	Water	122.5 $\pm$ 1.4	97.6 $\pm$ 1.2	6.80 $\pm$ 0.29	4.37 $\pm$ 0.07	57.6 $\pm$ 1.7

the gutless non-hydrated rat paralleled those for the intact animal.

B. Hydrated Animals

It is seen from Table 4 that the adrenalectomized rats retained over twice as much of the ingested water as did their controls. This difference in retention was due in part to both an impaired gastric motility and to a delayed absorption of water from the gastro-intestinal tracts of the adrenalectomized animals. Over six times as much water remained unabsorbed. However, this is not the major factor in respect to water retention for, while approximately two-thirds of the ingested water was absorbed from the gut, very little was excreted. In contrast, the controls, which absorbed most of the ingested water, were able to excrete over one-half of it.

The distribution of the total water retained is shown in Table 1 and 5. The total body water of both the hydrated control and adrenalectomized rats was very close to the expected value calculated on the basis of retained water.

Most of the ingested water which was not excreted appeared to be retained in the intracellular space. About three-fourths and two-thirds, respectively, of the retained water was located in the extra-sodium space of the control and adrenalectomized rats. The reason for this difference

TABLE 4

The Retention of Water in Hydrated Normal and

Adrenalectomized Rats

	No.	Urine excreted cc./100 gm. $\pm$ S.E.	Total water retained in the animal cc./100 gm. $\pm$ S.E.	Total water retained in G-I tract cc./100 gm. $\pm$ S.E.	Total water absorbed but not excreted cc./100 gm. $\pm$ S.E.
Normal rats	18	6.2 $\pm$ 0.4	4.6 $\pm$ 0.3	0.6 $\pm$ 0.2	4.0 $\pm$ 0.2
Adrenal- ectomized rats	17	1.2 $\pm$ 0.1	10.1 $\pm$ 0.3	3.8 $\pm$ 0.3	6.4 $\pm$ 0.3

An average of 10.1 cc/100 gm. of water was administered. Total water retained was the sum of the increase in body weight plus the average weight loss during the two-hour period of the nonhydrated animals.

was due to the greater amount of water still in the gut of the adrenalectomized animal, which is part of the sodium space.

To determine the sites of absorbed but unexcreted water, it was necessary to measure the fluid compartment values for the gutless rat (Table 2 and 6). Total body water values of the gutless rats were slightly lower than those for the intact animals because of the high percentage of water in the gastro-intestinal tracts of the latter. Again the total body water values for the hydrated animals were very close to the expected values calculated on the basis of the water absorbed but not excreted. It was also observed that in the gutless animal nearly all of the retained water was found to be located in the extra-sodium space. This was particularly true in the case of the adrenalectomized animal in which over ninety per cent of the absorbed but unexcreted water had shifted into the cells.

To determine what might account for these fluid shifts, certain changes in electrolyte balance were determined (Table 3). Hydration of the adrenalectomized rats reduced the low serum sodium and chloride values still further by dilution. In spite of this dilution the concentration of serum potassium increased. Nagareda and Gaunt (1951) have shown, however, that there may be an increased output of

TABLE 5

Percentage Distribution of Retained Water in the Hydrated Intact Rat

	No.	Percentage of retained water in sodium space	Percentage of retained water in extra- sodium space
Control rats	18	23	77
Adrenalectomized rats	17	32	68

TABLE 6

Percentage Distribution of Absorbed Water in the Hydrated Gutless Rat

	No.	Percentage of absorbed water in sodium space	Percentage of absorbed water in extra- sodium space
Control rats	18	15	85
Adrenalectomized rats	17	8	92

adrenal cortical hormones in excessively hydrated rats; and since these hormones have been considered to regulate the distribution of potassium between the tissues and the blood plasma, the increased serum potassium of the hydrated adrenal-ectomized rats may be due in part to the absence of this hormonal mechanism (Ingle et al., 1937; Marenzi, 1938).

The total sodium in the two-hour sodium space of the intact animals did not change significantly during hydration. The total sodium of the hydrated gutless animals, of course, decreased because the guts had a larger sodium space than those that were non-hydrated.

The radiosodium activity of the gut from hydrated adrenal-ectomized rats was much higher than that of the hydrated controls, but this was due primarily to the increased sodium space of the gut. Therefore, it was not possible with the methods used, to detect specifically a decreased rate of sodium absorption from the gut of hydrated animals during adrenal insufficiency, although this would be expected from the work of other investigators (Clark, 1939; Stein and Wertheimer, 1942).

II Nephrectomized Rats

Fluid and electrolyte shifts in the nephrectomized animals were qualitatively similar to those changes which

occured in the non-nephrectomized group.

A. Non-hydrated Animals

As is indicated in Table 7 and 8, the total body water of animals which had their second adrenal removed two days before complete nephrectomy was not significantly different from that of the nephrectomized controls. The sodium space decreased however, and a significant shift of fluid to the cells was indicated by the increased extra-sodium space.

Electrolyte changes which occurred during adrenal insufficiency are shown in Table 9. The expected rise in serum potassium and decrease in serum sodium were observed. Likewise, the total sodium in the two-hour sodium space decreased. Changes in the gutless non-hydrated nephrectomized rat paralleled those of the intact animal.

It is seen, therefore, that the fluid and electrolyte shifts which occurred in the nephrectomized adrenalectomized rat which had not undergone hydration, followed both qualitatively and quantitatively those changes which occurred in the non-nephrectomized animal.

B. Hydrated Animals

The retention of water in hydrated normal and adrenal-ectomized nephrectomized rats is shown in Table 10. Equivalent

TABLE 7

The Distribution of Water in the Intact Nephrectomized Rat

	No.	Total body water cc./100 gm. $\pm$ S.E.	Expected total body water cc./100 gm.	Sodium space cc./100 gm. $\pm$ S.E.	Water retained in sodium space cc./100 gm.	Extra- sodium space cc./100 gm. $\pm$ S.E.	Water retained in extra- sodium space cc./100 gm.
Control rats	No water	11	70.9 $\pm$ 0.4	-	-	37.6 $\pm$ 0.5	-
	Water	13	79.9 $\pm$ 0.4	80.3	37.8 $\pm$ 0.4	42.1 $\pm$ 0.4	4.5
Adrenal- ectomized rats	No water	11	70.8 $\pm$ 0.4	-	-	39.9 $\pm$ 0.3	-
	Water	12	79.8 $\pm$ 0.2	80.1	35.5 $\pm$ 0.6	44.3 $\pm$ 0.6	4.4
	Water & DCA	12	80.0 $\pm$ 0.4	80.1	35.9 $\pm$ 0.6	44.1 $\pm$ 0.5	4.2

TABLE 8

The Distribution of Absorbed Water in the Gutless Nephrectomized Rat

		No.	Total body water cc./100 gm. ± S.E.	Expected total body water cc./100 gm.	Sodium space cc./100 gm. ± S.E.	Water retained in sodium space cc./100 gm.	Extra- sodium space cc./100 gm. ± S.E.	Water retained in extra- sodium space cc./100 gm.
Control rats	No water	11	70.2 ± 0.4	-	32.6 ± 0.7	-	37.6 ± 0.5	-
	Water	13	78.4 ± 0.5	78.1	36.0 ± 0.5	3.4	42.4 ± 0.5	4.8
Adrenal- ectomized rats	No water	11	69.7 ± 0.5	-	29.6 ± 0.5	-	40.1 ± 0.6	-
	Water	12	74.5 ± 0.4	74.1	30.7 ± 0.6	1.1	43.8 ± 0.7	3.7
	Water & DCA	12	75.3 ± 0.7	74.7	32.1 ± 0.8	2.5	43.2 ± 0.4	3.1

TABLE 9

Electrolyte Changes in Normal and Adrenalectomized
Nephrectomized Rats

	No.	Serum K m. eq./liter ± S.E.	No.	Serum Na m. eq./liter ± S.E.	No.	Total sodium in 2-hour sodium space in m. eq./100 gm. ± S.E. Intact Gutless	
Control rats	No water	11	3.95 ± 0.11	11	139.0 ± 0.7	11	4.63 ± 0.07 4.53 ± 0.10
	Water	13	4.72 ± 0.19	13	126.0 ± 1.3	13	4.77 ± 0.06 4.53 ± 0.07
Adrenal- ectomized rats	No water	11	6.53 ± 0.21	11	134.7 ± 1.1	11	4.15 ± 0.05 3.99 ± 0.06
	Water	9	6.83 ± 0.39	12	122.7 ± 1.1	12	4.36 ± 0.07 3.78 ± 0.06
	Water & DCA	9	5.69 ± 0.24	12	122.5 ± 0.7	12	4.39 ± 0.10 3.93 ± 0.10

TABLE 10

The Retention of Water in Hydrated Normal and
Adrenalectomized Nephrectomized Rats

	No.	Total water retained in the animal cc./100 gm. ± S.E.	Total water retained in the G-I tract cc./100 gm. ± S.E.	Total water absorbed from the G-I tract cc./100 gm. ± S.E.
Control rats				
Water	13	9.4 ± 0.1	1.5 ± 0.2	7.9 ± 0.3
Adrenal- ectomized rats				
Water	12	9.3 ± 0.1	4.9 ± 0.2	4.4 ± 0.2
Water & DCA	12	9.3 ± 0.1	4.3 ± 0.5	5.0 ± 0.5

Total water retained was the difference between the non-hydrated body weight and the hydrated body weight at the end of the two-hour period.

amounts of water were retained in both the control and adrenalectomized rats. The adrenalectomized animals withheld over three times as much of the ingested water in their gastrointestinal tracts as did the controls. This difference was due to both a decreased gastric motility and to a delayed intestinal absorption. There was a tendency, however, for the absorption of water from the intestinal tracts of the adrenalectomized rats to increase after treatment with desoxycorticosterone acetate (DCA). This apparent difference was not significantly established.

These observations are qualitatively similar to those for the non-nephrectomized animals. Quantitatively, less water appeared to be absorbed from the intestines of the nephrectomized animals. This was particularly true in the adrenalectomized rats.

Distribution of the ingested water in the intact nephrectomized animal is indicated in Table 6 and 11. The total body water of both the hydrated control and adrenalectomized rats was very close to the expected value calculated on the basis of retained water. Approximately one-half of the ingested water was contained in the extra-sodium space of all of the nephrectomized animals regardless of experimental treatment. Water which is retained in the lumen of the gastro-intestinal tract is still part of the sodium space.

Since less water was absorbed from the intestines of the adrenalectomized animal, it would appear that the sodium space of this group should have been proportionally larger than that of the controls. The reason that it was not was because, even though almost twice as much water was absorbed from the intestines of the controls, only about one-half as much of the absorbed water shifted to the cells in contrast to that of the adrenalectomized group. Thus, the two phenomena effectively canceled each other.

To determine the sites of the absorbed water, it was necessary to measure the fluid compartment values of the gutless nephrectomized rat (Table 8 and 12). As in the non-nephrectomized animals, total body water values of the gutless rats were slightly lower than those for the intact animals because of the high percentage of water in the gastro-intestinal tracts of the latter. Likewise, the total body water values for the hydrated animals were fairly close to the expected values calculated on the basis of the absorbed water.

In general, proportionally, more of the absorbed water was found to be located in the extra-sodium space than in the sodium space of the gutless animals. Again, as was also observed in the non-nephrectomized animal, this was particularly true in the case of the adrenalectomized rat. Approximately three-fourths of the absorbed water had shifted to the cells.

TABLE 11

Percentage Distribution of Retained Water in the Hydrated Intact
Nephrectomized Rat

	No.	Percentage of retained water in sodium space	Percentage of retained water in extra- sodium space
Control rats	13	50	50
Adrenalectomized rats	12	51	49
Adrenalectomized rats treated with DCA	12	54	46

TABLE 12

Percentage Distribution of Absorbed Water in the Hydrated Gutless
Nephrectomized Rat

	No.	Percentage of absorbed water in sodium space	Percentage of absorbed water in extra-sodium space
Control rats	13.	41	59
Adrenalectomized rats	12	23	77
Adrenalectomized rats treated with DCA	12	45	55

DCA treatment appeared to produce some interesting changes in the adrenalectomized gutless rat. The tendency for an increased absorption of water from the intestinal tract is reflected in the increased total body water. However, as before, this increase was not significantly established. Likewise, the apparent action of DCA to prevent cellular hydration is well demonstrated percentage-wise in Table 12. The distribution of absorbed water seemed to be returned essentially to normal. It is regrettable that there was not time to confirm these DCA-induced tendencies by using additional carefully controlled DCA-treated animals.

Changes in the electrolyte balance of the hydrated nephrectomized rats are given in Table 9. The serum sodium values as was expected were reduced by dilution. As in the non-nephrectomized animals, however, there was not observed a dilution of serum potassium in the hydrated controls. In fact, the serum potassium of these nephrectomized animals increased. It is possible that the stress of excessive hydration may induce a general systemic liberation of cellular potassium which might in part explain the absence of potassium dilution (Selye, 1950). A change in the serum potassium of the adrenalectomized rats after hydration was not significantly established with the number of animals used.

Little change occurred in the total sodium of the two-hour

sodium space in the intact nephrectomized animal after hydration. Because of the large amount of fluid which remains unabsorbed in the gastro-intestinal tract (as part of the sodium space) during adrenal insufficiency, the total sodium of the outless adrenalectomized rat decreased after hydration. These observations for total sodium are similar to those which were found in the non-nephrectomized rats.

Thus it is apparent that the fluid and electrolyte shifts which occurred in both the control and adrenalectomized hydrated nephrectomized rats qualitatively confirm those changes which occurred in the non-nephrectomized animals.

GENERAL CONCLUSIONS

As discussed previously, there are several factors which are responsible for the increased retention of ingested water during adrenal insufficiency:

1. An increased renal tubular reabsorption of water which has been seen by all recent workers who have looked for it. Gaunt (1951) and his co-workers consider this the most important factor in explaining water retention.
2. A decreased renal plasma flow and decreased glomerular filtration rate which have also been observed by most investigators. Boss et al., (1950) have demonstrated that while these factors are of no great relative importance, they may become quite significant when heavy water loads are imposed.
3. A decreased emptying time of the stomach and a delayed intestinal absorption of water and sodium salts which has been demonstrated by Gaunt (1944) but denied by Shipley (1945).

This study has indicated in regard to the latter factor that an impaired gastric motility and a delayed reabsorption from the gut does play an extra-renal role in connection with

water retention during adrenal insufficiency. This fact was significantly established in both the non-nephrectomized and nephrectomized animals.

It was not established, however, that a retention of sodium salts in the intestinal tract plays a role in this regard, although the results do not deny such a possibility. Additional studies in this laboratory have indicated that two hours after the oral administration of radiosodium to hydrated rats, the serum activity is significantly higher in normal animals than in those that were adrenalectomized. While this suggests an impaired intestinal absorption of sodium in adrenalectomized rats, it could be partially due to a depressed gastric motility which would lessen the opportunity for absorption.

In addition to a delayed intestinal absorption, Gaunt (1944) also reported a shift of salt from the extracellular fluid to the unabsorbed water in the intestines of the hydrated adrenalectomized rats. This decrease in the electrolyte concentration of the extracellular space would result in a shift of water to the cells and a consequent increase in the intracellular space. For reasons previously discussed, Shipley (1945) was unable to confirm these fluid compartment changes (See Introduction).

This investigation shows that the absorbed but unexcreted

water was retained largely in the cells of the adrenalectomized animals. Even the adrenalectomized nephrectomized rats which were unable to excrete any of the ingested water, retained over three-fourths of the absorbed water in the extrasodium space.

A study of the changes in electrolyte balance suggest the reasons for these fluid shifts. Over a ten per cent loss of total sodium from the two hour sodium space was observed two days after adrenalectomy. This was true for both the non-nephrectomized animals and those that underwent subsequent nephrectomy. In an endeavor to maintain the extracellular body fluids in a state of isotonicity, there was then a compensatory loss of fluid from the extracellular space. This was detected as a decrease in the sodium space. Since the total body water of both the non-nephrectomized and nephrectomized two-day adrenalectomized rats did not change, there was implied a shift of fluid into the intracellular compartment. This loss of fluid from the extracellular space, however, did not entirely compensate for the loss in total body sodium, for a decrease in serum sodium was observed after the two-day period of adrenal insufficiency. It was therefore assumed that loss of salt and the subsequent fluid shifts were still in a dynamic state of change.

Proportionally, more of the absorbed but unexcreted

water shifted to the cells of both the non-nephrectomized and nephrectomized hydrated adrenalectomized animals than it did in their controls, probably because the loss of extracellular salt produced a higher osmotic gradient.

Since water intoxication is generally considered to be due to cellular hydration, these observations help to explain the sensitivity of adrenalectomized animals to excessive ingestion of water. The adrenal steroids have been shown to afford some extra-renal protection against water intoxication in hydrated adrenalectomized rats. As mentioned previously, Hays and Mathieson (1945) and Birnie et al., (1948) suggest that this extra-renal protection is due to the effect of these hormones on the distribution of water between extracellular and intracellular compartments, to prevent excessive intracellular hydration.

In this study the tendency of DCA to prevent the shift of water into the cells of hydrated adrenalectomized rats was percentage-wise most apparent and is in agreement with the hypothesis stated above. This tendency however, was not significantly established and additional experiments will be necessary to confirm this extra-renal hormonal action.

Although changes in serum potassium were followed in this investigation merely as an index of adrenal insufficiency, several interesting observations were made during the course

of the experiments. For instance, serum potassium unlike sodium did not appear to be diluted upon hydration. In fact, under some experimental conditions it actually increased. Also, there was some indication that adrenal insufficiency tended to exag⁴erate this phenomenon.

.It is suggested that hydration may act in the same way as other stressing agents by inducing a general systemic release of intracellular potassium. If, as Seize (1950) suggests, the release of potassium during the shock phase of the alarm reaction is conditioned by a state of relative cortical insufficiency, one would also expect that these changes in serum potassium would be exag⁵erated after adrenal-ectomy.

Further speculation is not warranted until additional experimental information is obtained. At the present time carefully controlled experiments especially designed to investigate the meaning of such changes in potassium metabolism are under consideration.

SUMMARY

I Non-nephrectomized Animals

Normal and two-day adrenalectomized rats received two doses of water (5 cc./100 gms.) an hour apart. Two hours after the first dose, the adrenalectomized rats had retained over twice as much water as their controls. This retention was due in part to a delayed intestinal absorption, but primarily, to a delayed water diuresis.

The absorbed but unexcreted water was found to be located almost entirely in the cells. The loss of salt from the extracellular fluid during the two-day period of adrenal insufficiency was suggested as the cause of the increased cellular hydration in these animals.

The tendency for fluid to shift to the cells during adrenal insufficiency may explain in part the sensitivity of the adrenalectomized animal to excessive hydration.

II Nephrectomized Animals

Normal and two-day adrenalectomized nephrectomized rats underwent the same experimental treatment as the non-nephrectomized animals. In addition DCA was administered to a group of hydrated adrenalectomized rats.

Qualitatively, changes in fluid and electrolyte balance were similar to those observed in the non-nephrectomized group. There was a delayed intestinal absorption of water in the hydrated adrenalectomized rats. Likewise, a larger percentage of absorbed water shifted to the cells of the adrenalectomized animals as a result of previous salt depletion.

DCA appeared to prevent the excessive intracellular hydration of the adrenalectomized animals. This extra-renal hormonal action, however, was not significantly established and additional experimental data will be necessary to confirm this tendency.

BIBLIOGRAPHY

- Addison, T., 1855. On the Constitutional and Local Effects of Disease of the Suprarenal Capsules. London, Highley.
- Allers, W. D. and E. C. Kendall, 1937. Maintenance of adrenalectomized dogs without cortin through control of the mineral constituents of the diet. Am. J. Physiol., 118:87.
- Banting, F. G. and S. Cairns, 1926. Adrenal insufficiency in dogs. Am. J. Physiol., 77:100.
- Baumann, E. J. and S. Kurland, 1927. Changes in the inorganic constituents of blood in suprarenalectomized cats and rabbits. J. Biol. Chem., 71:281.
- Biedl, A., 1913. Internal Secretory Organs. William Wood and Company, New York.
- Birnie, J. H., 1950. Inactivation of posterior pituitary antidiuretic hormone by liver extracts. Federation Proc., 9:12.
- Birnie, J. H., W. J. Eversole, W. R. Boss, C. M. Osborn and R. Gaunt, 1950. An antidiuretic substance in the blood of normal and adrenalectomized rats. Endocrinology, 47:1.
- Birnie, J. H., W. J. Eversole and R. Gaunt, 1946. The extra-renal action of desoxycorticosterone: survival and water intoxication studies. Endocrinology, 42:412.
- Boss, W. R., J. H. Birnie and R. Gaunt, 1950. Renal factors in adrenal cortical control of water metabolism. Endocrinology, 46:307.
- Boss, W. R. and C. M. Osborn, 1950. Effects of adrenal cortical extract on renal function in adrenalectomized rats. Anat. Rec., 108:598.
- Bradley, S. E., editor, 1951. Renal Function. Transactions of the second conference, October 19-20, 1950. Josiah Macy, Jr. Foundation. Corlies, Macy and Company Inc., Printer, New York.

- Chambers, G. H., E. V. Melville, R. S. Hare and K. Hare, 1945. Regulation of the release of pituitrin by changes in the osmotic pressure of the plasma. Am. J. Physiol., 144:311.
- Christman, R. C., editor, 1950. Pituitary-Adrenal Function. A.A.A.S. Symposium, December 28-29, 1949. Horn-Shafer Company, Printer, Baltimore.
- Danowski, T. S. and J. R. Elkington, 1951. Exchanges of potassium related to organs and systems. Pharmacol. Rev., 3:42.
- Doxiadis, S. A. and D. Gairdner, 1946-8. The estimation of extra-cellular fluid volume by the thiocyanate method in children and adults. Clin. Sc., 6:257.
- Earle, Jr., D. P., R. C. deBodo, I. L. Schwartz, S. J. Farber, M. Kurtz, and J. Greenberg, 1951. Effect of hypophysectomy on electrolyte and water metabolism in the dog. Proc. Soc. Exptl. Biol. Med., 76:608.
- Elkington, J. R., 1950. Water metabolism, Annual Rev. Physiol., 12:145.
- Eversole, W. J., J. F. Birnie and R. Gaunt, 1949. Inactivation of the posterior pituitary antidiuretic hormone by the liver. Endocrinology, 45:378.
- Flanagan, J. B., A. K. Davis and R. R. Overman, 1950. Mechanism of extracellular sodium and chloride depletion in the adrenalectomized dog. Am. J. Physiol., 160:89.
- Frost, R. C. and R. V. Talmage, 1951. Fluid and electrolyte shifts in the hydrated adrenalectomized rat. Endocrinology, 49:606.
- Gaudino, M. and M. F. Levitt, 1949. Influence of the adrenal cortex on body water distribution and renal function. J. Clin. Invest. 28:1487.
- Gaunt, R., 1944. Water diuresis and water intoxication in relation to the adrenal cortex. Endocrinology, 34:400.
- Gaunt, R., 1950. Hormones of the Adrenal Cortex. Cyclopedia of Medicine, Surgery, Specialties. F. A. Davis Company, Philadelphia.

- Gaunt, R., 1951. The adrenal cortex in salt and water metabolism. Recent Progress Hormone Research, 6:247.
- Gaunt, R., and J. H. Birnie, 1951. Hormones and Body Water. Charles C. Thomas, Springfield.
- Gaunt, R., J. H. Birnie and W. J. Eversole, 1949. Adrenal cortex and water metabolism. Physiol. Rev., 29:281.
- Gaunt, R., J. H. Birnie, W. R. Boss, W. J. Eversole and C. M. Osborn, 1950. The adrenal cortex in water metabolism. Pituitary-Adrenal Function. A.A.A.S. Symposium, December 28-29, 1949. R. C. Christman, editor. Horn-Schafer Company, Printer, Baltimore.
- Gaunt, R. and W. J. Eversole, 1949. Notes on the history of the adrenal cortical problem. Ann. New York Acad. Sci., 50:511.
- Gaunt, R., M. Liling and M. Jordsen, 1945. Adrenal medulla in water diuresis and water intoxication. Endocrinology, 37:136.
- Gordon, E. S., editor, 1950. A Symposium on Steroid Hormones. The University of Wisconsin Press. Business Press, Inc., Lancaster, Pennsylvania.
- Harrison, H. E. and D. C. Darrow, 1939. Renal function in experimental adrenal insufficiency. Am. J. Physiol., 125:631.
- Harrop, G. A., A. Weinstein, L. J. Soffer, and J. E. Trescher, 1933a. (1) Studies on the suprarenal cortex II. Metabolism, circulation, and blood concentration during suprarenal insufficiency in the dog. J. Exptl. Med., 58:1.
- Harrop, G. A., L. J. Soffer and R. Ellsworth and J. E. Trescher, 1933b. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. J. Exptl. Med., 58:17.
- Hartman, F. A. and K. A. Brownell, 1949. The Adrenal Gland. Lea and Febiger, Philadelphia.
- Hartman, F. A., K. A. Brownell and W. E. Hartman, 1930. A further study of the hormone of the adrenal cortex. Am. J. Physiol., 95:670.

- Hastings, A. B. and E. L. Compere, 1931. Effect of bilateral suprarenalectomy on certain constituents of the blood of dogs. Proc. Soc. Exptl. Biol. Med., 28:376.
- Hawk, P. B., B. L. Oser and W. H. Summerson, 1947. Practical Physiological Chemistry, 12th Edition. The Blakiston Company, Philadelphia.
- Hays, H. W. and D. R. Mathieson, 1945. Studies on water intoxication in adrenalectomized rats and effects of DCA and epinephrine in water diuresis. Endocrinology, 37:147.
- Ingle, D. J., H. W. Wilson and E. C. Kendall, 1937. The effect of cortin on the concentrations of some constituents of the blood of adrenalectomized rats. Am. J. Physiol., 118:302.
- Kendall, E. C., 1942. The influence of the adrenal cortex on the metabolism of water and electrolytes. Vitamins and Hormones, 6:277.
- Kuizenga, M. H., 1944. Chemistry and Physiology of the Hormones, 57. A.A.A.S. Washington, D. C.
- Loeb, R. F., 1933. Effect of sodium chloride in treatment of a patient with Addison's disease. Proc. Soc. Exptl. Biol. Med., 30:808.
- Loeb, R. F., D. M. Atchley, E. M. Benedict and J. Leland, 1935. Electrolyte balance studies in adrenalectomized dogs with particular reference to the excretion of sodium. J. Exp. Med., 67:775.
- Lucas, G. H. W., 1926. Blood and urine findings in desuprarenalized dogs. Am. J. Physiol., 77:114.
- Marenzi, D., 1936. Blood potassium and suprarenal glands. Endocrinology, 23:330.
- Mosher, R. E., A. J. Boyle, E. J. Bird, S. D. Jacobson, T. M. Batchelor, L. T. Iseri and G. B. Meyers, 1949. The use of the flame photometry for the quantitative determination of sodium and potassium in blood and urine. Am. J. Clin. Path., 19:461.
- Note, J. R., editor, 1951. Proceedings of the Second ACTH Conference. The Blakiston Company, Philadelphia.

- Nagareda, C. S. and R. Gaunt, 1951. Functional relationship between the adrenal cortex and the posterior pituitary. Endocrinology, 48:560.
- Noble, R. L., 1950. Physiology of the adrenal cortex, Chapter III. The Hormones, Vol. II. G. Pincus and K. V. Thimann, editors. Academic Press, Inc., New York.
- Newton, W. H., 1949. Recent Advances in Physiology. Chapter II, Water diuresis. The Blakiston Company, Philadelphia.
- Overman, R. R., 1951. Sodium, potassium and chloride alterations in disease. Physiol. Rev., 31:285.
- Pfiffner, J. J., 1942. The adrenal cortical hormones. Advances in Enzymology, 2:325.
- Pincus, G., editor, 1951. Chemistry, physiology and clinical endocrinology of the adrenal cortex. Recent Progress Hormone Research, 6:247.
- Pincus, G. and K. V. Thimann, editors, 1950. The Hormones, Vol. II. Academic Press, Inc., New York.
- Ralli, E. P., editor, 1950. Adrenal Cortex. Transactions of the first conference. November 21-22, 1949. Sponsored by Josiah Macy, Jr. Foundation. Progress Associates, printer, Caldwell, New Jersey.
- Reichstein, T. and C. W. Shoppe, 1943. The hormones of the adrenal cortex. Vitamins and Hormones, 1:345.
- Rommelt, J. C., Jr., O. W. Sartorius and R. Pitts, 1949. Excretion and reabsorption of sodium and water in the adrenalectomized dog. Am. J. Physiol., 159:124.
- Sayers, G., 1950. The adrenal cortex and homeostasis. Physiol. Rev., 30:241.
- Schienberg, I. H., H. J. Kowalski and G. M. Latshaw, 1950. Binding of thiocyanate to albumin in normal human serum and defibrinate blood with reference to the determination of thiocyanate space. J. Clin. Investigation, 29:475.
- Selye, H., 1946. The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol., 6:117.

- Selye, H., 1950. The Physiology and the Pathology of the Exposure to Stress. Acta, Inc., Montreal, Canada.
- Selye, H. and H. Stone, 1950. On the Experimental Morphology of the Adrenal Cortex. Charles C. Thomas, Springfield, Illinois.
- Shipley, R. A., 1945. The cause of abnormal retention of ingested water in adrenalectomized rats. Endocrinology, 36:118.
- Smith, Homer W., 1951. The Kidney, Structure and Function in Health and Disease. Oxford University Press, New York.
- Stehle, R. L., 1949a. The chemistry of the hormones of the posterior lobe of the pituitary gland. Vitamins and Hormones, 8:383.
- Stehle, R. L., 1949b. The actions of the hormones of the posterior lobe of the pituitary gland upon the circulation and secretion of urine. Vitamins and Hormones, 8:390.
- Stein, L. and E. Wertheimer, 1944. Disturbances of water balance in the rat on removal of the adrenal medulla. J. Endocrinol., 3:356.
- Swingle, W. W. and J. J. Pfiffner, 1929. Experiments with an active extract of the suprarenal cortex. Anat. Rec., 44:225.
- Swingle, W. W. and J. J. Pfiffner, 1930. An aqueous extract of the suprarenal cortex which maintains the life of bilaterally adrenalectomized cats. Science, 71:321.
- Swingle, W. W., J. J. Pfiffner, N. M. Vars, P. A. Bott and W. M. Parkins, 1933. The function of the adrenal cortical hormone and the cause of death from adrenal insufficiency. Science, 77:58.
- Swingle, W. W. and J. W. Remington, 1944. The role of the adrenal cortex in physiological processes. Physiol. Rev., 24:89.
- Verney, E. B., 1946. Absorption and excretion of water; antidiuretic hormone. Lancet, 2:739.

Verney, E. B., 1947. The antidiuretic hormone and the factors which determine its release. Proc. Roy. Soc. London, s. B., 135:25.

Waring, H. and F. W. Landgrebe, 1950. Hormones of the posterior pituitary, Chapter VIII. The Hormones, Vol. II. G. Pincus and K. V. Thimann, editors. Academic Press, New York.

White, A., editor, 1951. Symposium on Steroids in Experimental and Clinical Practice. The Blakiston Company, Philadelphia.

White, H. L., Heinbecker, P. and D. Rolf, 1949. Enhancing effects of growth hormone on renal function. Am. J. Physiol., 157:47.

Wolff, A. V., 1950. The Urinary Function of the Kidney. Grune and Stratton, Inc., New York.

Zaffaroni, A., 1950. The adrenal cortex and its secretory products, Chapter 6. Symposium on Steroids in Experimental and Clinical Practice. A. White, editor. The Blakiston Company, Philadelphia.

Zwemer, R. L., 1927. (1) An experimental study of the adrenal cortex. I. The survival value of the adrenal cortex. Am. J. Physiol., 79:641.

Zwemer, R. L., 1927. (2) An experimental study of the adrenal cortex. II Prolongation of life after complete epinephrectomy. Am. J. Physiol., 79:658.