Changes in the osmotic pressure of the plasma after adrenalectomy in the Long-Evans rat

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CHANGES IN THE OSMOTIC PRESSURE OF THE PLASMA
AFTER ADRENALECTOMY IN THE LONG-EVANS RAT

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I wish to acknowledge my indebtedness to my loving parents, Mr. and Mrs. Willie Wright.

I wish further to express gratitude to my most inspiring teachers, Dr. M. L. Reddick, Dr. J. H. Birnie and Miss Fronnie M. Whitehurst, who have given me the benefits of their advice, criticism and experience.
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CHAPTER I

INTRODUCTION

According to studies by Swingle and Pfiffner ('30), Britton and Silvette ('37), Long, Katzin and Fry ('40) and Ingle ('42), the adrenal cortex elaborates one or more hormones essential to the maintenance of life. Various fractions serve in the maintenance of the physiological "steady states"; the regulation of the distribution of water and electrolytes, and many aspects of carbohydrate metabolism.

It was demonstrated by Muntwyler, Mellors and Mautz ('40a) that adrenalectomy caused an alteration in the normal sodium and potassium levels in the body, thus causing alterations in electrolytes, disturbances in the acid-base balance, hemoconcentration and water diuresis. Silvette and Britton ('33), Zwemer and Truszkowski ('36) and Corey, Britton, Kline and French ('41) considered the adrenal-cortical tissue as being responsible for the control of these electrolyte concentrations in the body. Harrison and Darrow ('39), Clarke and Cleghorn ('42) and Gaunt ('44) considered the kidney as the site of the primary control mechanism, whereas Ingle, Nilson and Kendall ('37) considered it to be extra-renal. According to Ingle ('42), these differences of opinions arose during the period when most investigators believed that the adrenal cortex produced only a single hormone which regulated electrolyte and water metabolism. He reported that they also postulated that when this hormone was altered all the symptoms of the adrenal insufficiency syndrome could be explained. The present concept of Ingle ('42) that the adrenal glands produce several hormones, each of which may have a different function, has done much to clarify some of the earlier controversies.
The present investigation was undertaken to determine whether or not changes in the osmotic pressure of the plasma take place after adrenalectomy in Long-Evans rats.
CHAPTER II

REVIEW OF LITERATURE

Studies by Stewart ('21) revealed that when both adrenal glands were removed from most laboratory animals death speedily ensued. However, it was observed that the fatal result was delayed when the operation was performed in two stages and time was allowed to elapse between the removal of the two glands.

In laboratory animals which have been deprived of their adrenal glands a number of biochemical changes supervene in the tissues. These changes, for the most part, involve electrolyte and water metabolism. Among the first investigators to analyze the blood of animals in adrenal insufficiency were Muntwyler, Mellors and Mautz ('41a). They analyzed the content of serum and cells of eleven dogs in adrenal insufficiency and of four dogs in adrenal insufficiency following the administration of cortical extract. The analysis of the serum of the eleven dogs in adrenal insufficiency revealed a fall in the water content and a gain in the plasma proteins. There were also a fall in phosphoric acid, decreases in chloride and sodium ions, and an increase in potassium. The analysis further revealed that the red blood cells usually gained water. From these observations, they concluded that the acidosis encountered was due to the great loss of sodium over chloride. The analysis of the serum of the four dogs in adrenal insufficiency following the administration of cortical extract showed marked improvement. No evidence of blood dilution was observed, since there was little if any change in serum water. They interpreted their findings as indicating that the increase in the serum chloride resulted from the entrance of interstitial fluid into the circulation followed by the excretion of water and some electrolytes with the
partial retention of sodium. From the blood changes observed following
cortical extract administration they concluded that these results tended
to minimize the importance of electrolyte changes as the function of the
cortical hormones. As a result, they suggested that the action of the
adrenal cortical hormone was either on the nervous system or the capillaries.

Muntwyler et al. ('40a) also observed that the chemical pattern of the
red blood cells underwent wide changes when they came into contact with or
into equilibrium with the serum. Evidence was presented to show that the
establishment of osmotic equilibrium between the serum and cells of dogs in
adrenal insufficiency was associated with the loss of base or sodium from
the cells and with anion and water exchange. After cortical extract adminis-
tration, the equilibrium was associated with a gain of cell sodium and with
anion and water exchange.

Silvette and Britton ('36) and Britton and Silvette ('37) performed simi-
lar experiments and found that in the marmot and the opossum the sodium level
of the blood was increased rather than decreased following the removal of the
adrenal glands.

Marine and Bauman ('27) administered physiological solutions such as
Ringer's and isotonic sodium acetate to experimental cats from which both
adrenal glands had been removed at one operation. They observed that the
duration of life in these cats was about three times that of the controls. By
feeding salt solutions after the administration of cortical hormones Swingle,
Vars, Pfiffner and Parkins ('34) were able to lengthen greatly the lives of
dogs in adrenal insufficiency. Gaunt, Tobin and Gaunt ('35) used rats in
different stages of adrenalectomy and were able to maintain them in an appa-
rent state of good health for a long period of time by feeding them sodium
chloride. They concluded that it was possible to rescue rats in the terminal
stages of adrenal insufficiency with salt solutions and prolong their period of survival. Allers and Kendall ('37) used a mixture of sodium chloride and sodium citrate and were able not only to prolong the life of adrenalectomized dogs, but were also able to maintain the electrolyte balance of the blood within normal limits. Even though the administration of salt solutions may lengthen the lives of animals in adrenal insufficiency for an extensive period of time, Kendall ('37) pointed out that the concentration of the electrolytes in the blood at this time was not normal. He demonstrated that only by the use of both cortical extract and salt in adequate amounts could an entirely normal electrolyte balance be maintained in totally adrenalectomized animals.

Following adrenalectomy in dogs, Harrison and Darrow ('37) observed marked disturbances in renal function. They interpreted these disturbances as due to a failure of the tubules to reabsorb sodium adequately from the glomerular filtrate at a time when the concentration of the sodium in the plasma was low and to excrete potassium, phosphates and perhaps other ions of high concentration. They concluded that the disturbance in renal function may have caused the decrease in plasma sodium and the increase in plasma potassium and phosphate found in animals in adrenal insufficiency. Thron, Engel and Lewis ('41) injected a normal and an adrenalectomized dog with 25 mgs. of 11-dehydro-17 hydroxy corticosterone and noted a marked increase in sodium and chloride excretion in both animals. They concluded that the adrenal cortical steroids with a hydroxyl group on C17 induced an increased excretion of sodium in contrast to the well known "retaining effect" of other steroids such as corticosterone and desoxycorticosterone.

Studies by Eversole ('45) demonstrated that small doses of desoxycorticosterone acetate maintained life and growth in adrenalectomized rats which
had been fed purified diets free from sodium chloride. Later studies by Ferrebee, Parker, Carnes, Gerity, Atchley and Loeb ('45) revealed that normal dogs which received daily injections of 25 mgs. of desoxycorticosterone acetate developed attacks of muscular weakness and a syndrome resembling diabetes-insipidus. From these observations, they concluded that the prolonged administration of desoxycorticosterone acetate may cause such a retention of sodium so as to bring about the replacement of potassium in the skeletal muscles by sodium and thus create an upset in the electrolyte balance. This upset might have caused this "diabetes-insipidus" like condition.

Many investigators have postulated that the function of the adrenal gland may be the regulation of sodium metabolism. From a number of different abdominal operations performed on cats, Britton and Silvette ('37) observed a reduction in serum sodium two or three times as great as that found in adrenal insufficiency. They found that the salt loss after adrenalectomy was relatively small and non-cortical. Muntwyler, Mellors, Mautz and Mangun ('40b) demonstrated that adrenalectomized animals showed improvement before any change in serum sodium could be observed. They concluded that electrolyte changes may not be the main function of the adrenal cortical hormone.

The role of sodium in adrenal insufficiency and its physiological function caused many controversies among early investigators. During these controversies, it was thought that the adrenal glands produced only one hormone, which when affected caused adrenal insufficiency. According to Turner ('55) investigators have extracted approximately thirty different steroids from the adrenal cortex. These extracts have been divided into three groups which have different functions. The location of the structure which becomes impaired upon cortical deprivation is still debatable. It is believed by some
that the kidney is the regulatory structure while others believe that extrarenal factors regulate the sodium level of the blood.

Another change observed in the serum of animals in adrenal insufficiency, in addition to a decrease in sodium, was a rise in the potassium concentration. Studies by Harrison and Darrow ('38) and Hartman, Lewis, Thatcher and Street ('42) demonstrated that the potassium level of the blood of dogs in adrenal insufficiency increased. Zwemer and Truszkowski ('36) had observed the same increase in the potassium level of the blood of adrenalectomized cats. They had previously concluded that all of the various known symptoms of cortico-adrenal insufficiency may be explained in terms of a cortico-adrenal-potassium interrelationship. Studies by Nilson ('37), demonstrated that adrenalectomized dogs may be maintained without cortical extract on a diet rich in sodium citrate but low in potassium. He also observed that such dogs required a greater balance of sodium and chloride than did intact dogs and that they were not able to maintain a daily uniform balance of electrolytes. He concluded that the acute symptoms of adrenal insufficiency were due to the low intake of sodium which would have stimulated the production of potassium.

Zwemer and Truszkowski ('36) contributed fundamental descriptions of animals in adrenal insufficiency. Allers and Kendall ('37) and Nilson ('37) demonstrated that dogs in adrenal insufficiency may be kept for some time on diets high in sodium chloride and sodium citrate but low in potassium. According to Allers ('37) the clinical significance of these results have been applied to Addisonian patients.

Muntwyler et al. (40b) found that the potassium content of muscle tissue per unit of dry weight increased in dogs during adrenal insufficiency. The studies of Clark and Cleghorn ('42) demonstrated that tissue analysis of rats
and dogs in adrenal insufficiency revealed an increase in the per cent of potassium in the muscles, liver and small intestine. They concluded that the rise in potassium could be due to its liberation from other sources.

Nilson ('37) has proposed that the adrenal cortical hormone may be concerned with the permeability of the cell membrane to potassium. Ingle and Kendall ('36) demonstrated that cortical extract would prevent the rise of potassium in adrenalectomized-nephrectomized rats. This was an indication to them that extra-renal factors may be involved in maintaining a normal electrolyte balance. McKay, Bergman and McKay ('37) observed that bilateral adrenalectomy decreased by half the duration of life of uremic doubley nephrectomized rats due to the retention in the serum of some toxic substance. According to them, this toxic substance did not reach the serum in the presence of the adrenals and was excreted in the urine when the kidneys but not the adrenals were present. From their observation on the serum potassium of these adrenalectomized-nephrectomized rats, they concluded that the increased potassium was not responsible for the deleterious influence of adrenalectomy upon experimental uremia. Studies by Marenzi ('38) demonstrated that suprarenalectomy in the dog caused a decrease in sodium chloride and an increase in the potassium of the blood. In other studies he injected cortical extract into the dog and observed that the plasma potassium returned to normal levels within 10 to 15 minutes. From these observations, he concluded that the cortico-suprarenal hormone may be a factor which regulated the "fixation" of potassium in the body tissues. According to him, this hormone is absent in animals in adrenal insufficiency and accounts for the high level of potassium in the serum of adrenalectomized animals.

Nilson ('37), demonstrated that the administration of sodium salts made it possible for potassium to return to its normal level in the plasma. Buell
and Turner ('41) administered desoxycorticosterone to normal rats and observed the entrance of small quantities of sodium into the muscle cells. At the same time they administered sodium chloride to these animals, they observed that it too prevented the rise in muscle potassium. From these observations, they concluded that either desoxycorticosterone or sodium chloride would prevent the rise in potassium which would have occurred in their absence.

According to Harrison and Darrow ('38), Muntwyler et al. ('40a) and Turner ('55) the distribution of sodium and potassium ions on the cell membranes may become imbalanced due to the rise of potassium in the intracellular spaces and the lowering of sodium in the extracellular spaces. They concluded that those factors disturbed the osmotic pressure and required a shift of water to restore the equilibrium.
CHAPTER III

MATERIALS AND METHODS

In this experiment 34 Long-Evans rats were used. Both male and female rats were used. The rats were kept in cages, fed on Gaines Krunchon cubes and given water daily. Fourteen of these animals were used for controls while 20 were used for experimental.

The experimental animals were bilaterally adrenalectomized. The animals were anesthetized after which they were restrained. An incision was made through the right side of the abdominal wall at the anterior end of the kidney. With fine-pointed curved forceps the fatty tissue adjacent to the body wall was laid aside and the right adrenal gland was removed with another pair of fine-pointed curved forceps. The left adrenal gland was removed in the same manner as that on the right. The incisions were closed with wound clamps.

The control animals were operated on in the same manner as that above except that the adrenal glands were not removed. The control and the experimental animals were placed in separate cages and identified by the shape of the black hair which ran antero-posteriorly along the dorsal surface.

After ether anesthesia, samples of blood were taken from one control and one experimental animal on the first, third, 5th and 7th day after the operation by cardiac puncture. The samples of blood were centrifuged in order to obtain the plasma, the osmotic pressure of which was to be taken. The osmotic pressure of the plasma was determined by the cryoscopic method or freezing-point determination. Five cubic centimeters of the plasma were transferred to the inner jacket of the cryoscopic apparatus which contained a Beckman thermometer. The apparatus was then placed in an ice bath and the
ice was constantly stirred around the outer jacket in order to freeze the plasma. When the plasma had just frozen there was a sudden drop in temperature as registered by the thermometer; this was followed by a sudden rise in the temperature. From the sudden drop in the temperature the osmotic pressure of the plasma was calculated as follows:

\[ T_f = M K_f \]
\[ M = \frac{\Delta T_f}{K_f} \]
\[ P = \frac{\Delta T_f}{K_f} R T \]

\( T_f \) = freezing point of biological fluid or plasma
\( K_f \) = molal freezing point constant for water
\( M \) = molarity
\( R \) = universal gas constant
\( T \) = any temperature of \( T \) degrees on the centigrade scale plus 273 degrees on the absolute scale (273\( ^0 \) + \( T \))
\( P \) = osmotic pressure
CHAPTER IV

EXPERIMENTAL RESULTS

Experimental results were obtained from a total of 34 Long-Evans rats. The experiments were performed from March 18, 1960 to May 21, 1960. The average findings show that the osmotic pressure of the plasma of the control rats was 5.019. The average osmotic pressure of the plasma of the experimental rats was 4.820 on the first day after adrenalectomy; 3.670 on the third; 3.651 on the 5th, and 3.021 on the 7th. It was observed that there was only a small deviation of .041 between the osmotic pressure of the plasma of the experimental rats on the third and the 5th day after adrenalectomy. This deviation represented a loss of .0037 grams of sodium.

The results are recorded in Table 1 and Graph 1.
CHAPTER V

DISCUSSION

It was found that adrenalectomy caused reductions in the osmotic pressure of the plasma in Long-Evans rats and that these reductions depended upon the stage of adrenal insufficiency the animal was in. It was observed that there was only a small deviation of .041 between the osmotic pressure of the plasma of the experimental rats on the third and 5th day after adrenalectomy. The osmotic pressure changes of the plasma found in this investigation may be explained on the bases of several postulations which have been presented by other investigators. These are: stoppage of urine, circulatory disturbances and the gradual depletion of electrolytes.

These findings are in accord with the observations of Gaunt ('44) and Shipley ('45). They observed that adrenalectomized rats almost stopped urinating three to 4 days after adrenalectomy, thus only a fraction of the sodium was excreted by the kidneys. They further reported that after the leveling off of the urine in these animals, they began to urinate again and a sharp drop in the osmotic pressure was found. They found that this condition influenced the osmotic pressure of the plasma of the experimental rats on the 7th day. According to them, the lack of these animals to excrete water on the third and 4th day may have been due to a reduced emptying time of the stomach and to their inability to excrete the water that was absorbed. This idea is supported by the experiments of Gaunt ('44) and Shipley ('45). They observed that shortly after adrenalectomy animals in cortical insufficiency had delayed intestinal absorption and emptying time of the stomach. Similar conditions may have existed in the adrenalectomized rats in these experiments, thus influencing their osmotic pressure.
Harrison and Darrow ('37) demonstrated a definite relationship between cortical insufficiencies and circulatory inadequacies following bilateral adrenalectomy. Therefore, the changes found in the osmotic pressure of the plasma in these adrenalectomized animals may have been due to vascular abnormalities which resulted from an abnormal metabolism of electrolytes.

Muntwyler et al. ('40a) reported that under such conditions the loss of sodium and chloride ions and the retention of potassium ions contributed to a diminished blood volume and a fall in arterial pressure. They postulated that the permeability of the capillaries was increased, and a redistribution of water occurred throughout the body. It appears then that following adrenalectomy the loss of sodium ions causes the transfer of water into the intracellular spaces. Therefore, the changes in the osmotic pressure of the plasma in these adrenalectomized animals may have been due to fluid and electrolyte shifts from the blood and extracellular spaces into the body cells or intracellular spaces.

The variations found in the osmotic pressure of the plasma on different days following adrenalectomy may have been due to the fact that in the absence of the adrenal cortex the extracellular electrolytes were gradually depleted by renal elimination and partly by the gradual movement of sodium and chloride ions into the cells. This gradual unbalance in the distribution of ions on the cell membranes may have disturbed the osmotic equilibrium at different rates. As a result, the rate at which water shifted from the extracellular spaces and was taken up by the cells varied. The experiments and findings of Muntwyler et al. ('40) support this experimental evidence.

This investigation offers further evidence that following adrenalectomy alterations occur in the concentration of the electrolytes. These
alterations in electrolytes disturb the osmotic equilibrium, and, as a result, abnormal water shifts take place which disturb the normal osmotic pressure. This idea is supported by the experiments of Birnie, Eversole, Boss, Osborn and Gaunt ('50).
CHAPTER VI

SUMMARY AND CONCLUSIONS

1. This investigation was limited to the effects of bilateral adrenalectomy upon the osmotic pressure of the plasma.

2. The average osmotic pressure of the plasma of the control rats was found to be 5.019.

3. The average osmotic pressure of the plasma of the experimental rats was found to vary from 4.820 to 3.021.

4. The osmotic pressure of the plasma of the experimental rats varied according to the stage of adrenal insufficiency the animals were in.

5. A small deviation of .041 was found between the osmotic pressure of the plasma of the experimental rats on the third and 5th day after adrenalectomy.
LITERATURE CITED


Shipley, R. 1945 The causes of abnormal retention of ingested water in adrenalectomized rats. Endocrinology, 36: 118-123.


TABLE I

(Explaination of Table)
(Explanation of Table)

1. This is a table showing osmotic pressure changes of the plasma in control and adrenalectomized Long-Evans rats.
# TABLE 1

**Osmotic Pressure Changes of the Plasma in Control and Adrenalectomized Long-Evans Rats**

<table>
<thead>
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<th>GROUP I</th>
<th>Control of Control Rats</th>
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<td>1</td>
<td>5.059</td>
<td>4.820</td>
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| AVERAGE   |                         | 5.019 | 4.820 | 3.670 | 3.651 | 3.021 |
GRAPH I

(Explanation of Graph)
1. This is a graphic representation of the osmotic pressure of the plasma in the control rats as compared with the changes in the osmotic pressure of the plasma in adrenalectomized rats as recorded in Table 1.

Ordinate axis shows the osmotic pressure changes in the plasma after adrenalectomy. Abscissa axis shows the length of time in days after adrenalectomy.