other hand, with more extensive necrosis there is less uninjured tissue to absorb the additional potassium released from injured cells(1). More marked electrolyte changes therefore, seem to be related to the greater extent of the injury.

In rabbit No. 2124, there is an evident rise in sodium and a decrease in potassium concentration in injured tissue 6 hours after injury. After 24 hours, however, the damaged tissue shows values different from those in other experiments indicating that the analyzed tissue was limited to only a small number of injured cells, which can be confirmed in the histological sections. These experiments show the alterations in electrolyte distribution similar to the changes in electrolyte concentration in edema fluid subsequent to injury.

Finally, it is possible that the larger variations in electrolyte distribution in injured tissue might be influenced by the increased permeability of cell membranes in the adjacent, functionally damaged but morphologically intact, cells; further, varying degrees of re-establishment of circulation in injured areas and different levels of protein content in edema fluids, might accentuate or mask changes in local electrolyte concentrations.

Summary and conclusions. The redistribution of potassium and sodium in anterior tibial muscles of adult rabbits was investigated at different times after cold injury. The pathological changes were observed simultaneously by means of routine histological technics. Six hours after injury, an extensive necrosis in muscle tissue was observed with a simultaneous release of potassium from injured cells. The loss of potassium was progressive and parallel to the breakdown and subsequent disintegration of the cells during the 48-hour experiment. Conversely, the injured tissue progressively gained sodium despite the diminishing edema fluid. An increase of potassium and a decrease of sodium concentration in uninjured muscle tissues were observed. These events indicate the exchange of intracellular potassium for sodium in injured tissues. The variations in the electrolyte redistribution in injured tissues may be due to such factors as re-establishment of adequate circulation, protein content of edema fluid and ability of uninjured cells to absorb the additional potassium. The severity of the injury is apparently directly related to the electrolyte changes.

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Received March 13, 1952 P.S.E.B.M., 1952, v80.

Fluid and Electrolyte Shifts in the Hydrated Adrenalectomized Rat After Nephrectomy.* (19559)

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The normal diuresis which follows hydration is greatly impaired during adrenal insufficiency. This retention of water in the adrenalectomized rat is due in part to a decreased emptying time of the stomach and an impaired intestinal absorption of ingested water(1,2). Primarily, however, it is caused

* This investigation was supported in part by a grant from the Atomic Energy Commission.

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by an increased sensitivity of the renal tubules to an accumulating amount of an antidiuretic substance in the serum (references cited in 3). Frost and Talmage(2) have determined the sites of the retained fluid in hydrated adrenalectomized rats and correlated their observations with changes in electrolyte balance which occurred after adrenalectomy. They showed that the absorbed but unexcreted water was found to be located almost entirely in the cells. It was suggested that this tendency for water to shift to the intracellular space was caused by the loss of salt from the extracellular fluid during the previous 2-day period of adrenal insufficiency.

It was also observed that since adrenalectomized rats are unable to excrete rapidly large amounts of ingested water, more was retained for internal distribution in these animals than was retained in their controls. The present study was conducted in order to compare extrarenal fluid shifts in the hydrated control and adrenalectomized rat without the complication of renal excretion. This was accomplished by nephrectomizing the animals before hydration. An investigation of the internal partitioning of equivalent amounts of ingested water in the various experimental groups was thus made possible. An endeavor was also made to determine what extrarenal role, as suggested by other investigators(4,5), desoxycorticosterone acetate (DCA) treatment might have in altering the fluid shifts which occur in the adrenalectomized rat after hydration.

Materials and methods. Male rats weighing between 130-180 g were nephrectomized in 2 stages; the left kidney was removed 5 days before the experiment and the right one at least 2 hours before the experiment. The adrenalectomized rats also underwent a 2-stage operation; the left adrenal was removed at the same time as the left kidney, and the right adrenal was removed 2 days before the experiment. Both the control and adrenalectomized animals were starved approximately 22 hours before the experiment, and divided into non-hydrated and hydrated groups. In addition, part of the adrenalectomized hydrated rats received 2 mg of DCA subcutaneously and 1 mg intramuscularly when the last kidney was removed, and another 2 mg intramuscularly at the beginning of the experiment. Equivalent amounts of sesame oil were administered to the untreated hydrated animals. Total body water values were obtained by desiccation and the 2-hour sodium space, as measured with radiosodium[22], was used as an index of the extracellular fluid volume. The extra-sodium space, which afforded an index of the intracellular fluid volume, was obtained by subtraction. All fluid compartment values were made not only on the intact animal but also after the removal of the G-I tract in order to determine the location of the ingested water which had been absorbed. Serum sodium and potassium values were determined by means of a Beckman Flame Spectrophotometer according to the method described by Mosher *et al.*(6). Total sodium in the 2-hour sodium space was obtained by multiplying the serum sodium concentration by the sodium space. Details of the methods have been previously described(2).

Results. The fluid and electrolyte shifts, which occurred in the nephrectomized non-hydrated adrenalectomized rats, followed both quantitatively and qualitatively those changes which have been reported previously for non-nephrectomized animals(2). As is indicated in Tables I and II, the total body water of the non-hydrated animals did not change after adrenalectomy. The sodium space decreased and a significant shift of fluid to the cells was indicated by an increase in the extra-sodium space. Electrolyte changes in the non-hydrated animals are shown in Table III. The expected rise in serum potassium and decrease in serum sodium after adrenalectomy were observed. There was also a 10% loss of sodium from the 2-hour sodium space. This loss of extracellular sodium apparently caused the observed shift of fluid to the cells. Changes in the gutless non-hydrated rats paralleled those of the intact animals.

As is shown in Table IV the hydrated adrenalectomized animals withheld over 3 times as much of the ingested water in their G-I tracts as did their controls. This difference was due to both a decreased emptying time of the stomach and to an impaired intestinal absorption. Although it was not significantly established, DCA treatment tended to correct

TABLE I. Distribution of Water in Hydrated, Control and Adrenalectomized Rats after Nephrectomy.

		No.	Total body water, cc/100 g ± S.E.	Expected total body water, cc/100 g	Sodium space, cc/100 g ± S.E.	Water retained in sodium space, cc/100 g	Extra-sodium space, cc/100 g ± S.E.	Water retained in extra-sodium space, cc/100 g
Control rats	No water	11	70.9 ± .4	—	33.3 ± .7	—	37.6 ± .5	—
	Water	13	79.9 ± .4	80.3	37.8 ± .4	4.5	42.1 ± .4	4.5
Adrenalecto-mized rats	No water	11	70.8 ± .4	—	30.9 ± .4	—	39.9 ± .3	—
	Water	12	79.8 ± .2	80.1	35.5 ± .6	4.6	44.3 ± .6	4.4
	Water & DCA	12	80 ± .4	80.1	35.9 ± .6	5	44.1 ± .5	4.2

TABLE II. Distribution of Absorbed Water as Determined in Gutless Nephrectomized Rats.

		No.	Total body water, cc/100 g ± S.E.	Water absorbed from the G-I tract, cc/100 g	Sodium space, cc/100 g ± S.E.	Water retained in sodium space, cc/100 g	Extra-sodium space, cc/100 g ± S.E.	Water retained in extra-sodium space, cc/100 g
Control rats	No water	11	70.2 ± .4	—	32.6 ± .7	—	37.6 ± .5	—
	Water	13	78.4 ± .5	8.2	36 ± .5	3.4	42.4 ± .5	4.8
Adrenalecto-mized rats	No water	11	69.7 ± .5	—	29.6 ± .5	—	40.1 ± .6	—
	Water	12	74.5 ± .4	4.8	30.7 ± .6	1.1	43.8 ± .7	3.7
	Water & DCA	12	75.3 ± .7	5.6	32.1 ± .8	2.5	43.2 ± .4	3.1

TABLE III. Electrolyte Determinations in Control and Adrenalectomized Nephrectomized Rats.

		No.	Serum K, mEq/l ± S.E.	No.	Serum Na, mEq/l ± S.E.	No.	Total sodium in 2-hr sodium space, in mEq/100 g ± S.E. Intact	Gutless
Control rats	No water	11	3.95 ± .11	11	139 ± .7	11	4.63 ± .07	4.53 ± .10
	Water	13	4.72 ± .19	13	126 ± 1.3	13	4.77 ± .06	4.53 ± .07
Adrenalecto-mized rats	No water	11	6.53 ± .21	11	134.7 ± 1.1	11	4.15 ± .05	3.99 ± .06
	Water	9	6.83 ± .39	12	122.7 ± 1.1	12	4.36 ± .07	3.78 ± .06
	Water & DCA	9	5.69 ± .24	12	122.5 ± .7	12	4.39 ± .10	3.93 ± .10

this delayed intestinal absorption.

Distribution of the ingested water in animals with intact G-I tracts is indicated in Table I. 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 the animals regardless of experimental treatment. Water which is retained in the lumen of the G-I tract is still part of the sodium space. Since almost twice as much water was absorbed from the intestines of the controls as compared with the adrenalectomized animals, it would appear that the sodium space of the control group should have been proportionally smaller. The reason it was not smaller was because percentage-wise less of the absorbed water actually shifted to the extra-sodium space in the controls as compared with the adrenalectomized group. Therefore the lack of difference between the controls and adrenalectomized groups concerning the internal partitioning of ingested water was more apparent than real.

To determine the sites of absorbed water, it was therefore necessary to measure the fluid compartment values of the nephrectomized rats after the G-I tract had been removed (Table II). In the hydrated gutless

TABLE IV. Retention of Water in Hydrated, Control and Adrenalectomized Rats after Nephrectomy.

		No.	Total water retained in the animal, cc /100 g ± S.E.	Total water retained in the G-I tract, cc/ 100 g ± S.E.	Total water absorbed from the G-I tract, cc/ 100 g ± S.E.
Control rats	Water	13	9.4 ± .1	1.5 ± .2	7.9 ± .3
Adrenalecto-mized rats	Water	12	9.3 ± .1	4.9 ± .2	4.4 ± .2
	Water & DCA	12	9.3 ± .1	4.3 ± .5	5 ± .5

animals, the difference between the non-hydrated and hydrated total body water values afforded another index of the amount of water absorbed from the G-I tract. Within the limits of experimental error, the results are in good agreement with the values for total water absorbed which are given in Table I. It was also noticed by this method of determination that DCA again appeared to enhance the absorption of water from the G-I tracts of the adrenalectomized animals.

In all the experimental groups more of the absorbed water was found to be located in the extra-sodium space than in the sodium space. This was particularly true for the adrenalectomized rats in which over three-fourths of the absorbed water shifted to the cells. Less than 60% of the absorbed water shifted to the extra-sodium space of the controls. DCA treatment appeared to prevent this increased shift of fluid to the cells in the hydrated adrenalectomized animals, for the percentage distribution of absorbed water between the sodium and extra-sodium space was returned essentially to normal. Additional experiments, however, would be necessary to confirm this hormonal action.

The changes in electrolyte balance during the 2-day period of adrenal insufficiency suggest the reason for the fluid shifts observed in the hydrated adrenalectomized rats (Table III). As would be expected, hydration after nephrectomy did not significantly alter the amount of total sodium in the 2-hour sodium space of either the control or experimental animals. However, the low serum sodium values of the adrenalectomized rats were reduced still further by dilution after hydration. More of the absorbed water shifted to the cells of these animals than it did in the controls probably because the loss of salt from the extracellular fluid during the 2-day period of adrenal insufficiency resulted in a higher osmotic gradient.

Thus it is apparent that the fluid and electrolyte shifts which occurred in both the control and adrenalectomized hydrated nephrectomized rats are in agreement with the results which were reported earlier for non-nephrectomized animals(2).

Although changes in serum potassium were followed in these investigations merely as an index of adrenal insufficiency, several unexpected observations were made during the course of the experiments. Serum potassium unlike sodium was not diluted upon hydration and in fact, under some experimental conditions it actually increased. Also, there was some indication that adrenal insufficiency tended to exaggerate this phenomenon.

Possibly hydration may act in the same way as other stressing agents by inducing a general systemic release of intracellular potassium. If, as has been suggested(7), the release of potassium during the shock phase of the alarm reaction is conditioned in part by a state of relative cortical insufficiency, one would expect that these changes in serum potassium would be exaggerated after adrenalectomy.

Summary. Normal and 2-day adrenalectomized rats were nephrectomized and subsequently hydrated, thus making it possible to study the internal partitioning of relatively large amounts of ingested water. There was a delayed intestinal absorption of water in the adrenalectomized animals. A larger percentage of absorbed water shifted to the cells of these animals than was observed in the controls apparently as a result of salt depletion during the previous 2-day period of adrenal insufficiency. Although there was a tendency for desoxycorticosterone acetate therapy to enhance intestinal absorption and also to pre-

vent the excessive intracellular hydration of the adrenalectomized animals, these extrarenal hormonal actions were not significantly established. Alterations in potassium metabolism were also briefly discussed.

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Received May 6, 1952. P.S.E.B.M., 1952, v80.

Cortisone and Action of Exogenous Thyrotrophin in Hypophysectomized Rats.* (19560)

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Several workers have reported a depression of thyroid function by cortisone in man and in the rat(1-8). A block of the thyrotrophic function of the pituitary was suggested as a possible explanation of this phenomenon (5,6). Perry(4), however, presented data which are not in agreement with this concept and we made observations(9) which appeared to indicate that pituitary thyrotrophin (TSH) output is enhanced rather than depressed by cortisone. Woodbury *et al.*(10), on the other hand, described a striking modification of the action of exogenous TSH in hypophysectomized rats by cortisone. They found that the TSH induced increase in the thyroid I^{131} uptake of their animals was partly prevented by simultaneously administered cortisone. Since these findings do not necessarily indicate an interference of cortisone with TSH action *per se,* the present study was undertaken to assess the influence of the steroid on the effectiveness of exogenous TSH in hypophysectomized rats, using the change in thyroid cell height as the index of thyrotrophic stimulation. This technic has been used previously by Griesbach and Purves(11) for assay of TSH and recent observations by Ghosh *et al.*(12) have also shown a roughly rectilinear relationship between thyroid cell height increment and log dose of TSH administered to hypophysectomized rats.

Method. Hypophysectomized male rats weighing between 130-145 g were purchased from the Hormone Assay Laboratories, Chicago. Twelve days after the operation they were divided into 4 groups. Group I (14 rats) received no treatment, group II (15 rats) was injected with 5 mg of cortisone† daily for 10 days, group III (14 rats) received daily injections of .3 mg TSH‡ for 10 days, while group IV (16 rats) was treated for the same length of time with both cortisone and TSH in the aforementioned dosages. The rats were fed Purina Laboratory Chow supplemented with horse meat and fresh oranges. All treated animals, as well as the controls, were sacrificed 24 hours after the last injection. The thyroids were weighed on a torsion balance, fixed in SUSA, imbedded in paraffin and cut at 5 μ. Sections running through the center of the gland were stained with Gomori's trichrome method. The slides were projected and the height of one cell per follicle was measured in 50 follicles picked at random from each gland.

* Supported by a gift from the American Cancer Society and a grant from the Central Scientific Fund, State University of Iowa College of Medicine.

† Cortisone (Cortone acetate Merck) was generously supplied by Dr. Elmer Alpert of Merck and Co., Rahway, N. J.

‡ TSH (Parke Davis #50P4) was made available through the courtesy of Dr. D. A. McGinty and Mr. W. Donaldson of Parke, Davis & Co., Detroit, Mich.