# On the Role of Antidiuretic Hormone in the Inhibition of Acute Water Diuresis in Adrenal Insufficiency and the Effects of Gluco- and Mineralocorticoids in Reversing the Inhibition

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ABSTRACT In order to determine whether or not antidiuretic hormone (ADH) is essential to the inhibition of an acute water diuresis in adrenal insufficiency, the response to oral water loads was tested in rats with hereditary hypothalamic diabetes insipidus (DI) which lack ADH. It was found that 60 min after water loads of 3 or 5% of body weight urine flow was significantly lower and urine osmolality significantly higher in adrenalectomized DI rats than in the same DI rats before removal of their adrenal glands.

The efficacy of gluco- and mineralocorticoids in reversing the inhibition was then determined in the same adrenalectomized DI rats. Prednisolone alone, administered either acutely or chronically, restored the response in urine flow to that seen in the same rats before adrenalectomy, but failed to correct the defect in urinary dilution. Aldosterone when given alone tended to correct the diluting ability but not the response in urine flow. When these two adrenal cortical hormones were given simultaneously, both the urine flow and urine osmolality were nearly identical to what they had been in the same DI rats before adrenalectomy.

These studies strongly suggest (*a*) that ADH is not essential to the inhibition of an acute water diuresis in adrenal insufficiency, although it may abet the inhibition in individuals without diabetes insipidus, which can elaborate ADH; and (*b*) that both gluco- and mineralocorticoids are required in adrenal insufficiency in order to fully restore the water diuresis as judged by the dual criteria of urine flow and urine osmolality.

## INTRODUCTION

The mechanism by which an acute water diuresis is inhibited in adrenal insufficiency has been the subject of intensive investigation and debate. (For a list of references, see reference 1.) Although changes such as decreased extracellular fluid volume or decreased glomerular filtration rate (GFR) may contribute to the inhibition, it seems clear that these are not the only, or even the major, factors involved. The main current controversy centers about the role of the antidiuretic hormone (vasopressin or ADH). One view states that in adrenal insufficiency water diuresis is inhibited by sustained high blood concentrations of ADH because in the absence of adrenal corticoids, secretion of ADH from the posterior pituitary gland cannot be shut off (2). Proponents of the second view contend that ADH has little to do with the inhibited water diuresis, but rather, that the adrenal steroids alter the water permeability of the distal renal tubules and collecting ducts (3). Both views have been supported by results of bioassays, the first showing sustained, high concentration of ADH in the blood of patients with adrenal insufficiency even after an acute water load (2), the second showing undetectable blood levels of ADH under similar circumstances (3).

We saw the opportunity of possibly clarifying this controversy, without resorting to bioassays, by using rats with hereditary hypothalamic diabetes insipidus (DI). These animals have a defect for elaborating biologically active ADH (4). Hence, if water diuresis were inhibited also in these rats after they had been adrenalectomized, then ADH could not be essential to the inhibition. This is, in fact, what we have found.

It is generally believed that treatment with glucocorticoids alone suffices to fully restore the water diuresis in individuals with adrenal insufficiency (1–3). In most reported studies, this impression appears to be based on improvement rather than full correction of the diuretic response, mainly in patients, as compared to that which existed during adrenal insufficiency. In the present study in rats, we were able to observe the diuretic response to identical water loads in the same animals before adrenalectomy, after adrenalectomy, and after treatment with adrenal steroids. Our results in adrenalectomized DI rats suggest that while glucocorticoids alone can restore the response in urinary flow, the simultaneous addition of mineralocorticoids is required to reestablish the diluting ability.

## METHODS

Four groups of rats were tested. Group 1 consisted of six DI rats of the Brattleboro strain, and six normal Long-Evans hooded rats of the same strain (4). This group represented a pilot experiment. Normal rats were included to show that previously reported results could be reproduced by us. Group 2, summarized in Table I, consisted of 17 female and 17 male DI rats. This group was run in order to substantiate the results of the pilot study. Group 3, which is summarized in Table II, included 11 female and 13 male DI rats and was used to explore the efficacy of combinations of prednisolone and aldosterone at various doses in correcting the blunted water diuresis in adrenal

insufficiency. Group 4, summarized in Table III, consisted of 17 female DI rats and was run to determine the effect of aldosterone treatment alone.

Before being adrenalectomized, all rats except those of group 4 were maintained on Purina Labena rat pellets (Ralston Purina Co., St. Louis, Mo.) and tap water. After adrenalectomy, all animals received in addition to the rat pellets a choice of tap water and 0.9% NaCl as drinking solutions. Group 4 had the choice of these two drinking solutions even before adrenalectomy. At about 8 a.m. on the morning of each experiment, each rat received a subcutaneous injection of prednisolone and/or aldosterone, or a control injection of saline. 2 hr later each rat was given an oral load of tepid tap water amounting to either 3% (groups 1 and 2) or 5% (groups 3 and 4) of its body weight. Immediately after the water load each animal was placed in an individual metabolism cage (Acme Metal Products, Chicago, Ill.). In group 1 urine was then collected every 30 min by abdominal massage and lifting the tail, for a total of five collection periods. In groups 2, 3, and 4 a single urine specimen was collected 60 min after giving the water load. The rats did not have access to food or water while they were in the metabolism cages. The animals were acclimatized to the experimental procedure by administering water loads, placing them in metabolism cages, and collecting urine for at least three times during a period of 2 wk before the "intact" experiments.

Rats were adrenalectomized under ether anesthesia using a midline, dorsal skin incision. All animals were then maintained on a choice of tap water and 0.9% NaCl for at least 1 wk before they were retested in a manner identical to the one described above. The sequence of the experiments as well as the dosages of hormones used have been summarized in Tables I, II, and III. Within any one group, all experimental conditions were imposed on the same rats.

During the 24-hr collection periods conducted in groups 2 and 4, rats ate powdered Purina Labena chow, and their intakes of tap water and 0.9% NaCl were measured separately. Urine was collected under mineral oil.

Blood was taken from tail veins. Urine and serum osmolalities were determined by freezing point depression (Osmette, Precision Systems, Co., Inc., Boston, Mass., or Clifton nanoliter osmometer, Clifton Technical Physics, New York), sodium and potassium by flame photometry

TABLE I

*Group 2. Rats with Diabetes Insipidus. Values during the 60 min after an Oral Water Load Equal to 3% of Body Weight*

| Date | Condition | Sex | No. of rats | Body weight | Urine flow | | $U_{Osm}$ | $C_{Osm}$ | $C_{H_2O}$ | $C_{H_2O}/C_{Osm}$ |
|------|-----------|-----|-------------|-------------|------------|---|-----------|-----------|------------|--------------------|
| | | | | *g* | *ml/100 g body wt per hr* | *% of $H_2O$ load per hr* | *mOsm/kg* | *ml/100 g body wt per hr* | | |
| 6/13 | Intact | F | 17 | 180 ±6.0 | 2.4 ±0.13* | 80 ±4.5 | 82 ±3.6 | 0.65 ±0.035 | 1.77 ±0.110 | 2.78 ±0.145 |
| | | M | 17 | 279 ±8.9 | 1.8 ±0.13 | 59 ±4.4 | 89 ±2.8 | 0.52 ±0.041 | 1.25 ±0.093 | 2.44 ±0.118 |
| 7/5 | 17 days after adrenalectomy | F | 15 | 192 ±5.7 | 1.3 ±0.21‡ | 42 ±6.9‡ | 211 ±14.2‡ | 0.86 ±0.144 | 0.40 ±0.115‡ | 0.54 ±0.146‡ |
| | | M | 13 | 271 ±11.1 | 1.4 ±0.24 | 46 ±8.1 | 202 ±17.6‡ | 0.91 ±0.163‡ | 0.47 ±0.120‡ | 0.69 ±0.198‡ |
| 7/12 | Adx§ plus 1 mg of Pred sc 2 hr before $H_2O$ load | F | 15 | 191 ±5.2 | 3.0 ±0.21‡ | 97 ±6.4‡ | 154 ±6.9‡ | 1.53 ±0.096‡ | 1.51 ±0.147 | 1.01 ±0.095‡ |
| | | M | 12 | 274 ±11.7 | 2.2 ±0.34 | 73 ±11.4 | 196 ±19.3‡ | 1.27 ±0.159‡ | 0.92 ±0.260 | 0.74 ±0.208‡ |

All conditions were imposed on the same rats.
* Mean ±SEM.
‡ $P < 0.05$ when compared with intact rats of the same sex.
§ Abbreviations: sc = subcutaneously; Adx = adrenalectomized; Pred = prednisolone sodium succinate.

## TABLE II

*Group 3. Rats with Diabetes Insipidus. Values during the 60 min after an Oral Water Load Equal to 5% of Body Weight*

| Date | Condition | Sex | No. of rats | Body weight | Urine flow | | $U_{Osm}$ | $C_{Osm}$ | $C_{H_2O}$ | $C_{H_2O}/C_{Osm}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | g | ml/100 g body wt per hr | % of H₂O load per hr | mOsm/kg | ml/100 g body wt per hr | | |
| 9/23 | Intact | F | 11 | 178 ±3.4 | 4.0 ±0.43* | 80 ±8.6 | 93 ±4.3 | 1.25 ±0.158 | 2.77 ±0.286 | 2.30 ±0.153 |
| | | M | 13 | 264 ±7.3 | 2.5 ±0.40 | 51 ±7.9 | 107 ±3.4 | 0.89 ±0.128 | 1.64 ±0.272 | 1.83 ±0.093 |
| 10/12 | 7 days after adrenalectomy | F | 10 | 184 ±4.8 | 2.5 ±0.18‡ | 50 ±3.5‡ | 149 ±10.6‡ | 1.25 ±0.138 | 1.25 ±0.110‡ | 1.12 ±0.159‡ |
| | | M | 8 | 257 ±7.5 | 1.0 ±0.15‡ | 20 ±2.9‡ | 145 ±7.2‡ | 0.48 ±0.069‡ | 0.52 ±0.082‡ | 1.10 ±0.092‡ |
| 10/13 | Adx§ plus 0.1 mg of Pred sc 2 hr before H₂O load | F | 10 | 181 ±4.7 | 4.2 ±0.23 | 84 ±4.6 | 135 ±10.5‡ | 1.90 ±0.181‡ | 2.28 ±0.187 | 1.34 ±0.178‡ |
| | | M | 8 | 256 ±7.2 | 1.8 ±0.22 | 37 ±4.3 | 137 ±13.8‡ | 0.78 ±0.072 | 1.03 ±0.176 | 1.36 ±0.271‡ |
| 10/18 | Adx plus 0.1 mg of Pred daily × 6 days | F | 10 | 188 ±4.5 | 4.0 ±0.35 | 80 ±7.0 | 150 ±14.9‡ | 2.00 ±0.269‡ | 1.99 ±0.266 | 1.16 ±0.186‡ |
| | | M | 9 | 252 ±8.0 | 2.0 ±0.33 | 41 ±6.5 | 151 ±11.3‡ | 1.02 ±0.179 | 1.01 ±0.184 | 1.08 ±0.150‡ |
| 10/21 | Adx plus 0.1 mg of Pred daily × 9 days; 4 µg of Aldo 2 hr before H₂O load | F | 10 | 188 ±2.5‡ | 4.1 ±0.34 | 82 ±6.6 | 136 ±10.0‡ | 1.89 ±0.235‡ | 2.22 ±0.210 | 1.31 ±0.153‡ |
| | | M | 9 | 268 ±10.4 | 2.5 ±0.40 | 51 ±8.0 | 140 ±11.2‡ | 1.20 ±0.215 | 1.32 ±0.234 | 1.26 ±0.179‡ |
| 10/25 | Adx plus 0.1 mg of Pred daily × 13 days; 16 µg of Aldo daily × 5 days | F | 10 | 189 ±2.5‡ | 4.2 ±0.32 | 85 ±6.3 | 113 ±8.0‡ | 1.58 ±0.136 | 2.64 ±0.255 | 1.79 ±0.211 |
| 10/28 | Adx plus 0.1 mg of Pred daily × 16 days; 25 µg of Aldo daily × 3 more days | F | 9 | 192 ±2.4‡ | 4.4 ±0.51 | 87 ±10.1 | 112 ±6.4‡ | 1.62 ±0.192 | 2.73 ±0.346 | 1.75 ±0.157‡ |
| 11/4 | Adx plus 0.1 mg of Pred daily × 22 days; no Aldo × 7 days | F | 10 | 190 ±1.1‡ | 5.0 ±0.40 | 99 ±7.9 | 133 ±13.6‡ | 2.15 ±0.225‡ | 2.81 ±0.353 | 1.43 ±0.200‡ |
| | | M | 9 | 289 ±10.0 | 2.9 ±0.27 | 59 ±5.3 | 170 ±33.1‡ | 1.46 ±0.159‡ | 1.47 ±0.324 | 1.24 ±0.353 |
| 11/10 | Adx; no Pred × 6 days; no Aldo × 13 days | F | 10 | 200 ±2.4‡ | 3.5 ±0.30 | 71 ±6.1 | 146 ±17.7‡ | 1.68 ±0.226 | 1.85 ±0.299‡ | 1.30 ±0.230‡ |
| | | M | 9 | 298 ±10.4‡ | 1.9 ±0.25 | 37 ±5.1 | 184 ±38.9‡ | 1.01 ±0.189 | 0.84 ±0.222‡ | 1.07 ±0.288‡ |
| 11/18 | Adx; no Pred × 14 days; no Aldo × 21 days | F | 9 | 209 ±3.1‡ | 2.7 ±0.38‡ | 54 ±7.7‡ | 151 ±28.0‡ | 1.20 ±0.148 | 1.53 ±0.366‡ | 1.41 ±0.292‡ |
| 11/21 | Adx plus 23 µg of Aldo 2 hr before H₂O load | F | 9 | 205 ±3.0‡ | 3.0 ±0.38 | 60 ±7.6 | 119 ±13.6 | 1.13 ±0.395 | 1.87 ±0.345 | 1.76 ±0.282 |

All conditions were imposed on the same rats.

* Mean ±SEM.

‡ $P < 0.05$ when compared with intact rats of the same sex.

§ Abbreviations: sc = subcutaneously; Adx = adrenalectomized; Pred = prednisolone sodium succinate; Aldo = aldosterone.

(Instrumentation Laboratory Inc., Lexington, Mass.), and serum urea by the microdiffusion method of Conway (5).

Osmolal and free water clearances ($C_{osm}$ and $C_{H_2O}$, respectively) were calculated using a value for plasma osmolality of 300 mOsm/kg. Although we might have used the measured values (see below), a difference of even 30 mOsm/kg in the plasma value causes so little change in the calculation of these clearances that slightly greater accuracy was sacrificed for the sake of simplicity.

The following medications were used: prednisolone sodium succinate (Meticortelone Soluble, Schering Corp., Bloomfield, N. J.); and aldosterone (4-Pregnen-18-al-11B,21-Diol-3,20-Dione, Mann Research Labs., Inc., New York). The prednisolone was put into solution on the morning of each experiment while the aldosterone was prepared the night before.

All results were evaluated statistically by the methods for unpaired as well as for paired groups of equal variance, using the statistical computer programs of Dartmouth College.[1] There were no substantive differences in the results of the two types of analyses. Since the method for unpaired

TABLE III

*Group 4. Rats with Diabetes Insipidus. Values during the 60 min after an Oral Water Load Equal to 5% of Body Weight*

| Date | Condition | Sex | No. of rats | Body weight | Urine flow | | $U_{Osm}$ | $C_{Osm}$ | $C_{H_2O}$ | $C_{H_2O}/C_{Osm}$ |
|------|-----------|-----|-------------|-------------|------------|---|-----------|-----------|------------|---------------------|
| | | | | $g$ | $ml/100\ g$ $body\ wt$ $per\ hr$ | $\%\ of\ H_2O$ $load\ per\ hr$ | $mOsm/kg$ | $ml/100\ g\ body\ wt\ per\ hr$ | | |
| 2/11 | Intact | F | 17 | 155 ±5.0 | 3.9 ±0.30* | 78 ±5.9 | 135 ±7.7 | 1.77 ±0.178 | 2.10 ±0.186 | 1.34 ±0.129 |
| 3/5 | 8 days after adrenalectomy | F | 14 | 174 ±5.5 | 1.9 ±0.29‡ | 38 ±5.8‡ | 128 ±12.6 | 0.74 ±0.076‡ | 1.28 ±0.223‡ | 1.63 ±0.230 |
| 3/7 | Adx§ plus 25 µg of Aldo sc 2 hr before H₂O load | F | 14 | 176 ±5.6 | 2.5 ±0.31‡ | 50 ±6.1‡ | 117 ±9.6 | 0.90 ±0.076‡ | 1.62 ±0.260 | 2.00 ±0.213‡ |

All conditions were imposed on the same rats.
* Mean ±SEM.
‡ $P$ < 0.05 when compared with intact rats.
§ Abbreviations: sc = subcutaneously; Adx = adrenalectomized; Aldo = aldosterone.

groups permits inclusion of more animals, the results of this analysis have been portrayed in the tables and figures.

## RESULTS

*Inhibition of acute water diuresis in adrenalectomized normal and DI rats.* Fig. 1 a shows the course of an acute water diuresis in six normal rats before and after adrenalectomy. Within 150 min after receiving an oral water load amounting to 3% of body weight, the animals excreted 100% of the administered water. Their urine osmolality, which before the test averaged 1878 mOsm/kg, fell below that of plasma reaching a mean low of 145 mOsm/kg 60 min after the water load. 1 wk after bilateral adrenalectomy, the same rats excreted only 30% of an identical load after 150 min, and their urine remained hypertonic to plasma.

Fig. 1 b shows the results of a similar test, run simultaneously, in six DI rats. In the intact state, they excreted 194% of the water load after 150 min, and their urine osmolality, which averaged 131 mOsm/kg before the test, remained hypotonic throughout the collection period. 1 wk after bilateral adrenalectomy, the same DI rats, like the normals, showed a marked inhibition of the water diuresis. They now excreted only 79% of the administered load, and their urine became hypertonic to plasma during at least the last three collection periods. This important finding, that an acute water diuresis is inhibited in the absence of biologically active ADH, was substantiated in an additional 75 DI rats of both sexes (Tables I, II, and III). In the groups illustrated in these tables, a single urine specimen was collected 60 min after the water load.[2] Before being adrenalectomized,
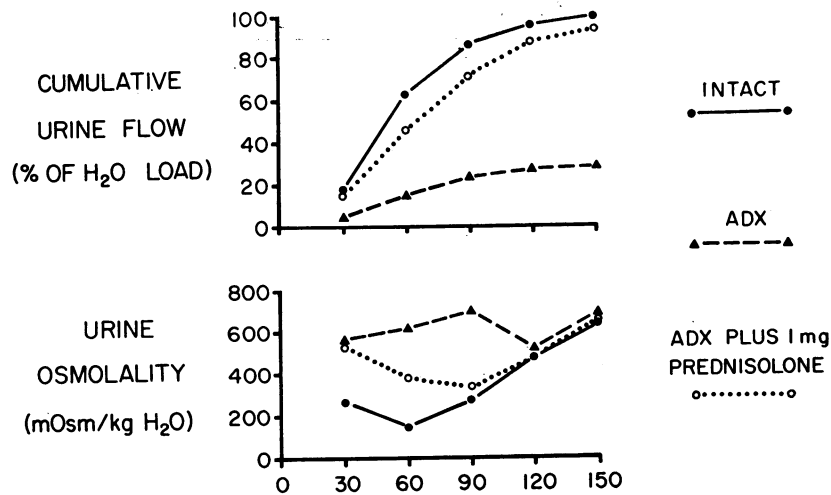
[2] It was apparent from the pilot experiment on group 1 that intact DI rats excreted about 100% of the administered water load after 60 min. In order to prevent dehydration which in itself can have an antidiuretic effect in DI rats (4), subsequent experiments were terminated after 60 min, and in groups 3 and 4 the water load was increased from 3 to 5% of body weight.

these 75 animals excreted 69.7 ±2.97% (mean ±SEM) of the load after 1 hr; the 60 rats which remained healthy after adrenalectomy excreted only 40.9 ±3.01% of an identical load ($P$ < 0.001). Corresponding urine osmolalities in these animals were 102 ±3.1 mOsm/kg before and 171 ±7.6 after adrenalectomy ($P$ < 0.001).

*Effect of glucocorticoid alone.* The efficacy of a glucocorticoid, prednisolone, in restoring the water diuresis in adrenalectomized rats is shown in Fig. 1 a and b. A single, subcutaneous injection of 1 mg of prednisolone given 2 hr before the water load very nearly returned the urine flow to the levels observed in the same normal and DI rats before removal of their adrenal glands, but it only partially restored the ability to dilute the urine. Again, this fact was confirmed in more DI rats. Neither in 27 DI rats of group 2 receiving 1 mg of prednisolone (Table I), nor in 18 DI rats of group 3 receiving what is considered to be a more physiological dose of 0.1 mg of prednisolone (Table II) was the diluting ability restored.

The data pertaining to this point have been summarized for all DI rats of groups 2 and 3 in Fig. 2. Prednisolone completely reversed the significant reduction in urine flow which was demonstrable in the same rats after adrenalectomy. But the urine osmolality, although it was decreased somewhat, remained significantly higher than it had been in the same animals before adrenalectomy. The three lower graphs in Fig. 2 show that while prednisolone increased the free water clearance, it simultaneously increased the osmolal clearance, so that the ratio of free water to osmolal clearances did not rise significantly over that seen in the adrenalectomized state. In the females of groups 2 and 3 (Tables I and II), prednisolone raised the osmolal clearance above that seen in the intact state, especially the higher dose of prednisolone used in group 2. That the concomitant inability to dilute the urine was not due to an over-

(a)

CUMULATIVE
URINE FLOW
(% OF $H_2O$ LOAD)

URINE
OSMOLALITY
(mOsm/kg $H_2O$)

INTACT

ADX

ADX PLUS 1 mg
PREDNISOLONE

(b)

CUMULATIVE
URINE FLOW
(% OF $H_2O$ LOAD)

URINE
OSMOLALITY
(mOsm/kg $H_2O$)

INTACT

ADX

ADX PLUS 1 mg
PREDNISOLONE

MINUTES AFTER WATER LOAD
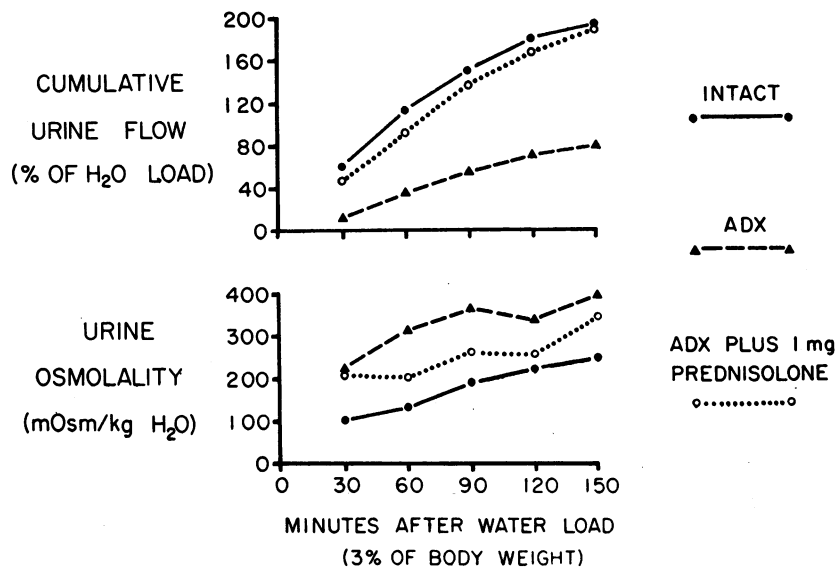(3% OF BODY WEIGHT)

FIGURE 1 Diuretic responses after water loads in six normal rats (a) and six DI rats (b). The same animals were tested in the intact state, after adrenalectomy, and then after a single injection of prednisolone. Tepid tap water amounting to 3% of body weight was given through a stomach tube at zero time. Since the diuretic response was inhibited in adrenalectomized DI rats, ADH cannot be essential to this inhibition. Treatment of adrenalectomized animals with prednisolone largely corrected the defect in urinary flow but not in urinary dilution. Each point represents the mean of five females and one male in each group. Note that the ordinate scales vary.

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whelming solute excretion is suggested by the males of group 3. As is shown in Table II, prednisolone in these males failed to return urine osmolality to that seen before adrenalectomy even though osmolal clearance remained lower than that seen in the intact state.

The effect of prolonged, daily treatment with prednisolone in both females and males of group 3 is depicted in Fig. 3. There clearly was no progressive rise in urine flow nor progressive fall in urine osmolality as treatment was prolonged from 1 to 6 to 22 days. Osmolal clearance did rise progressively, becoming significantly higher than that of the intact state after 6 and 22 days of treatment. But this was not accompanied by a progressive or disproportionate rise in free water clearance; hence the ratio of the two clearances was not corrected during prolonged treatment. Thus, while treatment of adrenalectomized DI rats with glucocorticoid alone appears to fully correct the deficiency of urine flow after an acute water load, it only partially corrects the defect in urinary dilution.

*Effect of glucocorticoid plus mineralocorticoid.* There is evidence that sodium reabsorption from the loops of Henle may be stimulated by aldosterone (16, 17). Since this is the area of the nephron in which free water is generated and hence in which free water clearance might be altered during water diuresis, we tested the efficacy of adding aldosterone as well as prednisolone in correcting the diluting ability of adrenalectomized DI rats.

Fig. 4 shows the effect of daily, subcutaneous injections of 0.1 mg of prednisolone to which daily injections of aldosterone in increasing doses have been added. All the data were obtained from the same female DI rats of group 3 (Table II). The addition of 4 $\mu$g of aldosterone resulted in a slight reduction of osmolal clearance over the value seen after 6 days of prednisolone only (Table II, 10/18), and a very slight rise in free water clearance. Hence, there was a small but insignificant reduction in the urine osmolality. Progressively increasing the dose of aldosterone from 4 to 25 $\mu$g daily during the ensuing 8 days resulted in further restoration of diluting ability. This occurred mainly because of a decrease in osmolal clearance and a further rise in free water clearance. Although this combined treatment with gluco- and mineralocorticoids restored free water clearance to that seen before adrenalectomy, slight though insignificant elevation of the osmolal clearances apparently prevented reduction of urine osmolality to that of the intact state.

The dose range of aldosterone over which increasing effects were seen in this experiment is considerably higher than what was required for maximal reduction of urinary sodium excretion in adrenalectomized rats of another study (6). Although a number of speculative
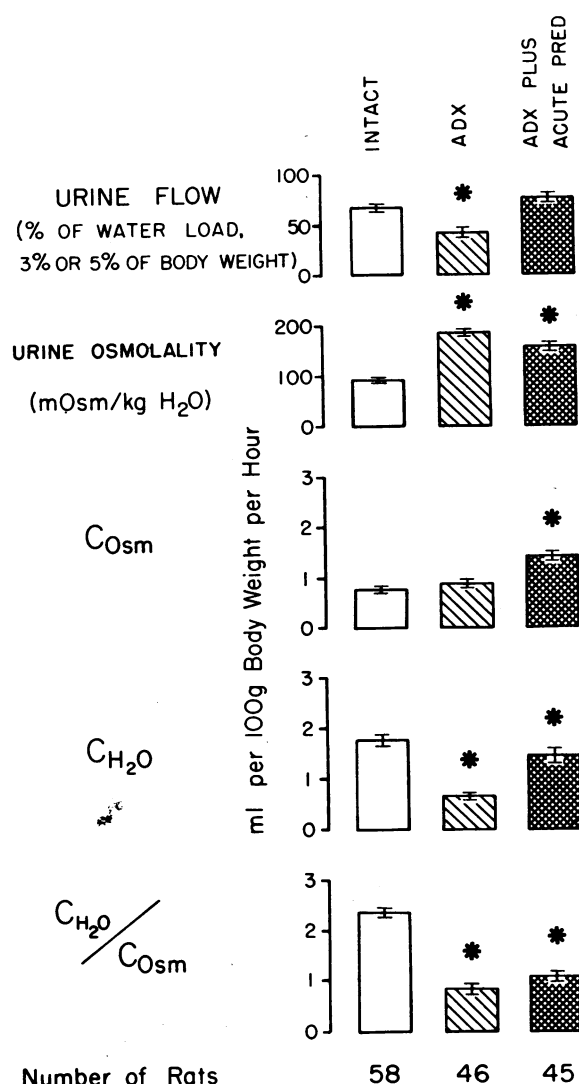


FIGURE 2 Restorative effect of acute glucocorticoid alone. Response in urine flow, urine osmolality, and osmolal and free water clearances during the 60 min after an oral water load. The data from females and males of groups 2 and 3 have been combined: intact, 6/13 and 9/23; adrenalectomized, 7/5 and 10/12; Adx plus prednisolone, 7/12 and 10/13. The columns and brackets represent the mean ±SEM. Asterisks indicate values that are significantly different from those of the intact state ($P < 0.05$).

interpretations might be offered, we have no conclusive explanation for this difference.

*Effect of mineralocorticoid alone.* Since, as shown above, aldosterone enhances the diluting ability over that seen with prednisolone alone, it seemed pertinent to ascertain the effect of aldosterone replacement alone. The results of such experiments in females of groups 3 and 4 are shown in Fig. 5. It is clear from this graph that when aldosterone alone is given to adrenalectomized
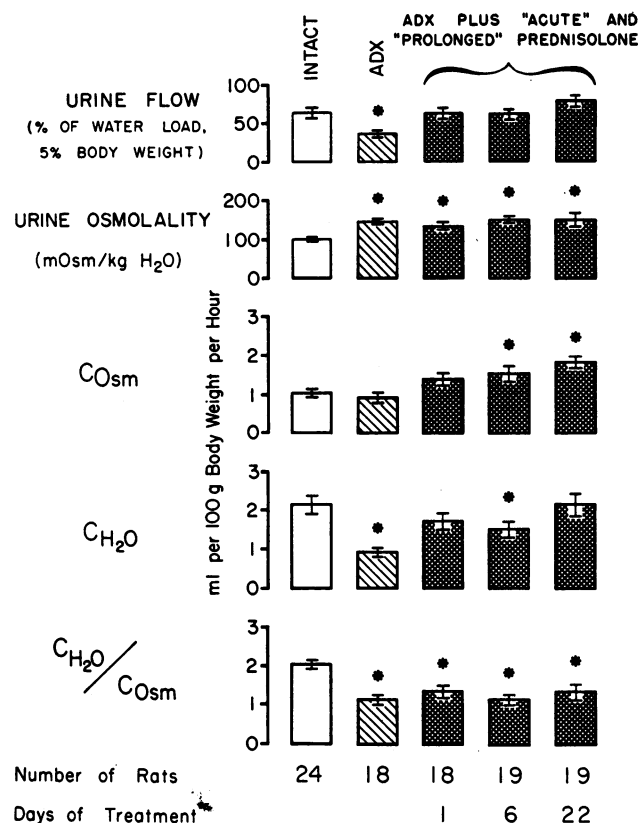
FIGURE 3 Restorative effect of prolonged glucocorticoid alone. Responses during the 60 min after an oral water load. The data from females and males of group 3 have been combined: intact, 9/23; adrenalectomized, 10/12; Adx plus prednisolone for 1 day, 10/13; Adx plus prednisolone for 6 days, 10/18; Adx plus prednisolone for 22 days, 11/4. The meaning of columns, brackets, and asterisks is the same as in Fig. 2.

animals their urine osmolality is restored to that of the intact state, but not their response in urine flow. Stolte, Brecht, Wiederholt, and Hierholzer (1) also found that aldosterone alone could not correct the urine flow of adrenalectomized rats. Phrased differently, aldosterone alone increases the free water clearance after an acute water load in adrenalectomized rats but it does not simultaneously raise the osmolal clearance. Only the combination of prednisolone, which increases the osmolal clearance, and of aldosterone, which enhances the free water clearance per unit of osmolal clearance, appears to correct the blunted water diuresis.

The urine osmolality and osmolal clearances after a water load in the intact state were unusually high in group 4 (Table III) as compared to all other groups. (This finding may be related to rats of group 4 having had access to salt water even before adrenalectomy.) Consequently the changes in urine osmolality and $C_{H_2O}/C_{Osm}$

which are depicted in Fig. 5, representing combined data from groups 3 and 4, are not statistically significant. Nevertheless, it is to be noted that the alterations which we have attributed to aldosterone alone were qualitatively identical in both groups and they had statistical significance in group 3 (Table II).

*Evidence that the adrenalectomized rats remained healthy.* It is frequently argued that animals may be so ill after bilateral adrenalectomy that their failure to respond normally to a water load reflects nonspecific changes. A number of data argue against such changes having been present in our series. A few rats did die, either during the operation or shortly after being adrenalectomized. Such sick animals were invariably identified because they looked and acted poorly and because they were noted to lose weight. Since each animal was weighed in order to determine its exact water load, a loss of weight could be detected and the rat ex-

URINE FLOW
(% OF WATER LOAD,
5% BODY WEIGHT)

URINE OSMOLALITY
(mOsm/kg $H_2O$)

$C_{Osm}$

$CH_2O$

$CH_2O / C_{Osm}$

ml per 100g Body Weight per Hour

Number of Rats 11 10 10 10 9
Date 9/23 10/12 10/21 10/25 10/28

INTACT
ADX
ADX PLUS PRED·9d PLUS 4μg ALDO·1d
ADX PLUS PRED·13d PLUS 16μg ALDO·5d
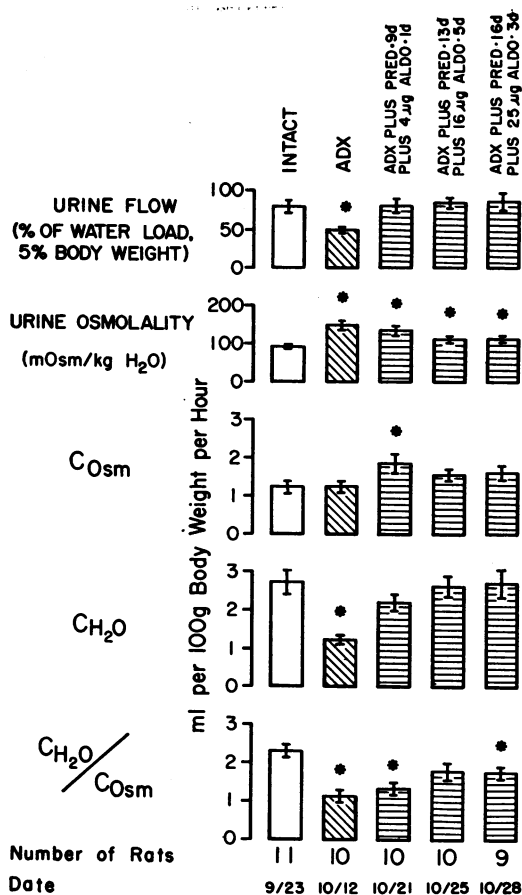ADX PLUS PRED·16d PLUS 25μg ALDO·3d

FIGURE 4 Restorative effect of simultaneous replacement with glucocorticoid and mineralocorticoid. Responses during the 60 min after an oral water load. The data are from the females of group 3; d stands for day. Between 10/21 and 10/28, the daily amount of aldosterone was given in two divided doses. The meaning of columns, brackets, and asterisks is the same as in Fig. 2.

cluded from the series before running an experiment on it. Thus no rat was excluded in retrospect.[3] It is noteworthy that the animals which were used continued to gain weight throughout the course of this study, even after their adrenal glands had been removed (Tables I, II, and III).

Serum osmolalities were determined on the rats of group 4. Before adrenalectomy, their osmolality was 308 ±5.1 mOsm/kg (mean ±SEM) without a water load and 300 ±2.3 mOsm/kg 30 min after the water load. 8 days after adrenalectomy and without a water load, the mean

[3] The exclusion of sick animals accounts for the number of rats being slightly smaller after adrenalectomy than before. Rarely, the administration of a water load did not go smoothly; in that event, the number of animals was greater on a subsequent than on a prior date.

osmolality was 312 ±2.7 mOsm/kg, a value not significantly different from that of the intact state. Furthermore, the average weight gain of 19 g 8 days after adrenalectomy (Table III) argues against the slightly higher osmolality having been due to severe illness or reduction in extracellular fluid.

Serum sodium and osmolal concentrations were also determined in a separate series of 14 DI rats. 7 days after adrenalectomy and without a water load, their serum osmolal and sodium concentrations were 302 ±1.4 mOsm/kg (mean ±SEM) and 148 ±1.3 mEq/liter, respectively; 30 min after a water load of 5% of body weight, these values were 286 mOsm/kg and 140 mEq/liter. The dilution of the serum after the water load is comparable to what we have measured in nonadrenalectomized normal and DI rats. This finding probably reflects integrity of the splanchnic circulation after adrenalectomy and argues against delay of water absorption from the gut or loculation of the water load in some

URINE FLOW
(% OF WATER LOAD,
5% BODY WEIGHT)

URINE OSMOLALITY
(mOsm/kg $H_2O$)

$C_{Osm}$

$CH_2O$

$CH_2O / C_{Osm}$

ml per 100g Body Weight per Hour

INTACT
ADX
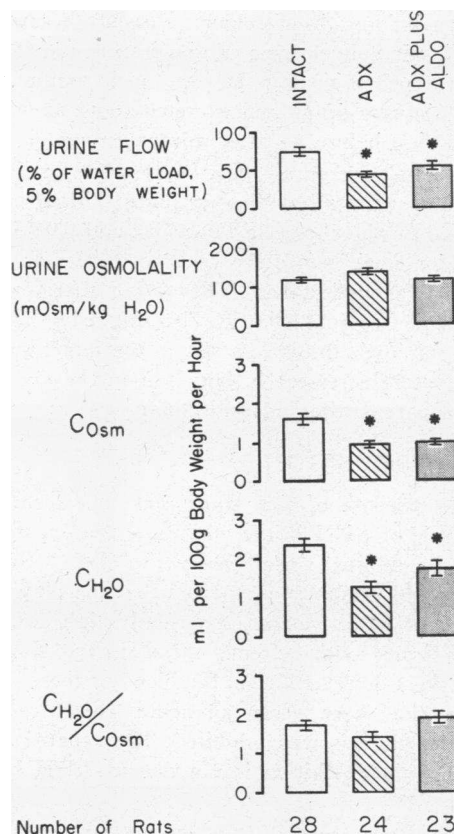ADX PLUS ALDO

Number of Rats 28 24 23

FIGURE 5 Restorative effect of aldosterone alone. Responses during the 60 min after an oral water load. The data from the females of groups 3 and 4 have been combined: intact, 9/23 and 2/11; adrenalectomized, 10/12 and 3/5; Adx plus aldosterone, 11/21 and 3/7. The meaning of the columns, brackets, and asterisks is the same as in Fig. 2.

fluid compartment other than the plasma as a factor in the delayed diuresis in the absence of adrenal steroids.

If nonspecific changes prevented the water diuresis, it seems doubtful that a single injection of prednisolone just 2 hr before the water load would fully restore the urine flow. Finally, although decreased GFR in adrenal insufficiency may contribute to the inhibition of water diuresis (see Discussion), such possible decrease could not be ascribed in our animals to nonspecific changes such as depletion of body fluid volumes.[4]

*Fluid balance without an acute water load.* 24-hr collections before and after adrenalectomy while eating and drinking ad lib. were obtained in groups 2 and 4. The results are illustrated in Fig. 6 *a* and *b*. Females and males of group 2 showed similar changes. After adrenalectomy, they decreased their solute excretion, fluid intake, and urine flow, and significantly increased their urine osmolalities. These animals had not been maintained on 0.9% sodium chloride before being adrenalectomized, although they were offered the choice of this fluid and tap water during the 24-hr collection periods even before adrenalectomy. Possibly because of a "natural" salt hunger even in the intact state (Purina Labena rat pellets contain half as much sodium as potassium) the rats drank and excreted about as much sodium chloride before as after adrenalectomy.

The results in group 4 (Fig. 6 *b*) were qualitatively similar, but quantitatively less striking. This group was maintained on the choice of tap water and 0.9% sodium chloride for 2 wk before the 24 hr collection in the intact state was undertaken. They were therefore in a steady state of sodium balance. This might be the reason that these animals drank less salt in the intact state and that a greater increment in both salt intake and output became apparent after adrenalectomy.

## DISCUSSION

The main purpose of this study was to determine the possible role of ADH in the inhibition of an acute water diuresis in adrenal insufficiency. In 1964, Kleeman, Czaczkes, and Cutler (3) reported that the plasma concentration of ADH was not abnormally high in humans, dogs, and rats with adrenal insufficiency. Nor could these workers detect ADH in the blood of these subjects after they had been given an acute water load, even though the diuresis was inhibited. They therefore concluded that the inhibition is not due to ADH but that

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[4] We did not measure GFR in the present study because all of our experiments were conducted on unanesthetized rats, and in the same animals within any one group. We wish to emphasize that while determining GFR may be important toward testing our postulated explanation (see Discussion), it is in no way essential toward validating the conclusions of the present work.

it results from an effect of the adrenal glucocorticoids on the water permeability of the distal nephron. Diametrically opposite results were reported by Ahmed, George, Gonzales-Auvert, and Dingman in 1967 (2). This group found abnormally high plasma concentrations of ADH in humans with adrenal insufficiency, even after these patients had been given large water loads. Treatment with glucocorticoids led to restoration of normal diuretic responses which were accompanied by falls in the plasma concentrations of ADH to undetectable levels. They therefore concluded that the inhibition of acute water diuresis in adrenal insufficiency is due to sustained high blood concentrations of ADH.

The controversy between these two groups seems to arise from the lack of a sufficiently sensitive, precise, and reliable method for assaying ADH in blood. Efforts to repeat the results of the assays which these groups used apparently have failed (7–9). It therefore appeared that the problem might be most conclusively resolved by an experimental design which would obviate the assay of ADH in blood; DI rats seemed to offer this opportunity. These animals cannot elaborate biologically active ADH. Hence, the marked inhibition of an acute water diuresis which we observed in all DI rats tested (Tables I, II, III) permits the conclusion that ADH cannot be essential for the expression of this phenomenon. We emphasize, however, that our experiments by no means exclude the possibility that high plasma concentrations of ADH may abet the inhibition in individuals with adrenal insufficiency which, unlike DI rats, can produce vasopressin.

There have been very few studies in the past which have assessed the adequacy of adrenal replacement therapy in fully restoring the water diuresis. Since by definition a water diuresis is the excretion of large amounts of dilute urine, we feel that full restoration should be judged quantitatively by the dual criteria of urine flow and urinary dilution. The impression that glucocorticoids alone suffice appears to be based on the observation, mainly in patients, that treatment with glucocorticoids results in the return of increased urine flow and some urinary dilution after a water load (1, 2). However, since for obvious reasons the same patients were not tested before they developed adrenal insufficiency, it is impossible to say whether the defect was entirely corrected. Similarly, in previous experiments on animals the diuretic responses before and after adrenalectomy were usually tested in groups composed of different individuals, the dual criteria of urinary flow and urinary dilution were usually not applied simultaneously, and the doses of glucocorticoid used were frequently so high that a significant mineralocorticoid effect could not be excluded. In the present study the diuretic responses after replacement therapy could be
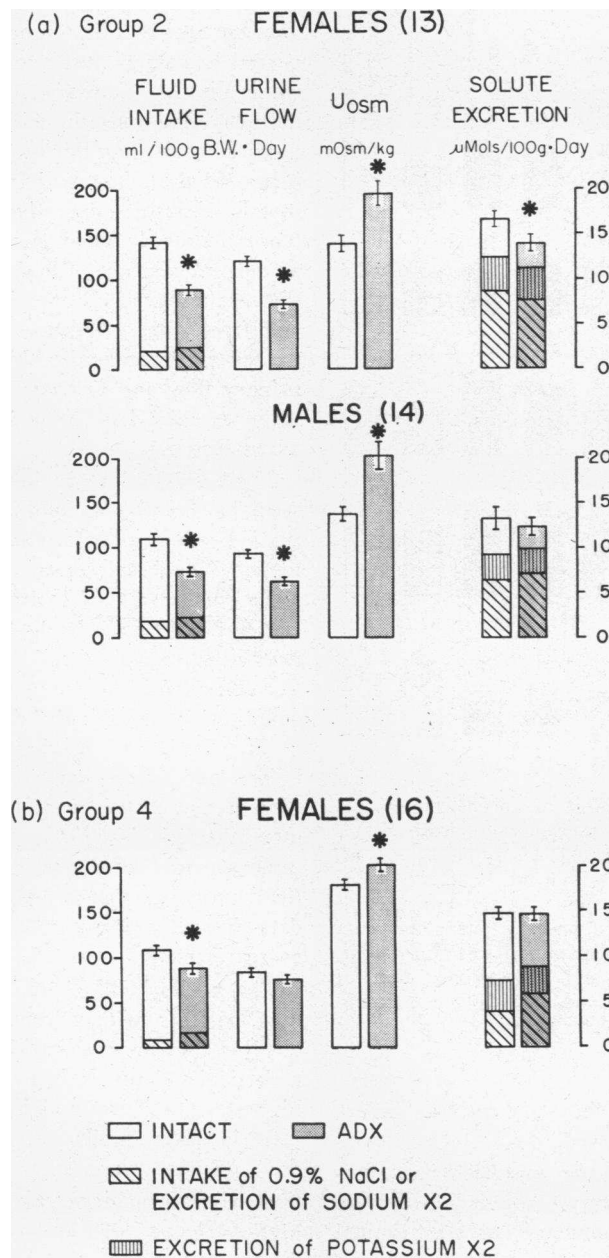
FIGURE 6 Effect of adrenalectomy on fluid economy of DI rats while drinking ad lib. and not given a water load. The columns and brackets represent the mean ±SEM of values obtained during 24-hr collection periods from the same animals before and after adrenalectomy. Asterisks denote values which are significantly different from those of the intact state ($P < 0.05$). Numbers in parentheses denote number of animals tested. The females and males of group 2 (a) were maintained on the choice of 0.9% sodium chloride and tap water after they had been adrenalectomized. In contrast, the rats of group 4 (b) received the choice of these two drinking solutions throughout the period of study, beginning 2 wk before the collections in the intact state.
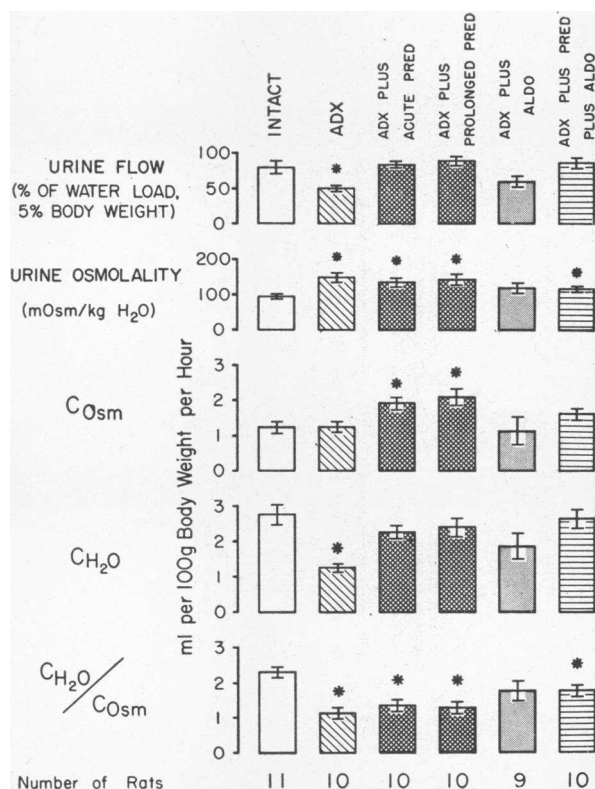
FIGURE 7 Summary, showing effect of adrenalectomy and varying hormonal replacement therapy on acute water diuresis in DI rats. All experimental conditions were imposed on the same female rats of group 3: intact, 9/23; adrenalectomized, 10/12; Adx plus acute prednisolone alone, 10/13; Adx plus prolonged prednisolone alone, data of 10/18 and 11/4 combined; Adx plus aldosterone alone, 11/21; Adx plus prednisolone and aldosterone, data of 10/25 and 10/28 combined. The meaning of the columns, brackets, and asterisks is the same as in Fig. 2.

compared to the responses in the same animals before their adrenal glands were removed.

The efficacy of replacement therapy has been summarized in Fig. 7, which portrays data obtained from the females of group 3. The summary has been limited to the results from 11 rats because this is the only group in which the same animals were studied under all experimental conditions. However, the conclusions which may be drawn from the results depicted in Fig. 7 have been verified in numerous other DI rats (Figs. 2–5).

Prednisolone alone, given either acutely as a single injection 2 hr before the water load, or chronically as continuous, daily injections, normalized the response in urine flow but failed to correct the defect in urinary dilution. The clearance patterns suggest that the diluting deficiency persisted because the amount of free water generated was insufficient in relation to the increased

solute excretion occasioned by the administration of glucocorticoid. Aldosterone when given alone also appeared to correct just one of the two components of a water diuresis. It tended to correct the diluting ability but not the response in urine flow, and the clearance patterns suggest that this was because, while aldosterone increased the amount of free water cleared per unit of osmolal clearance over that in the untreated adrenalectomized state, it failed to increase the osmolal clearance as did prednisolone. When both adrenal hormones were given simultaneously, the increases in both the osmolal and free water clearances over those seen in the untreated adrenalectomized state were such that both the urinary flow and urinary dilution were nearly identical to what they had been in the same animals before adrenalectomy.

The results of a previous and similar study on dogs seem in accord with our interpretation. Kleeman et al. studied four dogs before adrenalectomy, after adrenalectomy without replacement therapy, and after adrenalectomy plus 50 mg of hydrocortisone (Table III of reference 3). Their animals showed urine flows, urine osmolalities, osmolal and free water clearances, and $C_{H_2O}/C_{osm}$ which were not only qualitatively but also quantitatively strikingly similar to our values depicted in Fig. 7. Garrod, Davies, and Cahill studied two dogs before and after removal of the adrenal glands and compared the efficacy of treatment with gluco- and/or mineralocorticoids (10). Although they attributed the same separate effects to the two types of adrenal cortical hormones that we postulate, they did not publish data from the intact animals that permit quantitative comparison.

The work of Gill, Gann, and Bartter (11) on four human patients with adrenal insufficiency is frequently cited as showing restoration of water diuresis with glucocorticoid alone. It is to be noted, however, that the highest urine flows and lowest urine osmolalities were seen in these patients only when their extracellular fluid volumes were expanded. Furthermore, these patients did not achieve values of free water clearance as high as those which are seen in maximal water diuresis in normal humans given cortisol (12).

Studies on patients with adrenal insufficiency secondary to pituitary insufficiency also suggest that glucocorticoids alone restore water diuresis to normal (13). However, it has not been shown conclusively that such patients necessarily have a deficiency of mineralocorticoid activity.

Beyond the facts that ADH is not essential to the inhibition of acute water diuresis in adrenal insufficiency and that both gluco- and mineralocorticoids are required to correct the inhibition, the mechanism causing

this abnormality of water balance remains unknown. Nevertheless, we would like to present here an hypothesis for this mechanism, based on the present data and mainly on recent micropuncture studies. The following renal changes have been reported in adrenalectomized rats before and after replacement therapy: (a) glomerular filtration rate (GFR) appears to be markedly decreased after adrenalectomy and returned to normal or above normal by treatment with gloucocorticoids but not with mineralocorticoids (1, 14); (b) sodium reabsorption from the proximal tubules (15), the loops of Henle (16, 17), and from the distal convolutions (15, 16) and collecting ducts (18) is decreased after adrenalectomy and enhanced by aldosterone; and (c) osmotic water permeability of the distal tubules, but not of the proximal convolutions, is increased after adrenalectomy and restored toward that of intact rats by glucocorticoids (1).

In light of these results, some of which admittedly are still open to debate (14, 19), we speculate that the following mechanisms inhibit an acute water diuresis in adrenal insufficiency. Decreased GFR, coupled with increased osmotic water permeability of the distal nephron, leads to decreased urine flow. Although the proximal *fractional* reabsorption of sodium and water may actually be increased in adrenal insufficiency (15), it is difficult because of the simultaneous decrease in GFR to predict the absolute amount of sodium which may be delivered to the diluting segment of the nephron. In any case, it seems reasonable to assume that in the absence of aldosterone less sodium is reabsorbed from the diluting segment so that less free water is generated. This effect, coupled with increased reabsorption of free water as a result of increased osmotic water permeability of the distal nephron, would impair the diluting ability. These explanations presumably would apply not only to the diuretic response after an acute water load, but also to the partial amelioration of diabetes insipidus in adrenalectomized DI rats drinking salt and water ad lib. (Figs. 6 a and b).

Treatment with glucocorticoid alone tends to raise the GFR and decrease the osmotic water permeability of the distal tubule (1). While such therapy would be expected to restore the response in urine flow, it may not correct the diluting ability because in the absence of aldosterone there may be insufficient generation of free water in the diluting segment. Conversely, aldosterone alone may normalize the diluting ability in the loops of Henle; but since it may affect neither the glomerular filtration rate nor the osmotic water permeability of the distal nephron, it cannot by itself correct the response in urine flow. Thus, judging by the present state of our knowledge concerning the effects of adrenal steroids on

the nephron, it is not surprising that both gluco- and mineralocorticoids should be required to reverse the inhibition of acute water diuresis in adrenal insufficiency.

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