Impaired Urinary Concentration after Vasopressin and Its Gradual Correction in Hypothalamic Diabetes Insipidus

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ABSTRACT This study utilized rates with hereditary hypothalamic diabetes insipidus (D.I.) in order to explore possible mechanisms which prevent full urinary concentration after acute administration of vasopressin in hypothalamic D.I. and which correct this concentrating defect with prolonged therapy.

It was found: (*a*) that the concentrating defect persisted even when the urinary osmolal excretion of D. I. rats was reduced to that of normal animals; (*b*) that the defect was not corrected more rapidly if larger doses of vasopressin were given; (*c*) that it persisted even when the D.I. rats were deprived of drinking water after vasopressin was given; (*d*) that there was osmotic equilibration between urine and renal papilla at a time when the concentrating defect was still evident; and (*e*) that the correction of the defect was associated with progressive and significant rise of the papillary osmolality.

These studies appear to rule out osmotic diuresis, accumulation of exogenous vasopressin, persistent primary polydipsia, or delay in the induction of membrane permeability as causes for the concentrating defect. Rather, subnormal osmolality of the renal papilla, which can be corrected only gradually, accounts for the initial concentrating defect and the long time required for its correction. Reduction of water content and increase of urea content are primarily responsible for restoration of papillary osmolality to normal.

INTRODUCTION

It is well known that urinary concentration in response to supramaximal doses of vasopressin is impaired in both experimental (1, 2) and clinical (3) states of water diuresis. The gradual correction of this defect with prolonged administration of vasopressin has received less attention, although it has been pointed out by several authors (1, 4, 5), and convincing data demonstrating this correction were presented by Burka (6). A satisfactory explanation for these findings, however, has remained wanting (7).

Both the initial impairment and its gradual correction can be clearly demonstrated in rats with hereditary hypothalamic diabetes insipidus (D.I.) (8). In the experiments to be described, several possible mechanisms for this concentrating defect and its gradual response to treatment in D.I. rats have been explored. The studies suggest a gradual rise in the osmolality of the renal papillary interstitial tissue as the most plausible explanation.

METHODS

Each experiment will be described in relation to the hypothesis which it was designed to test.

All animals, both normal and D.I. of the Brattleboro strain (9), were obtained from our own breeding colony. Adult rats of both sexes were used, and in any given experiment, normal animals and rats with D.I. were

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matched for age and sex. Except when noted otherwise, rats had free access to Purina Labena rat pellets (Ralston, Purina Co., St. Louis, Mo.) and to drinking water.

Vasopressin tannate in oil [1] was used throughout and given subcutaneously. During collection periods, rats were kept in individual metabolism cages. Urine was collected under mineral oil at room temperature. Urine osmolality was determined in a Fiske osmometer. Osmolalities of papillary tissues were calculated as the sum of urea plus $2(Na^+ + K^+ + NH_4^+)$. The concentrations of these solutes were determined by methods previously described (10). Significance of the results was evaluated by Student's t test (11).

RESULTS

The concentrating defect and its gradual correction. Fig. 1 illustrates the progressive rise in urine osmolality as D.I. rats are given daily injections of vasopressin tannate in oil for 5 wk. In contrast, the urine osmolality of normal rats increased at the very onset of treatment and, except for one unexplained fluctuation, rose no further. After 5 wk of treatment, the mean urine osmolality of D.I. rats was about the same as that of normal rats given peanut oil (8), but not as great as that of normal rats treated with vasopressin (Fig. 1). In order to determine whether further treatment would completely normalize the urinary concentrating mechanism in D.I. rats, we continued injections of vasopressin in five D.I. rats for a total of 57 days. By this time their mean urine osmolality was 2657 mOsm/kg, which is not significantly different from the mean of the treated normal rats.

Urine flow decreased progressively during the course of treatment in the D.I. rats, but not in the normal animals.

Osmotic diuresis. The bottom of Fig. 1 indicates that urinary osmolal excretion was very much higher in untreated D.I. rats than in normals; this finding may be related to the greater food consumption of D.I. animals (12). When D.I. rats were treated with vasopressin, their osmolal excretion declined toward the levels seen in normal rats. This observation suggested that the initial subnormal response of D.I. rats to vasopressin might be due to osmotic diuresis, which is known to reduce maximum concentrating ability (13). The pattern of osmolal excretion during treatment of D.I. rats (Fig. 1) renders this explanation most unlikely. Even by the 2nd day of treatment, there had been a sharp decline, and although the total excretion remained higher than in the normals, this small difference in osmotic load is unlikely to have caused the very large differences in urinary concentration that were still apparent on days 2 and 9. Furthermore, by day 22, the urinary osmolal excretion of D.I. rats was less than that of normals, and yet the urinary concentration remained significantly lower than in normals by at least 500 mOsm/kg through the 28th day of treatment.

The role of solute excretion was explored further by depriving D.I. rats of food, thereby lowering their rate of osmolal excretion to that of normal rats (Fig. 2). Five normal rats (three females, two males) and six D.I. rats (three of each sex) which had previously been on food and water ad lib. were tested during the second 24 hr of treatment with vasopressin. During this test period the normal rats continued to have free access to food and water, but the D.I. rats had access only to water. Through this maneuver, the osmolal excretion in D.I. rats was reduced to the level seen in normal animals. Nevertheless, the mean urinary concentration remained some 1000 mOsm/kg lower in the former group.

The urine osmolality in fasted D.I. rats was slightly higher than we would have expected on the 2nd day of vasopressin treatment in fed D.I. rats. This phenomenon may have been due not only to decreased excretion of nonurea solutes, but also to dehydration. For unknown reasons, fasted D.I. rats drank less than normals, even though they had free access to water, and their urine output exceeded their fluid intake. This situation, plus insensible loss of water, must have resulted in a considerable negative fluid balance, and accounted for about 30% of the weight loss of fasted D.I. rats in this experiment.

One might suspect that the subnormal urine osmolality of the fasted D.I. rats was related to the known tendency of dietary nitrogen restriction to reduce urinary concentrating ability (14–16); but several findings suggest that this effect played no important role in this experiment. The urinary excretion of urea was almost halved in the fasted D.I. rats (Fig. 2). This phenomenon may have occurred mainly because of increased reabsorption of urea from the distal nephron under the influ-

[1] Pitressin tannate in oil, Parke, Davis & Co., Detroit, Mich.

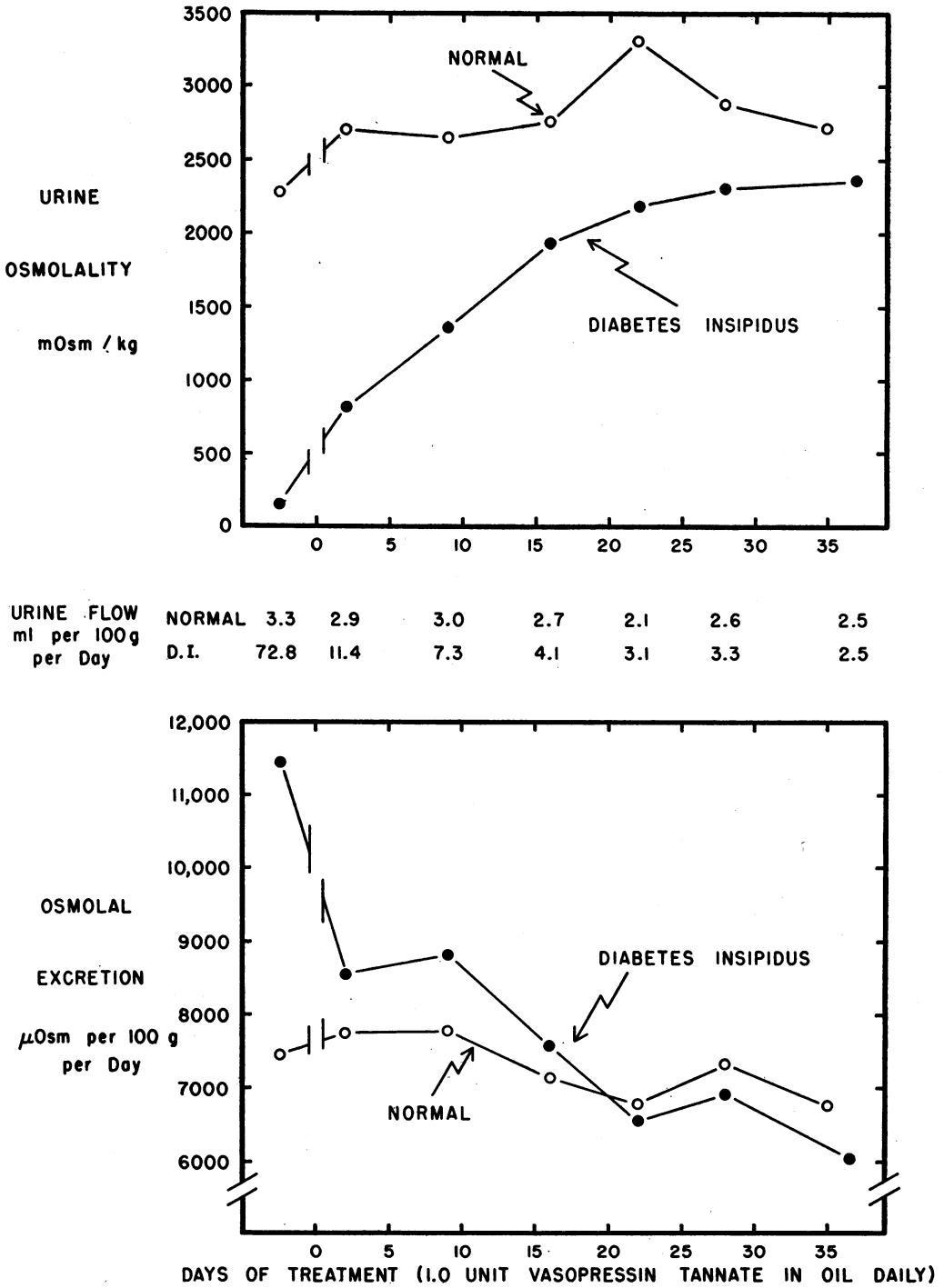

FIGURE 1 Mean values on eight normal and nine diabetes insipidus (D.I.) rats, before treatment (data to left of day 0) and during course of 37 daily, subcutaneous injections of vasopressin tannate in oil. All animals ate and drank ad lib. throughout the study. Except for the values at 35 and 37 days of treatment, all urine osmolalities in D.I. rats were significantly different from those in normal animals ($P < 0.05$). Urine flows in D.I. rats were also significantly different from those of normal rats ($P < 0.05$) except during the final two collection periods. During daily control injections of peanut oil vehicle, values did not deviate from those before treatment (8).

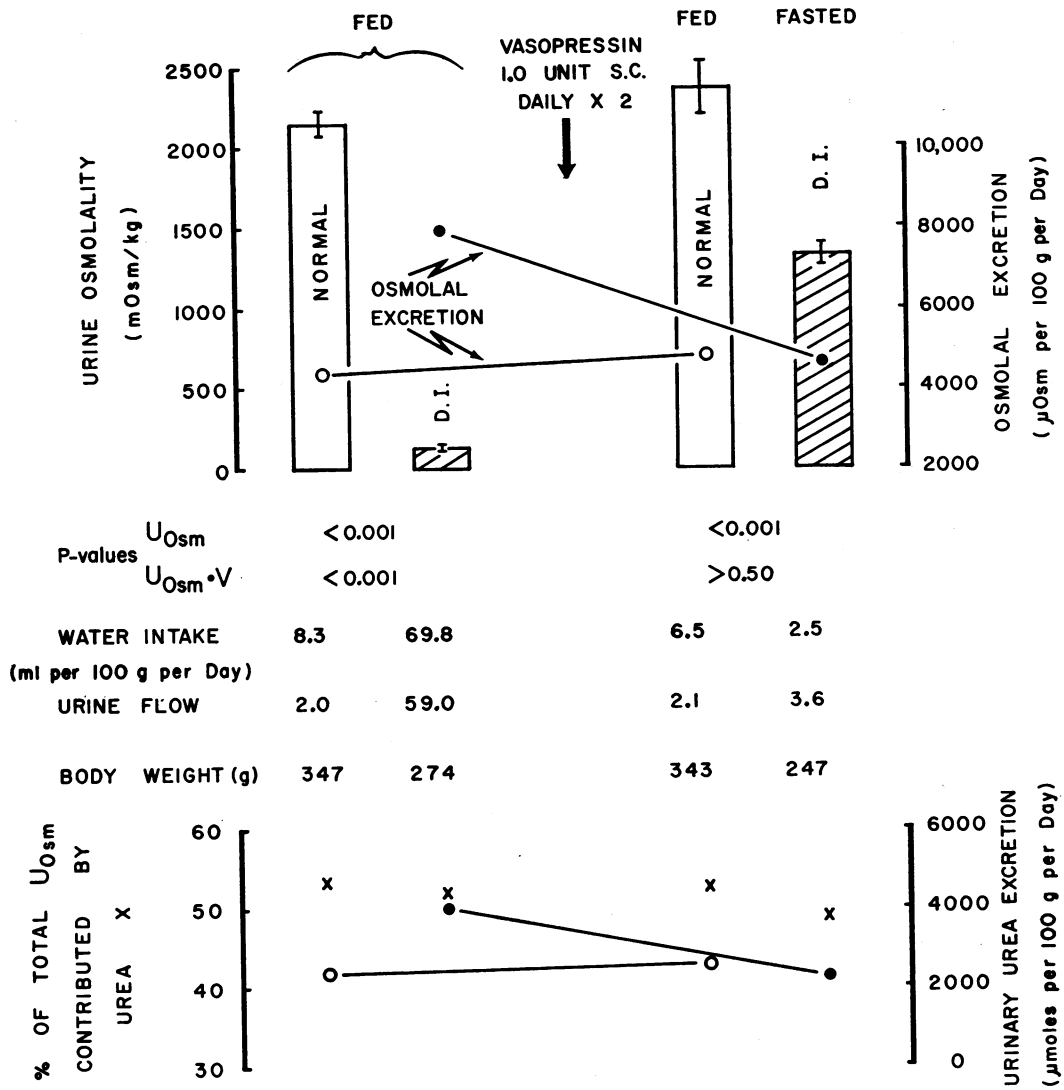

FIGURE 2 Data on five normal and six D.I. rats. The columns represent the mean ± SE of urine osmolalities. During the 24 hr immediately after the second injection of vasopressin tannate in oil, the D.I. rats were fasted in order to reduce their solute excretion to that of the normal rats. For unknown reasons, fasting also caused the D.I. rats to drink significantly less than the normals, even though they had free access to drinking water. Urinary excretion of urea was about the same in fasted D.I. rats as in normals, and the proportion of the total urine osmolality which was contributed by urea was only slightly and not significantly less in fasted D.I. rats than in fed normal and D.I. animals. For interpretation, see text. The differences in water intake, urine flow, and body weight between D.I. rats and normals were significant both before and after vasopressin. In the case of the urea values, only the difference in urea excretion between D.I. and normal rats before vasopressin was significant statistically ($P < 0.05$).

ence of vasopressin, rather than because of dietary nitrogen restriction, for the urea excretion in fasted D.I. rats did not drop significantly below that of the normals. Furthermore, the proportion of the total urine osmolality which was contributed by urea was 52–53% in normal and fed D.I. animals, and diminished to only 48% in fasted D.I. rats. The last value, which is not significantly lower than the others, compares with 49% in D.I. rats which had not been deprived of dietary protein and had been treated with vasopressin for 3 consecutive days (10). We conclude, therefore,

that the concentrating defect in question is accounted for very little, if at all, by osmotic diuresis.

Accumulation of exogenous vasopressin. The possibility was considered that gradual accumulation of exogenous vasopressin in D.I. rats given daily injections might have caused their increasing concentrating ability. It would follow from this argument that the dose of vasopressin we used (1.0 U/day) was not supramaximal and that higher urine concentrations could be achieved sooner if more vasopressin were used. The experiment illustrated in Fig. 3 was done in order to test this possibility.

Three groups of D.I. rats, each consisting of two females and two males, were given daily injections of 0.5 U, 1.0 U, and 2.5 U of vasopressin, respectively, for 3 days, and urine was collected for 24 hr on each day of treatment. There was no significant difference between the groups in the degree of urine concentration achieved on any one day, although the expected progressive rise in urine osmolality was seen in each group. The fact that a fivefold range in drug dosage caused no significant difference in urine concentration strongly suggests that a daily injection of even 0.5 U would have been supramaximal.

Habitual polydipsia. It seemed possible that D.I. rats, which consume an average of 80% of their body weight as drinking water each day (9), continue to drink excessively after replacement therapy with vasopressin is begun. In that case, primary polydipsia might be responsible for the initial concentrating defect.

This hypothesis was explored by testing the response to vasopressin of D.I. rats which were denied access to drinking water (Fig. 4). Three normal male rats and three D.I. males were allowed to eat and drink ad lib. during a 24 hr control period without treatment. The mean urine osmolality of the normal rats during this period was 2227 mOsm/kg, that of the D.I. rats was 116. Each animal, both normal and D.I., was then given 1.0 U of vasopressin tannate in oil. During the subsequent 24 hr normal rats were allowed food and water ad lib., while D.I. rats were allowed food but no water. During this final period urine was collected every 6 hr in order to avoid dilution of urine from the D.I. rats by urine formed immediately after the vasopressin was given.

The right-hand columns in Fig. 4 represent the mean urine osmolalities during the final 6 hr. Even though the D.I. rats were dehydrated and had lost an average of 10% of their body weight, their urine osmolality remained nearly 1100 mOsm/kg lower than that of the normal animals. Thus, habitual polydipsia cannot be responsible for the concentrating defect.

Progressive increase in membrane permeability vs. progressive rise in papillary osmolality. Vasopressin probably increases the permeability of the mammalian distal nephron to water (17–19). The biochemical reactions which bring about this change are not yet known, but it seems likely that these reactions involve one or more enzyme systems (20). If that is so, then the time required for adaptation of these systems (21) might account for the long delay before D.I. rats respond normally to vasopressin. According to this view,

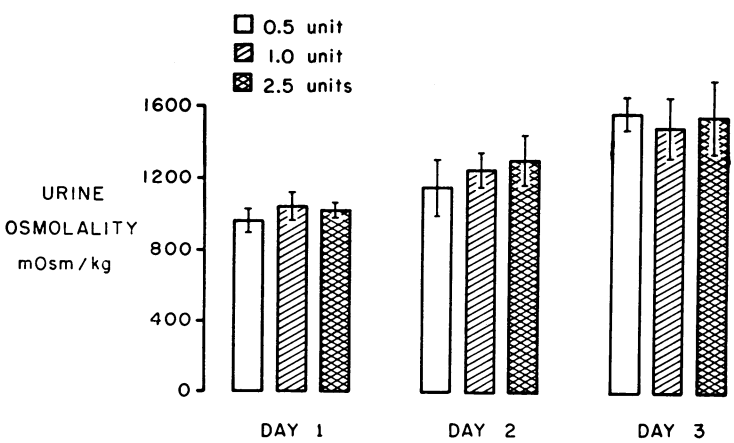

FIGURE 3 Effect of vasopressin dosage on urinary concentration in D.I. rats. The columns and brackets represent the mean ± SE of four rats. Numbered days refer to days of treatment with vasopressin tannate in oil, subcutaneously.

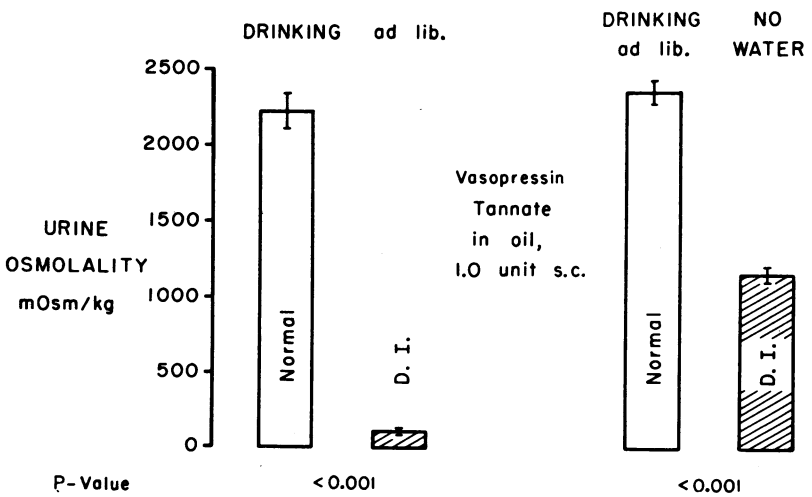

FIGURE 4 Effect of thirsting on vasopressin-induced urinary concentration in D.I. rats. Withholding drinking water, but not food, from D.I. rats during the 24 hr immediately after giving vasopressin did not restore urinary osmolality to that of normal rats. The columns and brackets represent the mean ± SE of three animals.

the membranes lining the distal nephrons of D.I. rats would not become fully permeable to water during the early period of treatment with vasopressin, and consequently osmotic equilibration between urine and papilla would not be achieved.

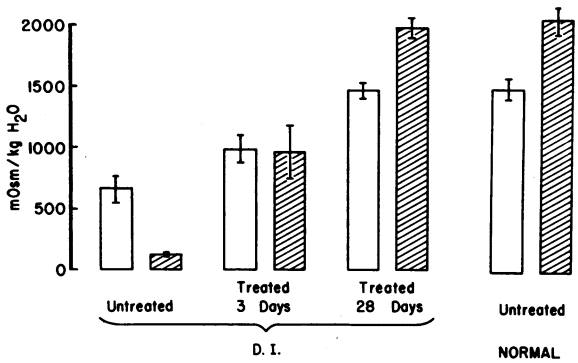

FIGURE 5 Simultaneous renal papillary osmolalities (open bars) (calculated as urea $+ 2[Na^+ + K^+ + NH_4^+]$) and urine osmolalities (striated bars) of D.I. rats before and after treatment with daily injections of vasopressin tannate in oil. Corresponding values for untreated normal rats are also shown. Each value represents the mean ± SE of six rats. Papillary and urinary osmolalities after 28 days of treatment were significantly different from those of untreated D.I. rats and D.I. rats treated for 3 days ($P < 0.01$), but not significantly different from those of untreated normal rats ($P > 0.40$). The data presented here and in Fig. 6 for untreated D.I. and normal rats and for D.I. rats treated for 3 days were published previously (10).

On the other hand, if the delay in reaching normal urinary concentration were due instead to a slow buildup of solute concentrations in the renal papilla, then osmotic equilibration between urine and papilla should be achieved early in the course of treatment, and a progressive rise in interstitial osmolality should be demonstrable.

The data depicted in Fig. 5 strongly suggest that when D.I. rats are treated with vasopressin, there is no delay in the hormonally induced increase in membrane permeability. Untreated rats with D.I. had a papillary osmolality of 661, but their simultaneous urine osmolality was only 124, presumably because in the absence of vasopressin relative impermeability of the distal nephron prevented osmotic equilibration. Already after 3 days of treatment there appeared to be osmotic equilibration of the urine, with papillary and urinary osmolalities of 985 and 969 mOsm/kg, respectively. After 28 days of treatment, the papillary osmolality was significantly greater than after 3 days of treatment and was essentially identical with that of normal animals. By this time, the urinary concentration was also comparable to that of normal rats, and now appeared to be greater than the papillary osmolality.[2]

[2] The calculated papillary osmolalities in untreated normals and in D.I. rats after 28 daily injections were lower than the urine osmolalities presumably because we had to

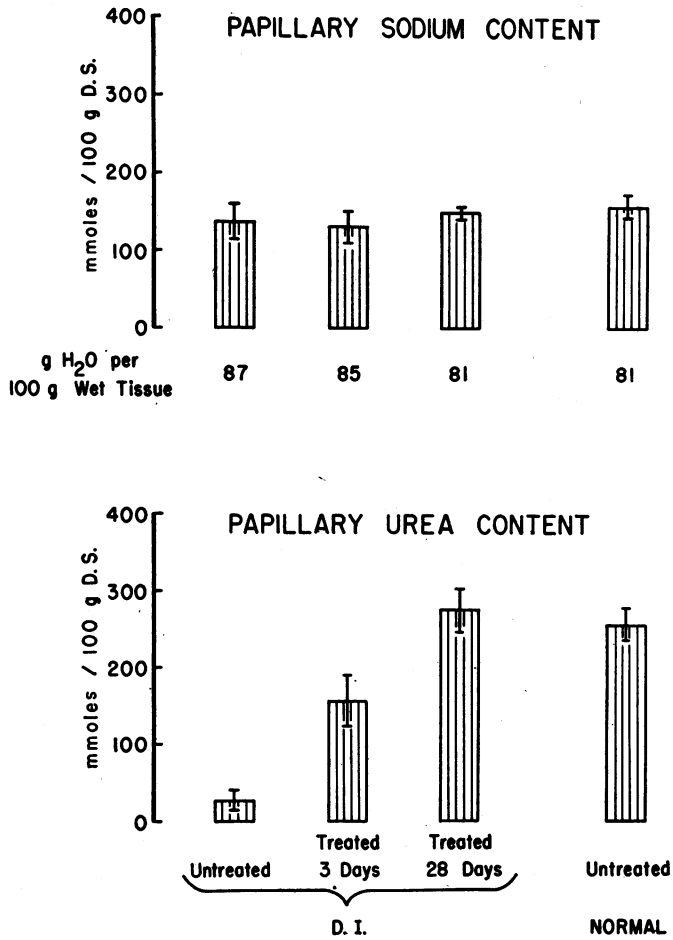

FIGURE 6 Renal papillary content of water, sodium (top), and urea (bottom) in D.I. rats before and after treatment with daily injections of vasopressin tannate in oil. Figures for untreated normal rats are also shown. The columns and brackets represent the mean ± SE of the same six animals for which values are given in Fig. 5. No significant difference of sodium content was found among these groups. The contents of both urea and water after 3 days of treatment were significantly different from those of untreated D.I. rats, from those of D.I. rats treated for 28 days, and from normal rats ($P < 0.05$). There were no significant differences between any values in D.I. rats treated for 28 days and those of untreated normal rats ($P > 0.50$). D.S. = dry solids.

Fig. 6 shows the main causes for the progressive rise in D.I. papillary osmolality which has been depicted in Fig. 5. Although previous work by us (10) had shown that the sequestration of papillary sodium did not increase in D.I. rats after three daily injections of vasopressin, there remained the possibility that more prolonged treatment might raise the papillary sodium content (mmoles/100 g of dry solids). As Fig. 6 shows, however, even 28 days of treatment failed to raise this variable significantly. It might be more meaningful to express contents as mmoles/100 g of urea-free dry solids. Although the slight rise which would occur in the sodium content if it were calculated in these units is still not significant, it must be admitted that the rise might reflect slightly increased sodium sequestration during prolonged treatment with vasopressin. However, this possible effect does not appear to contribute nearly so importantly to the rise in total papillary osmolality as do the significant and progressive rise in the content of urea and the progressive and significant decline in the content of water. The latter effect would of course tend to raise the concentrations (mmoles/kg of tissue water) of all solutes in the papilla. Thus, although tissue analyses admittedly do not provide direct measurements of the interstitial fluid volume and its contents, the striking differences between the behavior of

go down an osmotic gradient from the papillary tip toward the medulla in order to obtain enough tissue for analysis. This methodological error must have been minimized when papillary osmolality was determined after 3 days of treatment, for the interstitial osmotic gradient is much less steep when less concentrated urine is formed (22). Nevertheless, to the extent that the calculated papillary osmolality may have been an underestimate of the value at the very tip of the papilla, complete osmotic equilibration may not have occurred after 3 days of treatment.

sodium on the one hand, and urea and water on the other, strongly suggest that the progressive rise in papillary osmolality as D.I. rats are treated with vosopressin appears to be due principally, but not necessarily solely, to two factors: the decreasing content of water and the increasing content of urea.

DISCUSSION

There is now a fairly long list of conditions that can cause failure of the kidneys to concentrate urine, even in the presence of large amounts of endogenous or exogenous vasopressin (7). Most of these conditions can be ruled out as causes of the concentrating defect in hypothalamic D.I. in humans and in rats, because in these the kidneys are normal grossly and histologically (23), there are no known abnormalities of electrolytes, such as calcium or potassium (6, 12), and appreciable, albeit subnormal, concentration of the urine is usually achieved with the very first injection of vasopressin. The phenomenon being considered in this report is unique in that it represents only a partial unresponsiveness and one which can be corrected with the single measure of prolonged treatment with vasopressin.

Several possible explanations for the concentrating defect, such as solute diuresis, accumulation of exogenous vasopressin, habitual polydipsia, and enzyme adaptation appear to have been ruled out by the present experiments. The last, adaptation of enzymes involved in the action of vasopression, is further rendered most unlikely by the findings that the concentrating defect in humans cannot be prevented by giving large doses of exogenous vasopressin during the period of experimental overhydration (1), and that when D.I. rats are used as bioassay preparations they are more, rather than less, sensitive to intravenous vasopressin (24–26). Furthermore, contrary to the expectation if enzyme adaptation were involved, the sensitivity of the bioassay cannot be enhanced by prior prolonged treatment with exogenous vasopressin (25).

In an earlier report we suggested that the initial defect and its gradual correction in D.I. rats might be due to competitive inhibition by an abnormal polypeptide which these rats might produce instead of vasopressin (8). This hypothesis has since been disproved (27). It is conceivable that oxytocin might act as a competitive inhibitor of exogenous vasopressin in hypothalamic D.I. Such a mechanism seems unlikely in water-loaded rats and humans, however, since excessive release of oxytocin would not be expected in overhydrated, normal subjects.

A number of possible mechanisms have been postulated in the past to explain the concentrating defect during experimental and clinical water diuresis. Most of these have invoked an increase in the volume or decrease in the osmolality of the extracellular fluid, the ultimate effect possibly being cellular overhydration and consequent inability of vasopressin to act or to reach its site of action (1, 2, 28, 29). If rats with D.I. represent a comparable experimental model for the concentrating defect under discussion, there is considerable evidence against such explanations. The concentrating defect persisted in dehydrated D.I. rats (Fig. 4), which must have had contracted fluid volumes. Even in D.I. rats drinking ad lib., the serum osmolality and sodium concentrations are significantly higher than in normal rats(23). This finding suggests a state of mild dehydration and contraction of the extracellular fluid volume.[3]

Several authors have alluded to the possibility that the cause of the concentrating defect may lie in decreased concentration of the papillary interstitium. The data presented in Fig. 5 seem to leave little doubt that this explanation is correct in the case of D.I. rats. Although Fig. 6 shows that a slow and progressive rise in the papillary content of urea and a concomitant gradual and progressive decline in the content of water are primarily responsible for the slow buildup of papillary osmolality, these studies do not elucidate the mechanism(s) by which these changes are brought about.

[3] The Friedmans (12) found very slight changes in the opposite direction, but this may have been because they corrected extracellular fluid volumes to 100 g of body weight. Since D.I. rats are very much leaner than normals of the same age, one would expect the proportion of their body weight which is water to be greater than normal. This possible explanation is strengthened by the Friedmans' finding of no significant difference in extracellular fluid volumes between normal rats and those with surgically induced D.I. of similar body weights. There also appear to be no significant differences between rats with hereditary D.I. and normals in total and intracellular water content of gastrocnemius muscle and aorta.

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