Service of urea in renal water conservation

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Rats allowed water ad libitum were injected with vasopressin tannate in oil to insure formation of maximally concentrated urine. Low protein diet was fed to which were added varying amounts of sodium chloride with or without urea. Through a wide range of urinary excretion rates of nonurea solute the feeding of urea resulted in formation of lesser volumes of more highly concentrated urine. This reduction of the renal water requirement in the urea-fed rats was most marked when the ratio in urine between urea and nonurea solutes had a value of 0.3–0.5. When present in urine in amounts equal to or greater than osmoles of nonurea solute, urea increased the renal water requirement.

In 1934, Gamble et al. (1) studied the effect of ingestion of urea and of several inorganic electrolyte salts on the volume of urine in rats. It was shown that the osmolal concentration and hence amount of urine formed at a given rate of osmolar loading was approximately the same for the several inorganic electrolyte salts whether given alone or in various combinations. An equiosmolar load of urea, by contrast, was excreted in a substantially smaller urine volume. When the excretory solute was comprised of a mixture such that approximately one-third of the urine’s total osmotic activity was referable to urea and the remainder to inorganic salts, the volume of urine was found to be less than that predicted by summation of the renal water requirements of the components taken separately though greater than for an equiosmolar load comprised entirely of urea. A number of other organic compounds including glucose, galactose and creatinine were studied and shown not to share with urea this economy of water.

This phenomenon does not appear to have received further attention in the intervening years. Gilman in 1937 voiced the objection that since Gamble’s animals were allowed water ad libitum, the results might have reflected a basic difference in the influence of urea on thirst and water drinking of the rats rather than on the renal water requirement (2). Gilman’s conclusion with respect to his own observations (2) as well as those of Gamble (1) has been supported by Verney’s (3) demonstration that urea is inactive in stimulating the hypothalamic osmoreceptors. Thus, the renal economy of water might have been due to the fact that relative dehydration developed in the animals ingesting urea resulting in formation of urine of substantially higher osmolality than when other, more active osmotic stimulants were fed. Such an effect of bodily hydration on the renal concentrating mechanism has been shown by Epstein (4, 5).

The studies here reported were undertaken to see if an economy of water in renal function due to urea could be demonstrated in animals injected with a long acting vasopressin preparation. It was reasoned a priori that the hormone would insure at all times excretion of urine of maximal osmolality for the circumstance.

METHODS

Intact, male albino rats of the Sprague-Dawley strain weighing about 150 gm were used. For at least 3 days prior to and during metabolic study the animals were fed a low protein, low electrolyte diet2 supplemented with sodium chloride or urea or both. The sodium chloride supplement was designed to yield a

2 The composition per kilogram of the low protein, low electrolyte diet was as follows: corn oil, 57 gm; devitaminized casein (Sheffield Farms) 68 gm; dextrose, 185 gm; NaCl, 0.987 gm; KCl, 0.02 gm; NaHCO3, 2.13 gm; MgSO4·7 H2O, 1.45 gm; NaI, 0.004 gm; CuSO4·5 H2O, 0.006 gm; ZnSO4·7 H2O, 0.006 gm; MnSO4·4 H2O, 0.006 gm; Fe3(CO)2·3 H2O, 0.042 gm; CoSO4·4 H2O, 0.006 gm; cystine, 0.183 gm; thiamine, 0.001 gm; riboflavin, 0.004 gm; pyridoxine, 0.001 gm; calcium pantothenate, 0.008 gm; nicotinic acid, 0.015 gm; inositol, 0.142 gm; p-aminobenzoic acid, 0.285 gm; folic acid, 0.006 gm; menadione, 0.007 gm; a-tocopherol, 0.10 gm; vitamin A, 30,000 IU; vitamin D2, 5,500 IU. In addition, each animal’s daily ration was supplemented with a freshly prepared solution yielding 0.1 gm of choline dihydrogen citrate.
range of urinary osmolar excretion of these ions varying from 400 to 2000 mOsm/m²/day. When the diet was additionally supplemented with urea, the latter was given in quantities calculated to yield a range of osmolar values for urea excretion of 20-80% of those for sodium and chloride. In one experiment the results of which are included only in figure 3 a larger urea supplement was used designed to yield in urine a ratio of osmoles of urea to osmoles of sodium and chloride of 1.5. The animals were allowed free access to drinking water but received daily intramuscular injections of 0.5 U (0.1 ml) of vasopressin tannate in oil\(^3\) for 3 days prior to as well as on the day of urine collection. In most instances, a series of three to five observations were made under differing circumstances on the same animal. A given 'first' experiment usually involved use of eight rats all of which received the same dietary sodium supplement. Of this total, four received a preselected dietary addition of urea, the other four no urea. In the next subsequent experiment, ordinarily begun 1 week later, the same eight rats were used but the previous urea fed subgroup were now fed no urea and the four controls of the previous experiment became the urea-fed subgroup.

Carefully timed, approximately 24-hour urine collections were made in individual metabolism cages fitted with collection systems arranged to prevent admixture of urine and stool. Precautions were taken to prevent evaporation of urine from the collecting vessels which contained a few drops of toluene. Some loss of urine occurred as a result of incomplete drainage of all droplets into the collecting flasks. On the average, 85% of the sodium chloride fed was accounted for in the urine collected in fluid form. Virtually all of the remainder could be recovered in washings of the collecting systems. Urine volume and total solute, sodium, chloride and urea output rates were calculated from measurements made on urine collected without washdown of glassware and it has been assumed that the above described losses did not affect the measured concentration values.

Osmolarity was measured cryoscopically using a Fiske\(^4\) osmometer. Values reported are directly convertible to freezing point depression by multiplication by \(0.51186^\circ\text{C}\). Total solute output has been calculated as the product of volume and osmolarity. Urea was determined according to the method of Conway (6). Nonurea solute excretion has been taken as the remainder after subtraction of urea from the total solute output. Sodium was determined by internal standard flame photometry (7) and chloride by the Volhard titration (8). In the experimental circumstances, the sum of the separately determined urea, sodium and chloride excretion rates divided by the value for total solute output yielded a mean of 0.95; S.E. \(\pm 0.014; n = 28\).

Results have been expressed per square meter of body surface area computed from the formula:

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\text{Surface area (m}^2) = \frac{0.00092 \sqrt{\text{wt. in gm}}}{(9)}
\]

**RESULTS**

Figure 1 shows the influence of urea on the osmolarity of urine excreted by vasopressin injected rats. Nonurea solute output has been selected for the abscissa in preference to one showing output of total solute in order to permit visualization of the effect of urea by simple vertical comparison of data from animals excreting identical loads of solute other than urea. The open points represent osmolarity values for urine of rats fed diets contrived to keep urea output at a minimum and to make variations in total solute output a function primarily of sodium and chloride excretion. The urine of these animals was essentially constant at 71 \(\pm\) S.D. \(-82 \text{ mOsm/m²/24 hr.}\) The osmolar ratio of urea to other solutes had a mean value of 0.11 and varied inversely with sodium chloride loading between extremes of 0.03 and 0.19. The closed points represent the osmolarity of urine excreted by rats fed the same sodium chloride enriched diets with additional supplementation with various quantities of urea. The mean ratio of urea to other urinary solutes in these animals was 0.96. It is apparent that the osmolality of urine is distinctly greater at any given rate of nonurea solute output when the urine contains substantial quantities of urea in relation to other solutes. Thus, for example, at the same excretory rate of 400 mOsm of nonurea solute/m²/24 hr., rats on a low protein diet excreted urine containing 471 mOsm of total solute at 1580 mOsm/L while urea-fed rats excreted 560 mOsm of total solute at 2340 mOsm/L. Despite the extra solute load imposed by urea feeding, the increased urine

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\(^3\) Pitressin tannate in oil, Parke, Davis Co., Detroit, Mich.

concentration resulted in a total daily volume which was less for the urea-fed (240 ml/m²) than for the non-urea-fed animals (342 ml/m²).

The coordinates of figure 2 are designed to highlight the economy of water deriving from the increased osmolal concentration of urine in the urea-fed animals. Here the ordinate scale shows urine volume per unit time divided by the amount of nonurea solute excreted. The abscissa scale is the same as in figure 1. The open points refer to the nonurea-fed animals; the closed points to those fed urea. The data show a generally lower renal water requirement for excretion of a given load of nonurea solute in the animals fed urea. The regression curve drawn through the open points has been calculated from osmolality values computed from the equation for nonurea fed animals of figure 1. In deriving values for the curve it has been assumed that mean urine volume will be given by division of total solute excretion by urine osmolality, the numerator being taken as the sum of nonurea solute output and the mean value for urea excretion of the nonurea-fed animals (71 mOsm/m²/day). The curve drawn through the data of the urea-fed animals has been calculated as the product of values for milliliters of urine water per milliosmole of nonurea solute from this upper regression and a factor, 0.785, derived as follows: each of the values for urine water expenditure in milliliters per milliosmole of nonurea solute has been divided by the ordinate scale value of the upper regression at the corresponding rate of nonurea solute output. The mean of dividends obtained for the nonurea-fed animals is 1.00; that for the urea-fed animals, 0.785. The standard error of the difference between these means is 0.051 yielding a t value of 4.22. This factor of 0.785, significant according to its t value at the 0.001 probability level, has the meaning that the urea-fed animals required on the average only 78% as much urine water as did the nonurea-fed group for excretion of a given load of nonurea solute.

Since the data of figure 2 show considerable scatter and overlap, the development shown in figure 3 was undertaken. The ordinate scale here shows the individual values obtained as above by division of the observed urine water expenditure per milliosmole of nonurea solute by corresponding values from the upper regression of figure 2. These dividends are related on the abscissa scale to the ratio between urea and nonurea solute contained in the urine. As previously, the open points refer to the nonurea-fed animals, the closed points to those fed urea. In addition, data not included in the previous figures obtained by feeding rats urea loads sufficiently large to yield ratios in terms of the abscissa scale greater than 1.0 are shown by the group of seven points in the upper right-hand portion of the figure. It becomes apparent as suggested by the free hand curve that the optimal ratio in urine between urea and nonurea solutes for water conservation lies at about 0.4. Urea additions to the diet large relative to the load of other excretory solutes effect no reduction but rather an extension of the renal water requirement.

DISCUSSION

The effect of urea intake in enhancing urine osmolal concentration has been demonstrated both in rats (1, 5, 11, 12) and in the human being (4). However, only Gamble and his co-workers (1) have previously shown that urea acts to reduce the volume of urine formed and their work as mentioned earlier has been subject to the criticism that the effect may have been on water drinking of the animals rather than on the kidney. In the present study the use of depot vasopressin tends to counter this objection. Furthermore, differences in hydration according to whether or not urea was added to the diet were not detected by measurements of body weight, serum osmolality or serum sodium concentration. Had such differences been present, the results might have been interpreted according to the mechanism described by Epstein (4, 5). Hence, the present experiments support
the conclusion that the effect of urea feeding is exerted primarily at the kidney and on some mechanism other than that dependent on hydration. In addition, the fact that the animals were mature and were used as their own controls suggests that the influence of urea is functional rather than being dependent on the morphologic changes which can be induced by high protein or urea feeding especially in young animals (13).

It is now well established that sodium and chloride are concentrated in renal tissue water along a gradient rising from cortex to papilla (14–16). This gradient is presumably a function of active sodium transport coupled to a countercurrent exchange system (17). The accumulation of sodium and chloride in the renal papillary tissue which is independent of the concentration of the same ions in simultaneous urine (15, 16) appears to exert the osmotic force causing dehydration of the latter gradients, however, available data indicate equilibrium or, at least, very nearly so, with urine water (15–17). The fact that urea is concentrated along a gradient as are sodium and chloride yields insight into the phenomenon of enhancement of urine osmolal concentrations when the diet is high in protein or urea (15–17). Alternatively, several alternatives are available. If urea were subject only to passive transport, a reduction in the renal water requirement might be seen in its presence if urea in increasing concentrations accelerated the self-diffusion of water by alteration of its 'semi-ice' structure. Such an effect on these properties of water has been shown by Wang using a variety of electrolytes, though not urea, in water solution (18). Alternatively, were urea in sodium chloride solutions significantly to depress with respect to values calculated from the separate activities of the contained solutes the effective osmotic concentrations of the solutions as estimated from freezing point, an improvement in renal water economy would result. Urea fails so to act but does influence the passage of water across 'leaky' membranes in the phenomenon of anomalous osmosis (19, 20). Either of these two 'passive' phenomena, alteration of self-diffusion of water or anomalous osmosis, the latter being of greater appeal because of its experimental demonstration, could account for the observations recorded here.

Evidence for active transport of urea in mammals (presumably in the direction, renal parenchyma to tubular lumen) has recently been reviewed by Schmidt-Nielsen (13). Though direct proof for such transport is still lacking and equilibrium between urine urea and urea of papillary tissue water would constitute direct contrary evidence, it should also be understood that relative to the potential technical errors involved in the necessary analyses a very small gradient between simultaneous urine urea and urea of papillary tissue water would suffice to explain the effects observed in the present experiments. It should be further noted that if urea transport were involved in the mechanism underlying the phenomenon, the required direction of transport would appear to be such as to yield a rising concentration gradient from tubular lumen to renal parenchyma. The data here recorded should help in a further experimental attack perhaps better conceived to permit demonstration of such a gradient should it exist.

Urea administration has long been recognized in clinical medicine as a means of promoting diuresis (21). The present data suggest that the capacity for water conservation may be improved by appropriate adjustments in dietary urea or urea precursor content. In this connection, however, it is of interest to note that unpublished studies in this laboratory have shown no economy of water due to urea in patients unable as a result of chronic kidney disease to achieve an osmotic U/P ratio above 1.5 though the water conservation effect can be demonstrated in patients with normal kidney function. Furthermore, in patients with diabetes insipidus and in animals during maximal water diuresis urea loading causes a major extension of urine volume and minor increase in osmolality, effects identical with those observed after equal increments in total solute output due to sodium or potassium chloride.

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REFERENCES

2. Gilman, A. Am. J. Physiol. 120: 525, 1937.