EFFECT OF UREA ON URINE CONCENTRATION IN THE RAT

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(Submitted for publication May 17, 1961; accepted June 22, 1961)

Urea is the principal end-product of nitrogen metabolism in mammals and is excreted almost entirely through the kidney. Studies by Shannon (1, 2) in the dog and by Chasis and Smith (3, 4) in man have shown that the renal clearance of urea is less than the inulin or creatinine clearance at all rates of urine flow, and that the clearance of urea increases as the urine flow rises. These observations are consistent with the hypothesis that urea is filtered at the glomerulus and that a portion of this filtered load diffuses out of the nephron as the fluid passes from glomerulus to renal pelvis.

During the past few years a considerable amount of evidence has accumulated that urea has a unique role in the renal concentrating mechanism. The maximal urinary concentration achieved during antidiuresis is reduced by a low protein diet (5–8). The addition of urea to such a low protein diet will return the maximal urine concentration to near normal levels. In medullary tissue slices removed from the kidneys of antidiuretic dogs, it has been shown that urea is one of the principal solutes (9–11). Levinsky, Davidson, Berliner and Eden (11, 12) have proposed a mechanism by which urea, accumulated in the inner medulla passively, is able to add to the urine osmolality. During the passage of tubular fluid along the collecting duct, the urea concentration rises progressively as water moves into the medullary interstitial space along the osmotic gradient created by the active transport of sodium in the medulla. The rising urea concentration in the collecting duct establishes a concentration gradient along which urea moves into the inner medulla. The urea which diffuses into the inner medulla serves to balance an equiosmolal amount of urea in the collecting duct, leaving most of the medullary sodium free to balance other osmotic constituents in the urine. It has recently been demonstrated that the permeability of the collecting duct to urea is increased by vasopressin (13) and that the concentration achieved in the medullary tissue by this mechanism depends upon the fact that the collecting duct is less permeable to urea than to water (14, 15). This hypothesis is consistent with the concept proposed by Chasis and Smith and by Shannon, that urea is handled passively by the kidney.

The possibility that urea excretion is regulated at the tubular level has recently been re-examined by Schmidt-Nielsen (16). It has been shown that the regulation of urea excretion in the camel (17) and sheep (18, 19) is dependent largely upon the animal's requirements for protein and only partially upon the plasma urea level, the glomerular filtration or the urine flow. Thus, the sheep on a low protein diet reabsorbs a larger fraction of the filtered urea than does the same animal on a normal protein intake.

Studies by Gamble, McKhann, Butler and Tuthill (20) have shown that, by replacing sodium chloride with an equiosmolar amount of urea in the diet of rats, the volume of urine required to excrete a given solute load is reduced. However, as the volume of urine decreased in their experiments, so did the water intake. Thus, it is impossible to determine whether the drop in urine volume preceded or followed the decrease in water intake. Crawford, Doyle and Probst (21) have re-investigated the effect of urea on urine concentration in rats. In an attempt to avoid the problem of interpretation encountered in Gamble's work these authors gave their experimental animals a daily injection of vasopressin. They demonstrated that in rats on a low protein diet urea supplements decreased the urine volume and increased the urinary non-urea solute concentration. The decrease in urine volume might again be attributable to a primary effect on water intake. However, the effect of urea supplements on the non-urea solute concentration suggests that urea may influence the active transport mechanisms of the renal tubule.

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The studies reported here were done to re-evaluate the role of urea in the renal concentrating mechanism of the rat. It has been confirmed that urea supplements given to rats on a low protein diet increase the ability of rat kidneys to concentrate the non-urea solute of the urine, and it is further shown that rats with a low urine urea concentration, whether produced by a low protein diet or by a mannitol infusion, can have a significantly higher urea concentration in the renal papilla and inner medulla than in the urine.

METHODS AND MATERIALS

Rats were maintained on an artificial diet (22) modified to contain a low protein (5.8 per cent casein) and high salt (1.6 per cent sodium chloride) content or on Purina rat chow for at least 10 days before the experiments were begun. Rats receiving urea supplements were fed the low protein diet described above with 1.8 per cent urea added. Food and water were given *ad libitum*. No measurement of intake was made, but the body weight of almost all the rats was constant or showed a slow increase while on the diet. Vasopressin when used was given intramuscularly as Pitressin tannate in oil (Parke, Davis; lot 302), 500 mU daily.

Two kinds of experiments were performed. In the first group 24-hour urine collections were obtained in special cages constructed to immobilize the rat horizontally on wide mesh wire. In this way urine could be collected with no contamination by feces, food or water and yet rats could be allowed food and water. Urine was collected under mineral oil and toluene. At the end of every 24-hour collection period, urine was expressed from the bladder of each rat until the urinary bladder was no longer palpable. For tissue analysis, rats were sacrificed by exsanguination and the kidneys immediately removed. A slice was cut from the center of each kidney perpendicular to a line joining the poles of the kidney and placed between Parafilm in a refrigerator at +4° C. These slices were carefully dissected under a magnifying lens, weighed, and homogenized in water for analysis. Figure 1 is a schematic representation of the anatomy of the rat kidney and the approximate division between the various slices as dissected in these experiments. Less than 5 minutes elapsed between removing the kidney and homogenizing the slices. Tissue homogenates were not boiled or centrifuged but were promptly analyzed for urea or frozen until the analyses could be performed.

In the second group of studies experiments were performed by collecting urine from the ureters of anesthetized rats. Animals on a low protein diet as well as chow-fed rats were used. Under ether anesthesia, the abdomen was opened and a piece of no. 10 polyethylene catheter placed in one ureter. Upon completion of three or more collection periods, the kidney was removed, sliced as before, and analyzed for urea. Some rats on each diet received infusions of 15 per cent mannitol at rates varying from 0.25 to 4.8 ml per hour for 0.5 to 3.5 hours before samples were collected.

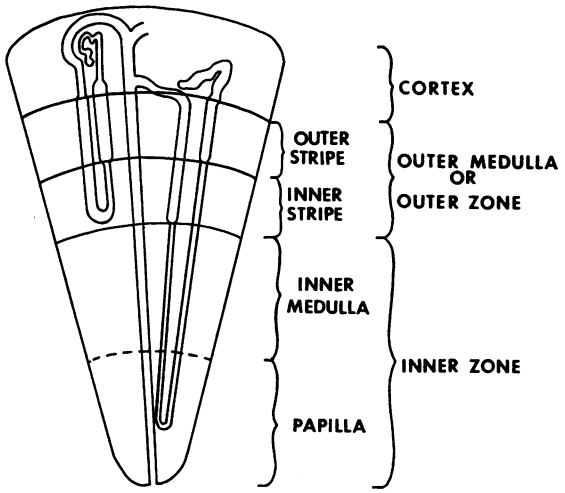

FIG. 1. A SCHEMATIC REPRESENTATION OF THE ANATOMY OF THE RAT KIDNEY TO INDICATE THE POSITION FROM WHICH VARIOUS SLICES WERE REMOVED.

Urea determinations were performed on tissue and urine by the microdiffusion method of Conway (23) and were corrected for any ammonia present by subtracting an ammonia blank. Sodium and potassium concentrations were determined with flame photometry (Baird-Atomic) with a lithium chloride internal standard. Osmolality was determined by freezing point depression (24). Tissue water was determined by drying slices from 3 groups of 2 rats each and averaging the values. All tissue concentrations were corrected for these water contents. The non-urea solute concentration of the urine was approximated by subtracting the determined urea concentration from the osmolality estimated from the freezing point.

RESULTS

The first set of experiments was performed to measure the effect of urea supplements on the urea and non-urea solute excretion of rats on a low protein, high salt diet. After 10 days on this diet collection periods were begun. There was considerable variation in the non-urea solute concentration achieved in consecutive collections from the same rat. In spite of this variation, however, it can be seen in Table I that five of the six rats receiving urea supplements were able to concentrate the urinary non-urea solute above 1,400 mOsm on at least one occasion. In 16 of the 29 collections from these rats the non-urea solute concentration was greater than 1,300 mOsm. In sharp con-

TABLE I

Effect of urea supplements on the non-urea solute concentration (NUSC) in 24-hour urine collections from rats on a low protein diet

With urea supplements			Without urea supplements		
Rat	Highest NUSC	Number collected	Rat	Highest NUSC	Number collected
A	1,462	4	a	1,156	4
B	1,521	5	b	1,214	5
F	1,557	5	c	1,084	2
H	1,339	6	d	1,301	4
K	1,411	5	h	1,130	5
V	1,614	4	t	1,162	6
			l	1,172	5
Average	1,484			1,174	

trast are the data from the seven rats not receiving urea supplements. Only one rat in this group had a non-urea solute concentration as high as 1,300 mOsm, while the others had non-urea solute concentrations at or below 1,214 mOsm on all occasions. Urea supplements thus enable rats on a low protein diet to increase the maximal non-urea solute concentration of their urine. These findings are in agreement with the observations of Gamble and co-workers (20) and Crawford, Doyle and Probst (21).

Tissue analysis. The results of tissue analysis from two groups of rats are shown in Figures 2 and 3. The urine values for sodium and urea plotted on the right-hand side of each figure were determined on 24-hour urine collections, ending at the time the animal was sacrificed. In Figure 2 the tissue values for sodium and urea in six normal rats not receiving exogenous vasopressin are presented. In these animals there is an in-

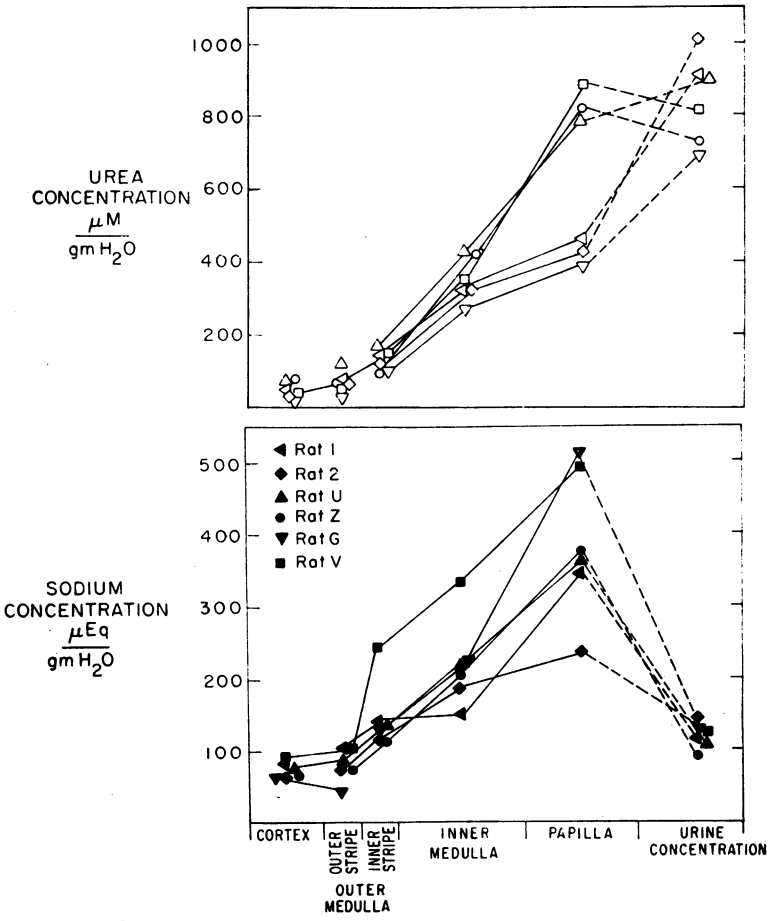

FIG. 2. TISSUE AND URINE ANALYSES ON SIX NORMAL RATS FED A CHOW DIET PRIOR TO URINE COLLECTION AND SACRIFICE. The upper frame contains the data for urea in tissue and urine, the lower frame the corresponding data for sodium. The concentration in μEq per g of water or per ml is plotted against the position of the tissue slice from which the analyses were obtained.

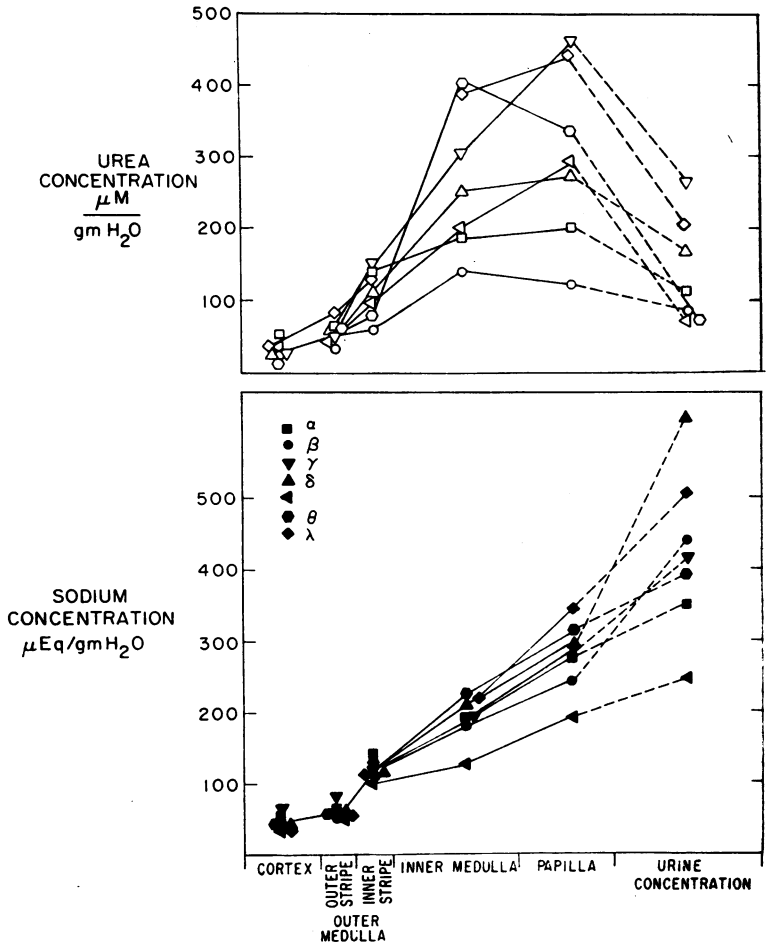

FIG. 3. TISSUE AND URINE ANALYSES FROM SEVEN PROTEIN-DEPLETED RATS. The upper frame contains the urea data, the lower frame the sodium data. The tissue concentrations per g of water are plotted against the position of the slice from which they were obtained.

crease in the tissue sodium concentration between the cortex and papilla, and in all cases the urine sodium concentration is less than the papillary sodium concentration. The tissue urea concentration also rises progressively from the cortex through the papilla. In all of these rats the urine urea concentrations are as high as or higher than the papillary urea concentration. A similar group of experiments was performed on six normal rats given vasopressin with essentially the same results.

The results of analyses of tissues from the rats on the low protein diet without urea supplements are shown in Figure 3. The rise in sodium concentration from cortex to papilla resembles that of normal rats, but in contrast with the previous analyses from normal rats, the urine sodium concentration is higher than the tissue sodium concentration. The tissue urea again shows the rise in concentration from cortex to inner medulla but only a small rise from inner medulla to papilla. In two rats the urea concentration in the inner medulla is actually higher than the papillary urea concentration. Even more striking is the fact that the urine urea concentrations are less than the papillary urea concentrations in all seven animals. The implication of these data and data from 16 other protein-depleted rats, which showed essentially the same findings, is that the rat on a low protein diet is able to accumulate urea in the medulla at a concentration up to two or three times greater than that in the urine.

TABLE II

Urea concentration in the urine and papilla of rats maintained on a low protein diet

Rat	Urine* flow	Urine urea	Papillary urea	Papillary urea	Papilla / Urine	Infusion Rate	Infusion Duration
	ml/hr	*μmoles/ml*	*μmoles/ g wet wt*	*μmoles/ g H_2O*		*ml/hr*	*min*
Group I, no infusion							
P-1	0.52	74	58	72	0.97		
P-2	0.50	76	70	84	1.11		
P-3	0.12	48	68	84	1.75		
P-5	0.60	45	83	104	2.34		
P-6	0.45	39	71	88	2.25		
P-7	0.50	133	151	189	1.42		
P-9	0.06	323	399	499	1.55		
P-10	0.12	85	102	128	1.50		
				Average	1.61		
Group II, 15% mannitol infusion							
P-13	0.12	43	107	134	3.10	0.25	30
P-14	0.14	44	95	119	2.71	0.25	30
P-15	0.18	104	140	174	1.68	0.25	30
P-16	0.12	8	86	107	13.2 †	0.25	30
P-17	0.16	60	65	81	1.36	0.25	30
P-18	0.40	33	64	80	2.47	0.25	30
P-19	2.8	28	47	59	2.08	4.8	100
P-20	2.6	21	47	58	2.80	4.8	100
				Average	2.31		

* Urine collected from catheter in ureter; flow from one kidney.

† Because of the aberrant value for urine urea concentration, data from this rat have been omitted from calculation of the averages.

In order to make certain that the urea concentrations found in papilla and medulla represented those present at the time that the urine was formed, urine was collected directly from the catheterized ureter of anesthetized (ether) rats. This procedure also served to exclude significant losses of urea during flow through ureter and bladder. When relative stability of urine flow and urea con-

TABLE III

Urea concentration in the urine and papilla of rats maintained on a chow diet and infused with 15% mannitol

Rat	Urine* flow	Urine urea	Papillary urea	Papillary urea	Papilla / Urine	Infusion Rate	Infusion Duration
	ml/hr	*μmoles/ml*	*μmoles/ g wet wt*	*μmoles/ g H_2O*		*ml/hr*	*min*
S-1	0.08	939	454	545	0.58	0.25	30
S-3	0.12	1,252	493	617	0.49	0.25	30
S-4	0.06	908	557	696	0.77	0.25	30
S-5	0.60	167	149	187	1.12	0.40	150
S-6	0.30	481	363	454	0.94	0.40	180
S-7	0.18	904	496	620	0.69	0.40	150
S-8	0.10	1,004	515	644	0.64	0.40	135
S-9	0.34	307	170	212	0.69	0.86	120
S-10	0.94	155	138	172	1.11	2.8	90
S-11	2.2	70	61	76	1.09	2.8	105
S-12	2.4	48	60	75	1.58	4.8	90
S-13	2.8	38	58	73	1.90	4.8	180
S-14	3.2	39	51	64	1.64	4.8	180
S-15	2.4	52	55	68	1.32	4.8	195
S-16	3.4	45	49†	61	1.34	4.8	210

* Flow from one kidney. Urine collected from catheterized ureter in ether-anesthetized animal.

† Value used is urea concentration in inner medulla. Papillary urea concentration = 36.0 μmoles per g wet weight.

centration had been established by successive urine collections, the animals were sacrificed and tissue analyses carried out as described above.

The results of experiments in rats maintained on a low protein, high salt diet are presented in Table II. Group 1 (Rats P-1 to P-10) received no infusion prior to collection of the urine. In all but one of the rats in this group, the urea concentration in the papilla was higher than that in the urine, with an average concentration ratio of 1.61. These findings establish more conclusively that, in the rat kidney, urea may be accumulated at a higher concentration in the medullary tissue than in the urine. Rats in group 2 were studied at two rates of mannitol infusion. In all rats in this group the tissue urea concentration was higher than the urea concentration in the urine, with an average papilla to urine ratio of 2.31 and a range of 1.36 to 3.10 (excluding a single aberrant value of 13.2).

The ability of the kidneys of rats on a low protein diet to maintain a significant papilla to urine gradient for urea during mannitol diuresis led to a series of experiments in which mannitol was infused into normal rats to test whether the low protein diet was essential to this phenomenon or whether a reduced urea concentration of the urine was itself the determining factor. Sixteen rats that had been fed a chow diet were anesthetized with ether, and one ureter was catheterized. They were infused with 15 per cent mannitol at rates varying from 0.25 to 4.8 ml per hour for periods varying from 30 to 210 minutes before urine was collected and the animals sacrificed for the performance of tissue analyses. The results are presented in Table III. As the rate of mannitol infusion was increased, the urinary urea concentration fell and, with the fall in urine urea concentration, the papillary urea concentration became equal to or higher than the urinary urea concentrations. In Rats S-5 and S-10 to S-16 in which the urea concentration of the papilla exceeded that in the urine, the urea concentration in the urine was less than 170 μmoles per ml.

DISCUSSION

The possibility that urea might be actively transported in the mammalian kidney has been discussed in detail by Schmidt-Nielsen (16) but direct evidence has been limited to the work in sheep. Studies of kidney slices from sheep which have been maintained on a low protein diet (1.9 per cent) show a higher urea concentration in the medulla than in the urine (25). The highest urea concentration in kidney slices from these sheep was regularly found in the inner zone of the outer medulla. The urea concentration in the inner medulla was always lower than in the outer medulla but still higher than in the urine. Schmidt-Nielsen and O'Dell (25) have suggested that urea might be actively transported by the thick portion of the ascending limb of Henle's loop and that the collecting duct of the sheep might be impermeable to urea. The findings in the rat differ from those of Schmidt-Nielsen and O'Dell in the sheep in several respects: in the rat, the highest tissue urea concentrations were found in the papilla or inner medulla, and the concentrations in both of these regions were higher than in the inner stripe of the outer medulla; and the urea concentration in the inner stripe of the outer medulla was usually lower than that in the urine.

There are two mechanisms by which the urea concentration in the medullary tissue might become higher than the urine urea concentration. The first of these involves transport of urea by the loop of Henle. The work of Wirz, Hargitay and Kuhn (26–28), Gottschalk and Mylle (29), and Berliner and co-workers (12) has focused attention on the importance of the countercurrent multiplier of the loop of Henle in producing hypertonicity in the medulla and consequently in the urine. The over-all effect of such a process is to deposit solute without water in the renal medulla by the active transport of sodium out of the loop of Henle and to remove the excess water as hypotonic fluid in the loop of Henle (27, 29, 30). Under most circumstances sodium is believed to be the solute which is actively transported in the loop. It is conceivable that urea could be actively transported by the loop of Henle and its concentration multiplied by the countercurrent flow in this structure.

The micropuncture studies of Lassiter, Gottschalk and Mylle (31) appear to eliminate the loop of Henle as the site of active urea transport. They have shown that the tubular fluid to plasma ratio for urea in the proximal tubule is 1.5 and for inulin 3.0. In the first portion of the distal tu-

bule, this ratio for urea is 7.7 and for inulin 6.9. Thus, in the normal rat kidney more urea leaves the medulla in the loop of Henle than enters from the proximal tubule, and this excludes a countercurrent multiplication of urea concentration by the loop as primarily responsible for the accumulation of urea under the conditons of their study.

The other structure that might transport urea is the collecting duct. Most of the evidence on the role of urea in the medullary concentrating mechanism in the dog implies that during antidiuresis, the collecting duct is permeable to urea, and that urea accumulates along a concentration gradient created by the removal of water from the collecting duct. Jaenike (13) has shown that, in the dog under the influence of vasopressin, the permeability of the collecting duct to urea is increased. However, in the experiments reported here the collecting duct of the rat on a low protein diet appears to be relatively impermeable to urea. In normal rats on a chow diet the urine urea concentration is invariably higher than the urea concentration in the papilla. Under these circumstances the medullary urea might rise by passive diffusion from the collecting duct. However, the infusion of 15 per cent mannitol into normal rats will lower the urea concentration in the urine and bring out the ability of the papilla to concentrate urea. Unless the permeability of the collecting duct changes during the infusion of mannitol, it would be reasonable to assume that the collecting duct of the rat is impermeable to urea on both normal and low protein diets. Thus, under most conditions, urea appears to enter the medulla by a carrier-mediated process of facilitated diffusion. Only by lowering the urea concentration in the collecting duct sufficiently would it be possible to bring out the ability of the rat kidney to concentrate urea.

Transport of urea across the collecting duct from urine to peritubular space would operate in two ways to concentrate the urine: *1*) by reducing the volume of fluid remaining in the collecting duct, and *2*) by raising the medullary urea concentration above that in the collecting duct. When the concentration of urea in the collecting duct is higher than that in the peritubular space, the effect of urea transport would be identical with that which could be achieved by the passive diffusion of urea, as in the mechanism proposed by Berliner and associates (12) and Jaenike (13). When the concentration of urea in the collecting duct is equal to that in the medullary interstitium, active transport of urea would raise the interstitial urea concentration at the expense of that in the urine. This would increase the concentration of non-urea solute in the urine in accord with the observation of Crawford and co-workers (21) but would not yield a further increase in total urine concentration. Furthermore, the reduction in urine volume resulting from urea transport would make possible a greater increase in urine concentration from a given amount of sodium transported by the loops.

The urea gradient that can be achieved between medullary interstitium and collecting duct fluid should depend upon a number of factors: *1*) the rate of transport of urea from tubular fluid to peritubular space; *2*) the rate of diffusion of urea from medulla back into the collecting duct; *3*) the rate at which urea is lost from the medulla by the countercurrent flow in the blood vessels and in the loop of Henle; and *4*) the proportion of non-urea solute in the urine. A high permeability of the collecting duct to water and a relative impermeability to urea assumed, the ability to demonstrate urea accumulation by the medulla will depend upon the rate at which urea is removed from the collecting duct, and the proportion of non-urea solute in the collecting duct. In the experiments with rats on a low protein diet where sodium chloride accounted for most of the solute in the collecting duct, or in the experiments with mannitol infusions in which mannitol was the principal solute, a low rate of urea transport by the collecting duct would have become apparent as a higher concentration of urea in the medulla than in the urine. In experiments where urea comprised a larger proportion of the solute in the collecting duct, a gradient for urea could not be demonstrated between medulla and urine, presumably because the rate of urea transport by the collecting duct was too low relative to the amount of urea in the collecting duct.

Klümper, Ullrich and Hilger (32) have demonstrated that urea is lost from the collecting duct of the golden hamster during antidiuresis. Although consistent with passive movement of urea

from collecting duct to interstitium, the observations reported here on the rat, and those of Lassiter and associates (31), introduce the possibility that the movement of urea out of the collecting duct of the hamster is due to active transport.

All micropuncture studies in mammals have used rodents as the experimental animal, but the data so obtained have been applied to other mammalian species under the assumption that the kidneys of all species behave in a similar way. The observations presented here which support the concept that urea is actively transported by the collecting duct of the rat differ from observations under similar conditions in the dog in which urea is thought to accumulate in the inner medulla by passive diffusion from the collecting duct. Such findings re-emphasize the need for caution in generalizing, to dogs or other species, micropuncture data obtained from rodents.

SUMMARY

Rats on a low protein, high salt diet receiving supplementary urea produced urine containing a significantly higher non-urea solute concentration than did rats on a similar diet but without urea supplements. The concentration of urea in the papilla and inner medulla from these and other rats on a low protein, high salt diet was as much as three times the concentration of urea in 24-hour urine collections. Experiments with normal and protein-depleted rats, in which urine was collected from a single ureter under ether anesthesia, further showed that the demonstration of urea accumulation in the medulla of the rat appears to be dependent upon the presence of a low urinary urea concentration and, hence, a considerable proportion of non-urea solute. The results of these experiments indicate that: *1*) urea supplements to a low protein, high salt diet increase the concentration of non-urea solute in rat urine; *2*) the rat kidney is able to concentrate urea in the medulla, presumably by an active transport mechanism located in the collecting duct; and *3*) under the conditions of these experiments the collecting duct of the rat must be relatively impermeable to urea.

ACKNOWLEDGMENT

The authors wish to thank Dr. Georg Peters for his helpful suggestions and Dr. Robert W. Berliner for his advice, suggestions and helpful criticisms throughout the course of this investigation.

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CORRECTION

On page 1287 of the article entitled "Glucagon Antibodies and an Immunoassay for Glucagon" by Roger H. Unger, Anna M. Eisentraut, M. S. McCall and Leonard L. Madison (J. clin. Invest. 1961, **40**, 1280), the units $\mu\mu$equivalents and μEq are incorrect in Table III and in the text below. In the table, the unit of measurement for B/F ratios and glucagon concentrations should be $\mu\mu$g equivalents per ml and the total glucagon content of organ at the bottom of column 1 should be in μg equivalents. In the text, lines 7–12 should read: "The estimated glucagon concentrations are expressed in micromicrogram equivalents of beef-pork glucagon per milliliter. Calculations of the total glucagon content of pancreas of both species range from 12.5 to 36.3 μg Eq of beef-pork glucagon."