Evidence of Restricted Permeability to Urea in Rat Distal Tubule.* (32216)

GASPAR CARRASQUER,† SIDNEY SOLOMON, AND HARALD SONNENBERG
Department of Medicine, University of Louisville, School of Medicine, Louisville, Ky., and Department of Physiology, University of New Mexico School of Medicine, Albuquerque, N.M.

Although urea is generally considered to be a highly permeant non-electrolyte, several lines of evidence have been produced to indicate that the distal part of the nephron has a restricted permeability to urea. The urea concentration of late distal samples collected by micropuncture of rat nephrons has been found to be highly variable, and it was suggested that the variability might be a reflection of varying degrees of permeability (1). When radio-inulin and radio-urea were injected into nephron segments, recovery in the urine was higher with distal tubular injection than with proximal(2). In the former study, interpretation is clouded by the fact that urea is added to the tubular fluid at the level of the thin loop of Henle(1). In addition, differences in contact time may contribute to explaining the results found in both studies. The studies of Capek *et al*(3) were of a more direct nature. These workers found that the permeability of the distal tubule to urea was 20 times less than the proximal tubule. The authors did not comment, however, on the physiological meaning of this observation and, in particular, did not evaluate if the reduced distal impermeability was low enough to account for accumulation of urea in distal samples.

In dogs, the renal tissue concentration of urea is increased during ureteral obstruction, but only in experiments characterized by high levels of stop-flow filtration and low medullary concentration of urea (*i.e.*, NaCl diuresis). The tissue concentration approached the free-flow levels shortly after release of the obstruction(4). In these experiments, the changes in concentration of urea followed the same pattern as those of creatinine. It was suggested that the stop-flow increase in concentration of urea could be due to stop-flow filtration and intraluminal trapping in the loops of Henle and distal convoluted tubules.

Intraluminal trapping during stop-flow would imply that the tubule would offer a restricted permeability to urea. Therefore, experiments using the micropuncture technique in rats were designed to test the hypothesis that the increase in the cortical concentration of urea is due to its intraluminal trapping in the distal tubules.

Methods. Following 24 hours of water and food deprivation, Sprague-Dawley rats (ca. 250 g) were anesthetized with Inaktin (100 mg/kg). The left kidney was exposed for micropuncture, its ureter was catheterized, and it was mounted in a lucite cup by conventional methods. C^{14}-urea (50 or 100 μc) and H^3-inulin (250 μc) were injected intramuscularly. Na^{22} (300 μc) was also injected in some experiments.

Osmotic diuresis was induced by intravenous infusion of 1.0 M mannitol or 0.5 M NaCl at a rate of approximately 6 ml/hr. The radioactive substances were administered as soon as a diuretic response was established in the exposed kidney. Almost steady-state plasma levels of these substances were achieved 30-40 minutes after injection.

Distal tubules were identified by intravenous injection of Lissamine Green(5). Following collection of 2 to 6 samples from the distal tubules by micropuncture technique, the ureter was clamped. Pooled samples (see below) were obtained for 50-80 minutes. The obstruction was then released and 2 to 6 more samples were collected. No attempt was made to localize the site of puncture.

The possibility that distal fluid was contaminated by retrograde flow from collecting ducts and pelvis during the period of ure-

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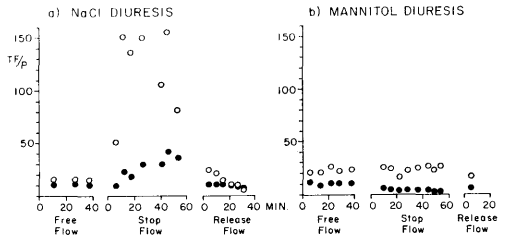

FIG. 1. Distal tubule fluid to plasma concentration ratios from a) one rat in NaCl diuresis and b) one rat in mannitol diuresis. Open circles = H^3-inulin; dots = C^{14}-urea. The time on the abscissa is: a) free-flow = *vs* time of injection; and b) stop-flow = *vs* time of obstruction; and c) release-flow = *vs* time of release of obstruction.

teral obstruction is considered unlikely because of the following: 1) When a tubule is punctured and collections are made, two phases of collection rate are seen; a very fast early phase followed by a slower phase. These two phases probably represent the rapid filling of the collection pipette by fluid trapped in the distal part of the nephron followed by delivery resulting from renewed filtration. Had the collection been retrograde, one would expect a constant rate of fluid collection. Only the early gush of fluid was collected. In order to get enough fluid for analysis, more than one tubule was punctured. 2) In mannitol loading experiments, it will be seen that $^{TF}/P$ of urea is less than that found in free-flow tubular fluid; a retrograde collection would lead to a change of $^{TF}/P$ in the opposite direction.

Two to three samples of urine were collected preceding ureteral obstruction and also following release of obstruction. Blood samples were obtained between each pair of micropuncture samples. C^{14} and H^3 were determined by differential counting in a liquid scintillation counter. Na^{22} was determined in a well-type scintillation counter.

Results. Sodium chloride diuresis. Fig. 1-a shows data obtained from one selected experiment in sodium chloride diuresis. The pre-obstruction urine flow was 2.0 ml/hr. The zero free-flow time represents the time of administration of the radioactive isotopes; the stop-flow time and release-flow time are presented in reference to time of obstruction and time of release of obstruction, respectively.

A marked increase in the fluid to plasma ($^{TF}/P$) ratios of H^3-inulin (open circles) is observed following ureteral obstruction, returning to free-flow levels following release of the obstruction. The change in the $^{TF}/P$ ratios of C^{14}-urea (dots) paralleled the change of the ratios of H^3-inulin. While the H^3-inulin increased about 10-fold during the period of obstruction, the C^{14}-urea ratios increased only 4-fold.

Table I presents compiled data obtained by micropuncture from 10 experiments in NaCl diuresis. The mean urine flow was 3.0 ml per hour (SEM = ± 0.4) in the exposed kidney. The values included in each

TABLE I. Effect of Ureteral Obstruction and Release of the Obstruction on Distal Tubule Inulin and Urea in Sodium Chloride Diuresis.

Rat	H^3-inulin F.F.	H^3-inulin St.F.	H^3-inulin R.F.	C^{14}-urea F.F.	C^{14}-urea St.F.	C^{14}-urea R.F.
A	5.0 (2)	102.9 (3)		3.1 (2)	8.8 (3)	
B	16.1 (2)	28.2 (1)		17.6 (2)	8.9 (1)	
C	2.3 (1)	31.6 (4)		2.1 (1)	10.7 (4)	
F	16.6 (2)	131.6 (2)		8.3 (2)	16.9 (2)	
G	5.8 (2)	98.4 (5)	6.2 (2)	5.7 (2)	13.7 (5)	8.6 (2)
H	2.4 (2)	27.3 (7)		3.5 (2)	14.8 (7)	
I	5.2 (4)	68.7 (9)	2.5 (2)	6.4 (4)	11.9 (9)	1.2 (2)
J	5.4 (5)	61.0 (10)		6.8 (5)	17.9 (10)	
O	16.8 (6)	60.2 (8)	10.4 (6)	8.7 (6)	15.9 (10)	9.3 (6)
Q	14.2 (3)	136.0 (9)	15.0 (6)	10.6 (3)	26.8 (9)	10.4 (6)
Rat mean	9.0 ± 1.9	74.6 ± 12.9	8.9 ± 2.3	7.3 ± 1.4	14.6 ± 1.7	7.4 ± 2.1
Sample mean	9.8 ± 1.3	74.9 ± 8.2	10.6 ± 1.8	7.7 ± .9	16.2 ± 1.1	8.6 ± .9

F.F. = free-flow period; St.F. = stop-flow period; R.F. = release-flow period.

Values are the fluid to plasma concentration ratios. The values on the rows headed by a letter are means of samples obtained during experimental period and number of samples. Rat mean = the mean of the means from each rat. Sample mean = the mean of all the samples collected during the experimental period.

TABLE II. Effect of Ureteral Obstruction and Release of the Obstruction on Distal Tubule Inulin and Urea in Mannitol Diuresis.

Rat	H^3-inulin			C^{14}-urea		
	F.F.	St.F.	R.F.	F.F.	St.F.	R.F.
D	6.6 (2)	19.6 (2)		3.5 (2)	1.8 (2)	
E	14.5 (1)	18.5 (4)		5.9 (1)	5.6 (4)	
K	9.6 (2)	9.4 (9)	9.2 (4)	6.8 (2)	2.4 (9)	3.2 (3)
L	21.6 (4)	17.2 (8)	7.8 (6)	11.7 (4)	6.8 (8)	3.9 (6)
P	23.3 (6)	21.6 (9)	17.4 (1)	10.7 (6)	3.7 (9)	5.6 (1)
Rat mean	15.1 ± 3.3	17.3 ± 2.0	11.5 ± 2.9	7.7 ± 1.6	4.1 ± .9	4.2 ± .8
Sample mean	18.2 ± 1.8	16.6 ± 1.2	9.2 ± 1.3	9.2 ± .9	4.2 ± .5	3.9 ± .2

Abbreviations and values same as in Table I.

of the 3 columns are the mean values of the fluid to plasma concentration ratios for the samples collected from each rat, before stop-flow, during stop-flow, and following release of the ureteral obstruction.

The "rat mean" was obtained from the mean value of each rat. The "sample mean" is the mean of all the samples obtained during the given experimental period.

The tubular fluid to plasma concentration ratios of H^3-inulin and C^{14}-urea were consistently higher in the samples obtained during the period of ureteral obstruction than in the preobstructive sample or postobstructive samples ($p<0.001$). The fluid to plasma concentration ratios of the preobstructive samples were not significantly different from those of the postobstructive samples.

The values of the $^{TF}/P$ ratios of Na^{22} were not changed significantly by stopping the ureteral flow or by releasing the obstruction in the two rats that were injected with Na^{22}.

Mannitol diuresis. Fig. 1-b presents data obtained from one selected experiment in mannitol diuresis. The preobstructive urine flow was 2.8 ml/hr.

No appreciable change in the $^{TF}/P$ ratios of H^3-inulin is observed during obstruction of the ureter or after release of the obstruction in the rat loaded with mannitol. An appreciable fall in the $^{TF}/P$ ratios of C^{14}-urea is observed during the period of obstruction.

Table II presents compiled data obtained from 5 experiments in mannitol diuresis. The mean urine flow was 4.1 ml per hour (SEM = ± 0.8) in the exposed kidney.

The $^{TF}/P$ ratios of H^3-inulin were not affected by stop-flow but a significant decrease in the ratios was observed following release of obstruction. This change is statistically significant when comparing the sample means ($p<0.001$). The $^{TF}/P$ ratios of C^{14}-urea decreased significantly during stop-flow ($p<0.05$),‡ remaining at the stop-flow level following release of the obstruction.

Discussion. How do the concentrations of H^3-inulin and C^{14}-urea increase in the distal tubules during stop-flow in sodium chloride loaded rats? At least the following possibilities exist: a) the substance could be secreted from the interstitium into the lumen, or b) shrinkage of the tubules due to water reabsorption, or c) stop-flow filtration following distal water reabsorption.

a) The fact that neither of these substances accumulated in the distal tubule during ureteral obstruction in the rats loaded with mannitol, rules out the secretory mechanism, unless an *ad hoc* assumption is made that secretion is inhibited by mannitol. This is unlikely because other substances (*i.e.,* PAH) are secreted by the renal tubule cells in the presence of mannitol and no evidence can be found in the literature for inulin secretion. If the *ad hoc* assumption of mannitol inhibition were limited to urea, this would still be unlikely since the increase in $^{TF}/P$ concentration ratios of C^{14}-urea were never higher than 50% of the increase of the concentration ratios of H^3-inulin.

b) The possibility that the tubules shrink during stop-flow can be easily ruled out since gross observation of the tubules showed that their diameter increased during the period of ureteral obstruction.

‡ A student's t-test using paired observations was applied in this case.

c) The third possibility is the most likely since reabsorption of water should depend on NaCl reabsorption. As a result of the reabsorption of water, a hydrostatic pressure difference may be produced between the site of reabsorption and the glomerulus. An increase in the concentration of non-reabsorbable substances (*i.e.*, H^3-inulin) would take place along the nephron proximal to the region where water is reabsorbed. In mannitol loaded rats the concentration of sodium chloride in the distal tubule and collecting duct is so low that no further reabsorption would take place during the period of ureteral obstruction. Therefore, the pre-stop-flow luminal fluid would remain there and the stop-flow filtration would be reduced to a minimum. In sodium chloride loaded rats the concentration of the salt is high even at the end of the collecting ducts. Therefore, relatively large amounts of sodium chloride and water continue to be reabsorbed during the period of ureteral obstruction. This would induce a relatively large movement of stop-flow filtrate to be carried out to the distal tubule, with the result of an increase in the concentration of non-reabsorbable substances as more sodium chloride and water are reabsorbed.

It can be deduced that the distal tubules offer a restricted permeability to urea regardless of which of the 3 possible mechanisms is operative. Nine of the samples reached ratios with values higher than 25. Assuming that the specific activity of urea was uniform in the lumen, renal cortex plasma, and in systemic plasma, concentration gradients of this magnitude across the tubule cell would be difficult to explain if the cells were readily permeable to urea. The increase in the C^{14}-urea concentration observed in the distal tubule seems to take place only in the lumen since as soon as the obstruction was released its concentration fell down to free-flow levels as fast or faster than the concentration of H^3-inulin (Fig. 1).

How can one relate the hypothesis of restricted permeability to C^{14}-urea to findings in mannitol loaded rats? In these experiments the $^{TF}/P$ ratios of C^{14}-urea decreased during stop-flow without further change following release of the obstruction. While the $^{TF}/P$ ratios of H^3-inulin were not changed by stop-flow they were reduced following release of the obstruction.

Perhaps in mannitol loaded rats there is a finite stop-flow filtration not due to sodium chloride and water reabsorption but due to distension of the tubules. The distal tubules could be supplied with extra inulin and perhaps somewhat diluted urea due to a more complete reabsorption of urea from the proximal tubules. As soon as the flow is released the distal tubules would remain distended for a while. This would explain the fall in concentration of H^3-inulin perhaps due to an influx of water from the peritubular space, or to a postobstructive supply from the proximal nephron of diluted H^3-inulin. In fact this would explain the fact that in dogs there is an increase in the tissue concentration of creatinine during stop-flow(7) while the tissue concentration of creatinine is not changed shortly after release of the obstruction(4).

Perhaps the distal tubule offers a finite permeability. The small leakage of urea could be higher than the low supply during stop-flow in mannitol loaded rats resulting in a net decrease in intraluminal concentration. The leak of urea would be lower than the stop-flow supply in NaCl loaded rats in order for the $^{TF}/P$ ratios of C^{14}-urea to increase. In any event if a finite permeability to urea exists in the distal tubules it has to be very low.

Support of the hypothesis of restricted permeability to urea in distal tubules may be inferred from other micropuncture studies (1,2,3). In addition, Lassiter *et al*(6) found a lack of movement of C^{14}-urea across the wall of the nephron beyond the proximal tubule in rats loaded with NaCl. They explained these results on the basis that small concentration gradients existed between the lumen of the nephron and surrounding tissue. These data are also consistent with the alternative hypothesis that the nephron offers a restricted permeability to the urea in regions beyond the proximal convoluted tubule including the distal tubule.

In essence, an increase in the $^{TF}/P$ ratios of C^{14}-urea in the distal tubules of NaCl

loaded rats, induced by ureteral obstruction, and accompanied by an increase of H^3-inulin ratios without change in the Na^{22} ratios is very strongly suggestive that C^{14}-urea is trapped in the lumen of the distal tubule, together with H^3-inulin, while Na^{22} is being reabsorbed. This together with the rapid fall in the $^{TF}/P$ ratios of C^{14}-urea (and H^3-inulin) following release of the obstruction is strongly suggestive that urea is retained in the distal tubules because of their restricted permeability. These data suggest then that the restricted permeability of urea is adequate to explain increased distal tubular fluid urea concentrations at least under the conditions of ureteral obstruction.

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Role of Growth Hormone and Dietary Adaptation on Incorporation Of Leucine into a Mitochondrial Protein.* (32217)

PATRICK F. DELANEY AND PAUL F. FENTON
Department of Biology, College of the Holy Cross, Worcester, Mass., and the Division of Biological and Medical Sciences, Brown University, Providence, R. I.

Nitrogen excretion studies on intact animals(1) led us to postulate that one function of pituitary growth hormone might be to speed the adaptation of the organism to the rapid utilization of fats to provide energy for protein synthesis under conditions under which little carbohydrate is available. Increasingly the metabolic effects of hormones are being sought at the subcellular level. Korner(2) has shown that incorporation of labeled amino acids into proteins of nuclei, mitochondria, microscomes and soluble fraction of liver was diminished by hypophysectomy and restored toward normal by growth hormone administration. In more recent work he has concentrated his attention on the growth hormone effect on protein synthesis in the ribosomal system. However, in the light of our observations on the effect of growth hormone on the nitrogen-sparing action of fat, we turned our attention to the well known protein synthesizing system of mitochondria. Since these organelles oxidize both carbohydrates and lipids, they might possibly be a site of adaptive changes which we postulated to occur after high fat feeding and after growth hormone administration.

Methods. Male mice of the C57/Fn strain, 3 to 5 months old, were fed either diet #343 containing 60% fat or diet #307 containing 60% carbohydrate(1) for at least 2 weeks prior to the experiment. Growth hormone,† 10 mg/kg body weight, was injected intraperitoneally 5-5-1/2 hours before the animals were decapitated and exsanguinated. Several livers were pooled and the mitochondria isolated by the method of Truman and Korner (3). The incubation medium was essentially medium B of Truman and Korner using either succinate or octanoate and β-hydroxybutyrate as substrate. When using the latter substrate, the mitochondria were washed 4 times with 0.25 M sucrose and twice with 0.15 M KCl. Each ml of the incubation medium contained the mitochondria from 1 g of liver, 0.5 μc of

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