Permeability of Superficial Proximal Tubules and Loops of Henle to Urea in Rats[1] (35105)

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The distal convoluted tubule has been found to be less permeable to urea than the proximal tubule (1–3). Although the prevailing view (1, 4–6) has been that urea enters the loop of Henle as it is reabsorbed from the collecting duct, we (7) have suggested that the loop of Henle should be relatively impermeable to urea. In the latter case urea could still enter the loops of Henle perhaps by an active mechanism. Recently Morgan and Berliner (8) have found that in the isolated renal papilla of the rat the loops were more permeable to urea than cortical collecting ducts of rabbits by an order of magnitude.

Present experiments were designed to study the relative permeability of the proximal tubules and loops of Henle to urea in the intact kidney of the rat.

Methods. Sprague-Dawley rats (av wt 275 g) were anesthetized with Inactin (90–100 mg/kg). The left kidney was exposed through a flank incision and mounted in a Lucite cup following the conventional micropuncture method. Animals were infused intravenously with isotonic saline at 0.1 ml/min throughout the experiment.

Microperfusions. Proximal tubule. The apparatus and technique of microperfusion of proximal tubules have been described in detail (9). The length of the perfused segment was estimated using the latex cast technique (10).

Loop of Henle. Loops of Henle were perfused from the last surface convolution of the proximal tubule to the first surface convolution of the distal tubule. The last convolution of the proximal tubule on the surface was identified as the one from which the color last disappeared following small injections of perfusate into any of the proximal convolutions. After establishing this, the tubule was perfused for 3–5 min in order to find if that tubule had a corresponding distal convolution on the surface. Usually one out of three selected proximal tubules had at least one distal convolution visible on the surface.

The portion proximal to the site of perfusion was blocked with warm lanolin dyed with Sudan black, in order to provide complete seal and prevent contamination with new filtrate. On terminating the experiment the loops were filled with India ink, as described by Oliver and Walker (11). The kidney was macerated in 50% HCl for 150 min at 45°, perfusion and collection sites were identified, and in some instances the whole loop of Henle was dissected and its length was measured.

The perfusion fluid consisted of isotonic Ringer's solution colored with lissamine green (0.25%) and contained the radioactive isotopes ^{3}H-inulin (125 μCi/ml), ^{14}C-urea (50 μCi/ml), and ^{22}Na (12.5 μCi/ml). Radioactivities in perfusate and collected fluid were determined with a three-channel liquid scintillation counter. Osmolality was determined by microcryoscopy using a Clifton microosmometer.

Results. Proximal tubule. Nineteen tubules were perfused in 11 rats. Figure 1 presents a

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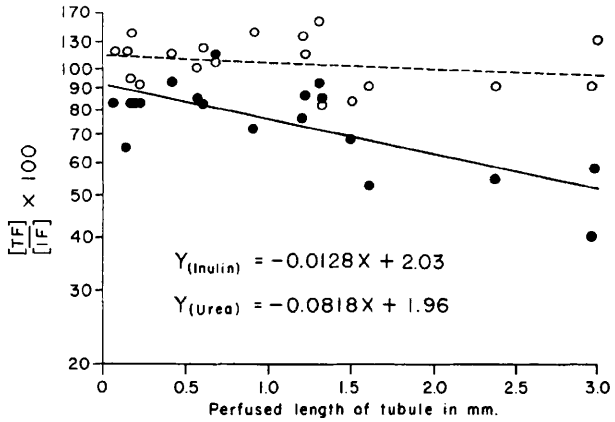

FIG. 1. Proximal tubular reabsorption of urea: *TF* = concentration in withdrawn sample; *IF* = concentration in perfusate; (O - - -) ^{3}H-inulin; (● —) ^{14}C-urea; lines are the calculated regression lines.

semilogarithmic plot of collected-fluid to perfused-fluid concentration ratios (*TF/IF*) of ^{3}H-inulin and ^{14}C-urea versus the length of the perfused tubule. Using linear regression analysis, straight-line relationships were obtained for the two sets of data. Correlation between the logarithm of the concentration ratios and perfused length was statistically significant for ^{14}C-urea ($p < 0.001$) but not for ^{3}H-inulin ($p > 0.40$). While the concentration of inulin did not change the concentration of urea decreased significantly indicating that no net movement of water took place in this experiment while urea was reabsorbed.

In the absence of a net movement of water, the transtubular permeability to urea can be estimated using the following equation derived previously (12):

$$P_{1,2} = \frac{v_l r_l}{2x} \ln \frac{C_i}{C_{l(x)}},$$

where $P_{1,2}$ is the transtubular permeability; v_l is the linear flow velocity; r_l is the radius of the tubule; x is the length of the perfused tubule; C_i is the concentration of the substance in the perfusate; and $C_{l(x)}$ is the concentration of the substance at point x. The value of v_l can be obtained from the perfusion rate (25 nl/min), and from the value of r_l. Assuming a value of r_l of 10^{-3} cm, a value for the permeability to ^{14}C-urea is obtained of 12.5×10^{-4} mm/sec.

Loop of Henle. Seventeen loops were perfused in 7 rats. Table I presents the *TF/IF* ratios of ^{3}H-inulin and ^{14}C-urea. The length of only three loops were accurately measured and they are marked with superscript a. The length values on the other 14 loops have been obtained from the relationship between rat weight–kidney weight–loop length published by Wahl and Schnermann (13). The length of several of these 14 loops was measured in part and none of these partial measurements was below 5 mm in length.

The *TF/IF* ^{3}H-inulin ratios were higher than one in all the collections indicating that water was reabsorbed. The *TF/IF* ratios of ^{14}C-urea were below one, suggesting reabsorption of ^{14}C-urea along the loops of Henle. Most of the *TF/IF* osmolality ratios were also lower than one, indicating that the perfusate was apparently diluted by NaCl reabsorption from the ascending limb of the loop of Henle. For the calculation of the transtubular urea permeability an equation had to be used different than that used for the proximal tubules because of the water reabsorption,

$$P_{1,2} = \frac{V_i}{2\pi r_l x}\left[1 - \frac{I_i}{I_{l(x)}}\right] \times \left[\frac{\log [U_{l(x)}/U_i}{\log [I_i/I_{l(x)}]} + 1\right],$$

where V_i is the perfusion rate (25 nl/min);

TABLE I. Water and Urea Reabsorption, and Change in Osmolality in Loops of Henle.

Rat	Body wt (g)	Length of loop (mm)	[TF]/[IF]			Urea permeability (mm/sec × 10^4)
			^{3}H-Inulin	^{14}C-Urea	Osmolality	
A	242	6.3	1.70	0.54	—	10.4
B	243	6.3	2.09	0.51	—	11.2
C	374	9.0[a]	1.19	0.38	—	9.9
		8.3	1.52	0.27	—	13.3
		8.5[a]	1.61	0.29	1.13	12.9
		8.3	1.47	0.46	0.92	9.1
D	349	8.0	1.57	0.34	0.74	12.1
		8.0	1.52	0.20	0.64	16.4
E	341	7.9	1.43	0.62	0.65	7.0
		7.9	1.35	0.44	0.79	9.7
		7.7[a]	1.67	0.50	0.52	11.5
F	277	6.7	1.19	0.34	0.80	10.3
		6.7	1.08	0.28	0.72	14.1
		6.7	1.44	0.49	1.05	9.9
G	271	6.6	1.19	0.57	0.59	7.6
		6.6	1.40	0.92	0.67	4.0
		6.6	1.27	0.65	0.74	6.7
	Mean		1.45	0.46	0.77	10.4
	SEM		±0.06	±0.04	±0.05	±0.7

[a] Measured lengths of loops. Other length values obtained from Wahl and Schnermann (13) (see text). *TF* and *IF* as in Fig. 1. The values in each row are obtained from microperfusion of one loop. Two or more loops were perfused in 5 rats.

r_l is the mean radius of the loop; x is the length of the loop; I and U are the ^{3}H-inulin and ^{14}C-urea concentrations respectively; subscript i refers to perfusate; and subscript $l^{(x)}$ refers to collected fluid at point x. This equation has been adapted from Curran and Solomon (14) and it is analogous to the equation used by Grantham and Burg (15) and Morgan and Berliner (8).

The values on permeability to ^{14}C-urea presented in Table I were obtained by assuming a mean loop radius (r_l) of 9×10^{-4} cm (8). The mean permeability was 10.4×10^{-4} (SEM $\pm$ 0.7×10^{-4}) mm/sec.

Discussion. Microperfusion studies in the intact rat kidney by Capek *et al.* (2) have shown that the proximal convoluted tubules are about 20 times more permeable to urea than the distal convoluted tubules. Grantham and Burg (15) found that the permeability to urea of the isolated cortical collecting ducts of rabbits was about the same as that found by Capek *et al.* in distal convoluted tubules of rats (about 10^{-4} mm/sec). Morgan and Berliner (8) have found that the permeability to both branches of the loop of Henle and collecting duct in the isolated renal papilla of the rat was about an order of magnitude higher than the permeability in distal convoluted tubules or cortical collecting ducts.

Present experiments were designed to study the permeability to urea of both the proximal convoluted tubules and loops of Henle in the intact rat kidney. In essence, the findings are similar to those previously reported, especially in reference to the loops of Henle. Thus, our calculated values are 10.5×10^{-4} mm/sec and those of Morgan and Berliner were 13–14 $\times 10^{-4}$ mm/sec. Our values for proximal convoluted tubules (12.5×10^{-4} mm/sec) are somewhat lower than those obtained by Capek *et al.* (19.1×10^{-4} mm/sec). In any event it seems that the permeability of the loops of Henle to urea is at least within the same order of

magnitude as that of the proximal convolutions.

How can we explain the small discrepancy of our findings with those of Capek? Dörge and Nagel (16) have found that addition of lissamine green (10 mg/100 ml or higher) to the epithelial bath medium of the isolated frog skin inhibits the active transport of sodium irreversibly. In the present experiments the net transport of sodium was inhibited in the proximal tubule as shown by the fact that the inulin concentration did not change along the perfused tubule. The lack of fluid reabsorption in perfused proximal tubules may be due to the concentration of lissamine green used, since perfusion with fluid of the same sodium concentration (145 mEq/liter) and osmolality (298 mOsm/kg), but without the dye, results in normal reabsorption (unpublished observation). On the other hand the effect of lissamine green, if present, was not as evident in the loops of Henle, since the inulin concentration increased in all perfused loops. It is possible that lissamine green decreases the permeability of the proximal tubule to urea which would explain the discrepancy of our findings with those of Capek *et al.* (2), who did not use the dye in their perfusates. This would be strongly suggested if the passive permeability to Na were affected by lissamine green, and this could be possible because of the lack in net water reabsorption which usually depends on the net reabsorption of NaCl. But ^{22}Na was present in the perfusate of all our experiments and the unidirectional permeability for Na, calculated in the same way as that for ^{14}C-urea gave values of 75.4×10^{-4} and 25.5×10^{-4} mm/sec or proximal convolutions and loops of Henle, respectively. Therefore it seems that the diffusivity of Na was higher in proximal tubules than in the loops of Henle, suggesting that if lissamine green affected the sodium transport in the proximal tubule it probably was at the level of the active pump, and not at the level of the passive movement.

Conclusion. The permeability of proximal convolutions and loops of Henle to ^{14}C-urea has been studied using the microperfusion technique in the intact rat kidney. The permeability coefficient of both structures is about the same, 12.5×10^{-4} and 10.4×10^{-4} mm/sec, respectively, and these values are very close to those obtained previously by other authors.

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