Site of Uptake of Nonfiltered Amino Acid in the Rabbit Kidney (42990)

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Abstract. **During passage from renal artery to vein, nonfiltered amino acids are known to be extracted by the kidney, an observation generally attributed to their basolateral uptake by tubular epithelium. This attribution is here tested in the rabbit, using the nonmetabolizable analogue cycloleucine as test compound. Uptake of cycloleucine is diffusion limited and could be maximized by lengthening its artery-to-vein transit time by short aortic occlusion. The transient anoxia did not abolish active solute transport in the kidney; the technique permits acute loading of the kidney with, for instance, a nephrotoxicant without excessive exposure of the whole animal. The major portion of cycloleucine taken up by nonfiltering kidneys during occlusion returned to renal venous plasma with a mean delay of 45 sec, as if it had accumulated in the same cellular transport pool through which reabsorbed cycloleucine has to pass. A fraction of the amino acid taken up also reached the tubular lumen. These results support the suggested role of tubule cells in the extraction of amino acids from postglomerular blood.** [P.S.E.B.M. 1990, Vol 193]

Silverman *et al.* (1) reported in 1970 that the recovery of certain monosaccharides in renal venous plasma, following their bolus injection along with creatinine into the renal artery of dogs, at first falls below that of the glomerular marker. Only in later fractions, when filtered and reabsorbed sugar reaches venous blood, does recovery of the sugar exceed that of the reference compound. The early venous deficit of the sugars was ascribed to their structure-specific transfer from postglomerular blood across basolateral membranes into tubule cells. Similar studies were subsequently reported on removal of nonfiltered amino acids from blood plasma in the rabbit kidney (2). In neither case, however, was direct evidence provided to support the suggested role of tubule cells in producing the venous deficit.

That tubule cells can actively extract amino acids from the peritubular medium was demonstrated in isolated and perfused proximal tubules from the rabbit (3) and in electrophysiologic studies on the rat kidney *in situ* (4). Furthermore, movement of amino acids across the basolateral cell membranes in the opposite direction, i.e., amino acid extrusion from the cells, has also been shown to be a mediated process (5).

In this work, attempts were made to evaluate the likelihood that the same cells are responsible for both reabsorption of amino acids from lumen to blood and their early venous deficits following arterial bolus injections. These experiments required maximizing the venous deficit, a result achieved by transiently trapping the bolus in the kidney. Such a technique is potentially useful in studies on acute renal intoxication.

Materials and Methods

The experiments were conducted with male New Zealand White rabbits (average body weight 2.5 kg) that had been maintained on commercial rabbit food (Purina) and tap water *ad libitum*. They were prepared for study by induction of anesthesia with pentobarbital sodium (Nembutal), followed by tracheotomy and positive pressure ventilation with oxygen. A catheter was inserted into a femoral artery for blood collection; another catheter was advanced through the contralateral femoral into the thoracic aorta for bolus injections. Catheters were also placed into the renal vein for blood collections with the aid of a rotary pump and into the ureters. A balloon catheter (Fogarty Arterial Embolectomy Catheter #12-040-3F; American Hospital Supply Corp., Chicago, IL) was advanced through the femoral artery into the abdominal aorta to a point just above the renal arteries to permit instantaneous abolition of

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renal blood flow. Throughout the study the animals received an intravenous sustaining infusion of 1 ml of 5% mannitol in saline/kg/min. The arterial bolus contained [^{3}H]methoxyinulin and [^{14}C]cycloleucine, both obtained from the New England Nuclear Corp.; the inulin here served as extracellular marker. We have previously shown (5) that (i) the best time resolution which could readily be achieved in rabbits weighing on the average 2.5 kg, and over a period of longer than 120 sec, without compromising circulatory homeostasis, was 4 sec/fraction; and (ii) under those conditions, the venous recovery curve of inulin closely resembles that of creatinine. Measurement of the uptake of nonfiltered cycloleucine by a standard indicator-dilution technique has been previously described (2); mean transit times ($\bar{t}$) were computed from the conventional formula

$$\bar{t} = \sum (C_t \cdot t) \cdot \Delta t / \sum C_t \cdot \Delta t$$

where C_t stands for recovery of solute in venous plasma at time t following bolus injection and Δt represents the length of each collection period. By analogy with the transepithelial transit time of filtered and reabsorbed cycloleucine (5), which reflects mostly its slow basolateral extrusion, the return to blood of cycloleucine previously extracted by the nonfiltering kidney was characterized by an extrusion delay equal to the difference between mean transit times of cycloleucine and inulin, as illustrated in Figure 2. Radioactivity was counted on a Packard model 2000CA liquid scintillation spectrometer.

Results

Validation of Aortic Occlusion Technique. To determine the extent to which 40 sec of aortic occlusion might interfere with normal solute transport in the anoxic kidney, renal extraction of *p*-aminohippurate (PAH) and cycloleucine were measured at steady-state arterial concentrations before and during 40-sec occlusion. Levels of PAH and cycloleucine were determined in arterial plasma, renal venous plasma before aortic occlusion, and plasma from the anoxic (dark) venous blood which had been trapped in the kidney and collected immediately after release of occlusion. Renal extraction of cycloleucine in four studies averaged −1% before as well as during occlusion; in other words, luminal cycloleucine present in the tubular lumen at the moment of occlusion continued to be completely reabsorbed. Similarly, in two studies, occlusion exerted little effect on extraction of PAH or on the fraction of trapped blood that had been filtered. These results are summarized in Table I.

Diffusion Limitation of Renal Extraction of Nonfiltered Cycloleucine. In three rabbits the effect of occlusion on the renal venous deficit of cycloleucine was determined as described in Materials and Methods. Results of one of these studies are illustrated in Figure 1. The shaded areas between the recovery curves of inulin and cycloleucine, as in earlier work (6), represent the venous deficit of nonfiltered cycloleucine. A first period with free perfusion was followed by a second period in which the aorta remained occluded for 40 sec, starting at 4 sec after bolus injection. The 4-sec delay was chosen in order to trap the bolus in the kidney. The mean ratio of the deficit with and without occlusion was 2.0 (range, 1.7–2.2). In five normally filtering kidneys transient aortic occlusion permitted uptake of 33 ± 12% of nonfiltered cycloleucine.

Table I. Renal Solute Transport during Short Anoxia[a]

Infusion	*n*	V^b (% of A, mean, and range)	
		Before occlusion	Trapped blood
Cycloleucine	4	101 (98–106)	101 (97–104)
PAH	2	32 (30–35)	37 (33–42)
Inulin	2	80 (73–86)	73 (66–79)

[a] The animals received sustaining infusions containing cycloleucine, PAH, and inulin.
[b] A, V: Arterial and renal venous plasma concentrations.

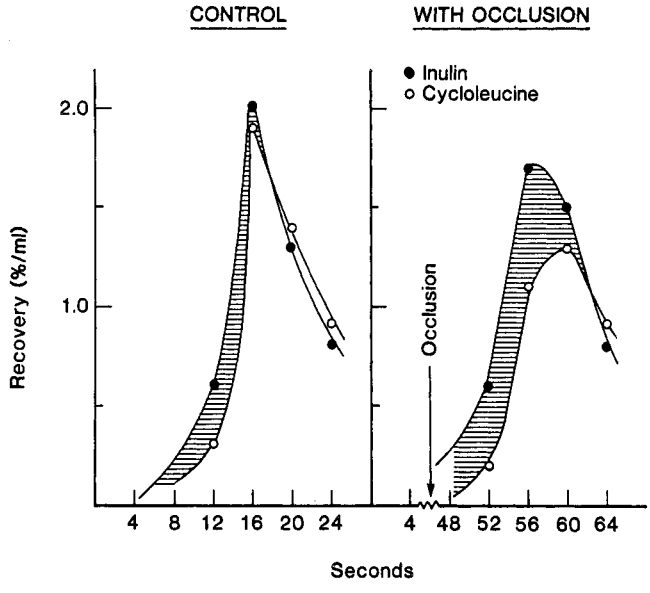

Figure 1. Recovery of cycloleucine and inulin in renal venous blood during control circulation and after transient aortic occlusion. For details, see text.

Extent and Rate of Correction of Cycloleucine Deficit. The occlusion technique permitted maximization of the renal venous deficit of cycloleucine, and subsequently the determination of the extent to which, and the rate at which, the missing cycloleucine returns to blood. In these experiments, filtration was minimized in the cannulated kidney by preliminary occlusion of the ureter (stop-flow kidney) so as to avoid the complication of filtered and reabsorbed cycloleucine returning to venous blood. Under these conditions, as shown in

Table II, the venous deficit of cycloleucine averaged 21% of the renal load, as calculated from the simultaneous recoveries of [^{3}H]inulin and of [^{14}C]cycloleucine. The major fraction (90%) of the missing cycloleucine subsequently returned to venous plasma, with a mean delay of 45 sec. Results of one of these experiments are shown in Figure 2 and illustrate in particular the calculation of the mean extrusion delay required for nonfiltered cycloleucine taken up during occlusion to return to blood. A factor which may well have contributed to the relatively large variability seen in the size of the deficit between individual animals (Table II) may arise from variation in precisely where in the kidney the arterial bolus had been trapped by occlusion.

Movement of Peritubular Cycloleucine into Urine. If the early venous deficit of cycloleucine resulted from its basolateral accumulation by tubular cells, then some cycloleucine movement from cell into lumen might also be anticipated during occlusion. Net flux, of course, would be low, given the greater than 90% fractional absorption of luminal cycloleucine. Seven experiments were carried out to test this prediction. In each case, filtration was minimized by ureteral occlusion for 8 min preceding the usual bolus injection and aortic occlusion. Six seconds after reestablishing circulation the ureteral occlusion was removed, and 32- × 0.1-ml urine fractions were obtained from each kidney. In six of seven animals (12 of 14 kidneys), clear evidence of transtubular movement of cycloleucine was obtained, as illustrated by results from one kidney in Figure 3.

Discussion

The hypothesis tested in this work was that the venous amino acid deficit observed during passage of a bolus from renal artery to vein results from transport of nonfiltered amino acid into the same cellular solute pool as that through which filtered amino acid passes during its reabsorption (5). Because the experimental conditions involved the presumed passage of the test amino acid through intracellular pools, it was important to choose a nonmetabolizable analogue (cycloleucine) as test compound. Now the transport of cycloleucine across basolateral cell membranes in the direction of reabsorption is a relatively slow process, requiring as long as 40 sec (5). If the venous deficit is indeed caused by basolateral uptake of cycloleucine, it seems likely that the size of the venous deficit should be diffusion dependent. In agreement with this prediction, Figure 1 shows that when the mean transit time of inulin from renal artery to vein is prolonged from its normal value of around 16 sec to 56 sec, by occluding the aorta and thus sec to trapping the injected bolus in the kidney for 40 sec, the size of the deficit is significantly increased.

The occlusion technique permitted maximization

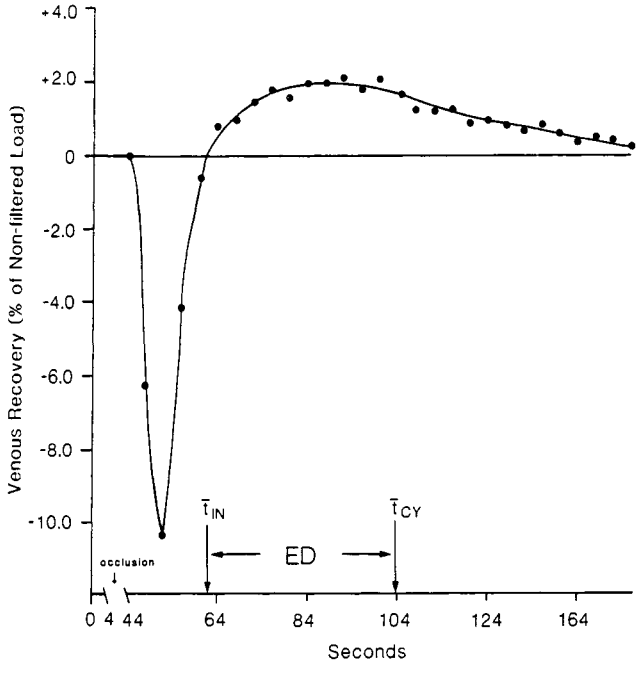

Figure 2. Early venous cycloleucine deficit in a nonfiltering kidney and its reversal. Rabbit 89/8, see Table II.

Table II. Venous Cycloleucine Deficit after Transient Interruption of Circulation through Nonfiltering Kidney[a]

Animal	Deficit		Extrusion delay[b] (sec)	Hematocrit (%)
	% of Non filtered load	Fraction returned to plasma		
88/31	20	0.5	48	—[c]
89/6	29	0.6	46	23[d]
89/8	21	1.3	42	34
89/9	14	1.2	34	33
89/10	19	1.2	48	34
89/12	24	0.9	52	22[d]
Mean ± SD	21 ± 5	0.9 ± 0.3	45 ± 6	

[a] In three additional animals, the recovery curve of cycloleucine showed a large variability between successive fractions and no clear-cut peak. No significant quantitative calculations could be performed and the animals were excluded.
[b] Defined as the mean time required for cycloleucine previously extracted from postglomerular blood during occlusion to return to blood.
[c] Hematocrit not measured, but normal value after mannitol infusion in a large number of animals averaged 34%.
[d] Hematocrit lowered by exchange transfusion with 20 ml of 5% dextran in saline/kg body weight.

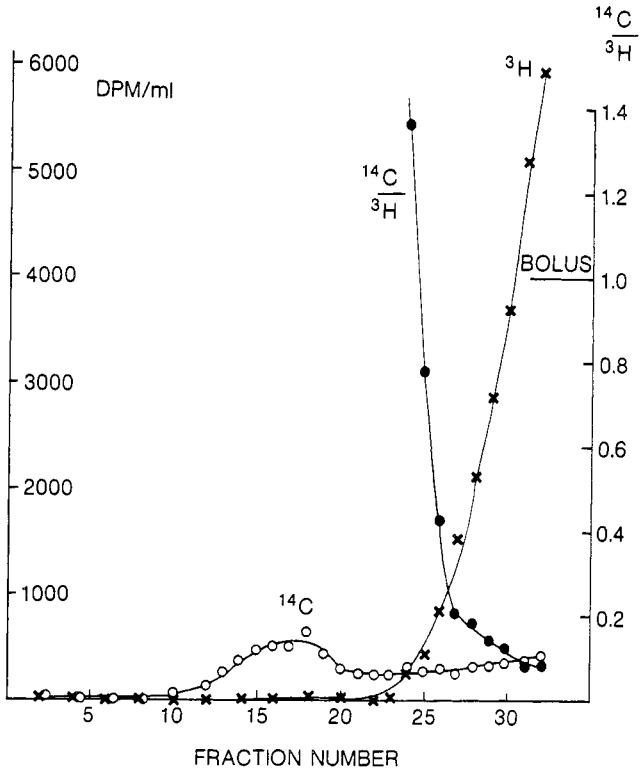

Figure 3. Transtubular movement of cycloleucine during occlusion. For details, see text. The arterial bolus contained 4 μCi of [^{14}C] cycloleucine (sp act, 0.13 μCi/μmol) and 2 μCi of ^{3}H; ^{14}C counts in urine were normalized for a ratio of ^{14}C:^{3}H in the bolus of 1.0.

of renal uptake, without any apparent effects of the transient anoxia on solute movement. This conclusion might perhaps have been expected on the basis of the work of Gerlach *et al.* (7) who observed that ATP levels in the anoxic kidney, in spite of their rapid fall, do not drop to zero within 40 sec. The work of Metaxas (8) had shown earlier that transient anoxia does not alter the ability of the kidney actively to transport solutes. Additional assurance of only minimal effects of short renal anoxia was provided by the present finding of continued active transport of cycloleucine and PAH during aortic occlusion. The occlusion technique, incidentally, has also proven useful in studies involving acute loading of the kidneys with relatively high nephrotoxicant concentrations, without introducing excessive amounts of these substances into the whole animal.

If the diffusion-limited uptake involves tubular cells, as suggested, then one would also predict that some cycloleucine should reach the lumen. Of course, net flux thus measured might be small, as active transport leads to greater than 90% fractional reabsorption from the lumen. The predicted small transtubular flux, from blood to urine, was observed in six of seven animals (see Fig. 3).

A third prediction of the hypothesis is that nonfiltered cycloleucine extracted from blood should all return to the vein, with a delay of around 40 sec. This prediction flows from the observation quoted above that basolateral extrusion of filtered cycloleucine during its reabsorption requires 40 sec (5). The results collected in Table II (see also Fig. 2) show that the major fraction of nonfiltered cycloleucine removed from plasma returned to plasma with a mean delay of 45 sec. Note that in two of these studies the hematocrit had been greatly reduced by exchange transfusion, without significant effect on the characteristics of the venous deficit.

The finding in two animals that the venous deficit remains unaffected by major reductions in hematocrit suggests that cycloleucine uptake by erythrocytes does not contribute appreciably to this deficit. This conclusion could already have been drawn from the fact that after release of occlusion, the major portion of cycloleucine taken up returns to renal venous plasma (Table II). This would obviously not be expected if the venous deficit after occlusion resulted to a large extent from cycloleucine uptake by blood cells; in that case the cellular amino acid would have disappeared into the general circulation and could not have been recovered in renal venous plasma.

Although the results presented here cannot prove that cycloleucine taken up from opposite sides of epithelial cells enters the same intracellular pool, they nevertheless fully support this hypothesis: to assume otherwise would necessitate the assumption of two separate pools exhibiting the same kinetic behavior. In addition, the experiments help validate a technique for acutely loading the kidneys with relatively high amounts of various solutes, as required in studies on the acute as opposed to delayed effects of nephrotoxicants.

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