Volume of Renal Cortical Cytoplasm in Rabbits, and Basolateral Transport Gradients of Cycloleucine and *p*-Aminohippurate (43539)

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Abstract. **The intracellular volume of distribution of the nonmetabolizable sugar derivative 3-O-methylglucose was determined in the renal cortex of diuresing rabbits. This sugar is not well reabsorbed from the lumen, but is readily taken up across the basolateral cell membranes by an apparently flow-limited, nonconcentrative process. The ratio of distribution volumes of nonfiltered 3-O-methylglucose and inulin, therefore, equals the ratio of their mean artery-vein transit times. An intracellular and presumably cytoplasmic volume for 3-O-methylglucose of 0.13 ml/g was thus determined in the cortex of rabbits undergoing mannitol diuresis; similar values were obtained with three other less direct approaches. Availability of a reliable value permitted calculation of the activity gradients against which *para*-aminohippurate and the neutral amino acid cycloleucine can be accumulated at the basolateral membrane *in vivo*; both gradients equal about 6:1. This finding underlines the active nature of basolateral amino acid uptake and points to a further characteristic common to the organic anion and the cycloleucine carrier systems.** [P.S.E.B.M. 1993, Vol 202]

Earlier work from this laboratory (1) confirmed that amino acids, like the extensively studied organic anion *para*-aminohippurate (PAH), are actively transported across basolateral membranes into tubular epithelial cells; the relationship between PAH and amino acid transport, however, remains in question. Moreover, in the absence of a reliable value in the diuresing rabbit for the intracellular volume in which the amino acid is accumulated, the conclusion on the active nature of the transport remains a qualitative one.

The first objective of the present work was, therefore, to obtain a reliable measure of the cytoplasmic volume of cortical tubule cells in the living animal. Earlier attempts to determine this volume were usually based on morphometric techniques with fixed tissue *in vitro*. For instance, Kruhoffer (2) counted a total of 150,000 nephrons/rabbit kidney, with a mean proximal length of 6 mm. Using values for internal and external diameters of the proximal tubule as seen in fixed kidneys, one thus arrives at a proximal cellular volume of 1.5 ml/kidney, equivalent to approximately 0.18 ml/g of cortex. A similar value was deduced somewhat indirectly *in vivo* from the ratio of the mean artery to ureter transit times of inulin and PAH in the presence of sufficient probenecid to inhibit basolateral PAH accumulation (3). A more direct functional approach to the measurement of the intracellular volume is reported here, based on the intrarenal distribution of the nonmetabolizable sugar derivative 3-O-methylglucose (3OM). Availability of this value made it possible to characterize more quantitatively the basolateral transport of cycloleucine, and to compare it with that of PAH.

Materials and Methods

The experiments were carried out with New Zealand White rabbits (males with an average body weight of 2.8 kg) purchased from Myrtle Rabbitry, Thompson Station, TN, and maintained on commercial rabbit

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food (Purina) and tap water *ad libitum*. They were prepared for study by induction of anesthesia with intravenous sodium pentobarbital (Nembutal), followed by tracheotomy and positive pressure breathing with 100% oxygen. Catheters were inserted into both ureters, the left renal vein, and the thoracic aorta via a femoral artery. Throughout the study, the animals received an intravenous infusion of 1 ml/kg·min of 5% mannitol in saline. Artery to vein transit times across either normal or essentially nonfiltering kidneys (stop flow kidneys whose ureters had been occluded 10 min earlier) were determined as described previously (4) with an arterial bolus injection of 1 μCi of [^{14}C]3OM and 2 μCi of [^{3}H]methoxyinulin. The volume of nonluminal inulin space in renal cortex approximates 0.24 ml/g, as routinely calculated in this laboratory from knowledge of the effective renal plasma flow (PAH clearance) and the mean artery to vein transit time (t) of inulin.

The apparent intracellular volume of distribution (ICF) of 3OM was obtained from the expression 0.24 (ratio of transit times of 3OM/inulin − 1.0). For measurement of ICF under steady state conditions, 3OM (0.1 mM), [^{14}C]3OM (0.1 μCi/ml), and [^{3}H]inulin (0.2 μCi/ml) were added to the infusion solution. Fractional excretion (FE) of 3OM, as determined after 1 hr from ^{14}C to ^{3}H concentration ratios in urine and arterial plasma, averaged 55 ± 9% (n = 6). The kidneys were then removed, and the cortex was weighed and extracted with 5% trichloroacetic acid at 100°C. In absence of active accumulation of 3OM, and for equal plasma concentrations (P) of ^{14}C and ^{3}H, ICF is given by the expression (^{14}C/g − 0.24P) = ICF × P + FE(luminal ^{3}H). Luminal ^{3}H is calculated as (^{3}H/g − 0.24P).

All labeled compounds were purchased from New England Nuclear Co., Boston, MA, and counted on a Packard 2000 liquid scintillation spectrometer. Results are presented as mean ± SD.

Results

Figure 1 illustrates the passage of inulin and 3OM from the renal artery into the renal vein of a normally filtering rabbit kidney. In order to avoid significant contributions of medullary blood flow, or of filtered and reabsorbed 3OM, mean transit times were computed as usual from exponential extrapolation of the inulin and 3OM peaks. Transient net uptake of 3OM was equated to the early venous deficit of ^{14}C, i.e., the area between the recovery curves up to the point where recovery of ^{14}C begins to exceed that of ^{3}H.

Results of nine such determinations in eight animals are summarized in Table I and yield a mean transit time ratio of 1.56 ± 0.06; this corresponds to a total volume of distribution of 3OM equal to the inulin space (0.24 ml/g) × 1.56 or 0.37 ml/g, and thus to a virtual cytoplasmic volume of 0.13 ± 0.01 ml/g cortex. This result implies a fractional uptake of nonfiltered 3OM of 0.13/0.37, or 35%, of the same order of magnitude as the value of 48% actually observed (Table I). As shown in Table II, the transit time ratio was not affected significantly by ureteral occlusion. Clearly, reabsorption does not influence t as calculated here. In addition, proximal tubular lumina presumably were fully dilated even during free flow; otherwise, the increased intraluminal pressure following occlusion would have tended to compress further the cytoplasmic volume.

A similar value for the cytoplasmic volume could be deduced from 3OM uptake under steady state, rather than transient, conditions; the average value for six kidneys was 0.15 ± .07 ml/g of cortex. However, because of its greater variance, this result is presumably less reliable than that calculated from the transients.

To reveal possible barrier limitations on 3OM uptake, three studies were carried out in which a first bolus contained 1 μmol of 3OM, followed 15 min later by a second bolus in which the 3OM concentration had been increased 25-fold. Net fractional uptake of 3OM under these conditions fell from 52 ± 7% to 48 ± 4%, an insignificant change.

In repeated experiments, attempts were made to demonstrate tissue swelling in animals injected intravenously 4 to 24 hr earlier with $HgCl_2$, as documented in Table III. No significant changes in overall volume of distribution of 3OM could be observed. Similar results were obtained in two animals that had received 10 μmol of $CdCl_2$/kg, together with 350 μmol of β-mercaptoethanol (5) 24 h before further study.

Discussion

The choice of the nonmetabolizable 3OM as probe for the determination of intracellular volumes was based on its ready basolateral uptake as contrasted, at least in the dog (6), with the absence of apical absorption. In the rabbit, we found evidence for partial reabsorption, but this fact does not influence the present calculations. Four different approaches, two of which are based on distribution of 3OM, all lead to similar intracellular volumes. The most direct and consistent results were obtained with the rapid transit procedure, and the value of 0.13 ml/g wet wt of cortex during mannitol diuresis will, therefore, be accepted for further discussion.

To the extent that the probe is excluded from subcellular organelles, its intracellular volume of distribution represents the cytoplasmic volume. The calculation is based on an earlier finding that 3OM is not actively accumulated by renal cortical slices of the rabbit (7). If it were, the actual cytoplasmic volume would be even smaller than 0.13 ml/g; this seems unlikely. The additional assumption that basolateral

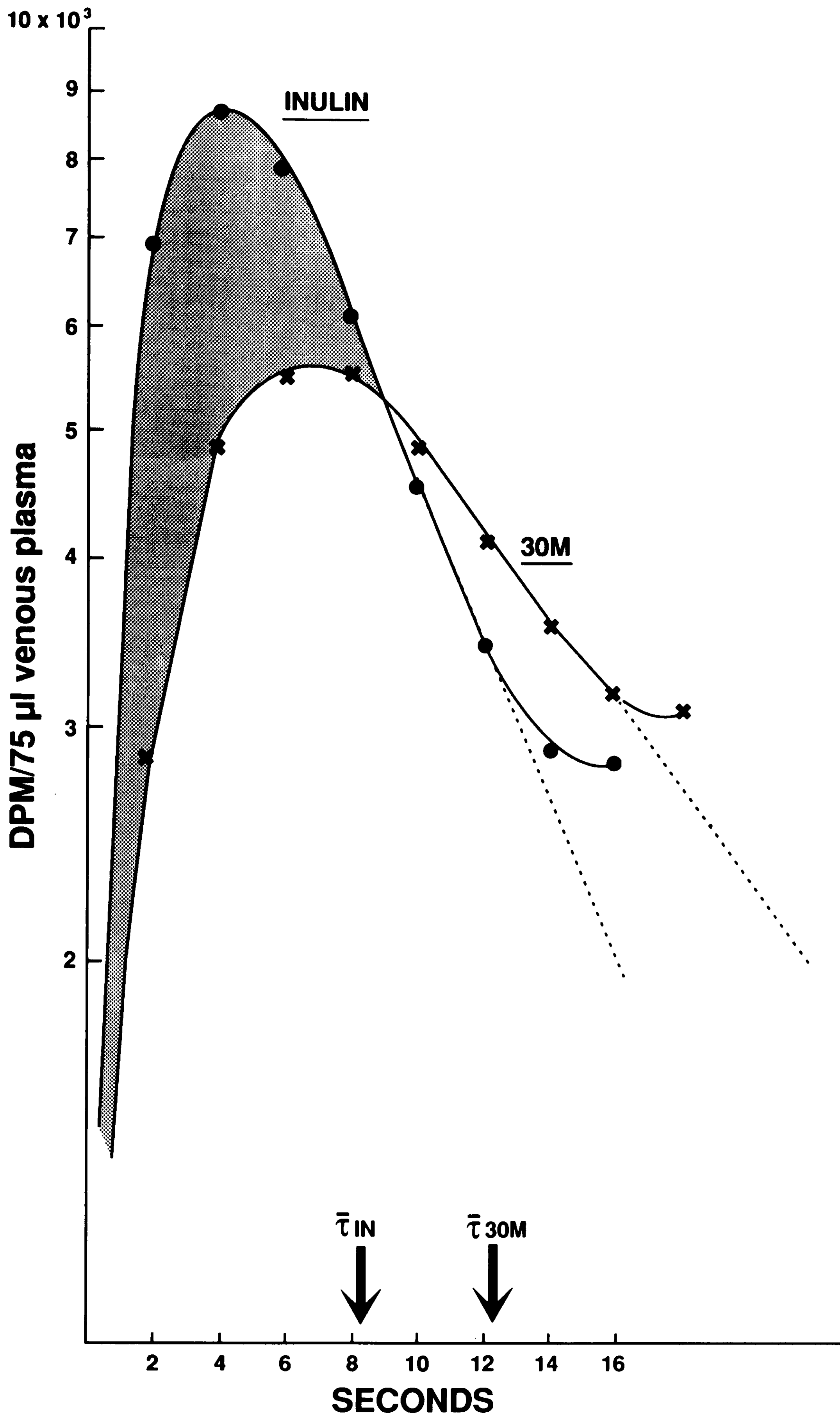

Figure 1. Renal transit of 3-O-methylglucose. Radioactivity in renal venous plasma is shown following an arterial bolus injection of [^{14}C]3OM and [^{3}H]inulin. The shaded area between the recovery curves measures postglomerular uptake of non-filtered 3OM. See Table I for a summary of nine similar experiments. *t* stands for the mean artery to vein transit time calculated as usual (4).

Table I. Vascular Transit of 3OM in Normal Kidneys

Rabbit	Fractional uptake[a]	t^b (sec)		
		^{14}C	^{3}H	^{14}C:^{3}H
15	49	14.7	9.0	1.63
16A	53	17.2	10.7	1.61
16B	39	11.4	7.3	1.56
24	48	11.6	8.0	1.45
27	52	14.9	9.3	1.60
28	51	17.1	11.0	1.55
35	51	14.3	9.3	1.54
40	41	15.1	10.4	1.45
43	—	25.2	16.1	1.57
Mean ± SD	48 ± 5			1.56 ± 0.06

[a] Fractional uptake is expressed as percentage of nonfiltered load.
[b] This refers to nonfiltered solutes only.

Table II. Vascular Transit of 3OM in Stop-Flow Kidneys

Rabbit	Fractional uptake[a]	t^b (sec)		
		^{14}C	^{3}H	^{14}C:^{3}H
45	38	14.3	9.7	1.47
2/1	31	14.4	10.2	1.41
2/2	42	19.3	12.1	1.60
2/4	37	12.4	7.8	1.59
2/5	31	10.1	7.4	1.36
Mean ± SD	36 ± 5			1.49 ± 0.11

[a] Fractional uptake is expressed as percentage of nonfiltered load.
[b] *t*, mean transit time.

Table III. Effect of Hg on 3OM Transit

Rabbit[a]	Dose[b]	Fractional uptake[c]	t^d (sec)		
			^{14}C	^{3}H	^{14}C:^{3}H
18	2.5 (24)	28	16.9	11.4	1.48
19	2.5 (24)	40	18.1	11.6	1.56
		42	15.9	10.6	1.50
20	2.5 (5)	43	18.6	12.3	1.51
36	10.0 (4)	53	27.2	17.6	1.54
38	10.0 (4)	41	15.1	9.3	1.62
Mean ± SD		41 ± 8			1.53 ± 0.05

[a] Studies were carried out on five rabbits injected intravenously with Hg as shown.
[b] Doses are expressed in μmol/kg; hours allowed to elapse between Hg injection and transit experiment are given in parentheses.
[c] Fractional uptake is expressed as percentage of nonfiltered load.
[d] *t*, mean transit time.

transfer of 3OM is flow limited rather than diffusion limited is supported by the finding that fractional uptake of nonfiltered 3OM is independent of plasma concentrations. Use of 3OM as volume probe should, therefore, theoretically yield correct values under present conditions.

The usefulness of the technique for demonstrating tubular volume changes of damaged cells *in vivo* is limited, however. Thus, cell swelling due to Hg has been observed by many investigators (see, e.g., [8]), and microscopic inspection of the kidneys from our Hg-injected rabbits showed a significant number of necrotic and swollen tubules. Nevertheless, no change could be observed in the volume of distribution of 3OM (Table III). This finding cannot be attributed to metal inhibition of 3OM transfer, because mean fractional 3OM uptake in the kidneys of mercury-treated animals remained essentially unchanged. Presumably, therefore, the swollen cells constituted too small a fraction of the total cell number to cause a significant increase in the total cytoplasmic volume.

Knowledge of the cytoplasmic volume in renal cortex now permits a restatement of the earlier conclusion (1) that the neutral amino acid cycloleucine is actively accumulated across basolateral cell membranes in rabbit kidney cortex *in vivo*. The steady state concentration ratio of cycloleucine in cell/plasma of nonfiltering kidneys was calculated as 1.5 ± 0.4, slightly, though significantly, exceeding the equilibrium value for an uncharged molecule of 1.0. This calculation was based on an assumed intracellular volume of 0.55 ml/g of cortex, a value now found to be much too high. Correction for a cytoplasmic volume of only 0.13 ml/g of cortex raises the concentration ratio to 6.3 ± 1.7, a more convincing reflection of basolateral uphill transport.

An activity gradient of similar magnitude can be calculated for PAH. Assuming that proximal tubule cells in absence of extensive Na transport are approximately 60 mV electronegative to both the lumen and the peritubular interstitium (9), and that uphill transport occurs only at the basolateral membrane (10), the concentration of PAH in trapped luminal fluid at steady state should be approximately 10 times higher than in the cell. The luminal vol/g cortex is computed as total water content (0.78 ml) minus the sum of the peritubular inulin space (0.24 ml) and the cytoplasmic volume (0.13 ml), or 0.41 ml. The PAH concentration/g cortex averaged 2.8 times the plasma concentration P (1). The corresponding cytoplasmic concentration (C) of PAH can then be calculated on the basis that total tissue PAH equals the sum of the interstitial + cytoplasmic + luminal PAH, i.e., $2.8\ P = 0.24\ P + 0.13\ C + 0.41 \times 10\ C$, or $C/P = 0.6$. Purely passive distribution of PAH across the basolateral membrane should have yielded a C/P value of only 0.1. In other words, PAH was transported against an activity gradient of around 6. Note that this gradient, calculated for the intact kidney *in vivo*, cannot be directly compared with the gradient against which PAH can be accumulated by thin cortical slices *in vitro*. Not only are such slices usually incubated in the presence of stimulating anions such as acetate (11), but it is likely that changes from normal physiologic fluid volumes and membrane po-

tentials make it difficult to compute accurate cell to medium gradients.

The finding of similar activity gradients *in vivo* for cycloleucine and PAH points to the magnitude of the amino acid transport. It also adds to the previously described similarities between the two transport processes. However, there is no reason to question the earlier conclusion that different systems are responsible for transport of the two solutes.

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