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DEVELOPMENTAL AND OTHER CHARACTERISTICS OF α-METHYL-D-GLUCOSIDE TRANSPORT BY RAT KIDNEY CORTEX SLICES

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SUMMARY

α-Methyl-D-glucoside has been shown to be a non-metabolizable sugar which is accumulated against a concentration gradient by a Na^+-dependent and phlorizin inhibited process by adult rat renal cortical slices incubated *in vitro* at 37 °C. (2) The velocity of accumulation increased linearly with substrate concentrations up to 1.5 mM, but at higher concentrations obeyed saturable kinetics with an apparent K_m of about 6 mM. (3) Uptake was enhanced as Na^+ was increased from 0 to 100 mequiv/l. Higher Na^+ concentrations caused no further effect. (4) A pH maximum of transport occurred between 7.35 and 8.0. (5) Glucoside uptake was inhibited by D-glucose, D-galactose, D-fructose, D-mannose and D-ribose. The inhibition by D-glucose and D-galactose was competitive with apparent K_i of 24 and 53 mM, respectively. (6) Both D-glucose and D-galactose accelerated the efflux of α-methyl-D-glucoside from preloaded cells. (7) Kidney cortex slices from 1-day-old rats were unable to accumulate α-methyl-D-glucoside to form a concentration gradient. The ability to concentrate the glucoside increased progressively after birth, reaching near normal in tissue from 15-day-old animals. The data indicate that the transport process in the newborn is rudimentary, failing also to display accelerated efflux phenomenon. (8) α-Methyl-D-glucoside is transported in rat kidney cortex by a mechanism similar in many ways to that of D-galactose.

INTRODUCTION

Although physiological evidence for proximal renal tubule reabsorption of sugars such as glucose and galactose has been known for many years, only recently has a detailed *in vitro* investigation of sugar transport by renal cortical cells been reported[1–6]. The latter investigators as well as Krane and Crane[7] employed rabbit renal cortex for their studies. Knowledge of the cellular processes of sugar accumulation by kidney cortex cells has been largely confined to their observations in rabbit tissue.

Our own concern with sugar transport by kidney cortical cells stems from two divergent yet interrelated interests. One is in the inherited human disorders

of sugar transport, renal glycosuria[8] and glucose–galactose malabsorption[9]. The other is in sugar induced aminoacidurias[10] and the inhibitory effects of sugars (glucose, galactose, α-methyl-D-glucoside) on amino acid transport *in vitro* by kidney cortical tissue[11,12]. We have, therefore, initiated experiments to characterize parameters of sugar transport of rat kidney cortex. Experiments *in vitro* with D-galactose as the transported substrate have shown that metabolism of this sugar by rat renal cortical cells is so great that adequate parameters of transport are difficult to determine[13]. Therefore, the intracellular accumulation of the sugar, α-methyl-D-glucoside, known to have similar transport properties to D-glucose and D-galactose in intestinal mucosa[14,15] has been evaluated in rat kidney cortex. Developmental and kinetic transport parameters have been assessed which indicate that this sugar is a non-metabolizable model representative of several aspects of the glucose–galactose transport process.

EXPERIMENTAL

Animals

Sprague–Dawley rats were used. Male adults weighing 150–200 g were fed *ad libitum* on Purina Chow. Newborn and other young rats under 21 days of age were permitted to suckle until killed by decapitation. No sex distinction was made for obtaining tissue from these young animals.

Chemicals

α-Methyl-D-[U-^{14}C]glucoside (73.4 Ci/mole) was obtained from Calbiochem, Los Angeles, Calif. This material was found to give only one radioactive area corresponding to α-methyl-D-glucoside on thin-layer chromatography of the trimethylsilylated compound on silica gel plates developed with dried benzene as described by Lehrfeld[16]. The radioactivity was assessed by scraping segments of the gel from the plates and determining the ^{14}C in the scrapings by liquid scintillation techniques.

Unlabeled α-methyl-D-glucoside was obtained from the Pfanstiehl Co., Waukegan, Ill. and found to be pure and free of glucose by analysis of silylated material by gas–liquid chromatography using a Packard instrument with a 6 ft × 0.25 inch column packed with 3 % OV-ion Gas Chrom Q at 170 °C at a carrier gas flow of 60 ml/min.

Other sugars were analytical grade obtained from either Sigma Chemical Co., St. Louis, Mo., Pfanstiehl Co., Waukegan, Ill. or Nutritional Biochem. Corp., Cleveland, Ohio except for 3-deoxyglucose which was a gift of Dr J. W. Pratt (deceased) of the National Institutes of Health, Bethesda, Md.

Determination of sugar accumulation

The technique for measuring sugar accumulation by rat kidney cortex slices was essentially that used previously for studies of amino acid accumulation[17–19]. Cortical slices of adult kidneys were made with a Stadie–Riggs microtome. Such slices could not be made of kidneys from rats below 15 days of age because the narrow width of the cortex along with compression of the tissue by the microtome and thickness of its blade made good slices with only cortical cells impossible to obtain. The best procedure for obtaining slices from newborn and other young rats was by use of a fine two-edged razor blade for taking small slices freehand directly

from the surface of the kidney. The validity for comparing these slices to that of adult cortex with regard to amino acid transport has been shown[20]. We have found similar results with α-methyl-D-glucoside, namely, that small tissue samples in the weight range used here have similar uptake to larger slices. The small slices were made from kidneys of an entire litter without sex distinction and pooled. Three slices, 3–6 mg total weight, were taken at random from the pool and placed together in the incubation flask. In each experiment at least three such flasks were incubated for determination of each data point. Adult tissues were incubated in triplicate, each flask containing a whole or segment of a slice from each of three animals with a total weight of 30–40 mg.

Incubations were carried out in 30-ml stoppered plastic bottles in 2 ml Krebs–Ringer bicarbonate buffer at 37 °C under O_2–CO_2 (95:5, v/v). The buffer constituents have been enumerated[21]. Each ml contained 0.2 μCi α-methyl-D-[U-^{14}C]glucoside with unlabeled sugar to give desired concentrations. The conditions have been published for anaerobiosis[17], Na^+ depletion by Tris substitution[22] and pH alteration[23].

The assay of tissue radioactivity and calculation of intracellular accumulation of sugar was essentially that of Rosenberg *et al.*[17] and Genel *et al.*[12], and used previously for α-methyl-D-glucoside uptake[21]. At the end of the incubation the tissue was quickly dipped in saline and placed in a tube containing 2 ml water which was placed in a boiling water bath to equilibrate the tissue contents. The water extract and incubation media were then assayed for ^{14}C by liquid scintillation techniques.

Results are expressed as the distribution ratio, the cpm/ml of intracellular fluid to cpm/ml of medium, using inulin space of slices to determine that portion of tissue radioactivity due to extracellular substrate and the difference of total tissue water and inulin space to assess the magnitude of the intracellular fluid[24]. The extracellular space of newborn and 5-day-old kidney cortex and total tissue water was found to be 23 % and 78.5 %, respectively, of wet tissue weight. These values for the adult are 25 % and 80 % (ref. 24). Values for these parameters in the absence of Na^+ (ref. 22) and alteration in buffer pH[23] were used when these experimental conditions were employed.

The distribution ratio represents a concentration gradient of α-methyl-D-glucoside since on silica gel chromatography of tissue extracts[16] only radioactive α-methyl-D-glucoside was found and studies to measure $^{14}CO_2$ production[25] from the labeled substrate reveal negligible amounts. The distribution ratio may thus be converted to concentration of the sugar by multiplying this value by the substrate concentration. This has been done to derive the velocity in concentration dependence experiments. In the latter experiments velocity was corrected for a diffusion component[26,27].

Efflux of sugar

The design of this type of study has been reported previously[28]. Slices were incubated for 60 min with 0.07 mM α-methyl-D-[U-^{14}C]glucoside. Tissue was then removed, quickly rinsed in physiological saline, blotted, and transferred to flasks containing 3 ml buffer or buffer with 20 mM unlabeled glucose or galactose. The vessels were then gassed and sealed. At 3-min intervals the flasks were opened and the medium sampled for radioactivity. At the end of 18 min the tissues were removed and the tissue content of ^{14}C assessed. The total counts effluxed into the medium

and the counts remaining in the tissue after 18 min were summed to determine the label present at the onset of the efflux phase.

RESULTS

Concentrative uptake of α-methyl-D-glucoside

This sugar is accumulated by renal cortical cells to concentrations greater than that in the incubation medium. This is demonstrated in Fig. 1 (upper panel). At a medium concentration of 0.2 mM, slices from adult rats show a distribution ratio of over 5 after 90 min of incubation without achieving a steady state level even at 120 min. The concentration within the cells at 90 min is 1 mM compared to 0.18 outside. When the medium concentration was 2 mM (lower panel, Fig. 1), the sugar was concentrated by adult tissue to a steady state level of 6 mM in the cells within 60 min of incubation.

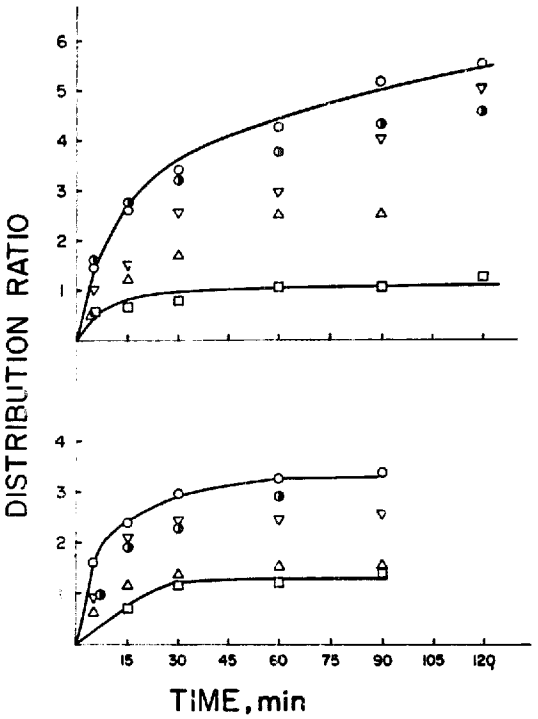


Fig. 1. Uptake of α-methyl-D-glucoside by rat kidney cortex slices. Slices were incubated in 2 ml Krebs–Ringer bicarbonate buffer (pH 7.35) containing 0.2 mM (upper panel) and 2 mM (lower panel) and 0.2 μCi/ml of label at 37 °C in a Dubnoff shaker. Uptake is designated by the distribution ratio, the ratio of cpm per ml intracellular fluid to cpm per ml medium. Each point is an average of 6–20 incubations, the tissues representing 18–60 animals. Rat ages in days: □, 1; △, 5; ▽, 10; ◑, 15; ○, adult.

The velocity of accumulation of α-methyl-D-glucoside by adult tissue increased linearly over the substrate range of 0.07–1.5 mM. Lineweaver–Burk plots of velocity determinations made both at 15 and 30 min reveal a line intersecting the $1/v$ and $1/S$ axes at the origin. Saturability of the uptake is demonstrated at concentrations above 1.5 mM. The apparent K_m of saturable transport was 4.5 mM in two experiments where velocity was measured at 15 min. The K_m ranged from 4.5 to 8.5 mM, average 6.6 mM, in seven experiments where velocity of uptake was measured after 30 min of incubation. (Two of these studies are shown in Fig. 4.)

TABLE I

EFFECT OF Na^+ DEPRIVATION, PHLORIZIN AND ANAEROBIOSIS ON ACCUMULATION OF α-METHYL-D-GLUCOSIDE BY RENAL CORTEX SLICES FROM ADULT AND NEWBORN RATS

The experimental procedure for determination of the distribution ratio, the ratio of cpm per ml intracellular water to cpm per ml medium was as described in Experimental. 10–40 mg of adult tissue and 3–6 mg of newborn tissue were incubated 30 min in 2 ml buffer containing 0.2 μCi/ml; substrate concentration was either 2 or 0.2 mM. For Na^+-free media, Tris at pH 7.4 replaced Na^+ in the Krebs–Ringer bicarbonate buffer. Anaerobiosis was produced by gassing Krebs buffer with N_2–CO_2 (95:5, v/v) and using the latter as the gas phase. Phlorizin concentration was 0.5 mM. Mean ± S.E. are given with number of analysis in parentheses.

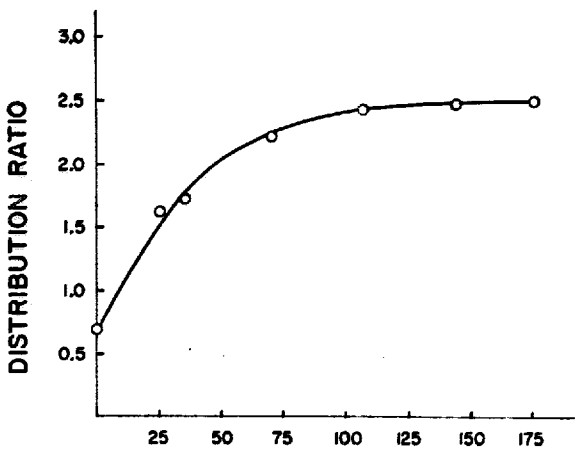
Condition	*Distribution ratio*		
	2 mM substrate		*0.2 mM substrate*
	Adult	*Newborn*	*Newborn*
Control	2.72 ± 0.15 (20)	0.74 ± 0.04 (6)	0.97 ± 0.06 (9)
Na^+-free medium	0.79 ± 0.05 (7)	0.38 ± 0.01 (4)	0.52 ± 0.07 (7)
Phlorizin	0.77 ± 0.02 (6)	0.34 ± 0.04 (4)	0.45 ± 0.07 (9)
Anaerobiosis	0.83 ± 0.03 (4)	0.55 ± 0.04 (4)	

Effect of anaerobiosis, Na^+ deprivation and phlorizin

The ability to form a concentration gradient was abolished under anaerobic conditions and when sodium was absent from the incubation medium (Table I). The extent of the Na^+ dependence was measured in adult slices with the results shown in Fig. 2. Very little difference in concentration gradients was observed between 108 and 170 mequiv/l of Na^+. Even at 72 mequiv/l there was only a 10% decrease from the distribution ratio seen at higher Na^+ concentrations. In fact, in some experiments there was no difference in uptake seen with routinely used Krebs–Ringer bicarbonate buffer with a 144 mequiv Na^+ concentration and 72 mequiv/l. The area of maximum Na^+ dependence is between 0 and 72 mequiv/l. If one views the curve of Fig. 2 as a concentration–velocity curve with Na^+ as a ligand for the transport process, the half maximum velocity (or K_m) is about 20 mequiv/l. The kinetic nature of the Na^+ dependence is under investigation and will be the subject of a future report. The uptake of the α-methyl-D-glucoside by adult tissue was markedly impaired by phlorizin, a known inhibitor of glucose and galactose transport in intestinal mucosa[29] and kidney cortex of both rabbit[7] and rat[30].

Effect of pH on cellular accumulation

The ability of adult kidney cortex to accumulate α-methyl-D-glucoside over the pH range of 6–8.4 for an incubation period of 30 min is shown in Fig. 3. Since Krebs–Ringer bicarbonate buffer routinely used at pH 7.3–7.4 may not be readily used over the wide pH range employed, phosphate buffer and Tris buffer with electrolyte composition similar to Krebs–Ringer bicarbonate buffer was employed for pH lower and higher than 7.4 (ref. 23). To determine whether these buffers influence uptake other than because of H^+ concentration, accumulation was compared at pH 7.35 in all three types of buffer and no real difference was observed (Fig. 3). Accumulation was found to be markedly reduced by the small change of pH from 7.35 to 7.0. This made the pH of routinely used Krebs–Ringer bicarbonate buffer a critical factor in obtaining reproducible results. On several occasions before we



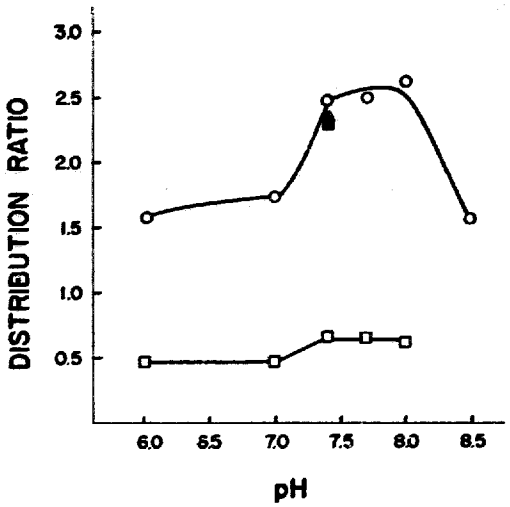


Fig. 2. Relation of α-methyl-D-glucoside uptake to medium Na^+ concentration. Conditions described in Table I. Incubation time was 30 min, substrate concentration 2 mM. Na^+ was replaced in the Krebs–Ringer bicarbonate buffer (145 mequiv/l) by Tris buffer to produce the desired Na^+ concentrations.

Fig. 3. Effect of pH on the uptake of α-methyl-D-glucoside. Slices were incubated at 37 °C for 30 min in 2 mM substrate; □, newborn; ○, adult; Krebs–Ringer bicarbonate was used for both at pH 7.35. Buffers were modified Krebs–Ringer solutions with phosphate at pH below and Tris at pH above 7.35. ■, adult tissue pH 7.35 phosphate buffer; ▲, adult tissue pH 7.4 Tris buffer. pH was determined both before and after the incubation. Each point is the average of 3–6 determinations.

were aware of this pH effect, low uptake of the sugar had been observed if the pH of the routinely used buffer was found to be below 7.3. The 30 min uptake was not as sensitive to pH above 7.4 with a constant high level of uptake being maintained until pH 8.0.

TABLE II

EFFECT OF MONOSACCHARIDES ON THE ACCUMULATION OF α-METHYL-D-GLUCOSIDE BY RAT KIDNEY CORTEX SLICES

Slices were incubated aerobically in Krebs–Ringer bicarbonate buffer containing 2 mM α-methyl-D-glucoside 0.1 μCi/ml (pH 7.35) for 30 min. The monosaccharides added were 10 mM. Mean ± S.E. are shown with the number of determinations in parentheses.

Monosaccharide	*Distribution ratio*	
	Adult	*Newborn*
None	2.46 ± 0.14 (14)	0.74 ± 0.03 (6)
D-Galactose	1.67 ± 0.05 (6)*	0.55 ± 0.03 (3)*
D-Glucose	0.89 ± 0.11 (4)*	0.37 ± 0.04 (3)*
D-Fructose	1.58 ± 0.09 (6)*	0.42 ± 0.02 (3)*
D-Mannose	2.12 ± 0.05 (9)*	
2-Deoxy-D-glucose	2.27 ± 0.10 (9)	
3-Deoxy-D-glucose	2.17 ± 0.09 (9)	
2-Deoxy-D-galactose	2.34 ± 0.10 (7)	
D-Xylose	2.20 ± 0.11 (7)	
D-Ribose	2.06 ± 0.12 (6)*	
L-Glucose	2.43 ± 0.07 (9)	

* Statistically significant difference from the control ($P < 0.05$ or less).

Inhibition by sugars

Table II shows that D-glucose, D-galactose and D-fructose severely inhibit α-methyl-D-glucoside accumulation while D-mannose and D-ribose cause moderate inhibition. 2-Deoxyglucose, 2-deoxygalactose and 3-deoxyglucose as well as L-glucose had no effect. The nature of the inhibition of 5 mM glucose and 10 mM galactose was studied in more detail with examination of the effects on α-methyl-D-glucoside uptake over a substrate range of 2–10 mM. These data are shown in Fig. 4. Both glucose and galactose appear to be competitive inhibitors of α-methyl-D-glucoside transport. The apparent K_t for glucose is 24 mM and that for galactose is 53 mM.

Accelerated efflux

In order to test the hypothesis that glucose and galactose interaction with α-methyl-D-glucoside involves a shared carrier system[31, 32] experiments were performed to determine the influence of these sugars on the efflux of cellular α-methyl-D-glucoside. In these studies adult slices were incubated for 60 min to accumulate the ^{14}C-

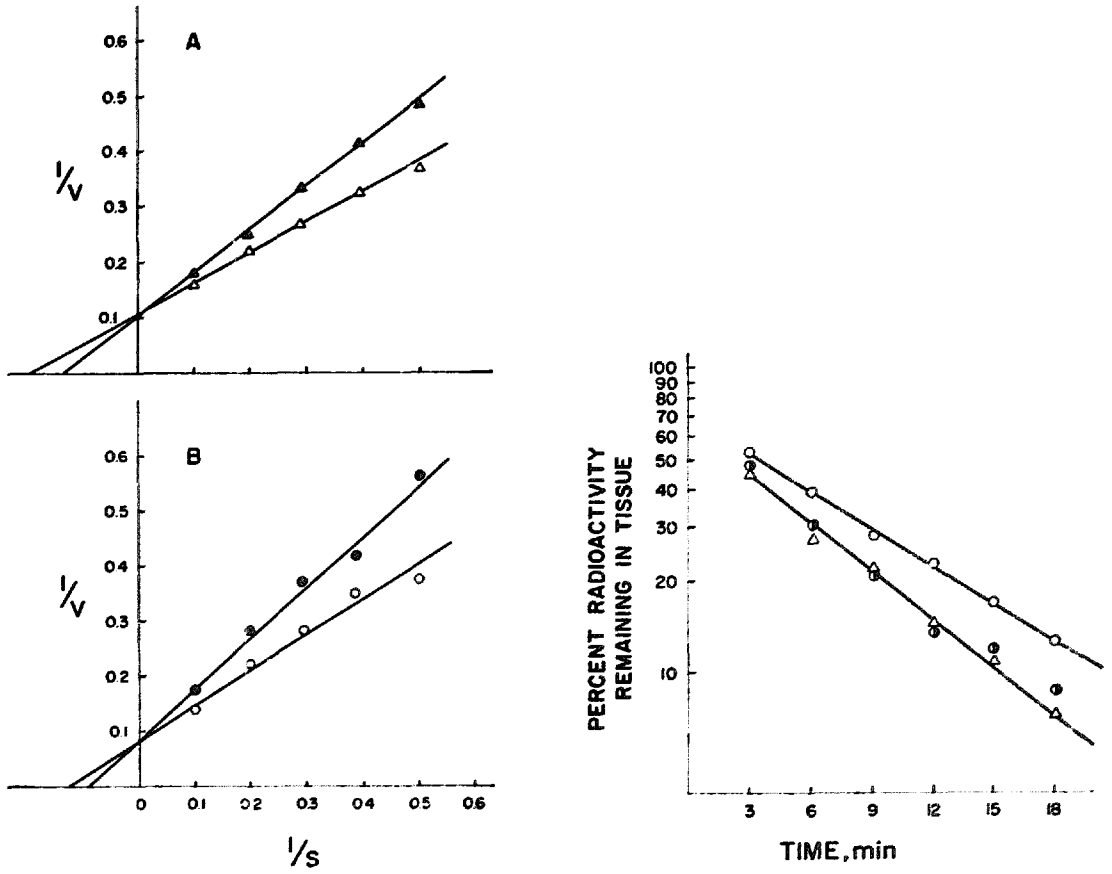


Fig. 4. Lineweaver–Burk plot of the effect of D-galactose (A) and D-glucose (B) on α-methyl-D-glucose uptake. v is velocity expressed as mmoles/l per 30 min and has been corrected for diffusion (equivalent to the sugar uptake not inhibited by phlorizin). S is α-methyl-D-glucoside concentration in mM. In Graph A galactose was 10 mM (▲). In Graph B glucose was 5 mM (●). △, ○ without inhibitor. Each point is the average of triplicate determinations.

Fig. 5. Effect of glucose and galactose on the efflux of α-methyl-D-glucoside efflux from rat kidney cortex slices. Slices accumulated radioactive glucoside for 60 min at 37 °C in Krebs–Ringer bicarbonate. Efflux of the glucoside was then followed in buffer alone (○) and in buffer containing 20 mM galactose (△) and 20 mM glucose (◑).

labeled glycoside to give an intracellular concentration of approx. 0.2 mM, after which the slices were transferred to flasks containing Krebs–Ringer bicarbonate buffer alone or buffer containing 20 mM glucose or galactose for measurement of loss of radioactive sugar from the slice. Previous experiments[12] have shown that extracellular unlabeled α-methyl-D-glucoside will accelerate the loss of the labeled compound from the slice. Results showing the effect of glucose and galactose on efflux are presented in Fig. 5. Both glucose and galactose were equally effective in promoting accelerated efflux of α-methyl-D-glucoside. The efflux K was 0.087 ± 0.003 min^{-1} in Krebs–Ringer bicarbonate buffer and 0.116 ± 0.004 min^{-1} in the buffer containing the sugars ($P < 0.01$ for difference of K in five studies).

Although these experiments are consistent with the concept of exchange diffusion and counterflow they have not been labeled as such. The possibility does exist that the external sugar inhibits re-entry of the glucoside effluxing into the medium producing an apparent faster loss of the latter from the cell. This would presuppose, however, that there is an entry process which operates against the concentration gradient imposed by the experimental design with an affinity many times higher than there is evidence for.

Effect of temperature on α-methyl-D-glucoside and galactose uptake

The paradoxical effect of reducing temperature below 37 °C on α-methyl-D-glucoside uptake by rat kidney cortex *in vitro* has been reported[21]. At lower temperatures the initial uptake is slower but the final steady state concentration of the glucoside is greater, a phenomenon due to a greater slowing of efflux than influx. The effect of reduced temperature on galactose uptake was compared with α-methyl-D-glucoside to determine if the galactose transport process behaved similarly. These results are shown in Table III. As reported previously, at 25 °C the uptake of α-methyl-D-glucoside at 15 min of incubation by rat cortex was reduced, but at 60 min it was greater

TABLE III

EFFECT OF TEMPERATURE ON UPTAKE OF α-METHYL-D-GLUCOSIDE AND GALACTOSE BY ADULT RAT AND RABBIT KIDNEY CORTEX

Three kidney cortex slices totaling about 40 mg, one from each of three adult rats or one slice of adult rabbit were incubated in 2 ml Krebs–Ringer bicarbonate buffer containing 2 mM and 0.2 μCi/ml of the sugars. Uptake is designated by the distribution ratio, ratio of cpm per ml intracellular fluid to cpm per ml medium calculated as described under Experimental. Triplicate determinations have been averaged.

Incubation conditions		*Distribution ratio*			
Time (min)	*Temp. (°C)*	*Rat*		*Rabbit*	
		α-Methyl-D-glucoside	*Galactose*	*α-Methyl-D-glucoside*	*Galactose*
15	37	2.11 ± 0.10	0.86 ± 0.06	1.64 ± 0.29	2.71 ± 0.16
15	25	1.70 ± 0.09*	0.68 ± 0.09	1.42 ± 0.10	1.88 ± 0.11*
60	37	2.50 ± 0.03	1.44 ± 0.06	1.72 ± 0.20	4.77 ± 0.23
60	25	3.90 ± 0.30*	1.38 ± 0.06	3.14 ± 0.43*	3.50 ± 0.19*

* Statistically significant at the 0.05 level or lower.

than at 37 °C. When galactose was taken up by rat kidney cortex, there appeared to be no significant effect of reduced temperature on the distribution ratio either at 15 or 60 min. It must be pointed out that the distribution ratio determined for α-methyl-D-glucoside is an indication of a concentration gradient but that the galactose distribution ratio as determined from a water extract of tissue is a ratio of cpm/ml cell water to cpm/ml medium. Our detailed studies of galactose uptake indicate the cell radioactivity includes galactose and phosphorylated derivatives and that reducing the temperature from 37 to 25 °C, although not affecting distribution ratio, does alter the partition amongst these compounds[13], lowering the neutral sugar while raising the phosphorylated metabolite content. Since transport of both galactose and α-methyl-D-glucoside have also been studied at 25 °C in rabbit tissue[1,2,6] a comparison of the effect of 37 and 25 °C temperatures was also determined in rabbit kidney cortex. These results are seen in Table III. As in the rat tissue, the distribution ratio at 25 °C of α-methyl-D-glucoside was somewhat reduced at 15 min but significantly increased above the 37 °C value at 60 min of incubation. Galactose behaved differently at 25 °C with a reduction in uptake at 15 min (ratio of 2.71 *vs* 1.88) which was also observed at 60 min when essentially a steady state is achieved. These data raise the possibility of differences in the transport systems involved for the two sugars, but the metabolism of galactose prevents definite conclusions.

Developmental aspects of kidney sugar transport

Kidney cortex slices from 1-day-old rats were unable to accumulate α-methyl-D-glucoside to form a concentration gradient either when incubated in 2 mM sugar or with 0.2 mM, a concentration in the range where the adult transport system is unsaturated (Fig. 1). By 5 days of age, a small ability to concentrate the sugar was observed. This progressively increased so that near normal values for adult tissue were observed with tissue from 15-day-old animals (Fig. 1).

The uptake process present in 1-day-old kidney cortex was characterized by study of those parameters enumerated for adult tissue. Table I shows that the distribution ratio for 2 mM glucoside accumulation was reduced from 0.74 to 0.38 by absence of Na^+ and 0.55 under anaerobic conditions. The process was inhibited by phlorizin (Table I) and the presence of glucose, galactose and fructose in the incubation medium (Table II). The latter monosaccharides inhibited uptake about 40%, the same extent as in adult tissue. Fig. 3 demonstrates that the transport process in the cortex from the young animal has the same pH dependence as that of the adult, the average of all values of distribution ratio at pH 7 or below being different statistically from the average of all values above 7.0 ($P < 0.05$).

The lack of ability to eventually accumulate sugar against a gradient could be due to rapid efflux since the formation of the steady state concentration gradient is dependent on both influx and efflux in this two compartment system consisting of intracellular fluid and medium[21]. Similar analysis of the uptake curves of Fig. 1 reveals that the influx rate constant in the 1-day-old tissue incubated with 0.2 mM glucoside is 0.0020 min^{-1}, while that of the adult is 0.0078 min^{-1}, a difference of about 75%. The efflux of α-methyl-D-glucoside was measured in both 1-day-old and adult cortex and found to be 0.063 min^{-1} in the former and 0.087 min^{-1} in the latter. The efflux rate constant of 1-day-old tissue is 30% lower than adult tissue, rather than higher. The disproportional flux changes in young tissue with proportio-

nally higher efflux rate constant is an important kinetic factor in the inability to form a concentration gradient.

That the transport process in the young 1-day-old tissue is rudimentary is supported by the fact that accelerated efflux could not be produced by addition of α-methyl-D-glucoside or glucose to the medium during efflux of ^{14}C-labeled glucoside. In addition, provision of an exogenous energy source such as 5 mM succinate did not increase the uptake rate in 1-day-old tissue suggesting the slow uptake is not related to diminished energy source for transport in the young.

DISCUSSION

Our experiments indicate that α-methyl-D-glucoside is a non-metabolizable sugar actively transported by adult rat kidney cortex cells *in vitro* by a Na^+- and energy-dependent system which conforms to the kinetics of a substrate-saturable process. The present findings have both differences and similarities to those published for the uptake of this sugar by rabbit kidney slices[2-6]. Some of the dissimilarities may be the result of somewhat different experimental conditions, the most prominent of which is the use of 25 °C compared to our 37 °C as the incubation temperature, or due to the different species examined. Effects of variation of temperature on renal cortical slices have been well documented. Reduction in temperature from 37 to 15 °C causes an 82% fall in O_2 consumption[33]. Maude[34] has shown that fluid reabsorption on microperfusion of the tubule segment of a renal cortex slice is slowed 10-fold by a change from 37 to 15 °C. Moreover, Whittembury and Proverbio[35] have observed that at 25 °C there are two mechanisms for Na^+ extrusion from the cell while at lower temperatures only one of these mechanisms persists.

In rat kidney cortex incubated with α-[^{14}C]methyl-D-glucoside, negligible radioactive CO_2 was produced nor were any labeled compounds other than the substrate found in the tissue. On the other hand, rabbit kidney incubated in 1 mM glucoside at 25 °C oxidized 1.04 μmoles/g per h, a value only 40% that of glucose oxidized by the same tissue[2]. The lack of metabolism of α-methyl-D-glucoside by rat renal tubule cells enables accurate assessment of tissue uptake by extracting the tissue pool in hot water or by any other method of preparing tissue extracts. This obviates the tedious determination of metabolites to relate metabolism to transport[13]. Our findings indicate that uptake of α-methyl-D-glucoside by rat kidney cortex cells is not associated with a process of phosphorylation as is seen in bacteria[36].

Whereas rabbit kidney cortex displayed little dependence of glycoside uptake on pH (ref. 5) rat kidney cortex showed a marked increase in uptake between pH 7 and 7.4 with a maximum between pH 7.4 and 8. Uptake of α-methyl-D-glucoside is Na^+ dependent in both species but in the rat the rate increases mainly as the concentration increases from 0 to 72 mequiv Na^+ per l. The apparent transport K_m in the rabbit was 1.2 mM compared to 6 mM in the rat. In both tissues galactose was a competitive inhibitor[3,6]. The K_i for galactose was about 7 mM in rabbit and about 50 mM in the rat.

Since the metabolism of galactose and glucose is so extensive in kidney cortex, transport parameters are difficult to determine. There would be a distinct advantage in having a non metabolizable model sugar which shares the glucose–galactose transport process and whose transport can be easily determined. The evidence

that α-methyl-D-glucoside shares the glucose–galactose transport process is compelling but there are some exceptions. α-Methyl-D-glucoside inhibits galactose uptake and both glucose and galactose are competitive inhibitors of glucoside uptake. Moreover, both sugars cause accelerated efflux of the glucoside which is consistent with the concept of a shared carrier system. The sugars and glucoside are inhibited by phlorizin. Deficiency of α-methyl-D-glucoside accumulation as seen in newborn rat kidney cortex has also been observed to exist for galactose uptake[37]. Although the uptake of glucoside is virtually absent when Na^+ is omitted from the medium, the uptake of galactose is only partially diminished, indicating there may be a Na^+-independent aspect of galactose uptake which is not shared by α-methyl-D-glucoside. Perhaps the most irreconcilable finding for a common system is the 0.2 mM apparent K_m for galactose uptake at low substrate concentrations[13] and the 50 mM apparent K_i for galactose for the glucoside. Kleinzeller[3] also reported a discrepancy between K_m and K_i for the competitive inhibition between α-methyl-D-glucoside and galactose.

The kidney cortex cells of newborn and young rats have been shown to possess only a rudimentary system for concentrating α-methyl-D-glucoside against a concentration gradient. The presence of a process other than passive diffusion is consistent with our data indicating that although a concentration gradient is not formed by newborn tissue even at very low substrate levels, the process is Na^+ dependent, phlorizin inhibited, and affected by glucose and galactose. The limited mechanism for active transport does not seem to be sufficient to participate in accelerated efflux or counterflow. Indeed, the efflux of sugar from the cell was slower than observed with adult tissue. Despite the impaired uptake of α-methyl-D-glucoside or galactose[37] by newborn rat kidney, no mellituria was observed when we analyzed bladder urine. Either the transport system was sufficiently functional to handle the filtered glucose, there is a separate glucose transport component or the very thin urinary bladder epithelium and prolonged urinary bladder stasis combined to facilitate reabsorption after urine formation. These possibilities deserve further investigation. The findings in the newborn also raise the question of the relationship of transepithelial transport in the intact kidney and accumulation by cortical cells *in vitro*. Recent studies with amino acids in the rat have indicated there may be a dissociation between the two processes[38].

Although kinetic analysis of α-methyl-D-glucoside uptake by rat kidney cortex slices is satisfied by a simple two compartment system where influx and efflux can be measured[21], the actual localization of sugar entry and exit to luminal or anti-luminal cell borders has not been determined. Such localization of sugar entry has been assessed by *in vivo* experiments reported by Silverman *et al.*[39,40] in which glucose and galactose were found to enter dog cortex cells *via* both cell membranes. Deetjen and Boylan[41] in their microperfusion studies of D-glucose in isolated rat nephrons demonstrate no actual glucose movement from peritubular capillary to tubule lumen. It may well be that the kidney cortex slice is a more complicated model for study of cellular accumulation of sugars or amino acids than heretofore believed likely. Asymmetry of transport properties of the membrane at the different poles of the tubule cell may be an explanation for the incompleteness of the correlation of α-methyl-D-glucoside and galactose transport characteristics. This seems most pertinent to the observed differences in the temperature effect on

accumulation of galactose and α-methyl-D-glucoside[13]. Nonetheless, because it is not metaboolized, α-methyl-D-glucoside appears to be a model sugar for evaluating transport characteristics of the rapidly metabolized sugars, glucose and galactose

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