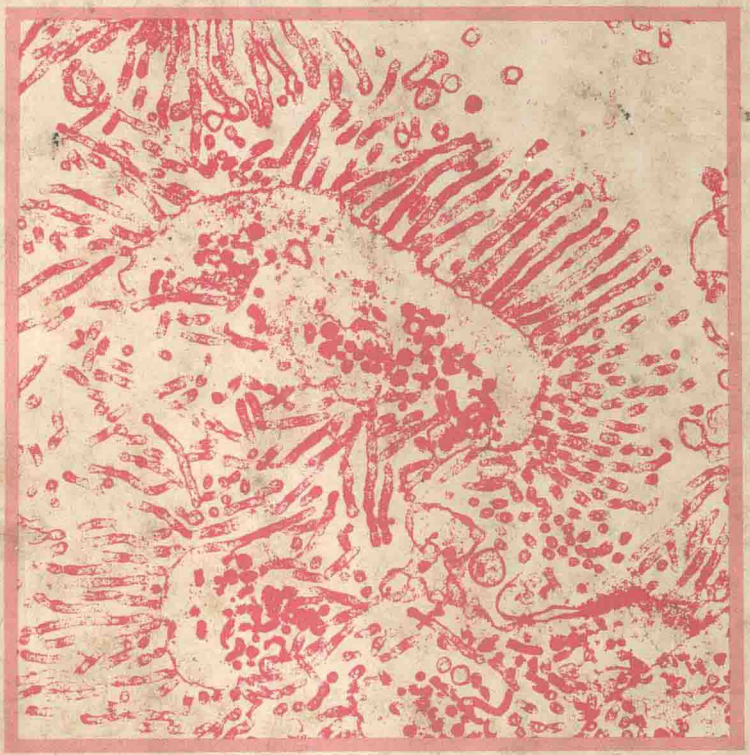
Recent Advances in Renal Physiology and Pharmacology

Edited by Laurence G. Wesson, M.D. and George M. Fanelli, Jr., Ph.D.



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Recent Advances in Renal Physiology and Pharmacology

Proceedings of the Fifteenth Annual A. N. Richards Symposium, held at Jefferson Medical College in Philadelphia, Pennsylvania, 14-15 May 1973

Sponsored by The Physiological Society of Philadelphia

Foreword

This volume, containing contributions to the proceedings of the Fifteenth Alfred Newton Richards Symposium, is the first published collection in the Symposium's brief history. Sponsored by The Physiological Society of Philadelphia, an organization which Dr. Richards strongly supported, the symposia are an expansion of the Richards Memorial Lecture Series. This series, presented annually since 1959, is represented in the present symposium by Dr. Gerhard Giebisch's lecture.

Appropriately, kidney function is the subject of this volume. Dr. Richards developed a keen interest in the study of the kidney and made monumental contributions to this field. Stimulated by the technical achievements of Robert Chambers, Dr. Richards and his associates created the subscience of micropuncture investigation of kidney function. The prominence of micropuncture technology is seen in this symposium.

This symposium, *Recent Advances in Renal Physiology and Pharmacology,* illuminates the advances of a broad spectrum comprising molecular structure, clinical applications of knowledge, homeostatic mechanisms, and the molecular biology of transport systems. Although the subject areas are familiar, the many technological advances and conceptual reversals to canonical renal physiology attest to the continuing vigor of this science as a significant area for research.

This symposium was made possible by generous contributions from Abbott Laboratories, Ayerst Laboratories, Hoechst Pharmaceuticals, Lakeside Laboratories, Lederle Laboratories, McNeil Laboratories, Merck Sharp and Dohme Research Laboratories, Penwalt Corporation, A. H. Robins Co., W. H. Rorer, Inc., Sandoz Pharmaceuticals, G. D. Searle and Co., Smith Kline Corp., Sterling-Winthrop Research Institute, The Upjohn Co., The Wellcome Research Laboratories, and Wyeth Laboratories. The editors and The Physiological Society of Philadelphia acknowledge with sincere thanks these contributions.

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Contents

Pathways of Sugar Transport in Renal Cells

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Evidence concerning pathways of sugar transport in renal cells was examined.

1. Phosphorylation of D-galactose, 2-deoxy-D-glucose, and 2-deoxy-D-galactose takes place within the cells and is not part of the transport step. Methyl glycosides are not phosphorylated.
2. The pathways of active sugar accumulation in slices of rabbit kidney cortex were studied on the basis of their electrolyte requirements and inhibition by selective reagents and competing sugars: (a) the Na-independent transport of 2-deoxy-D-hexoses differs from the Na-dependent transport by its lack of stimulation by K^+ and by its reduced sensitivity to phloridzin. D-galactose is simultaneously transported by both Na-dependent and Na-independent pathways; (b) the pattern of competition of sugars for transport sites allows thus far discrimination between two systems: the carrier shared by D-glucose and D-galactose requires hydroxyls on C_2 (in the position found in D-glucose), C_3, and C_6, and a free C_1-OH is not essential, while the D-galactose - 2-deoxy-D-galactose pathway requires a free C_1-OH as well as C_4-OH (in the position of D-galactose) and C_6-OH.
3. At the antiluminal face of flounder kidney tubules, at least three carriers with differing specificities were characterized: a pathway for α-methyl-D-glucoside; a D-glucose and 2-deoxy-D-glucose pathway; a D-galactose - 2-deoxy-D-galactose transport system. No conclusive evidence for active sugar transport at the antiluminal face was found.
4. In the flounder (and probably also the rat) kidney tubule α-methyl-D-glucoside is reabsorbed by an active step at the luminal cell face, followed by a carrier-mediated passive outflow at the antiluminal cell face.
5. Multiple transport pathways for sugars with overlapping specificities and (in part) different cellular localizations are postulated.

The concept of one pathway for the reabsorption of various sugars in the renal tubule stems from two sources. First, observations of Homer Smith and J. A. Shannon and their students (see Smith, 1951) showed that the tubular reabsorption of sugars such as galactose or xylose was inhibited by elevated glucose concentrations, suggesting the sharing of a common transport mechanism in the renal tubule. Second, the active cellular accumulation of some free sugars by intestinal (Crane, 1960; Csáky and Thale, 1960) and renal (Krane and Crane, 1959) epithelial cells led to the suggestion (Csáky and Fernald, 1961) of identical (or very similar) transport mechanisms in both tissues, illustrated by the following scheme (Fig. 1). Here, the active transport step is localized at the luminal brush border, producing a cellular accumulation of the free sugar. The defined structural requirements for active sugar transport by intestinal cells (Crane, 1960) implied one carrier of a rather wide specificity for various sugars. The sugars accumulated in the cells against their concentration gradient were then assumed to pass the antiluminal (serosal) face of the cells in accordance with their chemical gradient, possibly by a carrier-mediated process (e.g. facilitated diffusion). The observation that in renal cells *in vitro* the cellular accumulation of sugars such as D-galactose was Na-dependent (Kleinzeller and Kotyk, 1961), as previously demonstrated for intestinal sugar transport (Csáky and Thale, 1960), strengthened the analogy of transport mechanisms for sugars in both tissues and contributed to the view that one carrier with a rather wide specificity mediated the active transport of various sugars.

Experimental approaches available for an investigation of whether one or several pathways are involved in the transport of a solute in any cell are rather limited. If one pathway of active sugar transport were involved, one would expect identical electrolyte requirements as well as identical patterns of inhibition by reagents selective for the sugar transport system (such as phloridzin) or by other sugar species competing for the shared carrier. In renal cells, in addition to problems of cellular polarity (shared by all epithelial

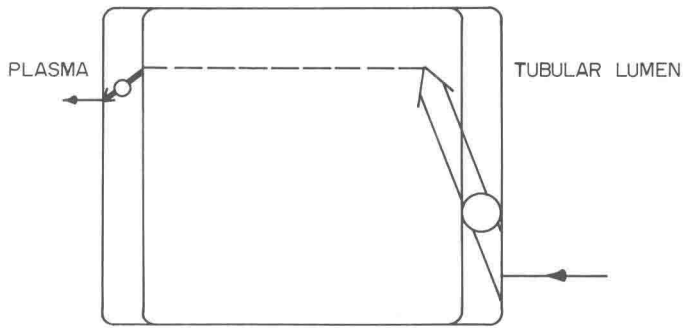

Fig. 1. Simple scheme of transepithelial active sugar transport: White arrow, active transport step; black arrow, carrier-mediated transport (e.g., facilitated diffusion).

cells), the experimental problems are compounded by the complex architecture of the tissue.

Before summarizing present evidence in favor of a multiplicity of transport pathways for sugars in renal tubular cells, it appears appropriate to re-examine the possible relationship between transport and metabolism of some sugars in renal cells.

TRANSPORT AND PHOSPHORYLATION OF SUGARS IN RENAL CELLS

Several years ago we observed major differences in values of free tissue sugars in slices of rabbit kidney cortex when employing different analytical procedures. In the procedure used hitherto in our and other laboratories the analyzed tissue was placed in a solution of $ZnSO_4$ and homogenized. Subsequently, proteins and sugar phosphates were precipitated by the addition of $Ba(OH)_2$. Values obtained for free sugars by this classical Somogyi (1945) procedure were considerably higher for many sugars, such as D-galactose or 2-deoxy-D-glucose, than those found when the tissue was deproteinized with trichloroacetic acid (TCA) and the TCA-free extract fractionated on a Dowex-1 ion exchange column (Kleinzeller, McAvoy, and Griffin, 1973). However, identical values for free tissue sugar were obtained by both methods if, prior to the $ZnSO_4$ + $Ba(OH)_2$ procedure, the tissue enzymes were first inactivated. In the absence of inactivation, the longer the tissue was at room temperature in contact with $ZnSO_4$, the higher values of free sugar were found in the Somogyi supernatant. Thus, as opposed to a widely held belief, a Zn-activated phosphatase catalyses the hydrolysis of sugar phosphates to free sugar. No indication of phosphorylation of α-methyl-D-glucoside (or methyl glycosides of other D-hexoses) was observed, as previously noted by Segal *et al.* (1973) concerning α-methyl-D-glucoside.

The following evidence suggests that the phosphorylation of model sugars such as D-galactose, 2-deoxy-D-glucose, and 2-deoxy-D-galactose takes place within the cells and does not constitute part of the transport step (Kleinzeller *et al.*, 1973). Figure 2 clearly shows that with increasing external pH, the near-steady-state distribution of total (i.e., free plus phosphorylated) tissue galactose in the cells increased while free tissue galactose remained constant. On the other hand, with increasing pH both free and total tissue 2-deoxy-D-galactose decreased. Using tissue homogenates, the same pH dependence could be demonstrated for the phosphorylation of both sugars, i.e., the phosphorylation of galactose increased whereas that of 2-deoxy-D-galactose decreased. Thus, the phosphorylation step is not restricted to preparations with intact cell membranes.

Second, efflux experiments showed that, during the outflow of free D-galactose from the cells, the tissue pool of D-galactose-1-phosphate also decreased with the same rate constants, suggesting that a steady state exists within the cells between free and phosphorylated D-galactose, the dephos-

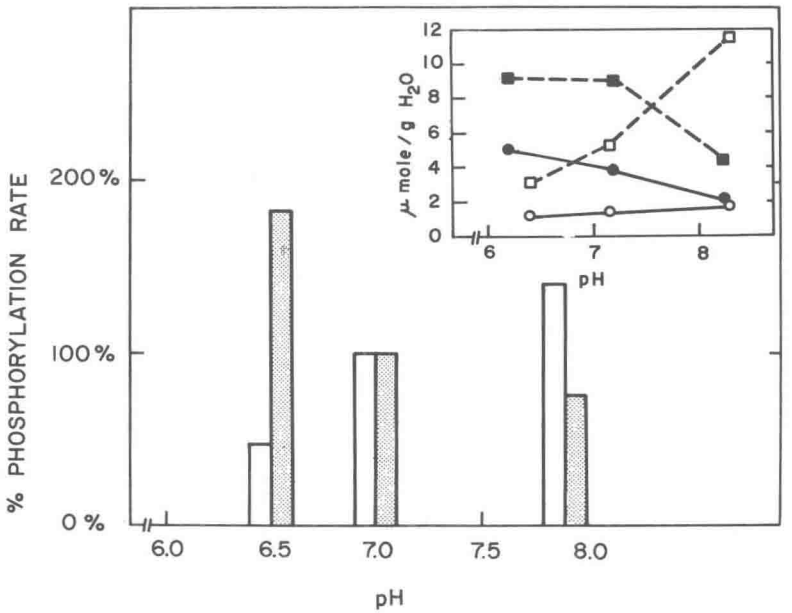

Fig. 2. Effect of pH on the uptake and phosphorylation of D-galactose and 2-deoxy-D-galactose in renal cortex tissue. Insert: Effect of pH on the sugar uptake by slices. Tissue was incubated aerobically (O_2) at 25°C for 60 min in Na-salines at varying pH. Substrates, 1 mM. Each point is the mean of three analyses: ○, □, D-galactose; ●, ■, 2-deoxy-D-galactose; ○, ●, free tissue sugar; □, ■, total (free plus phosphorylated) tissue sugar. Bar graph: Percentage rate of sugar phosphorylation in kidney cortex homogenate (pH 7.0 = 100%). White bars, D-galactose; shaded bars, 2-deoxy-D-galactose.

phorylation of galactose phosphate being the rate-limiting step for the efflux of the free sugar. Using slices of rabbit kidney cortex, the efflux of 2-deoxy-D-glucose or 2-deoxy-D-galactose was not associated with a decrease of the respective phosphates, confirming an observation of Elsas and Macdonell (1972) concerning the former sugar.

Finally, using galactose-1-phosphate as a model, the activation of the dephosphorylation step by Zn^{2+} was demonstrated in homogenates of kidney tissue.

These data are therefore compatible with the following scheme of events. Here the free external sugar (S_o) is transported across the cell membrane (M) by a carrier-mediated process, the active influx of the studied model sugars being energized directly (by ATP) or indirectly (via an electrolyte gradient) by the cell metabolism. Within the cell the free sugar (S_i) is phosphorylated (SP) by a kinase. The reverse process, i.e., hydrolysis of the cellular sugar phosphate, is brought about by a Zn^{2+}-activated phosphatase. In this scheme, K with the appropriate subscript represents the respective rate constants.

EVIDENCE FOR A MULTIPLICITY OF TRANSPORT PATHWAYS

The Electrolyte Requirement

Many of the actively transported sugars were shown (Kleinzeller, Kolínská, and Beneš, 1967; Kleinzeller, 1970b) to require external Na^+ for their cellular accumulation, and K^+ was found to stimulate the active sugar transport. On the other hand, the transport of 2-deoxy-D-sugars was independent of Na^+ and did not require external K^+ (Kleinzeller, 1970b). A considerable portion of D-galactose is also accumulated by renal tubular cells by a Na^+-independent mechanism (Kleinzeller, 1970b) as shown in Fig. 3. These results suggested the possibility of different direct energy sources for the active transport of both groups of sugars. Whereas the Na-dependent transport of sugars such as α-methyl-D-glucoside might be brought about by a mechanism of the Na-gradient type (Almendares and Kleinzeller, 1971), it had to be postulated that the Na-independent active transport of 2-deoxy-hexoses and some other sugars is directly coupled to cellular metabolism, since no evidence was found for other solute gradients capable of driving the up-hill transport of these sugars.

The pH dependence of the active transport of some model sugars (Kleinzeller *et al.*, 1970) suggested a further complication. It had been shown earlier (Fig. 2) that increasing pH had opposite effects on the uptake of sugars such

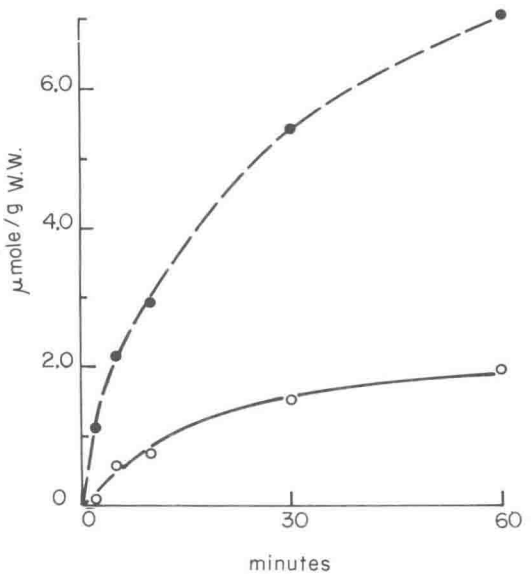

Fig. 3. Uptake of D-galactose by renal cortex slices. Tissue incubated aerobically (O_2) at 25°C for 60 min in Na-free saline (LiCl replacing NaCl) in the presence of 1 mM D-galactose. ○, free tissue sugar; ●, tissue galactose-1 phosphate.

Table 1. Effect of phloridzin and phloretin on sugar accumulation in slices of rabbit kidney cortex

Sugar	Saline	Phloridzin K_i (mM)	Phloretin K_i (mM)
α-Methyl-D-glucoside	Na^+	0.007	0.2
2-Deoxy-D-glucose	Na^+	0.6	0.2
2-Deoxy-D-galactose	Na^+	0.9	0.5
D-Galactose	Na^+	0.06	0.3
D-Galactose	Li^+	0.7	0.4

The tissue was incubated aerobically (O_2) in salines at 25°C for 20 min with 0.5 and 1 mM of the sugars and varying concentrations of the inhibitor. Values of K_i were computed using the Hunter-Downs plot.

as D-galactose and 2-deoxy-D-glucose, as opposed to 2-deoxy-D-galactose. Thus, the possibility of differing pathways for the Na-independent transport of 2-deoxy-glucose and 2-deoxy-galactose had to be considered.

Effect of Inhibitors

The inhibitory action of phloridzin and its aglycon, phloretin, on the active sugar accumulation in slices of rabbit kidney cortex allowed discrimination between two transport pathways (Kleinzeller, 1972). Based on measurements of the inhibition constants (Table 1), the Na-dependent transport pathway for α-methyl-D-glucoside is about 30 times more sensitive to phloridzin than to phloretin. On the other hand, the K_i for the phloridzin inhibition of the Na-independent transport of 2-deoxy-hexoses is two to three times higher than that for phloretin. A comparison of the K_i for the inhibition of D-galactose transport by phloridzin and phloretin in the presence and absence of Na^+ was of particular interest (unpublished data). In the presence of Na^+, the K_i for the phloridzin inhibition was intermediate between that for α-methyl-D-glucoside and the 2-deoxy-D-sugars. In the absence of Na^+, the values of K_i for both inhibitors were of the same order as those found for the inhibition of the 2-deoxy-hexoses. It follows that, in the presence of Na^+, D-galactose is transported simultaneously by both Na-dependent and Na-independent pathways.

Competition of Sugars for Transport Sites

Kinetic data obtained using slices of kidney cortex indicated some discrepancies between the values of transport K_m for a given sugar and the K_i for its competitive inhibitory effect on the transport of another sugar species; such discrepancies would not be expected if one carrier were involved in the transport of both sugars. Therefore, multiple transport pathways with some overlapping specificities have been suggested (Kleinzeller, 1970a). A similar conclusion was independently drawn on the basis of competition of sugars for transport pathways in renal tubules *in vivo* (Silverman, Aganon, and Chinard, 1970). A more detailed investigation of the patterns of specificity of sugar

transport in slices of rabbit kidney cortex is now yielding new information (Kleinzeller *et al.*, unpublished). Using [^{3}H] 2-deoxy-D-galactose, no competitive inhibition of the transport of this sugar by 2-deoxy-D-glucose was observed, although the cellular accumulation of 2-deoxy-D-glucose was inhibited by its galactose anomer. This observation provided further indication, in addition to that arising from differing patterns of pH effects on the transport of these two sugars, in favor of different pathways for the Na-independent transport of these two 2-deoxy-D-hexoses.

An active, Na-dependent accumulation of β-methyl-D-galactoside by renal cells was found. Figure 4 shows that the cellular accumulation of this sugar is competitively inhibited by the methyl glycosides of D-glucose, D-galactose, and L-arabinose (pentose homolog of D-galactose), as well as by the hexoses D-glucose and D-galactose. Since the reverse is also true, i.e., β-methyl-D-galactoside inhibits the transport of the above sugars, a single carrier is shared for their transport. Furthermore, it follows that a free hydroxyl on C_1 and its steric position is not essential for the interaction of these sugars with the carrier. On the other hand, α-methyl-D-mannoside and α-methyl-2-deoxy-D-

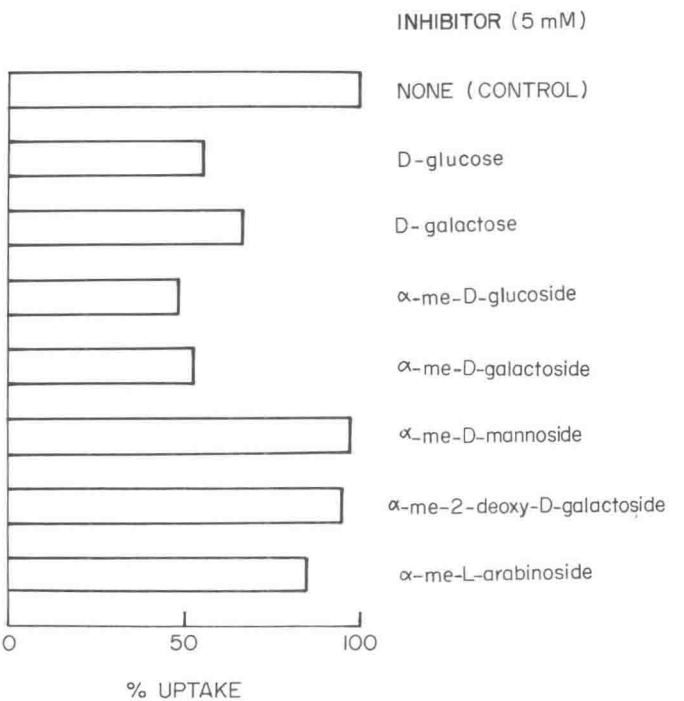

Fig. 4. Competitive inhibition of β-methyl-D-galactoside accumulation in kidney cortex slices. Tissue incubated aerobically (O_2) at 25°C for 60 min in the presence of 1 mM substrate, without (control, 100%) or with 5 mM other sugars (percentage of uptake of control). Each result represents the mean of at least five analyses. Mean accumulation ratio for β-methyl-D-galactoside is 3.0.