Transport of Glucose and Galactose in Kidney-Cortex Cells

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1. The aerobic transport of D-glucose and D-galactose in rabbit kidney tissue at 25° was studied. 2. In slices forming glucose from added substrates an accumulation of glucose against its concentration gradient was found. The apparent ratio of intracellular ($[S]_i$) and extracellular ($[S]_o$) glucose concentrations was increased by 0·4 mM-phlorrhizin and 0·3mM-ouabain. 3. Slices and isolated renal tubules actively accumulated glucose from the saline; the apparent $[S]_i/[S]_o$ fell below 1·0 only at $[S]_o$ higher than 0·5mM. 4. The rate of glucose oxidation by slices was characterized by the following parameters: K_m 1·16mM; $V_{max.}$ 4·5μmoles/g. wet wt./hr. 5. The active accumulation of glucose from the saline was decreased by 0·1 mM-2,4-dinitrophenol, 0·4mM-phlorrhizin and by the absence of external Na^+. 6. The kinetic parameters of galactose entry into the cells were: K_m 1·5mM; V_{max} 10μmoles/g. wet wt./hr. 7. The efflux kinetics from slices indicated two intracellular compartments for D-galactose. The galactose efflux was greatly diminished at 0°, was inhibited by 0·4mM-phlorrhizin, but was insensitive to ouabain. 8. The following mechanism of glucose and galactose transport in renal tubular cells is suggested: (*a*) at the tubular membrane, these sugars are actively transported into the cells by a metabolically- and Na^+-dependent phlorrhizin-sensitive mechanism; (*b*) at the basal cell membrane, these sugars are transported in accordance with their concentration gradient by a phlorrhizin-sensitive Na^+-independent facilitated diffusion. The steady-state intracellular sugar concentration is determined by the kinetic parameters of active entry, passive outflow and intracellular utilization.

Krane & Crane (1959) showed that rabbit kidney-cortex slices accumulate D-galactose against a concentration gradient by a metabolically dependent phlorrhizin-sensitive mechanism. Subsequently Kleinzeller & Kotyk (1961) demonstrated this active galactose transport to be Na^+-dependent.

Krane & Crane (1959) did not find an accumulation of D-glucose in kidney-cortex slices, although this sugar competitively inhibited the accumulation of galactose, suggesting a common transport mechanism. A high rate of intracellular glucose utilization was postulated by these authors as an explanation for the lack of glucose accumulation in this tissue.

The present study was started by an attempt to demonstrate an active accumulation of D-glucose in kidney-cortex slices, and was subsequently extended to a more detailed investigation of the mechanism of glucose and galactose transport in kidney-cortex cells.

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METHODS

Healthy adult rabbits (2–3kg.) were used. Slices of kidney cortex were cut by the method of Deutsch (1963). In some of the experiments isolated renal tubules, prepared as described by Burg & Orloff (1962), were employed.

Incubation saline media. In most experiments the bicarbonate saline of Krebs & Henseleit (1932) served as the suspending medium for slices or tubules. For technical reasons, in some instances a balanced phosphate saline [similar to that of medium IIA of Krebs (1950), but containing a physiological concentration of Ca^{2+} and 10mM-phosphate buffer, pH 7·4, the organic constituents being omitted] was employed. No marked differences between sugar transport in these two media were observed. Unless otherwise stated, 10mM-sodium acetate was used as metabolic substrate for the tissue (Kleinzeller, 1943), thus also decreasing the rate of utilization of metabolizable sugars.

Na^+-free media were prepared by complete replacement of Na^+ by tris$^+$ ion; this ion was found to be innocuous to the sugar transport function in hamster intestinal mucosa (Bosáčková & Crane, 1965).

Sugar accumulation in the tissue. The standard procedure was as follows (see also Krane & Crane, 1959): approx. 100mg. wet wt. of slices was placed into each 25ml. conical

flask containing 3 ml. of saline with or without the monosaccharide studied; the flasks were filled with the appropriate gaseous phase [$O_2 + CO_2$ (95:5) for bicarbonate saline; O_2 for phosphate saline], and then closed with rubber stoppers and agitated at 25° in a Dubnoff metabolic incubator.

For the study of the effect of Na^+ on sugar transport, the above procedure was somewhat modified: the slices were first soaked for 2 hr. at 0° in the appropriate saline (without sugar added) and only then incubated aerobically at 25° as described in the standard procedure; in this way most of the tissue Na^+ was washed out by the Na^+-free saline, the apparent intracellular Na^+ concentration being 3·5 mM compared with a value of about 80 mM in Na^+ media.

At the end of the incubation period the slices were removed, blotted between hardened filter paper and weighed on a torsion balance. For sugar determination a deproteinized (Somogyi, 1945) extract of the tissue or incubation saline was used.

In experiments with renal tubules, suspensions of approx. 150 mg. wet wt. of tissue in 3 ml. of saline were incubated. At the end of the experiment the tubules were centrifuged off at 20000 g, the supernatant was decanted, the walls of the tubes were wiped with filter paper to remove adhering medium and the weighed pellet was then treated as described for the slices.

In the deproteinized supernatants the sugars being examined were estimated. Glucose was determined with glucose oxidase essentially as described by Huggett & Nixon (1957). The values had to be corrected for tissue blanks found after incubation in saline media without added sugar. This blank could be considerably decreased by aerobic preincubation of the tissue for 30 min. at 25° in a sugar-free saline and only then following the standard incubation procedure. For the estimation of ^{14}C-labelled glucose and galactose, a portion of the supernatant after deproteinization was pipetted into flasks, the water was evaporated at about 80°, 2 ml. of ethanol was added to each flask and the flasks were gently agitated for at least 20 min.; after the addition of 10 ml. of scintillation liquid, the radioactivity was measured with a Tracerlab scintillation counter.

By the above procedure free sugars in the tissue are determined: this has been established directly for galactose by Krane & Crane (1959); for glucose, the use of glucose oxidase for the estimation of this sugar excludes the possibility of sugar phosphates being assayed (see Keilin & Hartree, 1948) even if these were present in the tissue extract after the $ZnSO_4$–$Ba(OH)_2$ deproteinization (Somogyi, 1945), which also precipitates phosphorylated sugars (see e.g. Kipnis & Cori, 1959).

From the results thus obtained the apparent intracellular sugar concentration, $[S]_i$, was calculated: the water content of aerobically incubated slices was taken to be 3 kg. of water/kg. dry wt. of tissue (see e.g. Kleinzeller & Knotková, 1964); where required (e.g. when inhibitors were used or when the incubation temperature was 0°), the tissue water was separately determined. A correction for sugar in the extracellular fluid compartment had to be applied, making the following assumptions: (*a*) that the concentration of the analysed sugar in the extracellular compartment equals that in the external medium, $[S]_o$; (*b*) that the extracellular spaces occupied by inulin and the examined monosaccharides are identical. The inulin space in slices was taken to be 250 ml. of water/kg. of slices (see Whittam, 1956; Kleinzeller & Cort, 1960); for that in the pellet of renal tubules, the value given by Burg & Orloff (1962), i.e. 200 ml. of water/kg., was used. The intracellular sugar was assumed to be uniformly distributed in all the intracellular water. The validity of the above assumptions is considered in the Discussion section.

Estimations of $[S]_i$ carried out in triplicate usually agreed within 5–10%. The $[S]_i/[S]_o$ ratio expresses then the degree of intracellular accumulation; values above 1·0 indicate transport against the concentration gradient.

In some experiments the initial rate, v, of galactose accumulation in the cells (in μmoles/g./hr.) was determined as the amount of [^{14}C]galactose entering the cells during 20 min. incubation at 25° (after correction for the extracellular space).

Sugar oxidation. The rate of oxidation of the sugars by the tissue was followed by measuring the amount of $^{14}CO_2$ formed from U-^{14}C-labelled substrates (approx. 0·1 μc/ml. of saline). Slices were incubated in phosphate saline in conical flasks provided with a centre well containing a disk of Whatman no. 3 filter paper soaked with 0·1 ml. of N-NaOH and the radioactivity trapped in the paper was measured as described by Buhler (1962); checks performed in this Laboratory by Dr M. Höfer (personal communication) showed that 95–100% of the $^{14}CO_2$ liberated could thus be estimated. From the known specific activity of the substrate used the amount of sugar oxidized during the incubation period (30–60 min.) was calculated. Results are expressed in μmoles of sugar/g. wet wt. of tissue/hr.

Galactose efflux. The technique of Kleinzeller, Janáček & Knotková (1962) was used. Slices were aerobically preincubated for 2 hr. at 25° in saline media containing [^{14}C]galactose (0·1 μc/ml.) to ensure a steady-state concentration of the sugar in the cells. The efflux was then followed for 60 min. either with or without galactose in the washing-out medium. Such procedure is feasible with kidney-cortex slices, which maintain steady values of tissue water and electrolytes during the whole experimental period. From the kinetics the efflux rate constants and corresponding sugar spaces were calculated.

Materials. Collagenase for the preparation of renal tubules was a product of Sigma Chemical Co., St Louis, Mo., U.S.A. (a gift from Dr J. Orloff, Bethesda, Md., U.S.A.). D-[U-^{14}C]Glucose and D-[U-^{14}C]galactose were obtained from the Institute for Research, Production and Application of Radioisotopes, Prague, Czechoslovakia; D-[1-^{14}C]-galactose was purchased from The Radiochemical Centre, Amersham, Bucks. All other reagents were commercial preparations of A.R. grade.

RESULTS

Evidence for glucose accumulation in kidney-cortex slices

Under conditions in which kidney-cortex slices produce glucose from added substrates (see Krebs, Bennett, de Gasquet, Gascoyne & Yoshida, 1963), the cells maintained a concentration gradient of glucose against the external medium (see Table 1). The values also suggested that the higher the glucose formation (and consequently the glucose concentra-

tion in the saline, $[S]_o$) the lower the concentration gradient ($[S]_i/[S]_o$ ratio).

Dependence of the $[S]_i/[S]_o$ ratio for glucose on the external sugar concentration

The above experiment suggested the possibility that Krane & Crane (1959) could not observe an accumulation of glucose against its concentration gradient in kidney-cortex slices because of the relatively high external concentration of this sugar used. Therefore the dependence of the $[S]_i/[S]_o$ ratio on the external glucose concentration after 60min. incubation with glucose added to the medium was examined (see Fig. 1); this incubation period was usually sufficient to obtain steady-state concentrations for tissue glucose. Fig. 1 also gives the values for galactose.

At low external concentrations of glucose (below 0·5mM) the cells established an $[S]_i/[S]_o$ ratio higher than 1·0, whereas at higher $[S]_o$ the apparent intracellular glucose concentration fell below that in the saline.

From the experiments described in Table 1 and Fig. 1 it follows that glucose accumulation in kidney-cortex cells may be studied under two sets of conditions, i.e. under conditions of gluconeogenesis when the uptake step of glucose is not operative, and with external glucose added to the medium when the cells have to transport glucose against the concentration gradient.

Steady-state $[S]_i/[S]_o$ ratios lower than 1·0 may be due to an increased intracellular utilization of glucose at higher concentrations of this sugar (i.e. the K_m for the uphill transport being lower than the K_m for intracellular glucose utilization). The apparent K_m for glucose utilization in the presence of 10mM-sodium acetate was 1·16mM (extrapolated from Fig. 2), V_{max} being 4·5μmoles/g. of tissue/hr.; the corresponding values for glucose uptake from the media established on the basis of the slopes of the uptake curve were: K_m 2·1mM; $V_{max.}$ 32μmoles/g./hr. The values of the kinetic parameters found do not appear to be consonant with the above assumption. However, the values of K_m and $V_{max.}$ for intracellular glucose utilization correspond to only one metabolic pathway of this substrate, i.e. oxidation, whereas glucose may have been metabolized within the cells by other pathways.

For galactose, the results of Krane & Crane (1959) were confirmed in that the $[S]_i/[S]_o$ ratio fell with increasing $[S]_o$; at $[S]_o$ 0·1mM values up to 20 were obtained, whereas at external concentrations of 20mM the $[S]_i/[S]_o$ ratio was close to 1·0 (1·08 ± 0·03 S.E.M., $n=8$). The rate of oxidation of [U-^{14}C]-galactose at $[S]_o$ 1mM was 0·02μmole/g./hr. From the results in Fig. 3 the extrapolated kinetic parameters of galactose entry into the cells were: K_m 1·5mM (a value close to that found by Krane & Crane, 1959); $V_{max.}$ 10μmoles/g./hr.

Glucose and galactose accumulation in isolated renal tubules

Since it has been shown by Burg & Orloff (1962) that the collagenous basal membrane of the tubule represents a permeability barrier, decreasing the

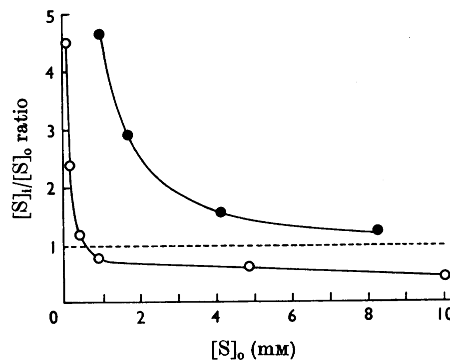

Fig. 1. Effect of sugar concentration on the accumulation of glucose and galactose in kidney-cortex slices. Slices were incubated aerobically [O_2+CO_2 (95:5)] for 60min. at 25° in bicarbonate saline containing 10mM-sodium acetate and various concentrations of glucose (○) or galactose (●). Each value is the mean of three analyses.

Table 1. *Glucose formation and accumulation in kidney-cortex slices*

Slices were incubated aerobically [O_2+CO_2 (95:5)] at 37° for 60min. in bicarbonate saline containing 10mM-glycerol or 10mM-fumarate or both. Each value is the mean of three analyses.

Substrate	Glucose formed (μmoles/g./hr.)	Final glucose concn. in medium (mM)	Intracellular glucose concn. (mM)	$[S]_i/[S]_o$ ratio
Glycerol	11·6	0·35	1·90	5·44
Fumarate	28·6	0·82	2·53	3·09
Glycerol + fumarate	101·0	3·25	4·26	1·31

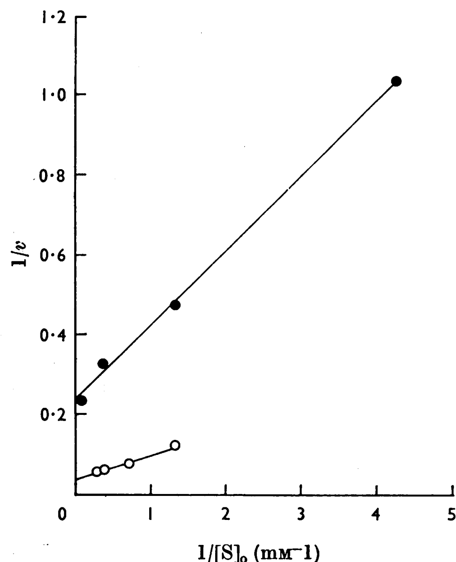

Fig. 2. Lineweaver–Burk plot of the rate of glucose utilization in kidney-cortex slices. Slices were incubated aerobically (O_2) at 25° in phosphate saline containing 10mM-sodium acetate and various concentrations of glucose. ○, Rate determined by measuring $^{14}CO_2$ formed in 30min. from [U-^{14}C]glucose; ●, rate determined from the slope of glucose removed from the saline. Initial velocity, v, is expressed in μmoles/g. wet wt./hr.

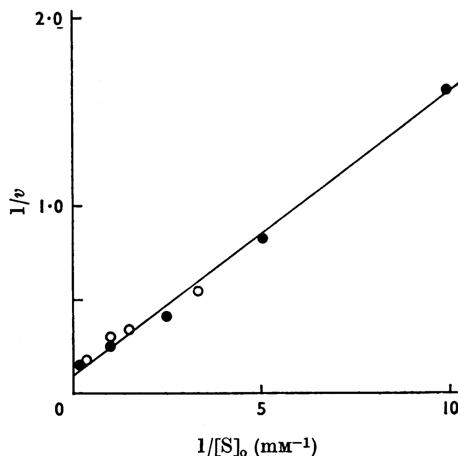

Fig. 3. Lineweaver–Burk plot of the rate of galactose transport into kidney-cortex slices. Slices were incubated aerobically [O_2+CO_2 (95:5)] at 25° in bicarbonate saline containing 10mM-sodium acetate and various concentrations of galactose. ○ and ● indicate two different experiments. Initial velocity, v, is expressed in μmoles/g. wet wt./hr.

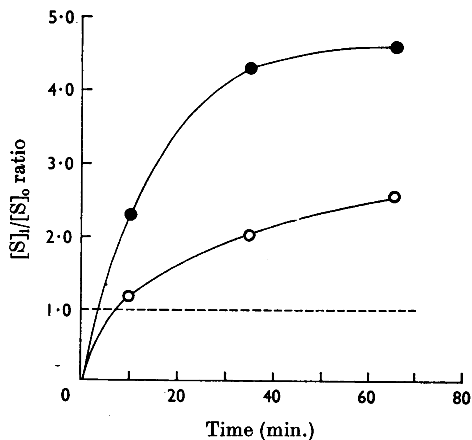

Fig. 4. Time-curve of galactose accumulation in kidney-cortex slices and isolated tubules. Tissue preparations were incubated aerobically [O_2+CO_2 (95:5)] at 25° in bicarbonate saline containing 10mM-sodium acetate and 1mM-galactose. ○, Slices; ●, tubules.

flow of solutes (Na^+, p-aminohippurate) threefold, the possibility was considered that the low $[S]_i/[S]_o$ ratio for glucose at higher $[S]_o$ might be due to a change of the rate of sugar outflow. First, the rates of entry of galactose into slices and isolated tubules were compared (Fig. 4). The rate of galactose entry into the cells was about three times as fast with tubules as with slices, the steady-state $[S]_i/[S]_o$ ratio being identical in both preparations.

The steady-state $[S]_i/[S]_o$ ratios for glucose (with external glucose added to the saline) accumulated in the slices and isolated renal tubules were also identical in both preparations (Fig. 5).

The above observations appear to indicate that the basal collagenous membrane of the tubules does not represent an important barrier for sugar outflow; the faster rate of sugar entry into the cells in the isolated tubule preparation, compared with slices, may be due to the shorter diffusion pathway from the saline to the transport site.

Effect of inhibitors on glucose transport

To define further the character of uphill glucose accumulation in renal cells, the effect of various inhibitors and Na^+ on this process was studied.

2,4-*Dinitrophenol.* At all external glucose concentrations tested (up to 5mM) the steady-state $[S]_i/[S]_o$ ratio was lower in the presence of 0·1mM-2,4-dinitrophenol (Fig. 6). This decrease of the glucose accumulation is obviously due to two factors: (a) an inhibition of the active transport mechanism (demonstrated for galactose accumulation by Krane & Crane, 1959), and (b) an increased

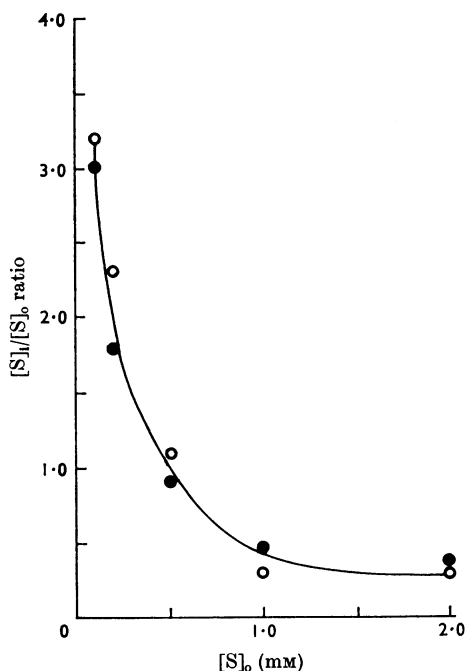

Fig. 5. Glucose accumulation in kidney-cortex slices and isolated renal tubules. Tissue preparations were incubated aerobically [O_2+CO_2 (95:5)] for 60min. at 25° in bicarbonate saline containing 10mM-sodium acetate and various glucose concentrations. ○, Slices, ●, tubules.

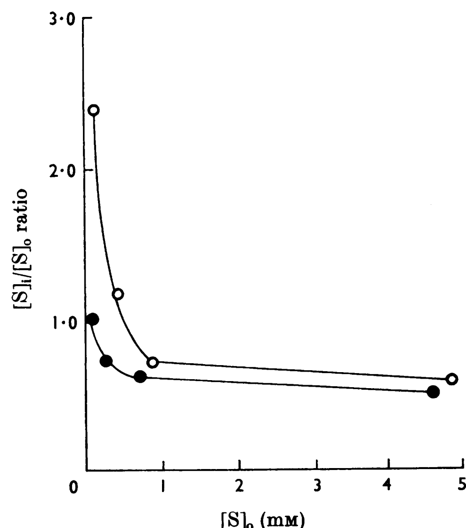

Fig. 6. Effect of 2,4-dinitrophenol on the accumulation of glucose in kidney-cortex slices. Slices were incubated aerobically [O_2+CO_2 (95:5)] for 60min. at 25° in bicarbonate saline without (control) and with 0·1mM-2,4-dinitrophenol. ○, Control; ●, 2,4-dinitrophenol.

Table 2. *Effect of phlorrhizin and* Na^+ *on the accumulation of glucose in kidney-cortex slices*

Slices were incubated aerobically [O_2+CO_2 (95:5)] for 60min. at 25° in bicarbonate saline media containing 10mM-sodium acetate (or tris) and 0·15mM-[U-^{14}C]glucose. Each value is the mean ± S.E.M. of six analyses.

Saline	Inhibitor	$[S]_i/[S]_o$ ratio
Na^+ saline	None (control)	1·90 ± 0·06
Na^+ saline	Phlorrhizin (0·5mM)	1·32 ± 0·04
Tris$^+$ saline	None	1·51 ± 0·05

utilization of glucose in the presence of uncoupling agents. Similar results were also obtained with other uncouplers, e.g. with carbonyl cyanide *m*-chlorophenylhydrazone.

Phlorrhizin. Phlorrhizin (0·5mM) inhibited glucose accumulation in kidney-cortex slices (Table 2). However, when the sugar entry step was not operative, the cells forming glucose from added substrates, the same concentration of the inhibitor produced a marked increase in the $[S]_i/[S]_o$ ratio (Table 3); simultaneously, the formation of glucose was impaired by the inhibitor. These differences in the action of phlorrhizin under both sets of experimental conditions might be due to: (*a*) a decrease of the glucose formation, which itself would produce an increase of the accumulation ratio (see Fig. 1), and/or (*b*) an inhibition of the sugar outflow from the cells. This latter aspect was tested by using galactose as the model sugar (see below).

Ouabain. The accumulation of glucose in the slices was inhibited by the absence of Na^+ in the incubating saline (Table 2), suggesting an Na^+-dependence of the process. This possibility was further examined by using ouabain as an inhibitor. It was found that 0·03mM-ouabain, which prevents the accumulation of galactose (Kleinzeller & Kotyk, 1961), actually increased the glucose $[S]_i/[S]_o$ ratio when this sugar was formed from added substrates (Table 3), but also markedly decreased gluconeogenesis. Since the glucose accumulation in the renal cells is a function of $[S]_o$ (see above), any factor decreasing glucose formation would also increase the $[S]_i/[S]_o$ ratio. Because of the many factors involved in glucose accumulation, a direct demonstration of the Na^+-dependence of this process in slices by using ouabain is difficult to establish.

The results reported above may be taken as evidence for an active accumulation of glucose by kidney-cortex cells, this process being Na^+-dependent and phlorrhizin-sensitive. These characteristics of the transport system for glucose coincide with those already established for galactose (Krane & Crane, 1959; Kleinzeller & Kotyk, 1961).

Table 3. *Effect of phlorrhizin and ouabain on glucose formation and accumulation in kidney-cortex slices*

Slices were incubated aerobically [O_2+CO_2 (95:5)] for 60min. at 25° in bicarbonate saline containing 10mM-glycerol and 10mM-fumarate. Each value is the mean of three analyses.

Inhibitor	Glucose formed (μmoles/g./hr.)	Final glucose concn. in medium (mM)	Intracellular glucose concn. (mM)	$[S]_i/[S]_o$ ratio
None (control)	14·8	0·50	1·03	2·06
Phlorrhizin (0·5mM)	9·5	0·25	1·53	5·45
Ouabain (0·03mM)	12·3	0·38	1·23	3·24

Galactose efflux

The study of efflux kinetics may provide some information on the mechanism by which the outflow is brought about. Though it would be desirable to have data for glucose efflux kinetics, such experiments might be seriously distorted by the not negligible glucose utilization. Therefore some experiments were directed to contribute to the understanding of the mechanism of [^{14}C]galactose efflux.

Time-course of galactose efflux. Fig. 7 shows the kinetics of galactose efflux from loaded slices into a sugar-free saline at 25° and 0°. The curve for 25° can be graphically resolved into two components: a relatively fast one with rate constant k_2 $0{\cdot}255 \pm 0{\cdot}056\,\text{min.}^{-1}$ ($n=4$) corresponding to $38 \pm 7\%$ of the tissue galactose, and a slow component with k_2' $0{\cdot}0125 \pm 0{\cdot}0035\,\text{min.}^{-1}$ corresponding to $62 \pm 7\%$ of the tissue galactose. Practically the same values for rate constants and spaces were obtained when labelled galactose was washed out of the slices into a saline containing 1mM-galactose. The amount of tissue galactose corresponding to the relatively fast efflux component by far exceeded that calculated to be present in the inulin extracellular space.

The efflux of galactose from the slices was considerably slowed by lowering the temperature to 0° (Fig. 7). The amount of galactose washed out also markedly exceeded that calculated to be present in the inulin extracellular space. The effect of phlorrhizin on the influx of galactose at 0° was studied (Fig. 8) to distinguish between the two feasible possibilities of this observation, i.e. (*a*) the extracellular space for galactose being identical with that for inulin, the extra galactose washed out coming from an intracellular compartment; (*b*) the extracellular space for galactose differing from that of inulin. The portion of galactose inflow into the tissue exceeding the inulin space of 25% of the tissue wet weight was very slow and only this component was inhibited by phlorrhizin.

It may thus be concluded that the relatively fast efflux component with rate constant k_2 is a composite one, corresponding to efflux from the extracellular compartment and also from an intracellular space.

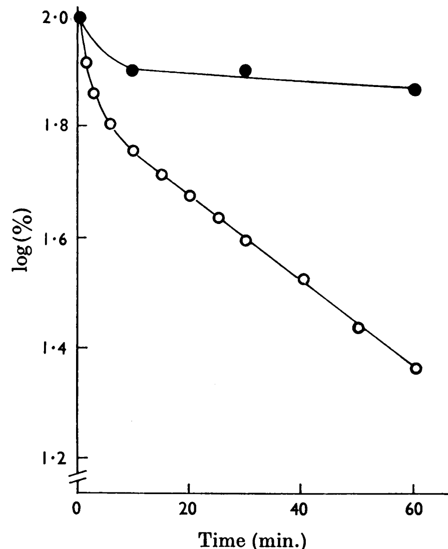

Fig. 7. Effect of temperature on the efflux of galactose. Kidney-cortex slices were loaded with [U-^{14}C]galactose (1mM) by incubation aerobically [O_2+CO_2 (95:5)] for 60min. at 25° in phosphate saline with 10mM-sodium acetate. The efflux of galactose into saline without sugar was then followed at 25° (○) and at 0° (●).

Effects of phlorrhizin and ouabain on galactose efflux. Phlorrhizin (0·4mM) markedly inhibited the galactose efflux at 25°, particularly the fast component, whereas 0·3mM-ouabain had practically no effect (Fig. 9). This latter result suggests that, as opposed to galactose entry into the cells, the mechanism responsible for the efflux of this sugar is not Na^+-dependent.

The sum of observations reported here on the galactose efflux, i.e. considerable decrease by lowering the temperature to 0°, sensitivity to phlorrhizin

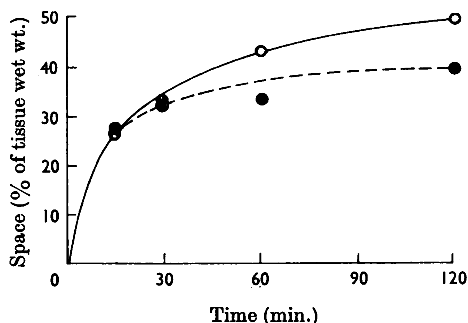

Fig. 8. Effect of phlorrhizin on galactose influx at 0°. Slices were first soaked in phosphate saline without added substrate at 0° for 2·5hr. to assure a balanced state of tissue constituents. The tissue was then transferred to ice-cold saline containing 1mM-[U-^{14}C]galactose without (control) and with 1mM-phlorrhizin. ○, Control; ●, phlorrhizin.

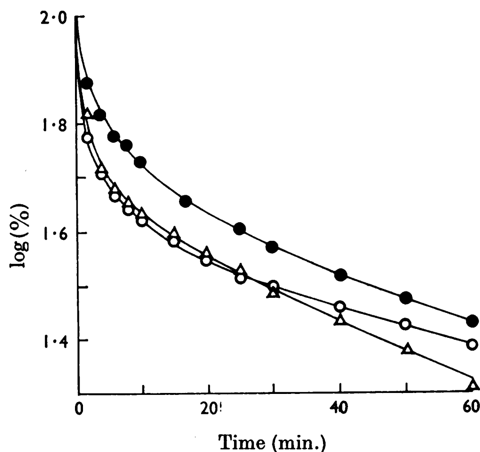

Fig. 9. Effect of phlorrhizin and ouabain on the efflux of galactose at 25°. Experimental details were as given in the legend to Fig. 4. The efflux of galactose was followed at 25° without (control) and with 0·4mM-phlorrhizin or 0·3mM-ouabain. ○, Control; ●, phlorrhizin; △, ouabain.

and insensitivity to ouabain, suggests that this transport is brought about by facilitated diffusion.

DISCUSSION

It has been shown above that an accumulation of glucose in kidney-cortex cells occurs under two sets of experimental conditions, i.e. when the slices were incubated in the presence of external glucose and under conditions of gluconeogenesis. Since the analytical method employed here determines free glucose, $[S]_i/[S]_o$ ratios higher than 1·0 indicate an active transport process.

After considering possible errors in the assessment of the $[S]_i/[S]_o$ ratios for sugars, the relevance of the findings to the following aspects is discussed below, i.e. (*a*) the mechanisms of the glucose and galactose transport systems in kidney-cortex cells, and (*b*) their localization.

Possible errors involved in the determination of the intracellular sugar concentration. The assessment of the $[S]_i/[S]_o$ ratio for sugar accumulation in the tissue (see the Methods section) may be distorted by several errors.

The observation that the extracellular spaces for inulin and galactose are within the limits of experimental error identical (Fig. 8) is consonant with those of Rosenberg, Downing & Segal (1962), who showed that the equilibrium spaces for saccharose and some polyols exceeded that of inulin, the extra entry of these compounds being inhibited by phlorrhizin. The result presented in Fig. 8 thus justifies the procedure adopted for the calculation of $[S]_i$.

In kidney-cortex slices, most of the intracellular water appears to be available as solvent, the equilibrium space for a small non-metabolizable and water-soluble molecule, i.e. propane-1,2-diol, being 81·4 ± 5·3% (Kleinzeller & Knotková, 1966). On the other hand, the assumption of a uniform distribution of sugars in the intracellular water may not hold, the data on efflux kinetics of galactose (Fig. 7) pointing in favour of intracellular 'compartmentation'; in this respect galactose does not differ from some other non-electrolytes, e.g. propane-1,2-diol (Kleinzeller, 1965).

Finally, the sugar utilization during the period of handling the tissue before deproteinization may affect the computed $[S]_i$. From data in Fig. 2 it was calculated that 0·03μmole of glucose was oxidized during the 2min. required for the blotting and weighing of the slices incubated at $[S]_o$ 0·3mM; thus at the moment of removal of the slices from the medium the $[S]_i/[S]_o$ ratio for glucose would in fact be about 10% higher than actually found. Such an error would be minimal for the very slowly oxidized galactose.

The factors enumerated here may thus affect the computed $[S]_i$ and the term 'apparent $[S]_i/[S]_o$ ratios' appears fully justified.

Mechanism of glucose and galactose transport in kidney-cortex cells. The entries of both D-glucose and D-galactose (see Krane & Crane, 1959; Kleinzeller & Kotyk, 1961) show identical characteristics, i.e. a metabolically dependent uphill transport, Na^+-dependence and inhibition by phlorrhizin; further, glucose competitively inhibits the galactose accumulation (Krane & Crane, 1959). Thus the criteria (Rosenberg & Wilbrandt, 1963) for an active

transport of these sugars by the same system are satisfied. This active accumulation may possibly be correlated with the mechanism responsible for the active reabsorption of these sugars in renal tubules *in vivo*, the latter showing saturation kinetics, phlorrhizin-sensitivity, competition between glucose and galactose (see Smith, 1951) and also Na^+-dependence (Vogel, Lauterbach & Kröger, 1965).

The mechanism of outflow of these sugars could be studied here directly only for galactose. It appears that the system responsible for the outflow differs from that for entry in being ouabain-insensitive and thus Na^+-independent. The results reported above point to a facilitated diffusion mechanism for the outflow. For glucose, information on the outflow mechanism could be obtained only indirectly by comparison of the effect of phlorrhizin and ouabain on the steady-state $[S]_i/[S]_o$ ratio under conditions of external glucose in the saline and of gluconeogenesis, when the entry mechanism was practically not operative. The phlorrhizin-sensitive mechanism of efflux (shown for galactose) may be responsible for the observation that this inhibitor increased the $[S]_i/[S]_o$ ratio for glucose under conditions of gluconeogenesis while inhibiting the entry of the sugar into the cells. Alvarado (1965) concluded from his experiments that in gut mucosa phlorrhizin competes with sugars for the transport site. If such a mechanism also applies to the system responsible for galactose outflow from renal cells, phlorrhizin would act on the inner face of the membrane; this is feasible, the results given in Table 3 indicating that phlorrhizin enters the cells. The increased $[S]_i/[S]_o$ ratio for glucose produced by ouabain under conditions of gluconeogenesis (Table 3) may be due only to an inhibition of glucose formation. This last observation suggests that ouabain, in addition to its known effect on the sodium pump (Schatzmann, 1953), also interferes with the intracellular glucose metabolism (cf. Gordon, 1965; Clausen, 1965).

The dependence of the steady-state $[S]_i/[S]_o$ ratio for both glucose and galactose on $[S]_o$ (Fig. 1) may be the result of an interplay of the mechanisms of entry and outflow as well as of the intracellular metabolism of the sugars. From equations derived for a model capable of accumulating substances against their concentration gradient (Rosenberg & Wilbrandt, 1963; Kotyk & Höfer, 1965; Silverman & Goresky, 1965) it follows that the $[S]_i/[S]_o$ ratio will tend to decrease with increasing $[S]_o$ if the affinities of the carrier for the sugar differ on both sides of the membrane; the $[S]_i/[S]_o$ ratio would approach 1·0 at high $[S]_o$ if the maximal rates of transport in both directions were equal. $[S]_i/[S]_o$ ratios below 1·0 may be then due to either (*a*) marked differences in the maximal rate of transport across the membrane, or (*b*) intracellular metabolism of the investigated sugar. It appears difficult at present to confirm or exclude the former possibility for glucose. However, the fact that for many actively accumulated sugars the $[S]_i/[S]_o$ ratio did not fall below 1·0 even at high external $[S]_o$ (see Fig. 1 for galactose and also Kleinzeller, Kolínská & Beneš, 1967) suggests that the appreciable rate of intracellular glucose utilization may be an essential factor responsible for $[S]_i/[S]_o$ ratios below 1·0 at $[S]_o$ higher than 0·5mM. The observation that 2,4-dinitrophenol actually still further decreased this ratio is in accordance with such a view.

Localization of the transport mechanism for sugars in the renal tubular cell. It has been repeatedly suggested (cf. Csáky & Fernald, 1961; Crane, 1965; Curran, 1965) that in cells known to absorb sugars actively *in vivo*, i.e. intestinal and renal tubular epithelia, the active transport process is localized in the brush border at the luminal face of these cells. The same mechanism may also hold for the epithelial cells of the choroid plexus (Csáky & Rigor, 1964). This view is based on the observation that in the gut and choroid plexus the Na^+-dependent transport of sugars from the lumen across the cells is accompanied by an accumulation of sugar within the cells. The sugar outflow would then occur at the basal cell membrane by a passive mechanism.

The close correlation between the characteristics of the sugar reabsorption mechanism in renal tubules *in vivo* and the active accumulation *in vitro*, mentioned above, suggests that the active Na^+-dependent and phlorrhizin-sensitive transport site is localized at the luminal face of the tubular cells, bringing about an accumulation of the sugars within the cells before their passive outflow at the basal membrane. If this is so, the notion that the tubular lumen of slices is not readily accessible to diffusion of solutes in the saline (cf. Murthy & Foulkes, 1967) will have to be modified. The observation that the accumulation of sugars is faster with preparations of isolated tubules than with kidney-cortex slices is consonant with such a view; the diffusion pathway of the sugar from the saline to the luminal face is considerably shorter in the isolated tubules than in slices, where it may limit the rate of accumulation (Fig. 2) without affecting the steady-state $[S]_i/[S]_o$ ratio (Fig. 3).

It appears difficult to design experiments *in vitro* that might throw light on the question whether an outflow of sugar may also occur at the tubular-cell membrane. In a slice most, if not all, of the sugar leaking out from the cells into the tubular lumen would again be reabsorbed by the active transport mechanism before diffusion into the external medium. It is therefore reasonable to postulate that the efflux kinetics with kidney slices describe predominantly the characteristics of the transport step at the basal cell membrane.

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