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HEXOSE TRANSPORT AND PHOSPHORYLATION BY HAMSTER KIDNEY CORTEX SLICES AND EVERTED JEJUNAL RINGS

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SUMMARY

1. Net influx and efflux of two model hexoses, 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose, were compared in hamster kidney cortex slices and everted jejunal rings.

2. The gut concentrated 3-*O*-methyl-D-glucose (0.01 mM) but equilibrium was not reached over a 3 h time course. By comparison the kidney was unable to accumulate this hexose and maximal uptake occurred in 30 min. Initial efflux was more rapid from kidney than intestine. No phosphorylated derivatives were found in either organ preparation.

3. The kidney concentrated 2-deoxy-D-glucose (0.01 mM) to levels 14 times that in the medium. Jejunal rings accumulated this hexose only 2-fold over the same 3-h time course. These differences in net influx were reflected by the more rapid efflux of 2-deoxy-D-glucose from gut than kidney. In contrast to the findings for 3-*O*-methyl-D-glucose, 2-deoxy-D-glucose-6-phosphate was found in both tissue preparations.

4. The relationship of phosphorylation to 2-deoxy-D-glucose transport was evaluated. In both kidney and intestine at initial times of uptake, intracellular free 2-deoxy-D-glucose was present in concentrations 5–6 times that of its phosphorylated derivative. When tissues were preloaded with 2-deoxy-D-glucose and placed in hexose-free medium the intracellular concentration of free hexose fell while the phosphate ester remained constant or rose. These observations indicate that phosphorylation of 2-deoxy-D-glucose occurs passively after active membrane transport; that phosphorylation is rate limiting allowing for the accumulation of free hexose; and that once phosphorylated, 2-deoxy-D-glucose does not add to the free hexose pool.

INTRODUCTION

The epithelial cells of kidney and intestine share morphologic and physiologic characteristics which reflect their ability to concentrate non-ionic substrates[1–3]. Previous studies of hexose uptake by kidney and intestine in human subjects with inherited disorders of glucose transport reveal differences in the genetic control of hexose transport by these two organs[4,5]. Renal glycosuria and glucose–galactose malabsorption syndrome are two disorders which conform to an autosomal recessive pattern of inheritance and therefore presumably are caused by a single mutant gene

of large effect. In renal glycosuria a severe defect in reabsorption of glucose by the kidney tubule is not accompanied by impaired intestinal glucose transport. In glucose-galactose malabsorption, on the other hand, absent hexose transport by the intestine is associated with a partial defect in renal glucose reabsorption. These observations in man suggest that the kidney and intestine possess both shared and unshared hexose transport mechanisms under separate genetic control. A more precise comparison of the control mechanisms of hexose transport by these two organs is difficult in man because of the infrequent availability of tissue. Comparison of lysine transport in rat kidney cortex slices and everted jejunal rings has clarified some of the biochemical mechanisms and dissimilarities of dibasic amino acid transport by these organs[6].

A large data base has accumulated defining the mechanisms of hexose transport by hamster intestine and by rabbit kidney cortex, but little work has been published comparing these systems within the same animal species. Two mechanisms are postulated by which monosaccharides enter the hamster jejunal epithelial cell[7]. The first "active" transport system requires a pyranose ring with a hydroxyl group in the β position like D-glucose and a C-6 carbon. Compounds in this group require Na^+ in the medium for concentration to occur within the tissue. A model hexose in this system is 3-*O*-methyl-D-glucose. By contrast, the second transport system does not exhibit similar steric requirements, does not require Na^+, and does not accumulate its substrate monosaccharides against a concentration gradient. A model sugar in this system is 2-deoxy-D-glucose. Quite different findings are observed in rabbit kidney cortex slices[8,9]. 3-*O*-Methyl-D-glucose is not concentrated, but 2-deoxy-D-glucose demonstrates marked accumulation against a concentration gradient. These data suggest different transport mechanisms in the two organs, but species differences prevent a definite conclusion.

These observations raise an additional important question. What are the "energy-linked" sources which allow the kidney, but not the intestine to accumulate 2-deoxy-D-glucose? Although several lines of evidence mitigate against a phosphorylation mechanism by mammalian intestine in the accumulation of hexoses, in yeast cells the phosphorylation of 2-deoxy-D-glucose to its 2-deoxy-D-glucose 6-phosphate derivative and subsequent dephosphorylation does provide such a mechanism[7,10]. The present study compares net influx and efflux of the two disparate model hexoses, 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose, in hamster kidney cortex slices and everted jejunal rings. The role of phosphorylation in the accumulation of 2-deoxy-D-glucose by these tissue preparations is also investigated.

METHODS

Male golden hamsters weighing 120–150 g were fasted for 48 h before sacrifice by stunning and decapitation. The techniques used for preparing everted jejunal rings (25–50 mg wet weight) and kidney cortex slices (30–60 mg wet weight) were similar to those previously described in the rat[6,11]. Two kidney cortex slices or gut rings were incubated in 2 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, at 37° in an atmosphere of 95 % O_2–5 % CO_2. Incubation in a Dubnoff metabolic shaker was performed in the presence of either 0.01 mM 2-deoxy-D-[U-^{14}C]-glucose or 0.01 mM 3-*O*-methyl-D-[1-^{14}C] glucose for the times indicated. At the termination of the incubation period, tissues were rinsed in 0.9 % NaCl, blotted, weighed and homogenized

in 1 ml of distilled water. The homogenate was centrifuged at 39100 × *g* in a Sorvall RC2B. This homogenate contained less than 5 % of total tissue counts. Radioactivity of the supernatant solution was then measured by liquid scintillation spectrometry in a Nuclear-Chicago Mark I scintillation counter with 92 % efficiency. An aliquot of supernatant fluid was also treated with equal volumes of warm (70°) aqueous $ZnSO_4$ solution (5.0 g/100 ml) followed by aqueous $Ba(OH)_2$ solution (4.5 g/100 ml) which removed soluble protein and ^{14}C-labelled phosphorylated hexose derivatives. Radioactivity in this aliquot which contained only free labelled hexose was then determined.

Inulin spaces in kidney cortex slices and everted jejunal rings were measured over a 3-h time course using [U-^{14}C]inulin by previously described techniques[12]. In the kidney, inulin spaces at 5, 15, 30, 60, 120, and 180 min were 11.7, 19.3, 20.4, 24.9, 28.7, and 30.8 % of wet weight, respectively. In the gut the inulin spaces were 5.9, 9.6, 10.8, 11.6, 17.5, and 19.4 % of wet weight at 5, 15, 30, 60, 120, and 180 min. Tissue water was 79.5 % wet kidney weight and 86.2 % of wet gut weight.

The accumulation of ^{14}C-labelled free hexoses was expressed as the distribution ratio: the ratio of counts/min per ml of intracellular fluid to counts/min per ml in the medium. A distribution ratio greater than one signified concentrative transport. Calculations of radioactivity in the intracellular spaces, the distribution ratio and efflux were performed as previously described[12]. Efflux was expressed as the percent of original tissue counts remaining at each time interval. The clear supernatant from tissue extracts was prepared for radiochromatography by lyophilization and extraction of lyophilate into 95 % ethanol–5 % water before application to the chromatograph. Labelled and unlabelled sugars were identified by descending chromatography using 1 % oxalic acid impregnated Whatman No. 1 paper in isopropanol–water (4:1, v/v.). Authentic compounds were located using an aniline–diphenylamine reagent[13]. ^{14}C-labelled compounds were identified by liquid scintillation of 1 cm × 3 cm strips cut from the chromatogram.

MATERIALS

3-*O*-[U-^{14}C]methyl-D-glucose (18.32 mC/mmole), 2-deoxy-D-[1-^{14}C]glucose (53.31 mC/mmole), [^{14}C]inulin (14.96 mC/mmole) and Liquifluor were purchased from New England Nuclear Corporation. Unlabelled 2-deoxy-D-glucose was purchased from Calbiochem. 3-*O*-Methyl-D-glucose, 2-deoxy-D-glucose 6-phosphate, and alkaline phosphatase type I, from calf intestinal mucosa (1.4 units/mg solid), were obtained from Sigma Chemical Company. Zinc sulfate ($ZnSO_4 \cdot 7\ H_2O$) and barium hydroxide ($Ba(OH)_2 \cdot 8\ H_2O$) were purchased from Mallinckrodt Chemical Works.

RESULTS

Transport of 3-O-methyl-D-glucose by hamster kidney and intestine

Comparison of net 3-*O*-methyl-D-glucose transport by hamster jejunum and kidney over a 3-h time course is shown in Fig. 1. The distribution ratio for everted jejunal rings at 5, 15, 30, 60, 120, and 180 min was 0.34 ± 0.02, 0.69 ± 0.03, 1.10 ± 0.05, 1.34 ± 0.05, 1.52 ± 0.06, and 1.89 ± 0.01, respectively. In kidney cortex slices, the distribution ratio at 5, 15, 30, 60, 120, and 180 min was 0.50 ± 0.02, 0.67 ± 0.01, 0.77 ± 0.02, 0.81 ± 0.02, 0.78 ± 0.01, and 0.86 ± 0.02, respectively. These data

indicate several differences between these two organ systems. The kidney attained higher distribution ratios than the gut at 5 min, reached equilibrium by 30 min, but failed to concentrate this model hexose over the 3-h time course. The gut, however, concentrated to levels 1.89 times that in the medium but failed to reach equilibrium throughout the 3-h incubation period.

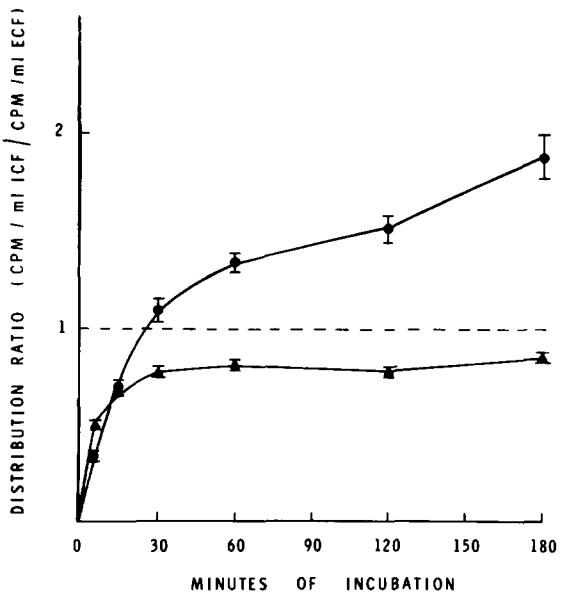

Fig. 1. Accumulation of 3-*O*-[U-^{14}C]methyl-D-glucose by hamster kidney and intestine: Flasks containing 2 kidney cortex slices (▲), or everted jejunal rings (●), were incubated aerobically at 37° in 2.0 ml Krebs–Ringer bicarbonate buffer (pH 7.4) containing 0.01 mM 3-*O*-[U-^{14}C]methyl-D-glucose (18.32 mC/mmole). Tissues incubated for the times specified on the abscissa were homogenized in 1.0 ml distilled water and centrifuged. An aliquot of tissue supernatant was treated with $ZnSO_4$ (5 %) and $Ba(OH)_2$ (4.5 %). Uptake is defined in this and subsequent figures as the distribution ratio of (counts/min per ml of intracellular fluid)/(counts/min per ml of extracellular fluid). The data are presented as the mean ± 1 S.E. of at least six observations at each time point.

These differences in net efflux were mirrored by the differences observed in the efflux of 3-*O*-methyl-D-glucose (Fig. 2). Whereas 72, 58, 49, 43, and 36 % of radioactivity remained in the kidney at 1, 2, 3, 4, and 5 min, the gut retained 82, 72, 64, 58, and 52 % at these same minutes of efflux. At the completion of the 30-min efflux period the jejunum retained 24 % of radioactivity, while the kidney had lost all but 9 % of its original 3-*O*-[^{14}C]-D-glucose. The inability of the kidney cortex slice to concentrate 3-*O*-methyl-D-glucose over a 3-h time period is associated with a rapid rate of movement from its intracellular to extracellular space. In contrast, the jejunal rings, which do accumulate this model hexose, efflux more slowly than the kidney preparation.

Transport of 2-deoxy-D-glucose by hamster kidney and intestine

0.01 mM 2-deoxy-D-glucose was actively accumulated by both kidney slices and jejunal rings over a 3-h time period (Fig. 3). This model hexose was concentrated by hamster intestine. Distribution ratios at 5 min rose from 0.06 ± 0.07 to 1.41 ± 0.10

and 1.97 ± 0.08 at 90 and 180 min of incubation. Net accumulation from 5–180 min was linear and did not reach equilibrium. By contrast, the kidney reached a distribution ratio greater than one within 15 min and concentrated 2-deoxy-D-glucose to a level 14 times that in the media by 2 h. The distribution ratios of 14.02 ± 0.83 and 14.03 ± 1.57, observed at 120 and 180 min, suggest that, unlike the intestine, 2-deoxy-D-glucose uptake by kidney slices reached equilibrium during the third hour of incubation.

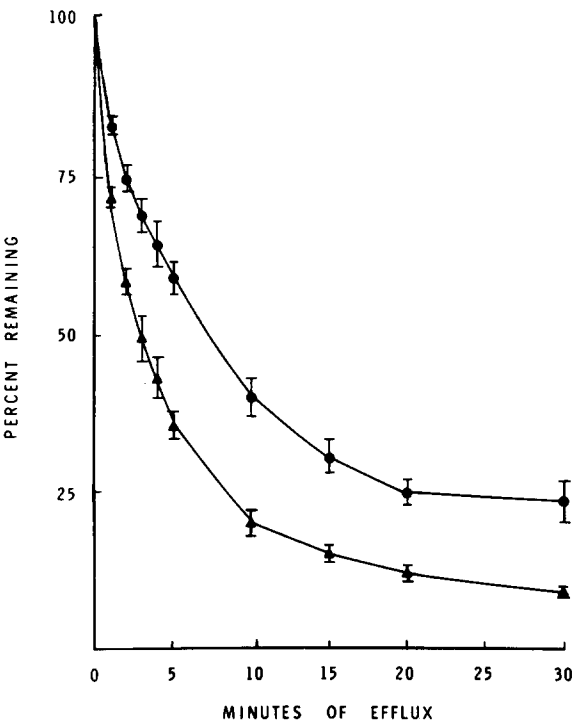

Fig. 2. Efflux of 3-*O*-[U-^{14}C]methyl-D-glucose from hamster kidney cortex slices and everted jejunal rings: Flasks containing 4 kidney cortex slices (▲), or jejunal rings (●), were incubated for 30 min in 0.01 mM 3-*O*-[U-^{14}C]methyl-D-glucose (18.32 mC/mmole). At the end of the incubation period, the tissues were transferred to flasks containing 4.0 ml of buffer and unlabelled 3-*O*-[U-^{14}C]methyl-D-glucose (0.01 mM). At the intervals shown small samples were removed from each flask and assayed for radioactivity. From the counts remaining in the tissues at the end of 30 min and the cumulative counts released into the media during the designated time intervals, the total counts/min present in the tissue at the beginning of the efflux study were calculated. The data are expressed as the mean percent counts remaining of quadruplicate observations ± 1 S.E.

The efflux of 2-deoxy-D-glucose described in Fig. 4 reflects the differences in net uptake by these two tissue preparations. Only free 2-deoxy-D-glucose appeared in the medium. The kidney retained 87, 82, 79, 76, and 74 % of radioactivity at 1, 2, 3, 4, and 5 min of efflux and by 30 min had eliminated only 50 % of the tissue counts originally present. In the jejunum, on the other hand, 82 % of original tissue radioactivity was transferred to the extracellular space by 30 min (18 % remaining). Since conversion of 2-deoxy-D-glucose to 2-deoxy-D-glucose 6-phosphate continued during

these efflux intervals in both tissue preparations, these observations do not allow quantitation of efflux, but do compare this outflow phenomenon in jejunum and kidney.

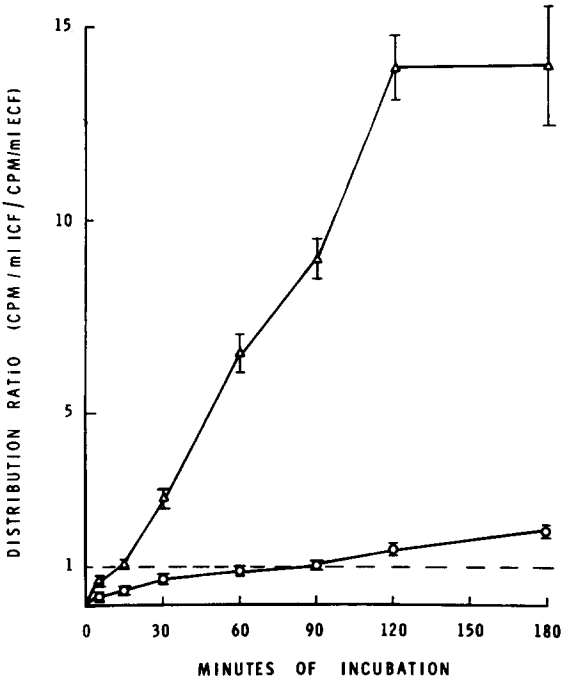

Fig. 3. Accumulation of 2-deoxy-D-[1-^{14}C]glucose by hamster kidney and intestine: The uptake of 0.01 mM 2-deoxy-D-[1-^{14}C]glucose (53.31 mC/mmole) by hamster kidney cortex slices (△), and everted jejunal rings (○), was studied. The data are presented as outlined in Fig. 1. Note scale difference.

The relatively low net uptake of 2-deoxy-D-glucose by the intestine evidenced by distribution ratios of 2 in 3 h, contrasts to the concentrating ability of the kidney (distribution ratio of 14). The observation that 2-deoxy-D-glucose is concentrated by the intestine at all is surprising, since previous investigations using shorter times of incubation and higher media concentrations failed to demonstrate accumulation of this model hexose[14].

Relationship of phosphorylation to 2-deoxy-D-glucose transport

The role of phosphorylation in the transport of these two model hexoses was investigated. After 3 h 3-*O*-[U-^{14}C]methyl-D-glucose was recovered unchanged from both kidney and intestine. No evidence of phosphorylation of this hexose was found utilizing differential precipitation and radiochromatography of tissue homogenates. However, 2-deoxy-D-[^{14}C]glucose 6-phosphate was recovered from both kidney cortex slices and jejunal rings. Results of a representative experiment in which kidney cortex slices were incubated in 2-deoxy-D-[1-^{14}C]glucose (0.01 mM) for 60 min is described in Table I. When tissue homogenates were extracted in an aqueous solution

containing 0.385 M Na_2CO_3–0.115 M $NaHCO_3$ (pH 10.4), two distinct areas were identified by radiochromatography. The area of slower mobility represented 23 % of radioactivity applied at the origin and co-chromatographed with authentic 2-deoxy-D-glucose 6-phosphate. The radioactive area of faster mobility represented 58 % of applied label and co-chromatographed with 2-deoxy-D-glucose. When this same tissue extract was precipitated with 5 % $ZnSO_4$ and 4.5 % $Ba(OH)_2$, 32.5 % fewer counts were recovered from equal volumes of the same aqueous supernatant, radioactivity in the area representing 2-deoxy-D-glucose 6-phosphate was no greater than that dispersed evenly throughout the remainder of the chromatogram, and significant

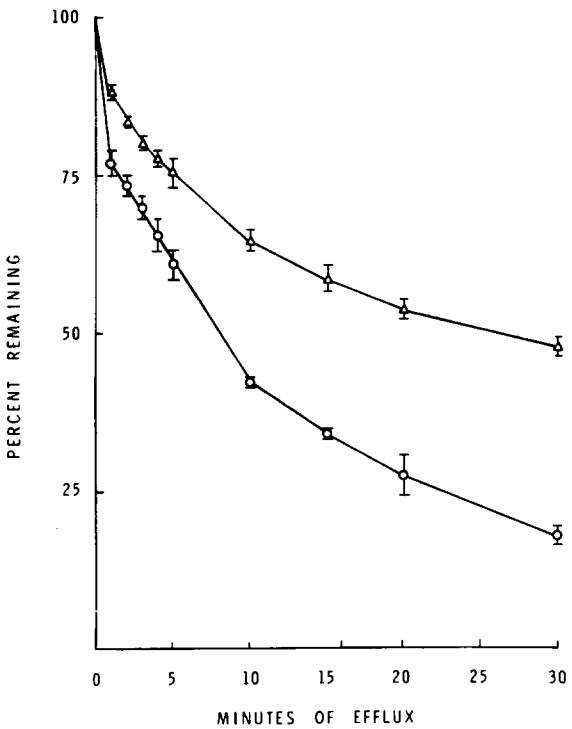

Fig. 4. Efflux of 2-deoxy-D-[1-^{14}C]glucose from hamster kidney and intestine: Flasks containing 4 kidney cortex slices (△), or jejunal rings (○), were incubated for 30 min in 0.01 mM 2-deoxy-D-[1-^{14}C]glucose (53.31 mC/mmole). At the end of the incubation period the tissues were transferred to flasks containing 4.0 ml of radioisotope-free buffer and unlabelled 2-deoxy-D-glucose (0.02 mM). The experimental procedures and presentation of data are identical to those outlined in Fig. 2. No 2-deoxy-D-[1-^{14}C]glucose 6-phosphate was present in the media.

counts were found only in the 2-deoxy-D-glucose area. When an equal aliquot from the original tissue extract was incubated with alkaline phosphatase but no Ba^{2+}/Zn^{2+} step was introduced, no significant radioactivity was found in the 2-deoxy-D-glucose 6-phosphate area, and 83 % of applied radioactivity was recovered as the free hexose. These data indicate that 2-deoxy-D-glucose 6-phosphate is formed during the accumulation of free 2-deoxy-D-glucose; that the use of $ZnSO_4$–$Ba(OH)_2$ precipitation removes the phosphorylated ester from the extract; and that alkaline phosphatase quantitatively converts the phosphorylated hexose back to its original free hexose form.

The relationship to time of 2-deoxy-D-glucose accumulation and conversion to 2-deoxy-D-glucose 6-phosphate was then investigated; the results are outlined in Table II. Both kidney and jejunum demonstrated more free hexose than the phosphorylated derivative at initial times of uptake. At 5 min of incubation in kidney and intestine respectively the concentration of 2-deoxy-D-glucose was 6.4 times and 5.2 times greater than that of 2-deoxy-D-glucose 6-phosphate. The intestine continued to accumulate free hexose and the phosphorylated ester, but the ratio of 2-deoxy-D-glucose to its phosphorylated derivative decreased. From 60 to 180 min the concentrations were essentially equal (ratio approximated one). In kidney cortex slices

TABLE I

CONVERSION OF 2-DEOXY-D-[1-^{14}C]GLUCOSE TO 2-DEOXY-D-[1-^{14}C]GLUCOSE 6-PHOSPHATE BY HAMSTER KIDNEY CORTEX SLICES

Six pairs of kidney cortex slices were incubated for 60 min with 0.01 mM 2-deoxy-D-[1-^{14}C]glucose (53.31 mC/mmole) as described in Fig. 3. Tissues were then homogenized and pooled in aqueous Na_2CO_3 (0.385 M)/$NaHCO_3$ (0.115 M) (pH 10.4). The supernatant was divided into three equal parts and incubated for an additional 60 min. *Control*, tissue supernatant was incubated alone, treated with 20% sulfosalycylic acid, concentrated and chromatographed. *Zn^{2+}/Ba^{2+}*, tissue supernatant was incubated alone, treated with 20% sulfosalycylic acid followed by 5% $ZnSO_4$–4.5% $Ba(OH)_2$, concentrated and chromatographed. *Phosphatase*, tissue supernatant was incubated with alkaline phosphatase (15 units/ml). The reaction was stopped with 20% sulfosalycylic acid and treated as in *Control*. Authentic 2-deoxy-D-glucose and 2-deoxy-D-glucose 6-phosphate were co-chromatographed with tissue extracts on 1% oxalic acid impregnated paper and evaluated by descending chromatography in an isopropanol–water (4:1, v/v) solvent system. The counts/min contained in the 2-deoxy-D-glucose or 2-deoxy-D-glucose 6-phosphate areas are designated with the percent of total radioactivity applied in parentheses. From 19 to 13% of total radioactivity applied was evenly distributed over the remaining chromatogram.

Radiochromatograph	*Conditions of tissue preparation*		
	Control	*Zn^{2+}/Ba^{2+}*	*Phosphatase*
Counts/min recovered as 2-deoxy-D-glucose 6-phosphate	499 (23%)	42 (3%)	76 (3%)
Counts/min recovered as 2-deoxy-D-glucose	1250 (58%)	1236 (84%)	2103 (83%)
Total counts/min applied	2167	2463	2534

2-deoxy-D-glucose 6-phosphate concentrations rose throughout the 3-h time course, but failed to equal that of its accumulating free 2-deoxy-D-glucose precursor. At 120 and 180 min 2-deoxy-D-glucose 6-phosphate rose from 917000 to 1949000 counts/min per ml intracellular fluid whereas the intracellular concentration of free hexose remained constant at 2539000. In contrast to the intestine, at no time were intracellular 2-deoxy-D-glucose 6-phosphate concentrations greater than 2-deoxy-D-glucose.

To determine whether 2-deoxy-D-glucose 6-phosphate might be dephosphorylated to free 2-deoxy-D-glucose, gut and kidney preparations were incubated with 2-deoxy-D-[^{14}C]glucose for 30 min and then placed in radioactive-free media for 30 min. The tissue ratio of 2-deoxy-D-glucose to 2-deoxy-D-glucose 6-phosphate was then quantitated (Table III). In the jejunal rings free hexose concentration fell from 1530000 to 54000 while concentrations of its phosphorylated derivative rose from 60000 to 94000 counts/min per ml intracellular fluid. The ratio of 2-deoxy-D-glucose/2-deoxy-D-glucose 6-phosphate thus fell from 2.6 to 0.6. The kidney cortex slices

TABLE II

RELATIONSHIP TO TIME OF 2-DEOXY-D-[1-^{14}C]GLUCOSE ACCUMULATION AND CONVERSION TO 2-DEOXY-D-[1-^{14}C]GLUCOSE 6-PHOSPHATE BY HAMSTER KIDNEY CORTEX SLICES AND EVERTED JEJUNAL RINGS

Hamster kidney cortex slices or everted jejunal rings were incubated with 0.01 mM 2-deoxy-D-[1-^{14}C]glucose (53.31 mC/mmole) in Krebs–Ringer bicarbonate buffer (pH 7.4) for the incubation times indicated. Tissues were then rinsed, blotted, weighed and homogenized in distilled water. Total counts/min per ml of intracellular fluid were quantitated directly. Aliquots of tissue supernatant were then treated with 5 % $ZnSO_4$–4.5 % $Ba(OH)_2$ to remove 2-deoxy-D-[1-^{14}C]glucose 6-phosphate. Radioactivity present as 2-deoxy-D-glucose 6-phosphate was obtained from the difference between total counts/min and counts/min present as 2-deoxy-D-glucose. The data were expressed as the mean ± 1 S.E. of at least five observations at each time point. P values were calculated using the paired t test. NS indicates $P>0.10$. Ratio is 2-deoxy-D-glucose/2-deoxy-D-glucose 6-phosphate.

Time of incubation (min)	*Counts/min × 10^{-5} per ml intracellular fluid*							
	Intestine				*Kidney*			
	2-Deoxy-D-glucose	*2-Deoxy-D-glucose 6-phosphate*	*Ratio*	*t*	*2-Deoxy-D-glucose*	*2-Deoxy-D-glucose 6-phosphate*	*Ratio*	*t*
5	0.51 ± 0.07	0.08 ± 0.01	6.4	$P<0.01$	1.40 ± 0.16	0.27 ± 0.07	5.2	$P<0.01$
15	0.86 ± 0.07	0.47 ± 0.21	1.8	$0.05<P<0.10$	2.30 ± 0.16	1.30 ± 0.20	1.8	$0.01<P<0.02$
30	1.53 ± 0.07	0.60 ± 0.11	2.6	$P<0.01$	6.14 ± 0.59	2.32 ± 0.44	2.6	$P<0.01$
60	1.92 ± 0.07	1.99 ± 0.41	1.0	NS	13.79 ± 1.05	2.79 ± 0.63	4.9	$P<0.01$
90	2.35 ± 0.18	3.21 ± 0.22	0.7	$0.02<P<0.05$	18.77 ± 0.97	9.29 ± 1.91	2.0	$P<0.01$
120	3.10 ± 0.20	3.62 ± 0.49	0.9	NS	25.39 ± 1.49	9.17 ± 0.92	2.8	$P<0.01$
180	4.22 ± 0.17	4.71 ± 0.71	0.9	NS	25.31 ± 1.57	19.49 ± 1.43	1.3	$P<0.01$

TABLE III

ABSENCE OF CONTRIBUTION BY 2-DEOXY-D-[1-^{14}C]GLUCOSE 6-PHOSPHATE TO THE INTRACELLULAR POOL OF 2-DEOXY-D-[1-^{14}C]GLUCOSE

Hamster kidney cortex slices or everted jejunal rings were incubated for 30 min with 0.01 mM 2-deoxy-D-[1-^{14}C]glucose (53.31 mC/mmole) under conditions identical to those described in Table II. "Media 2-deoxy-D-[1-^{14}C]glucose present": on completion of incubation tissues were immediately removed and the intracellular concentrations of 2-deoxy-D-glucose, 2-deoxy-D-glucose 6-phosphate, and the ratio 2-deoxy-D-glucose/2-deoxy-D-glucose 6-phosphate determined as outlined in Table II. "Media 2-deoxy-D-[1-^{14}C]glucose absent": on completion of incubation tissues were removed and re-incubated for 30 additional minutes in radioactive-free media. Following this re-incubation period the tissues were removed and the intracellular concentrations of radioactive 2-deoxy-D-glucose and 2-deoxy-D-glucose 6-phosphate were determined. The data are presented as the mean ± 1 S.E. with the number of observations in parentheses.

Media 2-deoxy-D-[1-^{14}C]glucose	*Counts/min × 10^{-5} per ml intracellular fluid*					
	Intestine			*Kidney*		
	2-Deoxy-D-glucose	*2-Deoxy-D-glucose 6-phosphate*	*Ratio*	*2-Deoxy-D-glucose*	*2-Deoxy-D-glucose 6-phosphate*	*Ratio*
Present	1.53 ± 0.07 (6)	0.60 ± 0.11 (6)	2.6	6.14 ± 0.59 (7)	2.32 ± 0.44	2.6
Absent	0.54 ± 0.11 (6)	0.94 ± 0.11 (6)	0.6	2.15 ± 0.91 (6)	2.35 ± 0.22	0.9

exhibited a similar fall in 2-deoxy-D-glucose from 6140000 to 2150000. Tissue 2-deoxy-D-glucose 6-phosphate, however, remained constant. The ratio of 2-deoxy-D-glucose/2-deoxy-D-glucose 6-phosphate decreased from 2.6 to 0.9. Thus the fall in tissue 2-deoxy-D-glucose but continued rise in its phosphorylated derivative suggested that 2-deoxy-D-glucose 6-phosphate does not contribute to the free hexose pool in either the kidney or intestine.

DISCUSSION

The present data compare the transport of two model hexoses by intestine and kidney from the same species. 3-*O*-Methyl-D-glucose was concentrated against a gradient by the hamster everted intestinal ring, while kidney cortex slices were unable to accumulate this hexose and transferred this monosaccharide from the intracellular spaces to the extracellular spaces more rapidly than the intestine. The kidney was able to concentrate a second model hexose, 2-deoxy-D-glucose, to levels 14-fold that in the media while the gut concentrated to less than 2-fold levels. Thus the hamster jejunal ring and kidney cortex preparations exhibit quantitatively different transmembrane transport characteristics for these model hexoses.

How can we explain the apparent concentration by the intestine of a model hexose which does not conform to the "minimal structural requirements for sugar active transport by the intestine?" Although 2-deoxy-D-glucose does contain a pyranose ring and a methyl group in the D-configuration at C-5 of that ring, it does not have a hydroxyl group at C-2. More recent observations demonstrated active transport by intestinal preparations of D-mannose and D-xylose, two hexoses also lacking "active transport" steric requirements[15,16]. In these studies as well as ours

the substrate hexoses were present in media concentrations below 1 mM. Perhaps there is no specific structural requirement for active transport? This hypothesis implies that essentially all sugars which enter the jejunal preparation do so by carrier mediation. However, intracellular accumulation may not be evident if medium substrate concentrations greater than transport capacity are used or if intracellular utilization occurs more rapidly than plasma membrane transport.

The present observation that 2-deoxy-D-glucose is phosphorylated by both kidney and gut reopened the speculation that phosphorylation followed by dephosphorylation might provide a mechanism for concentration of this hexose within cells. This hypothesis as previously stated suggested that hexokinase catalyzed phosphorylation of hexose and that a hexose 6-phosphatase dephosphorylated the hexose and allowed for its accumulation within the intestinal cell as the free hexose. Differences between the structural requirements for membrane transport and the hexokinase reaction were one of many objections to this phosphorylation–dephosphorylation theory for active intestinal hexose transport[17,18]. This phosphorylation theory is supported, however, by transport studies in yeast and bacterial membrane systems. 2-deoxy-D-glucose is actively absorbed by the yeast cell and recovered inside as both the free sugar and its phosphorylated derivative[10]. This concentrative mechanism involves a 20–30-fold intracellular increase in the free hexose and a simultaneous decrease in cellular ATP, orthophosphate, and polyphosphate. In these studies the phosphorylated 2-deoxy-D-glucose appeared in the intracellular space at initial times of uptake in higher concentration than the free hexose, an observation interpreted to indicate that accumulation was preceded by phosphorylation and subsequent hydrolysis within the cell. In bacterial systems a phosphotransferase system has been defined which involves two enzymes and a heat stable protein. This system catalyzes transmembrane transport and utilizes phosphoenolpyruvic acid as a phosphate donor[19–21]. Several observations from our studies suggest that phosphorylation and dephosphorylation do not provide a mechanism for 2-deoxy-D-glucose accumulation by hamster intestine or kidney. In these preparations as compared to yeast cells the free hexose was present in the intracellular fluid at initial times of uptake in concentrations 5 and 6 times that of 2-deoxy-D-glucose 6-phosphate. In the kidney cortex slices intracellular concentrations of the free hexose remained constant between 120 and 180 min of incubation while intracellular concentrations of 2-deoxy-D-glucose 6-phosphate continued to rise. Perhaps dephosphorylation of 2-deoxy-D-glucose 6-phosphate was slower than phosphorylation and contributed free hexose to the intracellular pool? Direct evidence against this hypothesis was obtained from the experiments in which preloaded tissues from both kidney and intestine rapidly transferred free hexose to the media, but continued to maintain or increase intracellular concentration of the phosphorylated derivative. A similar absence of 2-deoxy-D-glucose 6-phosphate dephosphorylation has been reported in human platelets[22]. This preparation transports the free hexose and rapidly converts 90% of it to 2-deoxy-D-glucose 6-phosphate. In both tissues it would appear that transport is followed by phosphorylation. Whether intracellular phosphorylated derivatives may in some way regulate free hexose membrane transport remains speculative. However, within the incubation time limits of our studies, increases in intracellular 2-deoxy-D-glucose 6-phosphate did not inhibit continued accumulation of free 2-deoxy-D-glucose by jejunal rings.

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