Transport and Metabolism of Galactose in Rat Kidney Cortex

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1. Analysis of transport of D-galactose was complicated by metabolism of the compound but appeared to have two components: a substrate-saturable component and a diffusion component. At low substrate concentration (<1 mM) active transport was observed. Accumulation of galactose was largely independent of Na⁺ concentration. The apparent K_m for this component was 0.2 mM. At substrate concentrations above 1 mM the active transport system appeared saturated and further increases in substrate concentration resulted in a linear increase in the rate of galactose accumulation, but no concentration gradient was formed. 2. D-[1-¹⁴C]Galactose (2 mM) was metabolized to ¹⁴CO₂ by rat kidney-cortex slices incubated at 37°C, at the rate of 68 nmol/h per 100 mg of tissue. 3. Intracellular components from such incubations were separated into a neutral fraction, the only major labelled component being galactose, and a phosphorylated fraction. 4. Phosphorylated metabolites found in galactose-incubated slices increased with increasing substrate concentration and achieved a limiting value of 0.42 mM after 60 min of incubation. 5. Galactose uptake was inhibited by anaerobiosis, dinitrophenol and phlorrhizin. 6. Methyl α -D-glucoside and D-glucose partially inhibited galactose uptake only at ratios of 100:1. 7. The presence of pyruvate did not decrease galactose metabolism although it did decrease production of ¹⁴CO₂ from [1-¹⁴C]galactose. Gluconeogenesis occurred in the presence of pyruvate and ¹⁴C from galactose was found in glucose. 8. Rat kidney-cortex slices metabolized 2 mM-[1-¹⁴C]galactonate to ¹⁴CO₂ at a rate of 20 nmol/h per 100 mg of tissue.

Accumulation of galactose *in vitro* has previously been reported in rabbit kidney-cortex slices and tubules and the kinetics of its transport have been studied (Krane & Crane, 1959; Kleinzeller *et al.*, 1967a; Kolínská, 1970). Further characterizations of the process from comparisons with other sugars were presented (Kleinzeller *et al.*, 1967b; Kleinzeller, 1970a,b; Kleinzeller *et al.*, 1970). All the data thus far available for kidney transport of galactose *in vitro* have been limited to uptake at 25°C by rabbit tissue with primarily the cellular neutral sugar pool being measured.

Evidence in the cat (Rasmussen, 1970) and in the chick (Wells *et al.*, 1970) shows the kidney is capable of metabolizing galactose and may contribute significantly to the overall metabolism of galactose in these animals. Indeed, previous communications from this laboratory have established the presence of galactokinase, galactose 1-phosphate uridylyltransferase and UDP-galactose 4-epimerase in the rat renal cortex (Cuatrecasas & Segal, 1965; Bertoli & Segal, 1966; Cohn & Segal, 1969). Our interest in abnormalities of mammalian galactose metabolism (Segal & Cuatrecasas, 1968), galactose toxicity (Rosenberg *et al.*, 1961a) and renal tubule transport mechanisms (Thier *et al.*, 1964; Genel *et al.*, 1971) prompted us to

determine the characteristics of galactose metabolism by rat kidney cortex *in vitro* and to relate this to mechanisms of galactose transport by cortical cells.

Experimental

Materials

Animals. Male Sprague-Dawley rats (Charles River Labs., Wilmington, Mass., U.S.A.), weighing 200–300 g and fed *ad libitum* on Purina Chow, were used.

Chemicals. D-[1-¹⁴C]Glucose (45 mCi/mmol) and methyl α -D-[U-¹⁴C]glucoside (73.4 mCi/mmol) were obtained from Calbiochem, Los Angeles, Calif., U.S.A. D-[1-¹⁴C]Galactose (6 mCi/mmol) was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. Unlabelled methyl- α -D-glucoside, galactitol, sorbitol and inositol were from Pfanstiehl Labs. Inc., Waukegan, Ill., U.S.A. D-Galactose and D-glucose were from Sigma Chemical Co., St. Louis, Mo., U.S.A. 2-Deoxygalactose and 2-deoxyglucose were purchased from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A. 3-Deoxyglucose was a gift from J. W. Pratt (deceased) of the National Institutes of Health, Bethesda, Md., U.S.A. Pyruvate was

purchased from Schwarz Bio-Research Inc., Orangeburg, N.Y., U.S.A., and phlorrhizin from Mann Research, New York, N.Y., U.S.A. Galactonate and [^{14}C]galactonate were prepared by the method of Cuatrecasas & Segal (1966).

The purity of all sugars and hexitols was confirmed by gas-liquid chromatography by the methods of Sweeley *et al.* (1963).

Determination of concentrative uptake

The techniques of preparation of slices for determination of intracellular accumulation of radioactive sugars by rat kidney-cortex slices in Krebs-Ringer bicarbonate buffer (Krebs & Henseleit, 1932), pH 7.4, have been described (Segal *et al.*, 1968; McNamara *et al.*, 1971). The uptake of substrates used is expressed as the distribution ratio, which is the ratio (c.p.m./ml of intracellular fluid)/(c.p.m./ml of medium). The tissue water spaces determined by use of [^{14}C]inulin agreed with those assessed previously (Rosenberg *et al.*, 1962). Unless otherwise stated, the distribution ratio was calculated from radioactivity in the heated (90°C) water extract of slices (Rosenberg *et al.*, 1961*b*). Barium hydroxide and ZnSO_4 were used to precipitate protein and phosphorylated compounds. The method used was that in Colowick & Kaplan (1955). For non-metabolizable methyl α -D-glucoside, the distribution ratio represents a true concentration gradient. For galactose (and for glucose) it is a ratio of radioactivity only and further characterization of the tissue extracts after transport is included. The distribution ratio is a derivative number, which, when multiplied by the substrate concentration in the medium, gives the concentration of the substrate and its metabolites in the tissue water.

Anaerobiosis was studied by bubbling the bicarbonate buffer with $\text{N}_2 + \text{CO}_2$ (95:5) instead of $\text{O}_2 + \text{CO}_2$ (95:5). The effects of 2,4-dinitrophenol and of sodium deprivation were explored by using the methods of Schwartzman *et al.* (1967). Tissue water spaces for various perturbations in the incubation conditions were also the same as those used by Schwartzman *et al.* (1967).

Evolution of $^{14}\text{CO}_2$

The evolution of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]galactose, [$1\text{-}^{14}\text{C}$]glucose and [$1\text{-}^{14}\text{C}$]galactonate was measured by the method of Weinstein & Segal (1968). Slices were incubated in freshly gassed bicarbonate buffer, pH 7.4, containing various concentrations of ^{14}C -labelled galactose, glucose or galactonate for various times in sealed vials with glass centrewells. Incubation conditions were identical with those used in uptake experiments. After the stated time-interval, 1 ml of Hyamine (Packard, La Grange, Ill., U.S.A.) was injected into the glass centre well and 0.3 ml of 3M-

H_2SO_4 into the medium. $^{14}\text{CO}_2$ formed after uptake was released and absorbed by the Hyamine over a 45 min period of shaking at 22°C. The Hyamine was then put in 14 ml of scintillation fluid (diluted Liquifluor) and the radioactivity counted in a Packard scintillation counter at 84% efficiency. Efficiency of counting and quenching was determined by addition of [^{14}C]toluene to samples and blanks. $^{14}\text{CO}_2$ evolved was expressed as c.p.m. released/g wet wt. of tissue after the stated time or in nmol/h per 100 mg wet wt.

Determination of neutral and phosphorylated sugars and sugar nucleotides

The percentages of ^{14}C -labelled phosphorylated intermediates (including sugar nucleotides) were determined by chromatography of tissue water extracts on DEAE-cellulose paper developed with either water for a separation of neutral sugars and phosphorylated intermediates (Cuatrecasas & Segal, 1965) or with 0.05M-LiCl for separation of UDP-glucose and UDP-galactose from glucose phosphate and galactose phosphate and from glucose and galactose, as reported previously (Rogers *et al.*, 1970).

Characterization of the neutral fraction

Neutral metabolites from $\text{Ba}(\text{OH})_2 + \text{ZnSO}_4$ filtrates of incubated tissue slices were characterized by gas-liquid chromatography with a Packard chromatogram (model 3372) on a 3% OV-1 column, a methyl silicone compound on Gas Chrom-Q (Applied Science Laboratories, State College, Pa., U.S.A.), at 165°C by employing trimethylsilyl derivatives according to the method of Sweeley *et al.* (1963). Specific radioactivity of hexoses and hexitols was determined by collection of trimethylsilyl derivative peaks according to the method of Wells & Wells (1967).

Results

Galactose uptake by kidney cortex slices

The uptake of galactose at 37°C over a period of 90 min was examined. The concentration was initially selected to be 2 mM since this is a physiological concentration found in plasma after milk ingestion in humans (Donnell *et al.*, 1967; Hartmann *et al.*, 1953) and it was in the range of saturable transport as found by study of the model sugar derivative methyl α -D-glucoside (S. Segal, M. Rosenhagen & C. Rea, unpublished work). The results are shown in Fig. 1, where uptake is expressed as the distribution ratio of radioactivity [(c.p.m./ml of intracellular fluid)/(c.p.m./ml of medium)] calculated in three ways. One is from radioassay of water extracts and represents ^{14}C in all neutral sugars and non-volatile metabolites in the tissue water. The second is from

Fig. 1. Time-course of uptake of D-galactose by rat kidney-cortex slices

Three kidney-cortex slices totalling 40–80mg, from three different rats, were incubated in 30ml plastic flasks containing 2ml of Krebs–Ringer bicarbonate buffer, pH7.4, and 0.1 μ Ci of [1-¹⁴C]galactose/ml under O₂+CO₂ (95:5) atmosphere in Dubnoff metabolic shakers at 37°C. Final galactose concentration was 2mM. Uptake is designated by distribution ratio [(c.p.m./ml of intracellular fluid)/(c.p.m./ml of medium)], by using tissue water and inulin spaces as described in the Experimental section. Values plotted are means of three to nine determinations for each point. Δ , Distribution ratio calculated from Ba(OH)₂+ZnSO₄ filtrates of water extracts; $\circ$, distribution ratio calculated from water extracts; $\square$, distribution ratio calculated by assuming ¹⁴CO₂ had not been released from water extracts; $\blacksquare$, ¹⁴CO₂ released during incubation (c.p.m./g of tissue).

Ba(OH)₂+ZnSO₄ filtrates of the water extract, which represents ¹⁴C-labelled neutral sugars demonstrated to be mainly galactose (see below). The third is by determining the ¹⁴CO₂ formed. For purposes of presentation of total uptake, ¹⁴CO₂ formed was treated as if the radioactivity remained in the intracellular water. The accumulation of free galactose reached a steady state with a distribution ratio slightly below 1.0 in about 30min. The distribution ratio in this instance is a measure of intracellular galactose and indicates the intracellular sugar concentration is about 2mM and equal to the medium or extracellular concentration. Thus at the 2mM substrate concentration there is no concentration gradient of galactose within the cell, which thereby fails to fulfil the criteria for active transport.

The difference between the curve derived from analysis of water extracts and Ba(OH)₂+ZnSO₄ filtrates represents the presence of phosphorylated or other polar intermediates (see below). These

substances are formed rapidly and their quantity continues to increase throughout the incubation. At 90min they would represent about 1.0mM-galactose. The curve with ¹⁴CO₂ included gives a picture of total uptake, which after about 30min continued to increase linearly. The curve of actual ¹⁴CO₂ liberation is included. There was a lag for about 30min, which corresponds to the build-up within the tissue of galactose and its metabolites before a linear liberation of ¹⁴CO₂.

An experiment similar to that whose results are shown in Fig. 1 was performed, except that 3mM-phlorrhizin was present in the incubation mixture. The phlorrhizin caused a decrease in the concentration of the neutral sugars and phosphorylated metabolites but had no significant effect on the distribution of radioactivity in the water extract or on the level of oxidation to ¹⁴CO₂ (Table 1).

Although the pool of neutral sugar achieved a steady state, the data indicate there is a large flux of galactose through this pool, which appears as metabolites and CO₂. The measure of the neutral fraction from tissues treated with Ba(OH)₂+ZnSO₄ for determination of galactose uptake presented in all studies reported thus far does not give an accurate picture of the process for rat tissue incubated at 37°C.

Nature of labelled compounds in water extracts

Phosphorylated fraction. Chromatography on DEAE-cellulose paper of the water extract of kidney slices incubated with 2mM-[1-¹⁴C]galactose served to fractionate the labelled compounds. The results of these fractionations are shown in Table 1. Over a 90min incubation, the percentage of ¹⁴C in the neutral fraction steadily decreased whereas that in the phosphorylated fraction concomitantly increased. The percentage of radioactivity in the phosphorylated metabolites was the same when the extract was chromatographed on a Dowex 1 column, as described by Oski & Rose (1967), and agreed closely with amounts precipitated by treatment of the extract with Ba(OH)₂+ZnSO₄. Chromatography on DEAE-cellulose of an extract from a 60min incubation to characterize the phosphorylated fraction further revealed compounds corresponding mainly to sugar phosphates and little radioactivity corresponding to nucleotide sugars. 3mM-Phlorrhizin added to the incubation flasks did not alter the pattern of labelled compounds (Table 1), a finding reflecting the negligible effect of phlorrhizin on total uptake.

Since previously reported galactose-transport studies were carried out at 25°C (Kleinzeller *et al.*, 1967a,b; Kolínská, 1970), an assessment was made of the effect of this temperature on localization of radioactivity. Table 1 shows that, after a 60min incubation, only a slight difference was obtained

Table 1. *Localization of radioactivity in the neutral sugar and phosphorylated fractions of water extracts of rat kidney-cortex slices incubated in the presence of [1-¹⁴C]galactose*

Incubations were performed as described in Fig. 1 with 2mM-galactose. Phlorrhizin was used at 3mM. After the stated time of incubation, tissue slices were extracted in water at 90°C and 20μl portions spotted on to a strip of DEAE-cellulose paper (Whatman DE-81) for assay by descending chromatography. For the total phosphate fraction the paper was run with water; for separation of sugar nucleotides, sugar phosphates and neutral sugars, the solvent was 0.05M-LiCl. Percentage distribution was calculated from means of three to nine determinations.

Incubation conditions			Tissue radioactivity (% in water extract)	
Substrate	Temp. (°C)	Incubation time (min)	Neutral fraction	Total phosphate fraction
[1- ¹⁴ C]Galactose	37	5	90	10
		15	82	18
		30	77	23
		60*	74	26
		90	68	32
[1- ¹⁴ C]Galactose + phlorrhizin	37	5	90	10
		15	83	17
		30	78	22
		60	69	31
		90	65	35
[1- ¹⁴ C]Galactose	25	60	65	35

* The percentage tissue radioactivity in sugar phosphates was 18 and in nucleotide sugars 3, determined only in this incubation.

from the results found after incubation at 37°C. Lowering of the temperature of the galactose incubation decreased the label in the neutral fraction and increased that in the phosphorylated metabolites slightly.

Neutral sugar fraction. The pattern of hexose components in the supernatant obtained from water extracts of tissue treated with Ba(OH)₂ + ZnSO₄ after gas-liquid-chromatographic analysis is shown in Fig. 2. Fig. 2(a) is a chromatogram obtained from cortical tissue extracted directly upon removal from the animal. Peak A is α-glucose, which is about 40% of total glucose, B is β-glucose and C is inositol. The small peak (region 3 of Fig. 2c) just before that of β-glucose was in the region expected for galactitol or sorbitol but did not correspond precisely to peaks obtained from either standard and was therefore not accurately identified. It was estimated by using galactitol as a standard. Table 2 reveals that the tissue analysed directly contains 50μg of glucose/100mg of tissue, 33μg of inositol and 4.5μg of unknown as the galactitol equivalent.

Fig. 2(b) represents the sugar pattern of tissue before the initiation of incubation with 2mM-galactose. These slices have been preincubated at room temperature in bicarbonate buffer for about 0.5h. The glucose peaks are markedly diminished and only about 11% remains of that present initially. Analysis of the preincubation buffer revealed the

presence of the glucose that had disappeared from the tissue. Diminution of the unknown substance (region 3) in the tissue also occurred during preincubation.

Fig. 2(c) represents the sugar pattern of tissue incubated with 2mM-galactose for 60min. The braced regions refer to six general regions of the chromatogram where collections were made for radioassay. Amounts of galactose, glucose, inositol and the galactitol equivalents of the peak in region 3 per 100mg of tissue incubated in 2mM-galactose for various lengths of time are presented in Table 2. As expected, the amount of galactose present increased with the time of incubation from 9μg/100mg of tissue after incubation for 5min to 16μg/100mg after incubation for 60min. The specific radioactivity of the galactose which accumulated remained relatively constant (Table 3) and always in close agreement with that of the starting incubation medium (580c.p.m./μg). Of the total radioactivity applied to the gas-chromatographic column, between 90 and 94% appeared in region 2, almost all of which was present as galactose. The amount of glucose in the incubated tissue decreased slightly during the course of the incubations from the already decreased value of 6 or 7μg/100mg of tissue of the preincubated slices to 3.6μg/100mg of tissue after 60min of incubation. During this time the specific radioactivity of glucose increased (Table 3) but never approached

Fig. 2. Sugar components of extracts from rat kidney-cortex slices incubated in various ways as determined by gas-liquid chromatography

Gas-liquid chromatography was run on fresh rat kidney-cortex slices and slices incubated as described for Fig. 1 in the presence and the absence of galactose. The tissue was prepared for chromatography as described in Table 4. (a) Fresh tissue; (b) zero time (no galactose); (c) tissue incubated for 60 min in 2 mM-galactose. The braces indicate six regions collected for determination of radioactivity. The regions were collected by using disposable Pasteur pipettes placed in the outlet of the stream-splitter while the region in question was passing from the column. Each region was collected in a separate pipette, the residue dissolved in 1 ml of toluene and the radioactivity counted by adding the toluene to 10 ml of diluted Liquifluor. Regions: 1 = solvent front; 2 = α -, β - and γ -galactose; 3 = unknown peak; 4 = β -glucose; 5 = unknown peak; 6 = inositol. (A) α -Glucose; (B) β -glucose; (C) inositol.

Table 2. Amounts of sugars in neutral fraction of rat kidney cortex incubated in the presence of galactose

Incubations were run as shown in Fig. 1. Tissue was extracted by heating in water and protein precipitated by $\text{Ba}(\text{OH})_2 + \text{ZnSO}_4$. The filtrate was deionized by passing through an Amberlite MB-3 column and evaporated *in vacuo*. Trimethylsilyl derivatives were made as stated in the Experimental section and run on a Packard gas-liquid chromatogram (model 3372) through a 3% OV-1 column at 165°C. Values given are from one representative experiment. Two to four determinations were performed for each incubation.

Tissue treatment at 37°C	Amount found ($\mu\text{g}/100\text{mg}$ of tissue)			Peak in region 3 (galactitol standard)
	Galactose	Glucose	Inositol	
Fresh tissue	0	50.0	33.1	4.5
Zero time		6.1	28.8	1.8
Incubation for 5 min; 2 mM-galactose	9.2	7.0	30.3	1.6
Incubation for 15 min; 2 mM-galactose	9.5	4.5	25.5	1.4
Incubation for 30 min; 2 mM-galactose	10.4	3.6	23.6	1.5
Incubation for 60 min; 2 mM-galactose	15.8	3.6	29.0	1.6
Incubation for 60 min; 2 mM-galactose + 10 mM-pyruvate	14	14	22	1.5

that of galactose. Inositol remained constant in the tissues and no evidence of its production from $[1-^{14}\text{C}]$ galactose was found. The unidentified peak in region 3 remained constant throughout the incubations, but the specific radioactivity of this

peak increased during the incubation from 55 c.p.m./ μg after 5 min to 190 c.p.m./ μg after 60 min. Incubating cortical tissue for 60 min in 2 mM-galactose with the addition of 10 mM-pyruvate resulted in the presence of 14 μg of galactose/100 mg of

Table 3. *Specific radioactivities of compounds isolated by gas-liquid chromatography after incubation of kidney-cortex slices with 2mM-[1-¹⁴C]galactose*

Experimental conditions were as described in Fig. 1. Values given are means of two determinations. The specific radioactivity of galactose showed the widest variation because in some cases the α , β and γ peaks were collected separately, which allows for greater loss of ¹⁴C while collection pipettes are changed (for method of determining specific radioactivity, see Fig. 2).

Incubation time (min)	Additions	Specific radioactivity (c.p.m./ μ g)			Unknown compound in region 3 (see the text and Fig. 2)
		Galactose	Glucose	Inositol	
5	None	518	21	1	55
15	None	593	30	1	95
30	None	572	50	1.3	180
60	None	545	62	1.8	190
30	10mM-Glucose	485	4	1	176
60	10mM-Galactonate	430	27	1	120
60	10mM-Pyruvate	451	36	1	198

Table 4. *Determination of ¹⁴CO₂ released from rat tissue incubated with various ¹⁴C-labelled substrates*

The incubations were performed under the conditions stated in Fig. 1. All incubations were for 60 min with the ¹⁴C-labelled sugars listed. For liver, one slice (weighing 80–90mg) per flask was used. ¹⁴CO₂ released was assayed as described in the Experimental section. Values given are means of 3–15 determinations $\pm$ S.E.M.

1- ¹⁴ C-labelled substrate (2mM)	Tissue	Secondary substrate (10mM)	¹⁴ CO ₂ released (nmol/h per 100mg of tissue)
Galactose	Kidney		68 $\pm$ 2
Galactose	Liver		27 $\pm$ 5
Glucose	Kidney		236 $\pm$ 18
Galactose	Kidney	Sodium acetate	51 $\pm$ 2
Galactose	Kidney	Pyruvate	23 $\pm$ 1
Glucose	Kidney	Pyruvate	35 $\pm$ 1
Galactose	Kidney	Galactonate	68 $\pm$ 2
Galactonate	Kidney		20 $\pm$ 1
Galactonate	Kidney	Galactose	19 $\pm$ 2

tissue, a value similar to that in tissue incubated in 2mM-galactose without added pyruvate. The specific radioactivity of the galactose was also the same as in a normal galactose incubation. The amount of pyruvate in the tissue was not determined, but the amount of glucose rose to 14 μ g/100mg, about four times that found in 2mM-galactose incubations. The specific radioactivity of the glucose was lower than in a normal galactose incubation. The addition of glucose to the incubation did not decrease the amount of radioactivity in the intracellular glucose pool but did result in a lower specific radioactivity of glucose because of increased pool size. Addition of galactonate, unlike that of glucose or pyruvate, resulted in a

decrease in radioactivity without an increase in glucose content.

Galactose oxidation to CO₂

Since extensive metabolism of galactose occurs in rat kidney cortex (Fig. 1) further aspects of production of ¹⁴CO₂ in the rat were investigated and are presented in Table 4. The data indicate that kidney cortex oxidized about twice the amount of galactose per unit weight as did the liver. From the relative weights of the two organs it would appear that the kidney can contribute substantially to overall galactose oxidation by the animal. Galactose was oxidized at about one-third the rate of glucose.

The effect of C_2 and C_3 compounds, acetate and pyruvate, on $^{14}CO_2$ production was explored, since Kleinzeller *et al.* (1967a) have employed acetate to decrease galactose metabolism in rabbit kidney cortex. The addition of 10mM-sodium acetate to the incubation decreased the $^{14}CO_2$ production by 25%, and 10mM-pyruvate caused a 66% decrease. Analysis of the tissue pools of glucose on pyruvate addition revealed a marked increase in label as well as total tissue glucose (Tables 2 and 3). In fact, the radioactivity trapped in glucose pools was increased about 50%. Pyruvate has an even more pronounced effect on glucose conversion into $^{14}CO_2$ (Table 4). It seems therefore that the addition of pyruvate and probably acetate merely diverts labelled carbon atoms from CO_2 to glucose but does not alter overall galactose metabolism.

Table 4 also shows that galactonate, an intermediate in a proposed alternative oxidative pathway (Cuatrecasas & Segal, 1966), was oxidized by kidney cortex. The exact contribution of this pathway to total galactose catabolism under normal circumstances is unknown but is probably not great (Segal & Cuatrecasas, 1968). The failure of galactose addition to alter $^{14}CO_2$ release from galactonate, and galactonate to alter $^{14}CO_2$ release from galactose, would support this conclusion.

Relationship of uptake to substrate concentration

The relationship of uptake to substrate concentration was studied by determining the accumulation of neutral sugar, of phosphorylated metabolites and the production of $^{14}CO_2$ after a 60min incubation over the range 0.08–4mM. The results shown in Fig. 3 are expressed in two ways.

The first (Fig. 3a) is presented from the transport point of view, with uptake measured as the distribution ratio as in Fig. 1. In Fig. 3(a) the curve corresponds to the formulation published by Kleinzeller *et al.* (1967a) for neutral sugar concentration by rabbit kidney slices on incubation with galactose. Our data on accumulation of neutral sugar [$Ba(OH)_2 + ZnSO_4$ tissue filtrates] show that at 0.08mM the distribution ratio is 2.7 and decreases with increasing substrate, though remaining above 1.0 until 1mM substrate is reached. In the range 0.08–1mM therefore a concentration gradient of galactose exists and the process appears saturated thereafter. Fig. 3(a) shows the curve of the distribution ratios derived from water extracts of tissues, which include neutral sugar and phosphorylated metabolite fractions, as well as the curve calculated by assuming that $^{14}CO_2$ formed from galactose remained in the tissue. These curves, by their decreasing values with increasing substrate concentration, also indicate saturability of the uptake process. It is noteworthy that at the low concentrations a significantly greater

proportion of galactose is phosphorylated and oxidized.

Fig. 3(b) presents the second or metabolic point of view. Here the data have been calculated in terms of intracellular concentration after the 60min incubation of neutral sugar, phosphorylated intermediates, galactose converted into $^{14}CO_2$ and the total uptake of galactose, which is a sum of the three fractions.

Fig. 3. Relationship of uptake of D-galactose to its concentration

Incubations were run for 60min as described in the legend to Fig. 1, with various galactose concentrations. (a) Three types of distribution ratios are calculated: ○, for water extracts; △, for $Ba(OH)_2 + ZnSO_4$ filtrates of water extracts; □, assuming $^{14}CO_2$ had not been released from water extracts. (b) The rate of uptake of galactose is designated in mmol/h of incubation per litre of intracellular fluid: △, concentration in the neutral fraction; ○, concentration in the phosphorylated fraction; ■, concentration in the $^{14}CO_2$; □, total of all three fractions.

The curve of neutral sugar accumulation shows two components: a non-linear increase at concentrations below 1 mM and a linear increase with concentrations between 1 and 4 mM. Over the concentration range studied, continuous increasing accumulation occurred. The curve of total galactose accumulation was similar in nature to that for neutral sugar. The curve of accumulation of phosphorylated metabolites increased with substrate concentration and achieved a limiting intracellular value at 0.42 mM. The fact that galactose uptake continued with limited formation of phosphorylated metabolites suggests that formation of these compounds is the limiting step in galactose metabolism. The curve for dependence of intracellular concentration of $^{14}\text{CO}_2$ formed from galactose on substrate concentration is also biphasic, with the linear phase ensuing at the galactose concentration where phosphorylated forms begin reaching their maximum pool size.

To derive the saturable component of the neutral sugar accumulation curve (Fig. 3b) the linear component was subtracted. The resulting concentration-dependency curve is shown in Fig. 4. A limiting value for accumulation of 0.36 mmol/h per litre is observed with an apparent K_m of 0.2 mM. Similar mathematical treatment of the total entry of galactose dependent on concentration (Fig. 3b) was performed and is also shown in Fig. 4, although the limiting value was greater than that of neutral sugar. As expected, the apparent K_m was remarkably similar, 0.1 mM. It thus appears that there may exist two processes for galactose entry into renal cortical cells, one at low concentration which is saturable and the other at a concentration exceeding 1 mM that resembles diffusion or a system with an extremely high K_m , unable to be determined from these experiments (Christensen & Liang, 1966).

Effect of anaerobiosis, dinitrophenol, sodium depletion and phlorrhizin on uptake

Results of various perturbations in the incubation conditions are presented in Table 5. At 0.2 mM-galactose, a concentration in the range where the transport system behaves as if saturable (Fig. 4), anaerobiosis and addition of 2,4-dinitrophenol decreased the uptake of galactose. Under these conditions water-soluble radioactive compounds did not accumulate above the concentration in the medium (i.e., distribution ratio ≤ 1.0). Of the accumulation of radioactive material at 0.2 mM, 73% was independent of the medium concentration of Na^+ . The presence

Fig. 4. *Saturable components of curves showing relationship of uptake to concentration of D-galactose*

Saturable components of curves in Fig. 3(b) of total uptake (■) and neutral fraction (▲) are derived by subtraction of the linear component from the curves. Apparent K_m for the curve for total uptake is 0.1 mM and for the curve for the neutral fraction is 0.2 mM.

Table 5. *Effect of anaerobiosis, sodium depletion, dinitrophenol and phlorrhizin on uptake of galactose by rat kidney cortex slices*

Incubations were performed as described in Fig. 1 and in the Experimental section. Distribution ratios were determined from water extracts in all cases. Preincubation under the same conditions as the incubation was carried out for 30 min at 37°C before addition of substrate. Results are the means of three determinations $\pm$ S.E.M. The significance of the difference between each group and its corresponding control was tested by using Student's *t* test.

Incubation conditions	With 0.2 mM-galactose		With 2 mM-galactose	
	Distribution ratio	<i>P</i>	Distribution ratio	<i>P</i>
Control	3.32 ± 0.15		1.29 ± 0.09	
Anaerobic	0.97 ± 0.11	<0.001	0.87 ± 0.03	<0.02
Na^+ -free	2.41 ± 0.19	<0.02	1.15 ± 0.06	<0.41
0.1 mM-2,4-Dinitrophenol	1.01 ± 0.10	<0.001	0.82 ± 0.11	<0.025
3 mM-Phlorrhizin	1.50 ± 0.04	<0.001	0.89 ± 0.03	<0.02

Table 6. *Effect of various sugars on the uptake of [1-¹⁴C]galactose by rat kidney cortex slices*

Incubations were performed as described in Fig. 1. Unlabelled sugars were added to the concentrations stated. Distribution ratios from water extracts were determined. Values given are means of three determinations $\pm$ S.E.M. The significance of the difference between each group and its corresponding control was tested by using Student's *t* test.

Concn. of [1- ¹⁴ C]galactose (mM)	Unlabelled sugar	Concn. (mM)	Distribution ratio	<i>P</i>
0.2	None		2.61 $\pm$ 0.13	—
	Galactonate	10	2.68 $\pm$ 0.39	>0.8
	Glucose	10	2.77 $\pm$ 0.25	>0.5
	Methyl α -D-glucoside	10	2.90 $\pm$ 0.04	>0.1
	2-Deoxygalactose	10	2.64 $\pm$ 0.14	>0.8
	2-Deoxyglucose	10	2.65 $\pm$ 0.11	>0.8
	3-Deoxyglucose	10	2.94 $\pm$ 0.27	>0.3
0.2	None		2.62 $\pm$ 0.12	—
	Methyl α -D-glucoside	20	2.00 $\pm$ 0.04	<0.01
	Glucose	20	1.66 $\pm$ 0.06	<0.01
2	None		1.21 $\pm$ 0.02	—
	Galactonate	10	1.25 $\pm$ 0.01	>0.2
	Glucose	10	1.04 $\pm$ 0.09	>0.2
	Methyl α -D-glucoside	10	1.05 $\pm$ 0.08	>0.1
	2-Deoxygalactose	10	1.27 $\pm$ 0.06	>0.3
	2-Deoxyglucose	10	1.08 $\pm$ 0.06	>0.1
	3-Deoxyglucose	10	1.20 $\pm$ 0.08	>0.9
2	None		1.21 $\pm$ 0.18	—
	Methyl α -D-glucoside	20	1.02 $\pm$ 0.07	>0.1
	Glucose	20	1.04 $\pm$ 0.11	>0.2

of 3mM-phlorrhizin decreased the uptake of 0.2mM-galactose by 55%. Fractionation of the water extract showed that the concentration of the neutral sugar component was less than the sugar concentration in the medium.

At 2.0mM-galactose, a concentration where most of the uptake does not occur by active transport, anaerobic incubations and the presence of dinitrophenol produced intracellular concentrations of water-soluble radioactive compounds less than the incubation medium (distribution ratio <1.0). Sodium depletion produced no significant change in uptake. Addition of 3mM-phlorrhizin decreased the uptake by about 30% to a value similar to that achieved during anaerobiosis. Over a 90min incubation period conversion of [1-¹⁴C]galactose into ¹⁴CO₂ was unaltered by phlorrhizin.

Effect of sugars on uptake

Table 6 shows the effect of various related sugars on uptake of [1-¹⁴C]galactose. At low concentrations (0.2mM) no effect could be observed at sugar:galactose ratios of 50:1. Only at ratios of 100:1 for glucose:galactose and for methyl α -D-glucoside:galactose was there any significant decrease in the uptake of galactose. At high substrate concentration (2.0mM) no significant effect of any of the sugars

tested was observed for sugar:galactose ratios of 5:1 or 10:1.

Discussion

We have seen in our studies with rat renal cortical slices that galactose is a metabolizable substrate. The metabolism of galactose complicates any kinetic analysis of transport, which must then be viewed in the light of total uptake and the behaviour of the various metabolic fractions. As far as characterization of galactose transport in reference to the neutral sugar pool and to total uptake is concerned, the system seems to consist of two components: (1) a substrate-saturable process, and (2) a diffusion process. The substrate-saturable component is observed at a range of low concentrations of substrate and is largely independent of concentration of Na⁺. Active transport criteria are fulfilled since intracellular concentration gradients greater than 1.0 are achieved. The apparent *K_m* for this component of the transport system is about 0.2mM. At substrate concentrations around 1mM the active transport system appears saturated. Further increase in substrate concentration results in a linear increase in the rate of intracellular galactose accumulation with no concentration gradients being achieved.

As we have previously established (Cuatrecasas & Segal, 1965; Bertoli & Segal, 1966; Cohn & Segal, 1969), all of the enzymes in the Leloir pathway for the conversion of galactose into glucose are present in the rat kidney. We have found phosphorylated intermediates in the tissues after incubation with galactose and have shown that the glucose isolated from cortical slices incubated in $[1-^{14}\text{C}]$ galactose does become labelled. The study of transport parameters of galactose might be facilitated if an effective metabolic blocking agent for galactose metabolism could be found. Evidence for suppression of UDP-galactose-4-epimerase in the liver in the presence of ethanol (Isselbacher & McCarthy, 1959) depends on increased production of NADH by the alcohol dehydrogenase reaction to block epimerization (Smith & Newman, 1959). Rat liver slices incubated in 2mM- $[1-^{14}\text{C}]$ galactose and 0.2M-ethanol show a 96% inhibition of $^{14}\text{CO}_2$ released whereas rat renal cortical slices show no inhibition, apparently owing to a lack of alcohol dehydrogenase (Gillette, 1959). A search for other agents that might act as a metabolic block could be of use.

The apparent use of the Leloir pathway leads to the conclusion that CO_2 produced comes from galactose that has been converted into glucose. A direct oxidative pathway may be present, as evidenced by ability of the tissue to metabolize galactonate. Experiments designed to trap radioactive galactonate, the proposed intermediate (Table 4), have failed to establish any considerable contribution of such a pathway to the overall metabolism of galactose.

Rasmussen (1970) has shown in the cat that at low filter loads the rate of metabolic conversion of galactose in the kidney considerably exceeds the rate of reabsorption. At increasing loads the metabolic rates approach a maximum value. Thus at low loads the metabolism of galactose in the kidney may determine its uptake. In this regard it would be interesting to study galactose accumulation in renal tissue of humans with galactokinase or galactose 1-phosphate uridyl transferase deficiency (Segal, 1972). To our knowledge, galactose clearance studies have not been made in patients with defects in galactose metabolism.

Some caution needs to be exercised in the interpretation of dynamic events in tissue slices, since the substrates have not been delivered in the physiological manner via the blood vessels. We think that the demonstration of a substrate-saturable process at low galactose concentrations would not be affected by the route of delivery of the substrate. However, the characteristics of the diffusion process may apply only to the system studied *in vitro* and should be verified under more physiological conditions.

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