BBA 25896

THE METABOLIC FATE OF [1-^{14}C]GALACTITOL IN MAMMALIAN TISSUE

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(Received August 15th, 1967)

SUMMARY

The metabolic fate of [1-^{14}C]galactitol in various rat tissues, intact rats and normal humans has been determined. Of the tissues examined kidney slices oxidized the galactitol to $^{14}CO_2$ to the greatest extent but this was only 3 % of the rate of oxidation of D-[1-^{14}C]galactose and [1-^{14}C]sorbitol. [1-^{14}C]Galactitol oxidation by kidney slices was inhibited 40 % by incubation with 5.5 mM D-glucose or D-galactose but not D-arabinose or D-xylose. Homogenates of renal cortex were capable of carrying out galactitol oxidation and the basal level was enhanced by addition of either ATP, DPN or TPN.

Studies of the penetration of galactitol into tissue slices *in vitro* revealed a volume of distribution for the polyol of about 60 % of wet tissue weight in most tissues. $3 \cdot 10^{-3}$ M phlorizin inhibited the penetration into kidney cortex slices. The volume of distribution of galactitol in tissues after injection of the compound into nephrectomized rats was found to vary from 10 % of wet tissue weight for brain to 108 % for liver. Chromatographic analysis of tissue extracts after penetration of the polyol revealed essentially all of the ^{14}C material to be galactitol itself.

Radioactive galactitol injected into rats or normal humans was almost entirely excreted unchanged in the urine with little or no detection of label in CO_2. These studies indicate that the formation of galactitol is a dead end alternate pathway of galactose metabolism and that this polyol's major fate in the animal is renal excretion.

INTRODUCTION

The sugar alcohol of galactose, galactitol, has become of increasing biological importance. This polyol has been shown to accumulate in the lens[1] and other tissues[2] of rats fed a high galactose diet. It has also been demonstrated in the urine[3] and various tissues[4] of patients with congenital galactosemia who lack the enzyme galactose-1-phosphate uridyltransferase. Galactitol has been considered to be the etiological factor in galactose cataract formation and to be responsible for a defect in inositide formation in rat brain[5].

Since the reduction of galactose to the hexitol is an alternative to the nucleotide sugar pathway of galactose metabolism the question arises of whether galactitol is an intermediate in a pathway for dissimilation of galactose or is a metabolite without a further metabolic fate. In the lens and liver galactitol is not a substrate for the enzyme

polyol dehydrogenase which normally oxidizes polyols to keto sugars[6], but other fates of galactitol have not been examined in mammalian tissues. The present report describes the results of studies of the metabolism of [1-^{14}C]galactitol by various rat tissues, the intact rat and the normal human subject.

MATERIALS AND METHODS

Experiments in vitro

Male Sprague–Dawley rats weighing about 150 g obtained from the Charles River Farms were used in all experiments. They were fed rat chow and water *ad libitum* until sacrifice by stunning and decapitation. Segments of everted jejunum weighing about 10 mg were prepared as described previously[7]. Slices of the cerebral cortex were made by hand with a razor blade. A Stadie-Riggs microtome was employed to make liver slices from the right lobe, kidney cortex slices from the poles and spleen slices. "Cut" hemidiaphragms were also employed. About 200 mg of tissue were used in tissue slice experiments. Homogenates of kidneys from which the medulla was removed were prepared in ground glass grinders with 2 vol. of cold 0.005 M sodium phosphate buffer (pH 6.5). Supernatant from these homogenates was obtained after centrifugation in a Sorvall SS-3 centrifuge at 34800 $\times$ *g* in the cold for 30 min. Determinations of protein in the homogenates were made using a modification of the method of LOWRY *et al.*[8].

Oxidation experiments for collection of $^{14}CO_2$ were carried out in modified Warburg flasks[9]. Tissues or homogenates were incubated in 2.0 ml Krebs–Ringer bicarbonate buffer (pH 7.4) *plus* 1.93 μmole containing 0.8 μC of appropriately labeled substrate. After gassing for 20 sec in 95 % O_2–5 % CO_2, the incubation flask was sealed and shaken in a Dubnoff Metabolic Shaker for 90 min at 37°. 1 ml of 1.0 M hydroxide of Hyamine was added to a small suspended center wall and 0.3 ml of 3 M H_2SO_4 was injected down the side of the sealed incubation flask. Labeled CO_2 was allowed to diffuse for 45 min at room temperature after which the Hyamine content of the center well was transfered to 15 ml of liquid scintillator, made as a 5 % solution of Liquiflor in toluene. Radioactivity was counted in the Packard Model 3365 liquid scintillation counter at 73 % efficiency.

The uptake of labeled galactitol into kidney, liver, brain, diaphragm and intestine was studied by incubating approx. 100 mg of tissue in 25-ml plastic flasks containing 2 ml Krebs–Ringer bicarbonate buffer containing 0.8 μC [1-^{14}C]galactitol (1.93 μmole) per flask under an atmosphere of 95 % O_2–5 % CO_2 for 90 min at 37°. After incubation the tissues were handled as previously described in transport studies from this laboratory[10]. Essentially, the radioactivity was extracted in 2 ml of hot water and assayed by the liquid scintillation technique[10]. The galactitol space in per cent of wet tissue weight was calculated as follows[11]:

$$\text{Galactitol space (per cent wet tissue wt.)} = \frac{\dfrac{\text{Counts/min [}^{14}\text{C]galactitol in total tissue water}}{\text{Counts/min [}^{14}\text{C]galactitol per ml medium}}}{\text{wet tissue weight}} \times 100$$

The identification of the radioactivity in the water extracts of the tissue was shown to be galactitol by thin-layer chromatography[12]. When phlorizin was used the concentration was $3 \cdot 10^{-3}$ M.

Experiments in vivo

Oxidation of [1-^{14}C]galactitol to $^{14}CO_2$ was determined in three normal volunteers, two males and one female, ages 18–22, after rapid intravenous injection of 5.0 ml of the radioactive material (0.92 μC/ml in isotonic saline) into an antecubital vein. Expired air was collected at 30-min intervals for 5 h and assayed for $^{14}CO_2$ by the method of FREDRICKSON AND ONO[13] as modified by SEGAL, BLAIR AND ROTH[14]. Urine was collected at intervals for 24 h and assayed for ^{14}C as previously described and chromatographed by thin-layer techniques to identify the nature of the urinary radioactivity[12].

Oxidation of galactitol to $^{14}CO_2$ was studied in rats after intraperitoneal injection of 1 ml of [1-^{14}C]galactitol (0.92 μC, 2.24 μmoles) and placement of the animal in a metabolic chamber through which CO_2-free air was drawn. Expired CO_2 was trapped in Hyamine for 10-min periods at 30-min intervals for 5 h by methods already described[14]. Urine was collected throughout the experiment. Both $^{14}CO_2$ in Hyamine and in the urine were assayed by a liquid scintillation technique as noted above.

Studies for the analysis of tissue distribution of radioactive galactitol were carried out in nephrectomized animals after recovery of the animal from surgery by a flank approach. 1 ml of [1-^{14}C]galactitol (0.92 μC/ml) in saline was injected intraperitoneally and 4 h later the animal was killed by exsanguination by cardiac puncture. Approx. 100 mg of brain, liver, diaphragm, intestine and spleen were removed and placed in 2 ml of water for extraction and assay of the tissue ^{14}C as reported in the preceding section. Radioactivity was determined in plasma by assaying a 0.2-ml aliquot of a 1:10 trichloroacetic acid filtrate. A galactitol space expressed as per cent of tissue weight was calculated as the ratio of the counts/min per ml of tissue water to counts/min per ml of plasma divided by the wet tissue weight multiplied by 100 as in the space experiments *in vitro*. All of the ^{14}C in the tissues and plasma was identified as galactitol[12].

[1-^{14}C]Galactitol, 0.4 μC/μmole was obtained from National Bureau of Standards. [1-^{14}C]Galactose and [1-^{14}C]sorbitol, both were diluted to 0.41 μC/μmole and were obtained from the Nuclear Research Corp. [1-^{14}C]Galactitol, 0.92 μC/ml, in normal saline was sterile and pyrogen-free. Unlabeled glucose and galactose were obtained from Pfanstiehl Laboratories, Inc. Hydroxide of Hyamine was purchased from Packard Instrument Co. and Liquifluor phosphor was obtained from New-England Nuclear. Dithiothreitol (Cleland's Reagent) was a product of Calbiochem. Phloridzin recrystallized before use was a product of the Mann Research Laboratories. [1-^{14}C]Galactitol, [1-^{14}C]galactose and sorbitol were found to be radiochemically pure using thin-layer chromatography[12].

RESULTS

Oxidation of [1-^{14}C]galactitol by various tissues

A comparison of the ability of tissue slices *in vitro* to oxidize [1-^{14}C]galactitol to $^{14}CO_2$ revealed that kidney and liver are able to perform this metabolic conversion (Table I). Diaphragm, spleen, brain and intestine showed very little activity in this regard. The extent of galactitol oxidation relative to that of the parent aldose, galactose, and the isomeric polyol, sorbitol, is also shown in Table I. Kidney oxidizes galactitol to CO_2 to only 3 % of the yield from either galactose or sorbitol, producing

TABLE I

OXIDATION OF GALACTITOL, SORBITOL AND GALACTOSE BY RAT TISSUE SLICES

Tissue slices were incubated in 2.0 ml Krebs–Ringer bicarbonate buffer (pH 7.4) containing 1.93 μmole and 0.8 μC of [1-^{14}C]galactitol for 90 min at 37°. $^{14}CO_2$ was measured as described in MATERIALS AND METHODS. Values reported represent a mean of 3 to 9 trials ± standard error. When less than 3 trials were made, individual values are given.

Tissue	*mμmoles CO_2 produced per g tissue wet weight*			
	[1-^{14}C]Galactitol		*[1-^{14}C]Sorbitol*	*[1-^{14}C]Galactose*
	No addition	*+ Glucose (5.5 mM)*		
Kidney	10.3 ± 0.8	7.3 ± 0.7 (6.4 ± 0.4)*	361 360	361 ± 21
Liver	3.7 ± 0.3	2.9 ± 0.2	259 250	237 ± 7
Diaphragm	0.91 ± 0.01	0.81 ± 0.1	16 17	19 ± 1
Spleen	1.7 1.8	0.94 1.1	105 0.07	339 ± 21
Brain	1.3 1.4	0.80 0.64	53 55	34 ± 3
Intestine	1.9 1.4	0.76 1.1	86 52	219 ± 26

* Indicates value obtained when 5.5 mM galactose was added instead of glucose.

10 mμmoles/g tissue wet weight compared to 360. The yield of $^{14}CO_2$ from liver metabolism of galactitol was only 1 % of $^{14}CO_2$ from either galactose or sorbitol. Preliminary studies with tissues from newborn rats revealed that the oxidation of galactitol to CO_2 by kidney slices is about two thirds the adult value.

The data in Table I indicate that the addition of 5.5 mM glucose or galactose causes a 30–40 % inhibition of galactitol oxidation by kidney. No inhibition was observed with 2.7 mM galactose but increasing the galactose to 11 mM caused a 50 % inhibition. No inhibition was observed if 5.5 mM D-arabinose on D-xylose were added to the incubation medium.

Oxidation of galactitol by kidney homogenates

Delineation of cofactor requirements for galactitol oxidation was sought by use of tissue homogenates. The results are shown in Table II. The addition of either ATP, DPN or TPN enhanced the capacity of both whole homogenates and a high speed supernatant to oxidize galactitol. In the latter preparation it appeared that the addition of ATP and TPN together increased the ability to metabolize the polyol to a greater extent than the addition of either cofactor alone.

Uptake of galactitol by various tissues

In order to determine whether penetration of galactitol into cellular water may have been a limiting step in tissue metabolism of the compound two types of experiments were performed to measure cellular accumulation. The first was an assessment *in vitro* of the galactitol "space" upon incubating slices of various tissues with the

TABLE II

OXIDATION OF [1-^{14}C]GALACTITOL BY RAT KIDNEY HOMOGENATE

Either 0.7 ml of whole kidney homogenate (approx. 95 mg protein/ml) or 0.6 of the 34800 × *g* supernatant (approx. 32 mg protein per ml) were incubated with Krebs–Ringer bicarbonate buffer to a final volume of 2.0 ml for 90 min at 37°. Substrate was added as in Table I. $^{14}CO_2$ was measured as described in MATERIALS AND METHODS. Values reported represent a mean of 4 to 6 trials ± standard error.

Additions	*mμmoles CO_2 produced per g protein*	
	Supernatant	*Whole homogenate*
None	2.1 ± 0.1	1.7 ± 0.2
ATP	8.0 ± 0.2	2.6 ± 0.4
DPN	6.2 ± 1	2.9 ± 0.4
TPN	7.5 ± 0.9	3.5 ± 0.5
ATP, DPN	8.1 ± 2	4.0 ± 0.4
ATP, TPN	11.7 ± 1	3.8 ± 0.3
ATP, TPN, DPN	14.5 ± 0.9	3.4 ± 0.3

TABLE III

CELLULAR PENETRATION OF GALACTITOL IN RAT TISSUES

In experiments *in vitro* 100 mg of tissue was incubated in 2.0 ml Krebs–Ringer bicarbonate buffer (pH 7.4) or in 2 ml phlorizin ($3 \cdot 10^{-3}$ M in Krebs–Ringer bicarbonate buffer (pH 7.4)) for 90 min at 37°. The tissue distribution of radioactive galactitol was measured *in vivo* after an intraperitonial injection of labeled galactitol into nephrectomized rats. The determination of radioactivity present in the tissues and the calculation of volume of distribution is described in MATERIALS AND METHODS. Values for experiments *in vitro* and *in vivo* are reported as a mean of 4 to 18 trials ± standard error.

Tissue	*Volume of distribution (% wet wt.)*		
	In vitro		*In vivo*
	Control	*Phlorizin ($3 \cdot 10^{-3}$ M)*	
Kidney	55 ± 0.5	46 ± 0.8	
Liver	64 ± 0.8	63 ± 1	108 ± 3
Diaphragm	61 ± 2	60 ± 2	44 ± 4
Intestine	49.5 ± 3	53 ± 3	72 ± 4
Brain			9.9 ± 1

polyol. These results are shown in Table III and are expressed as a volume of distribution in terms of per cent of wet tissue weight. A value of 100 would indicate a distribution of the material throughout both extracellular and intracellular fluid. (Extracellular fluid volume determined with inulin may vary with the tissue ranging from 12 % for intestine to 35 % for the cut diaphragm with kidney an intermediate 27 % as determined in this laboratory.) After a 90 min incubation the volume of galactitol distribution varied between 50 and 65 % of the wet tissue weight thus indicating penetration into intracellular fluid. These experiments were also performed in the presence of phlorizin which is known to inhibit the membrane transport mechanisms for certain sugars[15]. Of the tissues tested only accumulation by kidney

slices was inhibited. Fig. 1 shows the time course of uptake of galactitol and the significant inhibition by phlorizin.

The second type of experiment was an assessment of the galactitol space *in vivo* by analysis of the galactitol uptake into various tissues 4 h after injection of the ^{14}C-labeled polyol into nephrectomized rats. This determination is based on a comparison of tissue radioactivity with that in plasma as described under METHODS. The results in Table III indicate distribution in total liver cellular fluid and a large fraction of intestinal cell water. Interestingly, there was little or no penetration into brain. These results indicate that should galactitol exist in circulating plasma the material will be present in various tissues and need not be formed by metabolic conversion of galactose *in situ*.

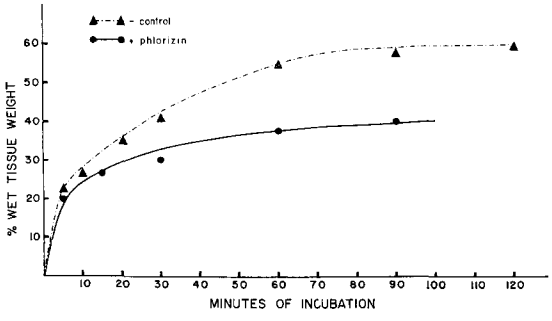

Fig. 1. Uptake of galactitol and inhibition by phlorizin with time. Methods as in Table III. Each point is an average of triplicate determinations.

Oxidation of [1-^{14}C]galactitol by rats and humans

The metabolic fate of [1-^{14}C]galactitol was determined after intraperitoneal injection of four normal rats and subsequent collection of urine and CO_2 for radioassay of ^{14}C. Between 74 and 87 % of the label was recovered in the urine as galactitol within 4 h. Radioactivity in expired CO_2 varied between 0.5 and 0.7 % of injected dose in the 4-h period. The latter results were also obtained after injection of labeled material into nephrectomized animals.

Results similar to those seen in rats were observed after intravenous administration of radioactive galactitol to three normal human volunteers. Between 75 and 88 % of the radioactivity was excreted in the urine in 5 h and 85 to 99 % within 24 h, all as unaltered galactitol. Collection of CO_2 from 2 of the normal subjects indicated less than 0.5 % excretion of the label in expired CO_2 within 5 h. It thus appears that the renal extraction of the polyol from blood is rapid and extensive. In the human, however, we have no knowledge of the metabolic capabilities of various tissues to handle endogenously formed galactitol.

DISCUSSION

In mammalian tissue there appears to be a widely distributed ability to produce sugar alcohols. In the lens, adrenal and muscle the enzyme aldose reductase seems responsible while in other tissues the enzyme L-hexonate dehydrogenase appears to be

implicated[16]. Comparison of the rates of formation of sugar alcohols in mammalian tissue[16] to the relatively slow rate of oxidation of galactitol demonstrated here produces a ready explanation for the accumulation of this polyol in tissues of animals fed a high galactose diet[2] and in the tissues of humans with impaired galactose metabolism[3].

The kidney and to some extent the liver have the ability to carry out the conversion of C-1 of galactitol to CO_2. The rate of [1-^{14}C]galactitol oxidation to CO_2 by kidney slices was, however, only 3 % that of galactose or the sugar alcohol sorbitol both labeled in the first carbon. Chromatography of tissue extracts after incubation with labeled galactitol did not reveal any large pool of intermediates which might account for delayed oxidation. Indeed, essentially all of the radioactivity in the tissue was galactitol itself.

The route by which [1-^{14}C]galactitol is oxidized to $^{14}CO_2$ by kidney and liver is open to speculation. The principal mammalian enzyme in the soluble fraction of liver and kidney for further metabolism of sugar alcohols is polyol dehydrogenase (L-iditol dehydrogenase)[6] which acts to convert sorbitol to fructose but which has little or no activity to form tagatose from galactitol. Our data with the supernatants of kidney homogenates shows the oxidation to be dependent on ATP, DPN or TPN. However, since these could act independently there may be both phosphorylation and dehydrogenation pathways involved. The formation of D-galactitol 1-phosphate is known to occur in bacteria[17]. The inhibition of galactitol oxidation by both D-glucose and D-galactose but not by D-xylose suggests the possibility that the usual oxidative pathways of the sugars are involved.

Penetration into the cell does not appear to be the limiting factor in metabolism of galactitol. In the experiments, both *in vivo* and *in vitro*, the polyol penetrated into liver cell water, yet only was metabolized to a fraction of the rate of galactose or sorbitol. In the distribution of galactitol *in vivo* the brain accumulated very little indicating a functional blood–brain barrier.

A central role for the fate of galactitol is played by the kidney since injection of the polyol to the rat and human leads to rapid excretion *via* the kidney. The observation that phlorizin affects penetration of galactitol into kidney slices in the face of poor reabsorption *in vivo* is enigmatic. The phlorizin effect indicates that a binding site for galactitol reabsorption is operative. It is possible, however, that phlorizin is inhibiting polyol uptake by the basal region of tubular cells while the brush border responsible for reabsorption *in vivo* does not participate in galactitol transport.

The oxidation of parenterally administered [1-^{14}C]galactitol by rats and humans is minute and corresponds with observations of [^{14}C]mannitol oxidation[18]. Radioactive sorbitol on the other hand is extensively oxidized to $^{14}CO_2$ by animals and man[19].

Results reported here support the idea that galactitol is essentially a "dead end" alternate pathway for galactose metabolism. Under conditions where galactose cannot be utilized normally by the conventional sugar nucleotide pathway, galactitol is formed in tissues, accumulates, exits into plasma and is cleared by the kidney for extensive urinary excretion. The conversion of galactose to galactitol is of no benefit to the organism, but on the contrary, leads to some of the severe manifestations of galactose toxicity.

ACKNOWLEDGMENTS

This work was supported by grants from the John A. Hartford Foundation and National Institutes of Health (AM10894).

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