ON THE BIOLOGICAL CONVERSION OF GALACTOSE TO GLUCOSE

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SUMMARY

Galactose, talose, gulose and idose labeled in carbon 2 with ^{14}C were synthesized. The conversion of [^{14}C]galactose to glucose isolated from liver glycogen was investigated in the fasted rat. It was found that the bulk of the isotope was in carbon 2 of glucose, indicating that an inositol intermediate could not be involved in the interconversion.

INTRODUCTION

The biological mechanism whereby D-galactose is converted to D-glucose has been the subject of numerous reports. Among these was that of TOPPER AND STETTEN[1] who administered [1-^{14}C]galactose to rats and determined the position of the isotope in glucose isolated from liver glycogen. By this procedure they were able to conclude that conversion had occurred without fragmentation of the carbon chain of galactose. Furthermore, the studies permitted exclusion of most forms of inositol as intermediates. However, mucoinositol, if formed from galactose, can be cleaved in four different positions to yield glucose. As pointed out by the above authors, the finding of isotope predominantly in carbon 1 of glucose eliminated three of these possibilities. The fourth possibility, however, could not be excluded if the assumption were made that the inositol intermediate was continually bound, either to an enzyme surface or in some other manner, thereby destroying the unique symmetry of the molecule.

Since publication of the above report it has been clearly established that the conversion of galactose to glucose occurs while the carbohydrates are bound as UDP derivatives[2]. It was not inconceivable, therefore, that the closing and opening of an inositol ring could have occurred while the carbohydrates were bound in this manner. A postulated mechanism is shown in Fig. 1. This mechanism is based on similar ring openings known to occur in the carbohydrate series[3].

In the present paper the results of an investigation made in order to test this hypothesis are reported. [2-^{14}C]galactose was administered to rats and glucose from liver glycogen was degraded to determine the location of the isotope. From Fig. 1

Abbreviations: UDP, uridine diphosphate; DPN, diphosphopyridine nucleotide.

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it can be seen that if the postulated mechanism were operative, the bulk of the isotope would be found in carbon 6 of glucose. It can also be seen that use of [1-^{14}C]-galactose would result in the formation of [1-^{14}C]glucose, in accordance with the results of TOPPER AND STETTEN[1]. Actual findings showed the bulk of the isotope to reside in carbon 2 of glucose, conclusively eliminating the postulated pathway as a possibility.

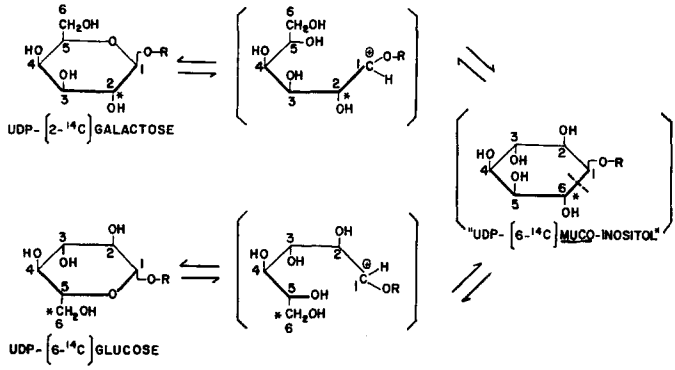

Fig. 1. Hypothetical conversion of UDP-[2-^{14}C]galactose to UDP-[2-^{14}C]glucose. The symbol R represents the uridine diphosphate moiety.

METHODS AND MATERIALS

Preparation of [2-^{14}C]*galactose, talose, gulose and idose*

Strontium xylonate was prepared from commercially obtained D-xylose by the electrolytic oxidation method of ISBELL AND FRUSH[4], and was decarboxylated to D-threose by HOCKETT's modification of the RUFF degradation[5]. Hexoses were prepared from this tetrose by two successive cyanohydrin syntheses[6] using the modifications of ISBELL *et al.*[7]. In the first cycle, Na^{14}CN, obtained from Tracerlab, Inc., Boston, Mass., was employed, and resulted in the formation of [1-^{14}C]xylose and [1-^{14}C]lyxose. The mixture was not separated, but was subjected to a second cyanohydrin synthesis using unlabeled NaCN. A mixture of [2-^{14}C]galactose, talose, gulose and idose was obtained.

The mixture of hexoses was separated by large scale paper chromatography and eluted from the paper. Radioautograms served to locate the sugars on the paper. An initial chromatogram was prepared using *n*-butanol–95 % ethanol–water (7:4:1, v/v/v) as the solvent and developed for 48–72 h. Clear separation of galactose and gulose was achieved, but the talose and idose moved too nearly alike to allow their separation from each other. All three areas were eluted from the paper with water.

The eluate containing [2-^{14}C]galactose was passed over a 1-ml column consisting of a mixture of equal parts of Amberlite IR-45 and Amberlite IR-120 to remove ionic materials eluted from the paper. The effluent and column washings were concentrated to a syrup under reduced pressure at 40°. An equal volume of methanol was added and the solution was refrigerated. Crystallization of galactose occurred, and the material was recrystallized from alcohol. Paper chromatography in three different solvent systems (*n*-butanol–95 % ethanol–water (7:4:1); *tert.*-amyl alcohol–water (11:2); water saturated phenol) and radioautography revealed only one substance

present, corresponding exactly in mobility with known galactose. The crystalline material had an equilibrium rotation of $[\alpha]_D^{25} + 79.6°$ ($c = 1$, water).

The eluate containing [2-^{14}C]gulose was treated in the same manner, but crystallization from the syrup could not be achieved. Examination by paper chromatography and radioautography as described above showed only one reducing substance to be present, which was also the only radioactive substance present, attesting to the purity of the carbohydrate.

The eluate containing the mixture of talose and idose was re-chromatogrammed for 24 h using water saturated phenol as the developing solvent. Clear separation of the two carbohydrates was obtained, and the two areas were eluted with water.

The eluate containing [2-^{14}C]talose was passed over a column consisting of a mixture of Amberlite IR-45 and Amberlite IR-120, and the effluent and column washings were concentrated to a thin syrup under reduced pressure at 40°. Absolute ethanol was added dropwise, with constant swirling, until a faint turbidity persisted. A seed crystal of D-talose was added and the flask was placed in a water bath at 9°. Crystallization began after 24 h. The crystals were collected and readily recrystallized from alcohol at refrigerator temperature. The purity of the [2-^{14}C]talose was established by paper chromatography and radioautography as described. The crystalline material had an equilibrium rotation of $[\alpha]_D^{25} + 20.5°$ ($c = 1$, water).

The eluate containing [2-^{14}C]idose was treated in the same manner, but crystallization could not be achieved. The syrup was stored at —15°. Idose was found to be quite unstable, and in other preparations of non-isotopic idose several changes were noted. Syrupy D-idose stored at 4° for some time spontaneously changed to D-sorbose which crystallized out of solution. This transformation is apparently analogous to the conversion of glucose to fructose. Paper chromatography of stored idose preparations frequently revealed the presence of a substance presumed to be a pentose. This assumption was based on the fact that in addition to the idose, a spot having a pink color when sprayed with aniline oxalate was observed, a reaction which has been reported to be typical for pentoses[8]. Idose can be fairly well separated from the pentose by paper chromatography using *tert.*-amyl alcohol–water (11:2), which can be allowed to develop for five days or more. Idose has also been reported to form idosan readily[9].

The rare aldohexoses thus prepared have been used in a variety of metabolic studies, the results of which will be the subject of future reports.

Administration of galactose to rats

Adult male rats of the Sprague-Dawley strain were used as the experimental animals. Prior to the experiment the animals were fasted for 24 h, 4 ml of a 50% aqueous solution of glucose was given by stomach tube, and the desired amount of radioactive galactose was injected intraperitoneally. The animal was immediately placed in a metabolism apparatus which enabled collection of expired CO_2 and urine. After 4 h the animal was sacrificed by a blow on the head and the liver quickly removed.

Isolation of glucose from liver glycogen

Glycogen was isolated from the liver by the trichloroacetic acid extraction method of KARP AND STETTEN[10]. The glycogen so obtained was dissolved in water,

dialyzed against water for 24 h at 4°, and precipitated by addition of an equal volume of absolute ethanol. A dry, white material was obtained by successive washings with alcohol and ether in the usual manner. Dialysis was found to facilitate crystallization of glucose in later steps.

Crystalline glucose was obtained by the hydrolytic and isolation procedures described by BELL AND YOUNG[11]. After recrystallization from ethanol, the purity of the product was established by paper chromatography and physical constants. The glucose so obtained was degraded by the method of KOHN AND DMUCHOWSKI[12].

RESULTS AND DISCUSSION

General experimental data are summarized in Tables I and II. Table III shows the results obtained on degradation of the glucose samples derived from liver glycogen. It can be seen that the bulk of the isotope was found in carbon 2, ranging in amount from about 75 to 92 % of the total. This finding made it unnecessary to degrade the molecule completely, since the data are sufficient to allow interpretation.

The findings indicate that the mechanism of conversion of galactose to glucose postulated earlier could not have been involved in the transformation. As pointed

TABLE I

COMPARISON OF ANIMALS ADMINISTERED [2-^{14}C]GALACTOSE

Expt. III was conducted for 2 h. Expts. I and II for 4 h.

	Expt. I	*Expt. II*	*Expt. III*
Animal weight, g	318	404	320
Liver weight, g	9.9	13.5	9.7
Liver glycogen, mg	250	323	105
Glucose from liver glycogen, mg	100	110	36
Expired CO_2 (as g $BaCO_3$)	8.4	9.2	4.9

TABLE II

INCORPORATION OF ^{14}C INTO METABOLIC PRODUCTS OF NORMAL RATS AFTER INTRAPERITONEAL ADMINISTRATION OF [2-^{14}C]GALACTOSE

Experiment number		*Total radioactivity counts/min*	*Radiochemical yield Per cent*
I	Galactose administered	$1.1 \cdot 10^6$	100
	Liver glycogen	$2.3 \cdot 10^5$	20.9
	Expired CO_2	$5.4 \cdot 10^4$	4.9
	Excreted in urine	$1.1 \cdot 10^4$	1.0
II	Galactose administered	$1.1 \cdot 10^6$	100
	Liver glycogen	$3.2 \cdot 10^5$	29.0
	Expired CO_2	$1.9 \cdot 10^5$	17.3
III	Galactose administered	$1.1 \cdot 10^6$	100
	Liver glycogen	$4.5 \cdot 10^5$	40.9
	Expired CO_2	$4.2 \cdot 10^4$	3.8

TABLE III

DISTRIBUTION OF ^{14}C IN GLUCOSE FROM LIVER GLYCOGEN OF NORMAL RATS AFTER INTRAPERITONEAL ADMINISTRATION OF [2-^{14}C]GALACTOSE

Experiment number	*Carbon source*	*Relative specific activity* Counts/min*	*Relative specific activity Per cent*
I	Glucose	2,233	100
	C-1	1,061	7.9
	C-2	10,079	75.3
	C-3,4,5,6**	2,258	16.8
II	Glucose	2,477	100
	C-1	976	6.6
	C-2	13,700	92.3
	C-3,4,5,6**	186	1.3
III	Glucose	10,575	100
	C-1	6,240	9.8
	C-2	54,900	86.6
	C-3,4,5,6**	2,310	3.6

* Specific activity of $BaCO_3$ counted at infinite thickness.
** Obtained by difference (see text).

out, if inositol were an intermediate, the bulk of the isotope would have been found in carbon 6 of glucose. These data are in accord with the more recent theories of the mechanism of interconversion of these two carbohydrates[13], but do not shed any further light on the role of DPN or the formation of a 4-keto intermediate. The findings are also in accord with those of SIU AND WOOD[14] and MOSER AND KARNOVSKY[15]. Similar inferences regarding the role of an inositol intermediate may be drawn from these reports.

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