ON THE BIOSYNTHESIS OF L-FUCOSE AND L-FUCOSE METABOLISM IN MAN

STANTON SEGAL AND YALE J. TOPPER

Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. (U.S.A.)

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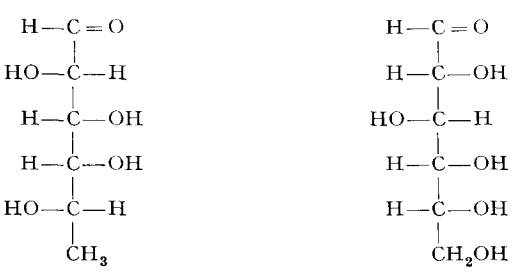
SUMMARY

Studies of the biosynthesis and metabolism of L-fucose in man have been performed. The ^{14}C labeling pattern of L-fucose in human milk subsequent to the intravenous administration of [6-^{14}C]glucose is compatible with the conversion of the intact hexose carbon chain of D-glucose to L-fucose. These results parallel those observed previously in studies of the bacterial synthesis of L-fucose. [1-^{14}C]fucose administered intravenously to a human subject was found to be rapidly and extensively converted to $^{14}CO_2$.

INTRODUCTION

The sugar, L-fucose, is known to occur in a variety of microorganisms, and in plant and animal tissues. It is a major component of the capsular polysaccharides of certain bacteria[1] and the polysaccharides of some marine algae[2]. In man, L-fucose is found as one of the principal sugars in blood group substances[3] and has been isolated both as the free sugar[4] and as a constituent of oligosaccharides in human milk[5,6].

The structure of L-fucose is shown in Fig. 1. Compared with the structure of other sugars found in man it is unusual because of its L-configuration and the absence of a hydroxyl group on carbon six. It differs from D-glucose in the configuration about carbons two, three and five and in the state of oxidation of carbon six. Previous reports from this laboratory[7] and elsewhere[8] have suggested that in *Aerobacter aerogenes*, strain A3S1, L-fucose of the capsular polysaccharide was synthesized by



L-Fucose D-Glucose

Fig. 1.

utilization of the intact carbon chain of D-glucose. Observations supporting this pathway of fucose biosynthesis have also been made by others studying a different microorganism[9].

An attempt has now been made to delineate the biosynthetic relationship between D-glucose and L-fucose in man by studying the formation of L-fucose in human milk. In addition, because of its presence in human milk and blood group polysaccharide, the metabolic fate of L-[^{14}C]fucose in man has also been determined.

MATERIALS AND METHODS

Four days *post partum*, after the mechanical emptying of each breast of milk, five microcuries of D-[6-^{14}C]glucose were administered intravenously to each of two lactating but non-nursing negro females on the obstetrical service of the District of Columbia General Hospital. 5 h later the breasts were again emptied and the milk obtained pooled for a total of 80 ml.

64.8 mg of L-fucose, determined by the method of DISCHE AND SHETTLES[10] were isolated from the milk by the extractive and chromatographic techniques of HEYNS, WALTER AND HEYDE[4]. Following the addition of 55.2 mg of pure L-fucose as carrier, crystalline derivatives were prepared and degraded by methods previously described[7].

Crystalline lactose obtained in the course of the fucose isolation was hydrolyzed in sulfuric acid. An aliquot of the hydrolysate was treated with glucose oxidase and gluconate isolated by the method of HEATH AND ROSEMAN[9]. Part of the gluconate was converted to the benzimidazole derivative according to MOORE AND LINK[11] and then combusted to CO_2 (see ref. 12) in order to obtain the glucose specific activity. Another aliquot of the gluconate was degraded and carbons one, two to five and six isolated as previously described[13]. An aliquot of the lactose hydrolysate was treated with periodate and, after treatment of the resulting formaldehyde with permanganate, C-6 of the combined glucose and galactose was isolated as CO_2.

Five microcuries of L-[1-^{14}C]fucose were administered intravenously to a 20 year old normal male volunteer weighing 80 kg. Expired CO_2 was collected and its radioactivity assayed by methods already reported[14]. Urine collected for 21 h after injection was counted for C^{14} (see ref. 14).

D-[6-^{14}C]glucose (1 μC/mg) and L-[1-^{14}C]fucose (3 μC/mg) were obtained from Dr. H. ISBELL of the National Bureau of Standards.

RESULTS

Biosynthesis

The distribution of ^{14}C in D-glucose and L-fucose in human milk after [6-^{14}C]-glucose administration is shown in Table I. Glucose in milk has much of the asymmetric labeling of the administered glucose. The ^{14}C pattern of L-fucose is similarly asymmetric with most of the label in the C-5,6 fragment of the methylpentose skeleton. This pattern of fucose labeling in human milk suggests that, like certain bacteria, man synthesizes L-fucose predominantly by direct conversion of D-glucose to L-fucose without rupture of the carbon chain[7–9]. The somewhat greater randomization of ^{14}C in fucose seen here has also been observed when bacteria are grown with

[6-^{14}C]glucose as the carbon source[7] and suggests the presence of a secondary pathway of fucose synthesis from smaller carbon fragments.

SCHAMBYE, WOOD AND KLIEBER[15] studying lactose synthesis in the cow have shown that the glucose in milk obtained 3 h after intravenous administration of [1-^{14}C] glucose has about 90 % of the radioactivity in carbon one. These investigators,

TABLE I

^{14}C DISTRIBUTION IN HEXOSES IN HUMAN MILK

Sugar	*Fragment*	*Specific activity counts/min/mM*	*Percent of radioactivity*
D-glucose	C-1,2,3,4,5,6	2780	
	C-1	150	4*
	C-2,3,4,5	300	10
	C-6	2700	86
L-fucose	C-1,2,3,4,5,6	2180	
	C-1	131	6
	C-2,3,4	459	22
	C-5,6	1510	72
Lactose hydrolysate	C-6	2700	

* These percentages are calculated on the basis of the sum of the fragments.

as well as others[16], suggest that glucose in milk is in equilibrium with blood glucose. Recent studies in man[17] have demonstrated that there is little randomization of ^{14}C in blood glucose for at least 3 h after intravenous injection of [6-^{14}C]glucose. It would thus appear that in man as well as the cow[15] and goat[16] glucose in milk is derived principally from blood glucose.

Studies of milk lactose of the cow[15], goat[16] and rabbit[18] after administration of [^{14}C]glucose have revealed an almost identical specific activity of galactose and glucose. If the assumption is made that these hexoses in human milk have similar specific activities, then the identical specific activity of C-6 in glucose with that of C-6 isolated from the equimolar mixture of glucose and galactose in the lactose hydrolysate implies that the pattern of labeling of the galactose in human milk is similar to that of the glucose. Such a conclusion is consistent with that of others[15] who have studied lactose formation in the cow and have observed that, after intravenous injection of a specifically labeled glucose, the galactose and glucose in milk had a similar pattern of isotope distribution.

Metabolism

The pattern of $^{14}CO_2$ excretion after L-[1-^{14}C]fucose administration is shown in Fig. 2. Integration of the area beneath the curve reveals that 39 % of the injected radioactivity was excreted as $^{14}CO_2$ in 6 h. 30 % of the ^{14}C given appeared in the urine within 6 h, virtually no ^{14}C being detectable in the urine after this time (Table II). If the administered ^{14}C be corrected for the urinary loss, the total $^{14}CO_2$ excreted in 6 h represents 56 % of the activity retained in the body.

Urine obtained in the first hour after the fucose injection was chromatographed

on paper (methyl-ethyl ketone–acetic acid–water (6:1:1); R_F of L-fucose = 0.17) and the area corresponding to L-fucose was eluted with water and assayed for ^{14}C. 83% of the radioactivity applied to the paper was recovered in this area (usual recovery of ^{14}C sugars so treated is about 93% (see ref. 19)). It thus appears that most of the urinary ^{14}C represents unaltered L-fucose. Fucose may be normally found in human urine[20].

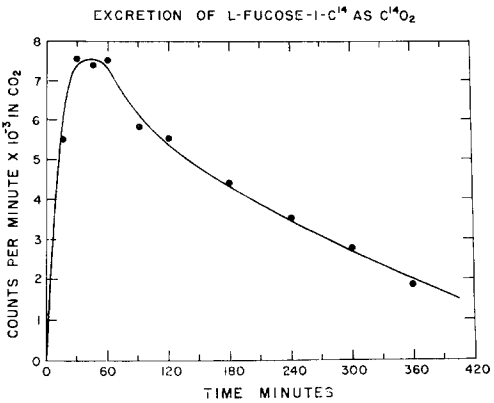


Fig. 2. The excretion of L-[1-^{14}C]fucose as $^{14}CO_2$ after the injection of 5 μC into a normal human.

TABLE II

URINARY EXCRETION OF ^{14}C

Hour	*Percent of dose*
1	25
2	28
3	29
4	30
5	30
6	30
7–21	31

DISCUSSION

L-Fucose comprises from 14 to 19% of various blood group polysaccharides[21]. Its presence is significant from an immunologic aspect, since it appears to be an important factor for the specificity of both H[22] and Le^a activity[23]. The destruction of blood group activity of A, B and H substance by mild acid hydrolysis is associated with liberation of fucose from the molecule.

Human milk contains about 128 mg percent L-fucose[6]. Except for the sheep, other animal milks tested thus far do not contain fucose[4]. Although fucose is of importance in the immunologic reactions involving blood group substances, the importance of L-fucose in human milk has not been ascertained. The quantity present in human milk, however, does not suggest a caloric importance of L-fucose.

The similarity in the pattern of labeling of L-fucose and D-glucose in human milk after the injection of [6-^{14}C]glucose is compatible with the interpretation that L-fucose is synthesized from D-glucose without rupture of the carbon chain. A recent report by GINSBURG AND KIRKMAN[24] has demonstrated in *Aerobacter aerogenes*, strain

A3S1, the presence of guanosine diphosphate fucose and the formation of the latter nucleotide from guanosine diphosphate mannose[25]. It thus appears that glucose is converted to guanosine diphosphate mannose with inversion of the configuration about carbon two. The latter nucleotide, by a series of reactions involving inversion of C-3 and C-5 plus reduction of C-6, is converted to guanosine diphosphate fucose. Guanosine diphosphate fucose has been isolated from sheep milk[26]. The results of the isotopic study here reported suggest the possibility of a similar mechanism of fucose biosynthesis in the human.

The fact that the fucose specific activity is less than the glucose specific activity, although the labeling pattern is similar, deserves some comment. Such a finding could be due to synthesis of fucose in part from unlabeled carbon fragments. An alternate explanation is that the fucose in milk has a slower turnover than the glucose moiety of lactose.

The present study reveals that carbon one of fucose is readily metabolized by man to CO_2. The $^{14}CO_2$ excretion curve presented here differs from that obtained from the oxidation of ^{14}C pentoses labeled in carbon one[19], as well as from [1-^{14}C]-glucose[17]. The extent and rapidity of the oxidation exceeds that of [1-^{14}C]glucose, as well as that of the pentoses, excepting ribose.

The oxidation of L-fucose appears to be extensive also in the intact rabbit and in rabbit liver and kidney slices[27]. Experiments employing *E. coli*[28] indicate that the initial steps in fucose metabolism involve the formation of the keto sugar phosphate, fuculose-1-phosphate, with subsequent conversion of the latter sugar to a product capable of being split by aldolase, presumably to dihydroxyacetone phosphate and lactaldehyde[28].

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