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Carbohydrate Metabolism in Leukocytes

VI. The Metabolism of Mannose and Fructose in Polymorphonuclear Leukocytes of Rabbit

VIGGO ESMANN,* ERNEST P. NOBLE** and RUNE L. STJERNHOLM***

Department of Biochemistry, School of Medicine Western Reserve University, Cleveland 6, Ohio, U.S.A.

The metabolic fate of ^{14}C-labeled mannose and fructose in the polymorphonuclear leukocyte of rabbit was studied by determination of the distribution of radioactivity in the glycogen and lactate. There is substantial incorporation of mannose into glycogen and lactate which occurs via the Embden-Meyerhof pathway. Fructose was also metabolized to glycogen and lactate via glycolytic reactions, but in this case the results indicate the presence of an alternative pathway.

Previous studies with rabbit polymorphonuclear leukocytes have been concerned with the pathways involved in the utilization of glucose and galactose. It has been shown that labeled glucose and galactose are incorporated into glycogen without alteration of the ^{14}C distribution in the 6 carbons and that two and three-carbon substrates such as acetate, lactate, and glycerol are incorporated into C-4, C-5 and C-6 positions of the glucose units of glycogen.[1-3] It was proved that there is no significant fructose-diphosphatase activity in leukocytes[2] and therefore no net hexose synthesis. The incorporation of ^{14}C from C_3 and C_2 compounds is explained by the transaldolase exchange reactions as outlined by Ljungdahl *et al.*[4] The presence of transaldolase and the participation of reversible exchange reactions catalyzed by transaldolase have been described previously.[5,6]

The present paper extends the above findings to the pathway involved in the metabolism of labeled mannose and fructose in polymorphonuclear leukocytes of rabbit. Further evidence is presented for the presence of alternative pathways in these cells based on the labeling patterns in glucose and lactate.

* Present address: Marselisborg Hospital, Aarhus, Denmark.

** Present address: Department of Psychiatry, Stanford University, Palo Alto, California.

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EXPERIMENTAL

Leukocytes were obtained from male rabbits as described previously.[3] The cells were washed in saline and added to siliconized flasks containing a mixture of 10 ml rabbit serum and 5 ml of Hank's buffer and 0.22 mmole of unlabeled glucose. In the fructose-6-^{14}C experiment the leukocytes were incubated in 10 ml of Krebs-Ringer bicarbonate buffer containing 1 % gelatine, 0.2 % sodium acetate and 0.055 mmole of unlabeled glucose. The addition of gelatine and acetate prevented the damage to the cells which is otherwise encountered when leukocytes are incubated in a serum-free medium.[7] All incubations were at 37°C under an atmosphere of 95 % oxygen and 5 % carbon dioxide. Radioactive substrates were added to the flasks and the reactions terminated after 4 h by addition of sulfuric acid to a final concentration of 0.02 N. The cells were recovered by centrifugation and used for isolation of glycogen. Lactic acid was isolated from the supernatant solution by ether extraction. The glycogen and the lactic acid were purified and degraded as described previously.[3] All ^{14}C determinations were made on CO_2 in a gas proportional counter. D-Mannose-1-^{14}C and D-fructose-1,6-^{14}C were obtained from the National Bureau of Standards. D-Fructose-2-^{14}C and D-fructose-6-^{14}C were gifts of B. R. Landau (Western Reserve University, Cleveland, Ohio).

RESULTS AND DISCUSSION

The incorporation of ^{14}C-labeled mannose and fructose into glycogen and lactate is recorded in Table 1. Recovery of ^{14}C when mannose was the labeled

Table 1. Incorporation of ^{14}C into glycogen and lactate of leukocytes after incubation with labeled hexoses. The main compartment of each flask contained 2×10^9 white blood cells (95–97 % polymorphonuclear leukocytes) suspended in 10 ml of rabbit serum and 5 ml of Hank's buffer containing 0.22 mmole of unlabeled glucose. In the fructose-^{14}C experiment 0.4×10^9 cells were suspended in 10 ml of serum-free Krebs-Ringer bicarbonate buffer containing 1 % gelatine, 0.2 % sodium acetate and 0.055 mmole of unlabeled glucose. The radioactive substrates were added in tracer amounts (2–10 µmoles) and the mixture incubated with shaking at 37° for 4 h.

Labeled substrate		Glycogen Amount isolated	Glycogen ^{14}C incorporated	Lactate Amount isolated	Lactate ^{14}C incorporated
	µC	mmoles	%	mmoles	%
D-Mannose-1-^{14}C	30	0.05	1.00	0.31	4.70
D-Fructose-2-^{14}C	3	0.04	0.11	0.36	0.45
D-Fructose-1,6-^{14}C	15	0.10	0.04	0.22	0.38
D-Fructose-6-^{14}C	6	—	0.04	—	1.00

substrate was 1.0 % in the glycogen and 4.7 % in the lactate; both values are approximately half of these reported previously for the incorporation of labeled glucose into glycogen and lactate.[1] The incorporation of mannose into glycogen as compared to the incorporation of glucose by leukocytes is consistent with the hexokinase activities on these substrates studied with erythrocytes,[9] epididymal fat pad,[10] liver,[10] and muscle of rat.[11]

The recovery of ^{14}C from fructose in glycogen was 0.04 to 0.11 % and in lactate 0.38 to 1.0 %. It has been reported that leukocytes metabolize fructose only to a small extent in the presence of glucose.[12,13] The increased yield of

labeled lactate observed in the fructose-6-^{14}C incubation may be due to a depletion of glucose, since the initial glucose concentration was much lower in this experiment, than in the fructose-2-^{14}C and fructose-1,6-^{14}C incubations.

Table 2. Distribution of ^{14}C in the glucose units of glycogen of leukocytes after incubation with labeled hexoses. Recovery of ^{14}C in the degradation ranged from 92 to 98 %.

	Distribution of ^{14}C in the glucose units					
Labeled substrate	C-1 %	C-2 %	C-3 %	C-4 %	C-5 %	C-6 %
D-Mannose-1-^{14}C	84	1	4	1	0	10
D-Fructose-2-^{14}C	14	65	8	1	8	4*
D-Fructose-1,6-^{14}C	48	2	6	2	1	41*
D-Fructose-6-^{14}C	9	3	0	2	0	86*

* The original fructose samples were degraded chemically.[8] In the fructose-2-^{14}C and fructose-6-^{14}C samples 99 % of the total activity was situated in C-2 and C-6, respectively. The fructose-1,6-^{14}C contained 49 % of its total activity in C-1 and 51 % in C-6.

The labeling pattern in the glucose units of glycogen is presented in Table 2. When the labeled substrate was mannose-1-^{14}C, 84 % of the radioactivity was retained in the C-1 position and 10 % was randomized to C-6, which is consistent with the values previously observed when glucose-1-^{14}C or galactose-1-^{14}C served as the labeled substrate.[1] Mannose is most likely metabolized by phosphorylation to mannose-6-phosphate followed by isomerization to fructose-6-phosphate, as has been observed in mammary gland.[14]

Fructose-2-^{14}C was incorporated into the glycogen after considerable randomization. The original labeled position (C-2) retained only 65 % of the total activity of the hexose units. The incorporation of isotope from C-2 into C-1 and C-3 and also the ratio of C-1 and C-3 is consistent with the calculated values for metabolism of 8 to 10 % of the hexose *via* a complete pentose cycle.[15]

With fructose-1,6-^{14}C as the labeled substrate, a preferential labeling of C-1, 2, 3, (56 %) over C-4, 5, 6 (44 %) occurred. The unequal labeling in the glucose units of glycogen may be due to an incorporation of unlabeled glyceraldehydephosphate from endogenous sources, into C-4, 5, 6 moiety *via* an exchange reaction with fructose-6-phosphate catalyzed by transaldolase.[4]

The labeling pattern in the glucose units of glycogen observed with fructose-6-^{14}C showed that 86 % of the ^{14}C-activity was situated in C-6 position, and 9 % was randomized into C-1. Previous results have shown a much smaller incorporation of ^{14}C into the C-1, 2, 3 moiety of the glucose units when the labeled substrate was glucose-6-^{14}C, xylose-5-^{14}C or arabinose-5-^{14}C, respectively.[1,6,16] In view of the very low fructose-1,6-diphosphatase in leukocytes,[2] this finding is unexpected, and may indicate that an additional minor pathway for fructose metabolism is operating in the leukocyte. It is possible that leukocytes possess an enzyme system capable of reducing fructose to the symmetrical compound mannitol as has been described in certain micro-

organisms,[17] with subsequent oxidation and metabolism via mannose. However, thus far no enzymatic proof for this reaction in white blood cells has been obtained.

Phosphorylation of fructose to fructose-1-phosphate [18,19] as observed in liver may occur. Further metabolism catabolized by fructaldolase would yield dihydroxyacetone phosphate and glyceraldehyde. This sequence, if reversible could introduce ^{14}C from the C-6 position into C-1 of fructose-1-phosphate provided that the glyceraldehyde is phosphorylated and isomerized to dihydroxyacetone phosphate. Dephosphorylation to fructose and rephosphorylation by hexokinase could then lead to fructose-6-phosphate, which by glycogenesis is incorporated into the glucose units of the glycogen. These reactions, if they occur, would not be affected by the absence of fructose-1,6-diphosphatase and thus explain the partial randomization observed with fructose-6-^{14}C.

The labeling pattern in the lactate are presented in Table 3 and a comparison with that predicted from the distribution of the ^{14}C in the glucose

Table 3. Distribution of ^{14}C in lactate produced by leukocytes from labeled hexoses and a comparison with the ^{14}C distribution in the glucose units of the glycogen. Recovery of ^{14}C in the degradation of lactate ranged from 95 to 99 %.

	Distribution of ^{14}C in the lactate			Distribution of ^{14}C in the glucose units		
Labeled substrate	CH_3	CHOH	COOH	C-1,6	C-2,5	C-3,4
	%	%	%	%	%	%
Mannose-1-^{14}C	91	1	8	94	1	5
Fructose-2-^{14}C	6	87	7	18	73	9
Fructose-1,6-^{14}C	93	1	6	89	3	8
Fructose-6-^{14}C	98	0	2	95	3	2

units of glycogen if catabolism was *via* the glycolytic pathway. There is fair agreement in all incubations, except in the fructose-2-^{14}C experiment. The reason for this discrepancy is not apparent.

These results, together with the observations made previously, indicate that the polymorphonuclear leukocyte possesses a number of enzyme systems capable of metabolizing various carbohydrates to lactate. The most outstanding function of the neutrophils is to protect the host from invading bacteria and foreign particles by phagocytosis. Neutrophils contain granules which lyse during the phagocytic event to release digestive enzymes which cause fragmentation and destruction of the bacterium. This digestion leads to a release of less common carbohydrates which are derived from the bacterial cell walls. The versatility in carbohydrate metabolism by the neutrophil may be of importance in protecting the host from harmful compounds produced by the lytic enzymes.

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