Studies on the Existence of a Pathway in Liver and Muscle for the Conversion of Glucose into Glycogen without Glucose 6-Phosphate as an Intermediate

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Mixtures of ^{14}C -labelled glucose plus pyruvate were incubated either with rat diaphragm or slices of rat liver. Incorporation of glucose carbon into glycogen was compared with its incorporation into glucose 6-phosphate relative to the incorporation of pyruvate carbon into these metabolic products. There was no preferential incorporation of glucose carbon relative to pyruvate carbon into glycogen compared with glucose 6-phosphate in the liver slices, but there was in diaphragm. On the assumption that glucose 6-phosphate is a necessary intermediate in the conversion of pyruvate carbon into glycogen, this is evidence for the existence in muscle, but not in liver, of more than one pool of glucose 6-phosphate or of a pathway from glucose to glycogen without glucose 6-phosphate as an intermediate. Galactose carbon, relative to pyruvate carbon, was preferentially incorporated into liver glycogen, so that a substrate converted in liver into glycogen without glucose 6-phosphate as an intermediate could be detected by this approach.

Figueroa, Pfeifer & Niemeyer (1962) found that, when a liver homogenate was incubated with $[^{14}\text{C}]$ glucose, the addition of hexokinase increased $^{14}\text{CO}_2$ formation, but did not alter incorporation of ^{14}C into glycogen. The addition of unlabelled glucose 6-phosphate decreased $^{14}\text{CO}_2$ formation, but increased incorporation into glycogen. From these findings they tentatively concluded that glucose 6-phosphate is not a required intermediate in the conversion by liver of glucose into glycogen. Nigam (1967), Petrova (1956) and Threlfall (1966) have also questioned the position of glucose 6-phosphate as a required intermediate. Beloff-Chain *et al.* (1955) arrived at a similar conclusion for muscle from their studies on the effect of insulin on the metabolism of $[^{14}\text{C}]$ glucose by rat diaphragm. Landau & Sims (1967) obtained results interpreted in terms of two pools of glucose 6-phosphate in muscle, but compatible with a single pool of glucose 6-phosphate and a pathway for the conversion of glucose into glycogen without glucose 6-phosphate as an intermediate.

We have designed experiments to help to

establish whether or not a pathway for the conversion of glucose into glycogen not involving glucose 6-phosphate does exist in liver and muscle. The experiments cannot, however, differentiate such a pathway from the existence of two pools of glucose 6-phosphate. Liver slices were incubated simultaneously with $[1\text{-}^{14}\text{C}]$ galactose, $[1\text{-}^{14}\text{C}]$ pyruvate and $[6\text{-}^{14}\text{C}]$ glucose, and the glucoses from glycogen and glucose 6-phosphate were isolated and degraded to obtain the relative ^{14}C radioactivity in each of their carbon atoms. $[1\text{-}^{14}\text{C}]$ Galactose labels C-1 of the glucose unit of glycogen and glucose 6-phosphate, $[1\text{-}^{14}\text{C}]$ pyruvate labels C-3 and C-4, and $[6\text{-}^{14}\text{C}]$ glucose labels C-6 (Scheme 1). Glucose 6-phosphate is assumed to be a necessary intermediate in the conversion of pyruvate into glycogen. If it also is a necessary intermediate in the conversion of glucose into glycogen and only one pool of glucose 6-phosphate exists, the ratio of the ^{14}C radioactivity in C-6 to that in C-3 and C-4 of glucose from glycogen should be identical with that in the corresponding carbon atoms from glucose 6-phosphate, i.e.:

^{14}C in C-6 of glycogen

^{14}C in C-3,4 of glycogen

$$= \frac{^{14}\text{C in C-6 of glucose 6-phosphate}}{^{14}\text{C in C-3,4 of glucose 6-phosphate}}$$

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Scheme 1. Pathways of incorporation of C-1 of galactose (○), C-1 of pyruvate (●) and C-6 of glucose (□) into glycogen and glucose 6-phosphate.

If [6-¹⁴C]glucose can be converted into glycogen without glucose 6-phosphate as an intermediate, the ratio of the radioactivity in C-6 to that in C-3 and C-4 of glycogen should exceed the corresponding ratio in glucose 6-phosphate:

$$\frac{^{14}\text{C in C-6 of glycogen}}{^{14}\text{C in C-3,4 of glycogen}} > \frac{^{14}\text{C in C-6 of glucose 6-phosphate}}{^{14}\text{C in C-3,4 of glucose 6-phosphate}}$$

This must be so unless the equilibration of the glucose units of glycogen with glucose 6-phosphate is so rapid that, even though there is conversion of glucose into glycogen without glucose 6-phosphate as an intermediate, the distribution of ¹⁴C in glycogen becomes identical with that in glucose 6-phosphate. Since [1-¹⁴C]galactose is converted into glycogen without glucose 6-phosphate as an intermediate, unless this isotopic equilibration occurs, [1-¹⁴C]galactose should label C-1 of the glucose unit of glycogen with more ¹⁴C, relative to the radioactivity in C-3 and C-4, than should be the case for glucose 6-phosphate, i.e.:

$$\frac{^{14}\text{C in C-1 of glycogen}}{^{14}\text{C in C-3,4 of glycogen}} > \frac{^{14}\text{C in C-1 of glucose 6-phosphate}}{^{14}\text{C in C-3,4 of glucose 6-phosphate}}$$

Therefore, if the radioactivity in C-1 is greater than that in C-3 and C-4 in glucose from glycogen than in glucose from glucose 6-phosphate, isotopic equilibration cannot have occurred.

Diaphragms were incubated with [1-¹⁴C]glucose and [1-¹⁴C]pyruvate, and the distributions of ¹⁴C from these substrates in glucose from glycogen and glucose 6-phosphate were compared.

MATERIALS AND METHODS

[1-¹⁴C]Galactose, [1-¹⁴C]glucose, [6-¹⁴C]glucose and sodium [1-¹⁴C]pyruvate were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. In preliminary experiments the quantities of the substrates to be added were established so that significant quantities would be present throughout the incubation and the ¹⁴C radioactivities in C-1 or C-6 or both of the glucose from glycogen and the glucose 6-phosphate would be of the same magnitude as in C-3 and C-4.

Male Wistar rats, weighing 180–220 g., fed *ad libitum*, were killed by stunning and then exsanguination. Liver slices, to a total weight of 3–4 g., were distributed alternately in each of two flasks containing 30 ml. of a medium of composition: K⁺, 110 m-equiv./l.; Na⁺, 0.5–1.5 m-equiv./l.; Mg²⁺, 40 m-equiv./l.; Ca²⁺, 20 m-equiv./l.; Cl[−], 90 m-equiv./l.; pyruvate, 40.5–41.5 m-equiv./l.; HCO₃[−], 40 m-equiv./l.; galactose, 20 mM; glucose, 20 mM (Landau, Hastings & Nesbitt, 1955; Landau, Hastings & Zottu, 1958). In Expts. 1 and 2 of Table 1 the galactose, pyruvate and glucose were labelled in both flasks; one flask was incubated for 45 min. and the other for 90 min. In Expts. 3, 4 and 5 of Table 1 both flasks contained labelled pyruvate and in one glucose and the other galactose was labelled; the incubation period for both flasks was 90 min. About 35–70 μC of [1-¹⁴C]galactose, 60–200 μC of [1-¹⁴C]pyruvate and 7–14 μC of [1-¹⁴C]glucose or [6-¹⁴C]glucose were added to a flask.

In Expts. 1 and 2 of Table 2 hemidiaphragms from each of ten rats were added to one flask and the contralateral hemidiaphragms to a second flask (total weight in each flask about 2.5 g.). In Expt. 3 of Table 2 three 'intact' diaphragms were added to each of two flasks. Each flask in these experiments contained 25 ml. of medium of composition: K⁺, 6 m-equiv./l.; Na⁺, 145 m-equiv./l.; Mg²⁺, 2.4 m-equiv./l.; Ca²⁺, 2.6 m-equiv./l. PO₄^{3−}, 3.6 m-equiv./l.; Cl[−], 100 m-equiv./l.; HCO₃[−], 25 m-equiv./l.; SO₄^{2−}, 2.4 m-equiv./l.; [1-¹⁴C]pyruvate, 25 m-equiv./l. (75 μC); [1-¹⁴C]glucose, 15 mM (7 μC); insulin (0.1 unit/ml.) (Landau & Sims, 1967). Incubation of one flask in each experiment was for 30 min. and the other for 60 min. Although galactokinase and galactose 1-phosphate uridylyltransferase have been reported to be present in rat diaphragm (Cuatrecasas & Segal, 1965; Bertoli & Segal, 1966), incorporation of ¹⁴C from [1-¹⁴C]galactose into glycogen of diaphragm was found to be too little in preliminary experiments to make its tracing feasible.

Flasks with their contents were gassed with O₂ + CO₂ (95:5) and then stoppered and incubated at 37° with shaking. The incubations were terminated by pouring the contents of each flask through a wire-mesh basket. The basket containing the liver slices or diaphragm was momentarily placed on absorbent paper to drain off adhering medium and then plunged into isopentane cooled to −150° in liquid N₂. This procedure was accomplished in about 5 sec. The medium passing through the mesh was collected in a beaker containing 3 ml. of 30% (w/v) HClO₄.

The frozen liver slices and diaphragms were powdered with a pestle in a chilled stainless-steel mortar and then each powder was homogenized in 20 ml. of 0.6 M-HClO₄ in an ice bath and at 15000 rev./min. for 2 min. (Omnimix homogenizer; Ivan Sorvall Inc., Norwalk, Conn., U.S.A.). Glycogen was added as carrier to the homogenate, which was then neutralized with KOH, and the KClO₄ that was

precipitated was removed by centrifugation. Ethanol (1.1 vol.) was added and the glycogen that was precipitated was collected by centrifugation. It was purified and hydrolysed, and the glucose from the glycogen was then degraded to yield the radioactivities in its individual carbon atoms (Landau & Katz, 1964).

Sodium glucose 6-phosphate (50 μ moles) (Sigma Chemical Co., St Louis, Mo., U.S.A.) was added as carrier to the supernatant after the collection of the glycogen and the supernatant was taken to 80% (v/v) ethanol and pH 8.2. Barium acetate was added and the barium glucose 6-phosphate that was precipitated with cooling was collected by centrifugation and washed with cold 80% (v/v) ethanol. Ba^{2+} was removed by treating the phosphate with Dowex 50 (H^+ form). The crude glucose 6-phosphate was then loaded on a column of 14 ml. of Dowex 1 (Cl^- form) (Dow Chemical Co., Midland, Mich., U.S.A.). Elution was as described by Khym & Cohn (1953). Borate in the eluent containing the glucose 6-phosphate was removed as methyl borate by repeated evaporations with the addition of methanol (Hansen, Wood, Peeters, Jacobson & Wilken, 1962).

The glucose 6-phosphate residue was then hydrolysed by incubating it with human prostatic acid phosphatase. The reaction mixture was passed through Duolite A-4 (OH^- form) and Duolite C-3 (H^+ form) (Diamond Alkali Co., Redwood City, Calif., U.S.A.) and the effluent was evaporated and applied to Whatman 3MM paper. Chromatography was in a butanol-ethanol-water system (Putnam, 1957) and the glucose area, localized with guide spots, was eluted and the eluate passed through another column of Duolite A-4 and Duolite C-3. The eluate was evaporated, and the glucose thus obtained was in several experiments further purified by using phenol-water, butanol-acetic acid-water and butanol-pyridine-water systems (Jeanes, Wise & Dimler, 1951; Putnam, 1957). The glucose was degraded after addition of carrier glucose. Each of the carbon atoms of glucoses was isolated as CO_2 and assayed for ^{14}C radioactivity. A sample of each of the glucoses was also burned to CO_2 and this CO_2 was assayed (Landau & Katz, 1964). The percentage of the radioactivity in the glucoses recovered in the individual carbon atoms, i.e. the percentage recovery, was then calculated. In control experiments the radioactive substrates were added to homogenates of muscle and liver, and carrier glucose 6-phosphate was added. Negligible radioactivity was found in the glucose from the glucose 6-phosphate isolated from these homogenates by the above procedures.

The glucose in the HClO_4 -treated medium at the completion of the incubations with liver slices was isolated by removal of the HClO_4 as KClO_4 and then deionization of the resulting solution by passage through a column of Duolite A-4 and Duolite C-3. The effluent was concentrated, acidified and then extracted with ether to remove any residual $[1-^{14}\text{C}]$ pyruvate. The aqueous phase was passed through additional columns of the ion-exchange resins. The effluent was chromatographed with the butanol-pyridine-water system (Jeanes *et al.* 1951) and the glucose was isolated from the paper and degraded.

RESULTS

The results of the experiments with liver slices are recorded in Table 1. The radioactivities in the

carbon atoms of the glucose from glycogen and glucose 6-phosphate are relative to the radioactivity in C-4, which has been set equal to 1.0. As expected, ^{14}C from $[1-^{14}\text{C}]$ galactose was concentrated in C-1, from $[1-^{14}\text{C}]$ pyruvate in C-3 and C-4 and from $[1-^{14}\text{C}]$ glucose and $[6-^{14}\text{C}]$ glucose in C-1 and C-6 respectively (Expts. 3, 4 and 5). The radioactivity in C-4 exceeds that in C-3. Distributions were similar for 45 min. and 90 min. incubations.

The incorporation of galactose carbon and glucose carbon into glucose of glycogen compared with glucose 6-phosphate has been calculated relative to C-4, the pyruvate carbon (last two columns of Table 1). About 1.3–2.9 times as much galactose carbon was incorporated into glycogen as into glucose 6-phosphate relative to pyruvate carbon. With glucose carbon there was 0.5–1.0 times as much.

Glucoses isolated from the medium at the completion of incubations with $[6-^{14}\text{C}]$ glucose had 2–14 times as much radioactivity in C-6 relative to C-4 as the radioactivity in C-6 relative to C-4 in the corresponding glucoses from glycogens and glucose 6-phosphates.

In Table 2 the results for diaphragm are presented in the same manner as for liver slices. Radioactivity, as expected, from $[1-^{14}\text{C}]$ glucose and $[1-^{14}\text{C}]$ pyruvate was negligible in C-2, C-5 and C-6. Radioactivity in C-4 exceeded that in C-3 to a greater extent than in liver. Relative to pyruvate carbon, 1.2–2.2 times as much glucose carbon was incorporated into glucose from glycogen as from glucose 6-phosphate. In Expts. 1 and 3 there was a preferential incorporation of glucose carbon relative to pyruvate carbon into glycogen glucose at 60 min. compared with that at 30 min. In Expt. 2 relative incorporation decreased, but incorporation of glucose carbon relative to pyruvate carbon into glucose 6-phosphate was also decreased.

DISCUSSION

In liver there is no preferential incorporation of glucose carbon into glycogen compared with pyruvate carbon, relative to their incorporation into glucose 6-phosphate. In muscle there is preferential incorporation. Therefore, under the conditions of incubation with liver slices, the results indicate the absence of a pathway from glucose to glycogen not involving glucose 6-phosphate as an intermediate. In muscle the results are in accord with the existence of such a pathway.

The approach resulting in these conclusions depends on the assumption that glucose 6-phosphate is an obligatory intermediate in the incorporation of pyruvate carbon into glycogen. There are no results indicating the contrary. Since ^{14}C from

Table 1. *Distribution of ^{14}C in glucose from glycogen and glucose 6-phosphate isolated from liver slices incubated with specifically ^{14}C -labelled galactose and glucose and pyruvate and the distribution in glucose from the medium at the end of incubation*

Expt. no.	Incubation time (min.)	Substrate	Glycogen	Glucose 6-phosphate	Glucose in medium	^{14}C relative to C-4 = 1.0 in						Recovery of ^{14}C (%)	Ratio glycogen/ glucose 6-phosphate	
						C-1	C-2	C-3	C-4	C-5	C-6		Galactose	Glucose
1	45	[^{14}C]Galactose	+			2.95	0.06	0.62	1.0	0.00	1.16	97	1.90	0.99
		[^{14}C]Glucose		+		1.55	0.24	0.34	1.0	0.01	1.17	96		
	90	[^{14}C]Galactose	+		+	0.70	0.85	0.51	1.0	0.11	16.10	93	1.34	0.95
		[^{14}C]Glucose		+		2.46	0.06	0.55	1.0	0.00	1.05	96		
	45	[^{14}C]Galactose	+			1.82	0.38	0.35	1.0	0.03	1.11	96	2.87	0.94
		[^{14}C]Glucose		+		0.50	0.60	0.49	1.0	0.02	6.75	89		
	90	[^{14}C]Galactose	+		+	2.01	0.08	0.62	1.0	0.01	1.14	89	1.55	0.75
		[^{14}C]Glucose		+		0.70	0.08	0.31	1.0	0.05	1.21	40		
	90	[^{14}C]Galactose	+			0.51	0.51	0.92	1.0	0.03	9.22	89	2.49	0.83
		[^{14}C]Glucose		+		1.92	0.08	0.54	1.0	0.01	1.18	91		
	90	[^{14}C]Galactose	+		+	1.24	0.13	0.34	1.0	0.05	1.57	60	1.40	0.52
		[^{14}C]Glucose		+		0.55	0.57	0.32	1.0	0.05	4.44	70 (78*)		
	90	[^{14}C]Galactose	+		+	2.69	0.12	0.42	1.0	0.01	0.05	91	1.97	0.86
		[^{14}C]Glucose		+		1.08	0.08	0.32	1.0	0.02	0.21	78		
	90	[^{14}C]Galactose	+			2.98	0.13	0.59	1.0	0.02	0.03	91	0.83	0.52
		[^{14}C]Glucose		+		3.61	0.08	0.47	1.0	0.14	0.18	96		
	90	[^{14}C]Galactose	+		+	1.37	0.02	0.76	1.0	0.00	0.01	95	1.40	0.52
		[^{14}C]Glucose		+		0.98	0.06	0.35	1.0	0.21	0.27	50 (96*)		
	90	[^{14}C]Galactose	+			0.14	0.06	0.72	1.0	0.01	1.72	89	0.83	0.52
		[^{14}C]Glucose		+		0.17	0.32	0.78	1.0	0.05	3.30	88		
	90	[^{14}C]Galactose	+		+	0.15	0.13	0.83	1.0	0.03	7.47	73	1.97	0.86
		[^{14}C]Glucose		+		3.62	0.05	0.66	1.0	0.01	0.03	92		
	90	[^{14}C]Galactose	+		+	1.84	Lost	0.74	1.0	0.07	0.00	98	0.86	0.52
		[^{14}C]Glucose		+		0.09	0.08	0.74	1.0	0.02	1.78	98		
	90	[^{14}C]Galactose	+		+	0.02	Lost	0.60	1.0	0.05	2.06	98	0.86	0.52
		[^{14}C]Glucose		+		0.12	0.60	0.60	1.0	0.07	9.15	98		

* Values in parentheses are percentage recoveries based on ^{14}C in glucosazones (Landau, *et al.* 1955). Recovery for glucose 6-phosphate was poor in several experiments owing to a radioactive contaminant that was inert in the degradations. This is evidence by recovery based on osazones and the fact that in Expt. 1 of Table 1 and Expt. 3 of Table 2 a portion of the glucoses from the glucose 6-phosphates on degradation gave recoveries of 39–61%, but distributions similar to those in the Tables. The distributions in the Tables and the recoveries of nearly 100% were obtained on repeated degradations after further purification of the glucoses.

Table 2. *Distribution of ^{14}C in glucose from glycogen and glucose 6-phosphate isolated from diaphragms incubated with $[1-^{14}\text{C}]$ glucose and $[1-^{14}\text{C}]$ pyruvate*

In Expts. 1 and 2 incubation was with hemidiaphragms, in Expt. 3 with intact diaphragms. The results are presented as in Table 1. The ratio glycogen/glucose 6-phosphate is for glucose carbon relative to pyruvate carbon.

Expt. no.	Incubation time (min.)	Glycogen	Glucose 6-phosphate	^{14}C relative to C-4=1.0 in						Recovery of ^{14}C (%)	Ratio glycogen/glucose 6-phosphate
				C-1	C-2	C-3	C-4	C-5	C-6		
1	30	+		1.57	0.01	0.24	1.0	0.00	0.02	97	1.17
			+	1.34	0.01	0.21	1.0	0.00	0.05	94	
	60	+		1.91	0.02	0.26	1.0	0.00	0.02	98	1.66
			+	1.15	0.02	0.26	1.0	0.00	0.04	88	
2	30	+		2.62	0.02	0.55	1.0	0.00	0.02	93	2.22
			+	1.18	0.00	0.38	1.0	0.00	0.03	94	
	60	+		1.31	0.01	0.31	1.0	0.00	0.05	99	1.68
			+	0.78	0.01	0.33	1.0	0.00	0.03	98	
3	30	+		0.80	0.01	0.36	1.0	0.00	0.00	101	1.40
			+	0.57	0.03	0.33	1.0	0.09	Lost		
	60	+		0.82	0.01	0.20	1.0	0.00	0.02	103	1.71
			+	0.48	0.17	0.23	1.0	0.02	0.02	117	

$[1-^{14}\text{C}]$ pyruvate is incorporated into C-3 and C-4 of glycogen, if glucose 6-phosphate is an obligatory intermediate, the ratio of incorporation into C-3 relative to C-4 in glycogen should be the same as that into the corresponding carbon atoms of glucose 6-phosphate. In muscle this was the case. In liver, incorporation into C-3 relative to C-4 of glucose 6-phosphate tended to be lower than that into glycogen. Whether this is a technical error or reflects an occurrence in metabolism is unknown. We have used C-4 as a basis for comparison. If C-3 were used it would still be concluded that in liver there was no preferential incorporation into glycogen of glucose carbon.

The greater incorporation into C-4 than into C-3 in liver has been shown to be due to isotopic non-equilibration of dihydroxyacetone phosphate with glyceraldehyde phosphate and the participation of the transaldolase reactions (Landau *et al.* 1955; Ljungdahl, Wood, Racker & Couri, 1961; Rose, Kellermeyer, Stjernholm & Wood, 1962). Since fructose 1,6-diphosphatase activity is negligible in muscle (Shonk & Boxer, 1964), synthesis of hexose 6-phosphate from triose phosphates must be very limited and incorporation was probably primarily via transaldolase. This would explain the greater incorporation into C-4 than into C-3 with muscle than liver. Hiatt, Goldstein, Lareau & Horecker (1958), however, did not observe such a degree of asymmetry with $[2-^{14}\text{C}]$ pyruvate as substrate.

The distributions in glycogen represent the

distributions consequent to deposition of ^{14}C in the glycogen and whatever breakdown of glycogen occurred during the incubations, whereas the distributions in glucose 6-phosphate are the distributions in glucose 6-phosphate at the instants when the incubations were terminated. Since the specific radioactivity of glucose 6-phosphate from glucose, an immediate precursor, probably arrives at steady state faster than that from pyruvate, the glycogen formed from glucose 6-phosphate in the early period of incubation would be expected to show preferential labelling from glucose carbon when compared with the distribution in glucose 6-phosphate at a later time. This could be an explanation for the results with muscle, but the results in Table 2 suggest that this was probably not the case. Under the conditions of the experiments incorporation of ^{14}C into glycogen increases in a linear fashion with time (Landau & Sims, 1967). This was checked in Expts. 2 and 3 of Table 2, where incorporation at 60 min. was respectively 1.6 and 2.0 times that at 30 min. In Expt. 1, if one assumes a mean specific radioactivity of C-1 of glucose 6-phosphate of $\frac{1}{2}(1.34 + 1.15) = 1.23$, then, if during the 30–60 min. period only the glucose unit of glucose 6-phosphate was converted into glycogen and incorporation was the same as during the 0–30 min. period, the relative specific radioactivity of C-1 of the glycogen should have been $\frac{1}{2}(1.57 + 1.23) = 1.40$. The greater specific radioactivity, 1.91, is in accord with incorporation other than via

glucose 6-phosphate in this experiment. This is also the case for Expt. 3, where a specific radio-activity of $\frac{1}{2}(0.80 + 0.53) = 0.67$ rather than 0.82 would have been predicted if incorporation in the 30–60 min. period had been solely via glucose 6-phosphate. However, for Expt. 2 a value of $(1/1.6)[2.62 + 0.6(0.98)] = 2.0$ rather than 1.31 would be predicted.

In liver the similar distributions in glycogen and glucose 6-phosphate would not require that glucose 6-phosphate be an obligatory intermediate, if there were relatively rapid equilibration between glucose 6-phosphate and an intermediate in a pathway for glucose conversion into glycogen not involving glucose 6-phosphate. The preferential incorporation of galactose carbon into glycogen compared with glucose 6-phosphate is evidence that this condition did not exist. This is particularly so with regard to UDP-glucose, presumably a necessary intermediate in the conversion of galactose into glycogen. However, since glucose 1-phosphate is not a necessary intermediate, these findings do not eliminate the possibility of conversion of glucose directly into glucose 1-phosphate and then rapid equilibration of the glucose 1-phosphate with glucose 6-phosphate as a possible pathway of glucose metabolism (Smith, Taylor & Whelan, 1967).

Also, the findings for liver slices would not remove the possibility of a pathway not requiring glucose 6-phosphate as an intermediate, if the glucose in the medium had a distribution similar to that in glucose 6-phosphate. This would be possible, if pyruvate conversion into glucose in the medium and the equilibration of the glucose in the medium with glucose 6-phosphate were sufficiently rapid. This was not the case, since the glucose in the medium at the completion of incubation had a distribution different from that in glucose 6-phosphate. That is, the glucose retained much of its specific labelling, although, in accord with the extensive conversion of pyruvate into glucose by liver slices (Landau *et al.* 1955), there was incorporation into C-3 and C-4. Although Figueroa *et al.* (1962) interpreted their results, obtained with liver homogenates, to indicate the existence of a pathway from glucose to glycogen not involving glucose 6-phosphate as an intermediate, an alternative explanation was proposed by Hers & Larnier (1964). They suggested that the observations of Figueroa *et al.* (1962) may have been consequent to exchange between [^{14}C]glucose and glycogen, catalysed by amylo-1,6-glucosidase. Metzger, Piskiewicz, Mayer, Mobley & Wick (1967) have also presented results on glucose oxidation by liver homogenates suggesting a pathway for glucose metabolism not involving glucose 6-phosphate.

A second assumption in our approach is that a

single pool of glucose 6-phosphate exists in the tissues. In muscle the findings may be interpreted to indicate two pools of glucose 6-phosphate rather than a pathway from glucose to glycogen not requiring glucose 6-phosphate as an intermediate. As such, the present approach, like that of Landau & Sims (1967), does not differentiate between these two possibilities. Thus, if one pool of glucose 6-phosphate had little ^{14}C relative to a second pool (i.e. rapid turnover of a small pool) and the first pool was that from which glycogen was derived, the glucose 6-phosphate isolated, reflecting primarily the second pool, could have a distribution markedly different from that of the glucose from glycogen. It must also be assumed that the distributions in glycogen and glucose 6-phosphate reflect accurately the distributions in the muscle cell and are not distributions from different cell types (Landau & Sims, 1967).

Pyruvate carbon relative to glucose carbon seems to some extent to have been preferentially incorporated into glycogen in liver (ratios 0.52–0.86 in Expts. 3–5 of Table 1). The explanation for this is unknown, but could be interpreted in terms of two pools of glucose 6-phosphate, one of which is preferentially formed from pyruvate relative to glucose, and which is a source for the glucose units of glycogen.

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