PRIMARY INCORPORATION OF U-^{14}C-GLUCOSE INTO GLUCOSE-1-PHOSPHATE AND GLUCOSE-6-PHOSPHATE BY INCUBATED PIGEON LIVER HOMOGENATE

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That glucose-6-P is a necessary step in the transformation of glucose to glycogen has been questioned in several reports using homogenates and slices from rat liver [1–3], or pigeon liver homogenate [4]. The work of Pocchiari [5] on the incubated rat diaphram has also led others [6] to suggest that glucose-6-P might not be the only immediate product of glucose phosphorylation.

Glycogen synthesis from glucose was shown by pigeon liver homogenate [4, 7]. This provides an ideal, cell-free system for following the incorporation of ^{14}C-glucose into glucose-6-P and glucose-1-P, which is described in the present communication.

The preparation of pigeon liver homogenate and of the incubation mixture was as described by Nigam and Fridland [7]. A total of 24 flasks were incubated for 5 min at 37°, each flask containing 1 μCi of radioactivity and 100 μmoles of glucose in 10 ml. The control flasks were incubated similarly without glucose. The reaction was stopped by the addition of an equal vol of 1.2 M perchloric acid. Following the enzymatic determination of the amount of glucose, glucose-6-P and glucose-1-P in neutralized perchloric acid supernate of both the experiments and controls [8], a known amount of glucose-6-P and glucose-1-P as carrier was added to the experimental perchloric acid supernate for further purification of the sugar phosphates.

Table 1
Amount of synthesis of glucose-1-P and glucose-6-P and their specific radioactivities in pigeon liver homogenate.

Experiment number	Amount synthesized (μmoles)		Specific radioactivity (cpm/μmole)		
	glucose -1-P	glucose -6-P	glucose	glucose -1-P	glucose -6-P
1	0.14	1.25	17,560	7,600	1,960
2	0.57	0.89	20,910	7,200	5,300
3	0.72	0.84	20,690	9,000	1,550

Liver homogenate (20%) in cold 0.15 M KCl in 50 mM glycylglycine buffer, pH 7.4, was incubated with ^{14}C-glucose (100 μmoles) in 0.15 M KCl in 50 mM glycylglycine buffer, pH 7.5, in a total vol of 10 ml, at 37° for 5 min. Reaction was stopped with an equal vol of 1.2 M perchloric acid. The neutralized perchloric acid extract was used for purification and enzymatic assay of glucose, glucose-1-P and glucose-6-P.

The separation of glucose, glucose-6-P and glucose-1-P was accomplished by Dowex-1-formate column chromatography [9]. They were purified by repeated chromatography in 0.01 M and 0.001 M borate washed paper strips [8]. The purified glucose-6-P and glucose-1-P were hydrolysed in acid and the free glucose extracted from the paper after chromatography in the aqueous phenolic solvent of Putman [10]. Specific radioactivity was determined by enzymatic assay of its glucose concentrations [8] and by the liquid scintillation counting of the same solution [11]. The amounts of glucose-6-P and glucose-1-P synthesized

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in 5 min by liver homogenate and their respective specific radioactivities together with that of the precursor glucose are shown on table 1.

The total amount of glucose-6-P formed is higher than that of glucose-1-P but the greater incorporation of ^{14}C-label from ^{14}C-glucose into glucose-1-P suggests the possible existence of a direct pathway from glucose to glucose-1-P in pigeon liver homogenate.

This is in agreement with our earlier *in vivo* demonstration of glucose-1-P as the earliest detectable product of glucose phosphorylation [8] and lends support to a number of other recent communications suggesting a new route of glucose metabolism in certain tissues (see Antony et al. [2]).

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