synthesis, whereas inhibition of nucleic acid synthesis is indirect.

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Role of Glucose 6-phosphate in the Synthesis of Glycogen by the Rat Liver *in vivo*

SINCE Leloir and Cardini[1] showed that glycogen was synthesized from uridine diphosphate–glucose (UDPG) by liver extracts, it has become commonly accepted[2,3] that the normal mechanism of glycogen synthesis in liver and muscle does not involve a reversal of the phosphorylase reaction but the following sequence of reactions:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{Glucose 6-PO}_4 + \text{ADP}$$

$$\text{Glucose 6-PO}_4 \underset{\leftarrow}{\xrightarrow{\text{phosphoglucomutase}}} \text{Glucose 1-PO}_4$$

$$\text{UTP} + \text{Glucose 1-PO}_4 \xrightarrow{\text{UDPG pyrophosphorylase}} \text{UDPG} + \text{pyrophosphate}$$

$$\text{UDPG} + \text{primer} \xrightarrow[\text{synthetase}]{\text{glycogen}} \text{Glycogen} + \text{UDP}$$

Figueroa *et al.*[4] (and others referred to in ref. 4) working with rat liver preparations have suggested that the hexokinase reaction is not an obligatory step in the synthesis of glycogen from glucose. Petrova[5] working with liver slices and Beloff-Chain *et al.*[6] studying the uptake of glucose by the rat diaphragm have reached similar conclusions. Experiments *in vivo* reported here lend support to these latter suggestions.

^{14}C-glucose was injected intravenously into rats and the livers rapidly removed into liquid nitrogen 10 min later. Fractionation of a perchloric acid extract of the liver on 'Dow 1'—formate resin[7] followed by paper chromatography enabled the specific activities of liver glucose, glucose 6-phosphate, UDPG glycerophosphate and lactic acid to be determined (Table 1).

Table 1. RATIO OF SPECIFIC ACTIVITY PER CARBON ATOM IN LIVER METABOLITES TO THAT IN LIVER GLUCOSE

	UDPG	Glucose 6-PO_4	Glycerophosphate	Lactic acid
Normal fed rats (5)†	0·646 ± 0·032*	0·291 ± 0·017	0·224 ± 0·012	0·244 ± 0·032
Fasted	0·597	0·392	—	—
Anaesthetized	0·770	0·371	—	—

* Results are means ± *S.E.*
† Number of rats in parenthesis.

If glucose 6-phosphate occupies the position in the sequence glucose→glycogen indicated above, then its specific activity should at least be equal to that of UDPG at this time. A conclusion in support of the claim made by the workers already mentioned that glucose 5-phosphate is not a direct intermediate in the synthetic route glucose to glycogen could be drawn from these data.

Other possibilities for consideration are:

(1) Glucose 6-phosphate compartmentation, that is, the glucose 6-phosphate initiating glycolysis, is separated from that involved in glycogen synthesis. This is pertinent because it has recently been shown[8] that two distinct enzymes are involved in the initial phosphorylation of glucose. The specific activities of α-glycerophosphate and lactic acid are only slightly lower than that of glucose 6-phosphate and it can be calculated from the data that 14 per cent of the glucose 6-phosphate pool should have a specific activity equal to that of UDPG and the remainder equal to that of the glycolytic intermediates, α-glycerophosphate and lactic acid. If phosphorylation of unlabelled glycerol from triglyceride degradation by liver glycerokinase were to occur, then the specific activity of α-glycerophosphate would be lowered relative to glucose 6-phosphate and the size of the glucose 6-phosphate pool involved in UDPG synthesis calculated here would be even lower.

(2) Breakdown of liver glycogen, when the animals are being killed, by phosphorylase to provide an unlabelled source of glucose 6-phosphate from glucose 1-phosphate by phosphoglucomutase. This possibility has been investigated in a rat fasted for 24 h the glycogen reserves of which are much lower than those in fed rats and by taking a liver sample from a rat in which the injection of ^{14}C-glucose and subsequent operations were performed under ether anaesthesia. The ratios of the specific activities of glucose 6-phosphate to UDPG were not grossly different in these rats from those in the fed unanaesthetized rats. Neither of these experiments suggested that glycogen provides unlabelled glucose 1-phosphate.

These results obtained from *in vivo* experiments lend some support to the suggestions based on evidence obtained *in vitro* that glucose 6-phosphate is not an obligatory intermediate in the direct pathway from glucose to UDPG in rat liver, and also that glucose 1-phosphate derived by the action of phosphoglucomutase on this glucose 6-phosphate is similarly not a precursor of UDPG. Because glucose 1-phosphate is the only known precursor of UDPG, at least two pools of glucose 1-phosphate should exist, the average specific activity of which is unpredictable because the contributions from the different sources are unknown.

The lability of glucose 1-phosphate prevented its isolation by the present technique; however, the relationship of the specific activity of glucose-1-phosphate to that of glucose 6-phosphate and UDPG requires investigation in order to resolve the problem of the initial reactions of glucose which occur before metabolic conversion to glycogen and carbon dioxide.

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Use of Sudan Black as an Indicator of Vitamin D on Thin-layer Chromatograms

THE chief problems in the accurate analysis of vitamin D in natural products by physico-chemical methods are: (*a*) the elimination of large quantities of interfering sterols, vitamin A and other carotenoids; (*b*) the need to