THE INFLUENCE OF PYRIDINE NUCLEOTIDES ON GALACTOSE-1-C^{14} OXIDATION TO $C^{14}O_2$ IN VITRO[1]

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Pyridine nucleotides have been observed to influence significantly the fate of catabolized glucose in mammalian liver preparations in vitro. Wenner, Dunn and Weinhouse (1953) have found that the addition of DPN[2] to whole tissue homogenates enhances the oxidation of glucose over the Embden-Meyerhof route, while the addition of TPN stimulates glucose oxidation via the pentose phosphate pathway (Wenner and Weinhouse, 1956). Presumably the TPN stimulation occurs at the glucose-6-phosphate dehydrogenase step. In the case of galactose metabolism there is an additional enzymatic site where pyridine nucleotides might possibly exert a controlling effect, namely at the UDP-galactose-4-epimerase step. This reaction has been shown to require DPN and to be inhibited by DPNH (Maxwell, 1957). We have been investigating the factors influencing and regulating galactose metabolism in mammalian tissues. We have observed that pyridine nucleotides and conditions affecting their oxidized or reduced state have a most pronounced influence on galactose oxidation in vitro.

Galactose oxidation was studied in cell-free preparations of rat liver by measuring the formation of $C^{14}O_2$ from galactose-1-C^{14}. Details of the incubations and the preparation of the tissue fractions are recorded in Table I. The liberated $C^{14}O_2$ was trapped as hyamine carbonate in the center wells of modified Warburg flasks and counted in a liquid scintillation spec-

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[2] Abbreviations: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; UDP, uridine diphosphate; GSSG, oxidized glutathione; GSH, reduced glutathione; LDH, lactic dehydrogenase; ATP, adenosine triphosphate.

Table I

Factors Influencing Galactose-1-C^{14} Oxidation to $C^{14}O_2$ in the Supernatant Fraction of Rat Liver Homogenates

Expt.	Additions	$C^{14}O_2$ Liberated*	Change in $C^{14}O_2$ Production
		c.p.m.	per cent
1	TPN (10^{-4}M)	13650	+ 125
2	TPN (10^{-3}M)	33400	+ 550
3	TPN (10^{-3}M) + isocitrate (0.1M)	5580	- 8
4	TPN (10^{-3}M); supernatant heated 50°, 1 min.	18800	+1150
5	GSSG (3 x 10^{-3}M)	42700	+ 710
6	GSH (3 x 10^{-3}M)	5520	+ 5
7	DPN (10^{-4}M)	6950	+ 22
8	Ethanol (0.15M)	2300	- 60
9	Ethanol (0.15M) + DPN (10^{-4}M)	1040	- 80
10	Ethanol (0.15M) + pyruvate (10^{-3}M) + LDH. .	8322	+ 46
11	Progesterone (6 x 10^{-5}M)	20350	+ 150

*Mean control values of $C^{14}O_2$ liberated without the respective additions were as follows: 6060 c.p.m. (expts. 1 - 3); 1500 c.p.m. (expt. 4); 5260 c.p.m. (expts. 5 - 6); 5700 c.p.m. (expts. 7 - 10); 8140 c.p.m. (expt. 11).

A 1:1 (w/v) homogenate of rat liver was prepared in 0.15 M KCl and 0.05 M nicotinamide using a Dounce homogenizer with a clearance of 0.5 to 1 mm. The supernatant fraction was obtained by ultracentrifugation at 100,000 x g. The incubation medium consisted of 80 μmoles potassium phosphate buffer, pH 7.4, 10 μmoles ATP, 10 μmoles $MgCl_2$ and 0.3 μmoles of galactose-1-C^{14} (specific activity 0.8 μcuries per μmole). Supernatant protein added was approximately 50 mg. and the total incubation volume was 1.6 ml. Where indicated, progesterone was added in 0.02 ml. dioxane, and LDH in the amount of 500 units. The incubations were carried out for 1 hr. at 37° in rubber-capped modified Warburg flasks. The reaction was stopped by the injection of 0.25 ml. 10 N H_2SO_4 into the incubation medium after the injection of 1 ml. hyamine in the center well to trap the $C^{14}O_2$.

trometer (Passman et al, 1956). The effects of added pyridine nucleotides on galactose oxidation were readily demonstrable in whole homogenates of rat liver but were even more striking in the 100,000 x g supernatant fraction. As indicated in Table I, added TPN at a final concentration of 10^{-4} M resulted in a 125 per cent stimulation of $C^{14}O_2$ liberation. At a TPN concentration of

10^{-3} M the increase was as much as 550 per cent. In order to make the UDP-galactose-4-epimerase limiting, the supernatant was heated for 1 minute at 50° (Maxwell, 1957). Under these conditions the baseline oxidation was reduced to about one-fourth but the TPN stimulation was more pronounced, namely 1150 per cent. It will be noted in Table I that when the added TPN was converted to its reduced state by a TPNH generating system (0.1 M isocitrate plus endogenous isocitric dehydrogenase) no stimulation of the added TPN was detected. Conversely, when the native TPN in the supernatant fraction was kept in its oxidized state by the addition of 0.3 M GSSG (in the presence of endogenous glutathione reductase) a 710 per cent increase of $C^{14}O_2$ liberation resulted. No stimulation was observed when GSH was added to the system. As might be anticipated from these data, the oxidation of galactose-1-C^{14} to $C^{14}O_2$ was considerably greater (more than tenfold) in homogenates prepared in 0.05 M nicotinamide and 0.15 M KCl than in those prepared in 0.15 M KCl without nicotinamide.

The addition of DPN to the supernatant fraction produced no significant stimulation. However, when DPNH was generated by DPN, ethanol and endogenous alcohol dehydrogenase, a depression of galactose oxidation of as much as 80 per cent occurred. The addition of ethanol alone was also inhibitory. This depression was in all likelihood due to the generation of DPNH from endogenous DPN, since the effect was enhanced by the further addition of DPN and was readily reversed by a DPNH-oxidizing system (10^{-3} M pyruvate and LDH). It is possible that the inhibition of hepatic galactose utilization which occurs promptly following ethanol ingestion (Tygstrup and Winkler, 1958) is also secondary to altered intracellular DPNH levels. The content of DPNH in rat liver has been found to increase more than two fold under such conditions (Smith and Newman, 1959; Krane, S.M. and Isselbacher, K.J., 1959).

Pesch and Topper (1958,1959) have reported an increase of galactose oxidation in the presence of steroids such as progesterone using preparations of rabbit liver and intestine. We have also observed such a progesterone effect in whole homogenates and the 100,000 x g supernatant fraction of rat liver.

However, in the rat tissue preparations, a significant steroid stimulation was demonstrable only when the homogenate was prepared with nicotinomide (0.05 M). This would suggest a pyridine nucleotide requirement in order to obtain the progesterone effect. We have also found that during the incubation of the liver supernatant fractions with progesterone and galactose-1-C^{14}, reduction of the α-β unsaturation of ring A of the steroid molecule occurs - a process known to require TPNH and to generate TPN (Tomkins, 1958). The fact that Pesch, Simon and Topper (1959) have observed progesterone stimulation only in those tissues known to metabolize steroids to any significant extent (viz. liver and intestine) supports the hypothesis that the steroid effects on galactose oxidation may be due to increased TPN levels resulting from the reductive metabolism of the steroids. We have not found the progesterone stimulation in the rat liver to be catalytic nor specific for galactose, since comparable stimulation of glucose-1-C^{14} oxidation to $C^{14}O_2$ has been observed in homogenates and supernatant fractions of rat liver.

In view of the cited observations of Maxwell (1957), it seems likely that the observed DPNH inhibition of galactose-1-C^{14} oxidation occurs at the UDP-galactose-4-epimerase step. The failure of added DPN (10^{-4}M) to cause significant stimulation in these experiments may be due to the fact that the endogenous DPN concentration (4.5×10^{-5}M) in the supernatant fraction of the rat liver homogenate was already comparable to the DPN activity (5×10^{-5}M) reported by Maxwell (1957) to be optimal for purified epimerase. The TPN stimulation of galactose oxidation probably occurs, as in the glucose oxidation experiments of Wenner and Weinhouse (1956), at the level of the glucose-6-phosphate dehydrogenase reaction.

The above results demonstrating effects of pyridine nucleotides on galactose metabolism in vitro suggest that the intracellular levels of TPN and DPN, as well as their oxidized and reduced state, may also be important in the regulation of galactose metabolism in vivo.

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