LOCALIZATION OF THE STEROID HORMONE EFFECT ON GALACTOSE METABOLISM

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It has been reported (Pesch and Topper, 1958) that the in vitro addition of certain steroids stimulates the oxidation of galactose 1-C^{14} to $C^{14}O_2$ by surviving liver slices. The only other tissue of the rabbit and rat in which such a hormone effect has been observed is small intestine. Progesterone, androsterone and testosterone are the most efficacious of the steroids tested (Pesch and Topper, 1958), in both tissues.

It is possible to show the same specific steroid stimulation of galactose 1-C^{14} conversion to $C^{14}O_2$ in the soluble fraction derived from rabbit liver homogenates. A locus of the steroid effect has been delineated. It is apparent from Table 1 that whereas heating to 50° decreases the capacity of the extracts to metabolize galactose, such treatment increases the steroid effect. This observation, and the reported (Maxwell, 1957) pronounced heat-lability of UDPGal-4-epimerase suggested that the epimerization of UDPGal to UDPG is the rate-determining step in the conversion of galactose to CO_2. This is verified by the following experiments. First, addition of UDPGal-4-epimerase[4] increased the activity of the liver extracts whereas the addition of galactokinase and galactose 1-phosphate uridyl transferase did not. Furthermore,

Abbreviations: TPN, oxidized triphosphopyridine nucleotide; DPN, oxidized diphosphopyridine nucleotide; UDPGal, uridine diphosphogalactose; UDPG, uridine diphosphoglucose; and G-6-P, glucose 6-phosphate.

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[4] The UDPGal-4-epimerase was purified from calf liver and was a gift from Dr. Elizabeth Maxwell.

in an 18,000 x g. supernatant fraction of rabbit liver homogenate, progesterone enhanced $C^{14}O_2$ production from UDPGal-1-C^{14}, but a comparable stimulation was not observed with UDPG-1-C^{14} (Table 2).

On the basis of the conventional pathways of galactose metabolism in the mammalian organism (Kalckar, 1958) these results indicate that progesterone, androsterone and testosterone stimulate the conversion of UDPGal into UDPG by liver slices and extracts. Failure to demonstrate a stimulation of purified calf liver UDPGal-4-epimerase by progesterone (Pesch and Topper, 1958) suggests that the effect on the epimerase reaction observed in liver extracts is indirect. Further studies are being conducted in an attempt to elucidate more precisely the mechanism of this steroid activation.

TABLE 1

PROGESTERONE EFFECT ON SOLUBLE FRACTIONS OF RABBIT LIVER HOMOGENATES

Liver Extract	Pyridine Nucleotide Addition	Total CPM in CO_2 (counted at 50% efficiency)		Progesterone Effect (%)
		No Progesterone	Progesterone	
Unheated	DPN	2037	2610	+ 28
	TPN	6226	14150	+125
Heated	-	140	134	-
	DPN	409	755	+ 85
	TPN	1121	3321	+200

The soluble fraction of rabbit liver was prepared from a 20% homogenate, made in water, by centrifugation for 30 minutes at 100,000 x g. The heated extract was prepared by heating whole homogenate to 50° by immersion in a boiling water bath, and maintaining the 50° temperature for one minute. After chilling in an ice bath, the preparation was centrifuged for 30 minutes at 100,000 x g. The incubation mixture contained 4.8 μM potassium

phosphate buffer, pH 7.4, 150 μM $KHCO_3$, 120 μM nicotinamide, 30 μM $MgCl_2$, 1.0 μM ATP, 0.6 μM pyridine nucleotide, 0.08 μM galactose 1-C^{14} (4.7 μC/mg.), 0.3 μM progesterone (dissolved in 0.03 ml. propylene glycol) and 0.5 ml. liver extract in a total volume of 3.0 ml. Incubations were carried out in stoppered vessels for one hour at 37° with constant shaking using air as the gas phase. $C^{14}O_2$ assay was performed as described previously (Pesch and Topper, 1958).

TABLE 2

PROGESTERONE EFFECTS ON UDPGALACTOSE 1-C^{14} AND UDPGLUCOSE 1-C^{14} METABOLISM BY HEATED LIVER EXTRACT

Incubation Time (min.)	Substrate	% Conversion of Substrate to $C^{14}O_2$		Progesterone Effect (%)
		No Progesterone	Progesterone	
30	UDPGal 1-C^{14}	0.92	2.6	+180
	UDPG 1-C^{14}	6.0	7.8	+ 30
	G-6-P 1-C^{14}*	27.0	-	-
60	UDPGal 1-C^{14}	7.0	15.0	+113
	UDPG 1-C^{14}	14.0	17.0	+ 21
	G-6-P 1-C^{14}*	27.0	-	-

* This substrate was employed to demonstrate that the phosphogluconic acid oxidative pathway was not limiting in this system.

Conditions as described in Table 1 except that the homogenate was centrifuged at 18,000 x g. for 20 minutes and the incubation mixture contained 0.6 μM TPN and 0.04 μM of the 1-C^{14} substrates.

References

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