INCORPORATION OF D-GALACTOSE INTO LIVER MICROSOMAL PROTEIN IN VITRO*

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In previous reports (Sarcione 1964, Sarcione et al 1964) evidence was presented that the microsomal fraction of liver is a major site of hexose and hexosamine incorporation into glycoproteins. In these experiments the kinetics of galactose and glucosamine incorporation into the submicrosomal fractions of rat liver were interpreted to indicate that in the biosynthesis of glycoproteins, a considerable portion of the galactose and glucosamine is incorporated into completed polypeptide chains within the membranes of the endoplasmic reticulum. Palade and Siekevitz (1956) have shown that the effect of deoxycholate is to disrupt the vesicles of the endoplasmic reticulum found in the microsome fraction of liver and to solubilize their limiting membranes while leaving the ribosomes apparently intact. From these observations it appeared reasonable to search for the enzymic system which catalyzes galactose incorporation into protein in the membranous fraction of liver microsomes. This report describes a cell-free system containing the deoxycholate-soluble fraction of microsomes and ribosomes obtained

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from rat liver which catalyzes incorporation of D-galactose, from UDP-galactose, into protein.

Materials and Methods: Microsomes were obtained by homogenizing rat liver in 0.35 M sucrose buffer followed by differential centrifugation as described by Keller and Zamecnik (1956). The microsomes were further fractionated into the deoxycholate (DOC)-soluble fraction and ribosomes by the addition of deoxycholate to a final concentration of 1% followed by sedimentation of the ribosomes at 100,000 X g for 1 hour. Both the deoxycholate-soluble fraction and the ribosomes were exhaustively dialyzed to remove deoxycholate, endogenous cofactors and nucleotides, and then lyophilized to dryness. UDP-galactose-C^{14} was prepared according to the method of Osborn et al (1962) or purchased from the International Nuclear Corp. Protein fractions were obtained by the addition of two volumes of cold 1.8 M perchloric acid to the reaction mixture. The acid insoluble proteins were washed repeatedly with cold 0.6 M perchloric acid and 95% ethanol, then defatted with an ethyl ether-ethanol-chloroform mixture (2:2:1) at 50° C for 30 min.

Results: Incubation of the deoxycholate-soluble fraction of liver microsomes with UDP-galactose-C^{14} resulted in relatively little incorporation of radioactivity into perchloric acid insoluble product (Table 1).

Incorporation of radioactivity was markedly increased by the addition of ribosomes isolated from liver microsomes. Heating the DOC-soluble fraction of liver microsomes at 100° for 5 minutes then combination with unheated ribosomes abolished incorporation of radioactivity in this

Table 1

INCORPORATION OF RADIOACTIVITY FROM UDP-GALACTOSE-C^{14} INTO MICROSOMAL PROTEIN FRACTIONS OF RAT LIVER

Reaction Mixture	Specific Activity dpm/mg	Total C^{14} incorporated dpm
DOC-soluble fraction	108	477
DOC-soluble fraction and ribosomes	797	7412
DOC-soluble and ribosomes (zero time)	23	214
Ribosomes	82	318
Heated DOC-soluble fraction and unheated ribosomes	28	260
Unheated DOC-soluble fraction and heated ribosomes	520	4836

The complete system contained 7.0 mg of lyophilized DOC-soluble preparation containing 2.5 mg protein and 25 mg ribosomes in 0.05 M tris buffer pH 8.0 containing 0.08 M $MgCl_2$, 0.05 M KCl, 0.0008 M Versene, 0.001 M Cysteine, 0.006 M mercaptoethanol and 0.5 μc UDP-Galactose-UL-C^{14} (14 mc/m Mole). Final volume 1.125 ml. Reaction mixtures were incubated at 37° C for 1 hr.

system. When ribosomes were preheated and combined with unheated DOC-soluble fraction, significant incorporation of radioactivity was observed. These results suggest that the enzymic activity catalyzing incorporation of galactose radioactivity is present in the DOC-soluble fraction of liver microsomes.

Figure 1 shows that incorporation of galactose radioactivity into product, was a linear function of added ribosomal protein up to 30 mg per assay tube.

In the presence of added ribosomes incorporation of radioactivity from UDP-galactose-C^{14} into the perchloric acid-insoluble product reached a maximum at 90 minutes and was proportional to the concen-

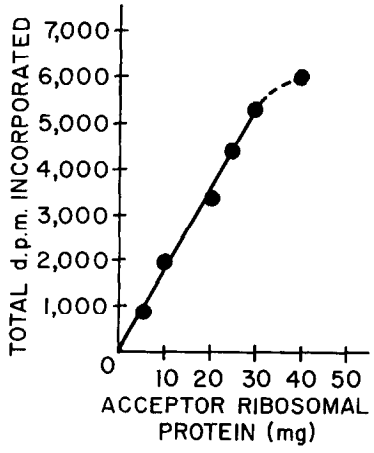

Figure 1. Relation between the rate of galactose-C^{14} incorporation and the concentration of ribosomal protein. Assay conditions as for Table 1, except ribosomes were preheated at 90° C for 5 minutes.

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tration of the DOC-soluble fraction up to 1.0 mg protein.

Evidence that UDP-galactose is the primary galactosyl donor in this reaction was obtained by the finding that when either galactose-1-phosphate-C^{14} or free galactose-C^{14} was substituted for UDP-galactose-C^{14}, no significant incorporation of radioactivity into product was observed. Since both the DOC-soluble fraction and ribosomes were exhaustively dialyzed prior to incubation to remove endogenous nucleotide sugars, these data suggest that the complete system can catalyze the incorporation of radioactivity from UDP-galactose-C^{14} into product in the absence of other nucleotide sugars.

Further characterization of the radioactive acid-insoluble product provided evidence that galactose radioactivity had been incorporated into proteins. Pronase digestion of the labeled reaction product was found to solubilize essentially all of the radioactivity. After complete acid hydrolysis of the defatted labeled product, paper chromatographic

THE EFFECTS OF PALMITYLCARNITINE ON HEPATIC FATTY ACID SYNTHESIS AND ON ACETYL CoA CARBOXYLASE ACTIVITY*

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During an investigation of the role of acylcarnitine formation in the regulation of the rate of long-chain fatty acid oxidation, we observed that (+)-palmitylcarnitine was a specific inhibitor for the carnitine palmityltransferase reaction (Fritz and Marquis, 1965), and that (+)-palmitylcarnitine competitively blocked the carnitine-induced increase of palmitate or palmityl CoA oxidation. It therefore appeared plausible that (+)-palmitylcarnitine might be employed to inhibit the carnitine-induced increase of fatty acid synthesis recently reported by Kipnis and Kalkhoff (1965). On the basis that palmityl CoA concentrations would probably be lowered by carnitine via operation of the carnitine palmityltransferase reaction, Kipnis and Kalkhoff (1965) suggested that decreased palmityl CoA concentrations would no longer inhibit fatty acid synthesis, thereby accounting for the observed stimulation of fatty acid synthesis by carnitine. Instead of using liver preparations from rats made acutely diabetic by injection of anti-insulin serum, we compared liver preparations from fed and starved animals. To our initial surprise, (+)-palmitylcarnitine did not inhibit fatty acid synthesis, but on the contrary greatly enhanced lipogenesis from labeled acetate. In contrast, carnitine addition stimulated lipogenesis only slightly. In this communication, we shall present our findings on the effects of (+)- and (-)-palmitylcarnitine on incorporation of acetate-1-C^{14} and malonyl-2-C^{14} CoA into fatty acids, and we shall report ancillary data indicating that the site of stimulation is probably at the level of acetyl CoA carboxylase (E. C. 6. 4. 1. 2).

METHODS

Fatty acid synthesis by 109,000 x g supernatant rat liver fractions from acetate-1-C^{14} was measured by procedures essentially identical to those reported by Abraham et al (1960). Acetate-1-C^{14} was purchased from Nuclear Chicago, and malonyl-2-C^{14} CoA was kindly provided by Dr. Roy Vagelos. Acetyl

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the DOC-soluble fraction markedly stimulates galactose incorporation into protein suggests that ribosomes provide additional suitable acceptor protein. It should be emphasized however, that this observation does not imply that ribosomes are the site of galactose incorporation into glycoproteins *in vivo*. Based on previous conclusions that *in vivo*, hexoses and hexosamine are incorporated into completed polypeptide chains during their migration through the membranes of the endoplasmic reticulum, it can be predicted that any proteins remaining bound to ribosomes isolated *in vitro* would be incompletely glycosylated. For these reasons, ribosomes would be expected to provide suitable protein acceptor activity for galactose incorporation in an appropriate enzymic system *in vitro*. These findings suggest that this cell-free system can serve as a useful tool for investigating the nature of the carbohydrate precursors and biosynthetic mechanisms involved in glycoprotein synthesis.

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