HETEROGENEITY IN THE COMPOSITION OF CHOLESTEROL ESTERS SYNTHESIZED BY LIVER CELL FRACTIONS OF FASTED AND FED RATS*

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Limited information is available on the enzymatic mechanisms involved in the formation of the liver cholesterol esters, and on the factor influencing the cholesterol esters synthesized. Liver microsomes have been shown to contain an enzyme which esterifies cholesterol in the presence of ATP and CoA (Goodman et al 1964, Swell et al 1964). The composition of the esters synthesized by this enzyme system closely reflects the cholesterol ester composition of the liver and consists of predominantly saturated and monounsaturated esters; a very small percentage (6-9%) of the total esters is present as arachidonate. Recent data (Swell and Law 1966) obtained in fasting rats injected with C^{14}-cholesterol suggested the presence in the liver of enzyme systems favoring the formation of cholesterol arachidonate. Liver cholesterol arachidonate was found to turnover more rapidly than the other esters, and a high percentage (30-40%) of the newly synthesized liver cholesterol esters was present as arachidonate. In the present report data are presented which indicate the presence of an enzyme system in the

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liver soluble cell fraction of the fasted rat which favors the formation of cholesterol arachidonate. It is also shown that the nature of the cholesterol esters synthesized by the liver enzyme systems reflects the nutritional status of the animal.

METHODS AND MATERIALS

Male rats (Wistar strain) weighing 125-150 gm fed on a stock pellet chow diet were used. Fed and fasting rats refers to those animals who had either access to food at all times, or were fasted 18 hours prior to sacrifice. The livers were homogenized with a phosphate-buffer medium (1 part liver to 2.5 parts medium) consisting of 0.004 M magnesium chloride, 0.03 M nicotinamide, 0.01 M glutathione, 0.12 M sucrose and 0.1 M phosphate buffer pH 7.4. The homogenate was centrifuged at 1000 X g for 10 minutes to remove nuclei and debris, and then fractionated into mitochondria (10,000 X g - 30 minutes) microsomes (104,000 X g - 60 minutes) and soluble cell fraction. The mitochondria and microsomes were washed once with buffer medium and re-centrifuged. The mitochondria and microsomal fractions were diluted with buffer medium to a volume equivalent to the homogenate from which those fractions were obtained. Incubations are described in Table I. The incubation mixtures were extracted with 2:1 chloroform-methanol. The free and esterified cholesterol fractions were isolated by silicic acid column chromatography. The C^{14}-activity of the cholesterol fractions was determined by liquid scintillation counting. The cholesterol esters were further fractionated by thin-layer chromatography on silica gel G impregnated with silver nitrate (Morris 1963). The C^{14}-activity of the cholesterol ester classes was determined by scraping the ester zones from the thin-layer plates into scintillation vials, and adding methanol to elute the esters. Further identification of the thin-layer

cholesterol ester zones was obtained with the aid of gas-liquid radiochromatography of the intact cholesterol esters (Swell 1966).

RESULTS

Esterification of cholesterol by liver cell fractions of fasting and fed rats is shown in Table I. In the fed rat the

Table I.

Cholesterol Ester Synthesis by Liver Cell Fractions

Liver Cell Fraction*	C^{14}-cholesterol Esterified CPM X 10^2	Distribution of C^{14}-activity in Cholesterol Esters, % of Total			
		Saturated	Monoun-saturated	Linoleate	Arachi-donate
Fed Rats					
Microsomes	391 ± 80	32 ± 1	33 ± 4	28 ± 3	7 ± 2
Mitochondria	120 ± 32	31 ± 2	29 ± 5	34 ± 5	6 ± 3
Soluble Fraction	100 ± 21	30 ± 4	28 ± 3	28 ± 1	14 ± 3
Fasting Rats					
Microsomes	257 ± 95	35 ± 2	22 ± 4	34 ± 5	9 ± 2
Mitochondria	93 ± 19	33 ± 2	19 ± 3	36 ± 6	12 ± 4
Soluble Fraction	231 ± 71	24 ± 3	13 ± 1	20 ± 1	43 ± 4

* Values represent the average ± standard deviation of 5 liver cell fraction preparations. Cell fractions prepared as described in text. One ml of microsomes, or mitochondria was incubated with 10 µmoles ATP + 1 µmole CoA. One ml soluble fraction was incubated with no additions. Cholesterol-4-C^{14} (0.5 µc) in 0.1 ml acetone was added to each digest. Final volume 2.5 ml with buffer-media pH 7.4. Incubations were for 1 hour at 37°C.

cholesterol esters synthesized by the liver cell fraction esterifying systems were chiefly saturated and monounsaturated. The esters synthesized by the soluble fraction system contained a significantly higher percentage of arachidonate than the esters synthesized by the mitochondrial and microsomal enzymes. Striking differences were noted in the pattern of cholesterol esters synthesized by the cell fractions of the fasted rat. The cholesterol esters synthesized by the microsomal and mitochondrial esterifying system were similar in composition and consisted principally of saturated and linoleate cholesterol esters. The soluble cell fraction esterifying system showed a marked preference for the formation of cholesterol arachidonate. This ester accounted for 43% of the total esters; linoleate plus arachidonate accounted for 63%. The C^{14}-cholesterol incorporation data indicate that in the fed rat the most active esterifying system was present in the microsomes, while in the fasted animal the microsomal and soluble esterifying systems were equally active. A large portion of the increased esterifying activity of the liver soluble fraction from the fasted rat could be accounted for as cholesterol arachidonate.

DISCUSSION

The data presented indicate that there are one or more enzyme systems associated with the liver particulate and soluble cell fractions which esterify cholesterol. Of particular significance is the finding that the pattern of the cholesterol esters synthesized by the liver enzyme systems can be altered by the nutritional status of the animal. This suggests the presence of a sensitive regulatory mechanism in the liver for determing the nature and extent of the cholesterol esters synthesized. The cholesterol esters synthesized may reflect the nature of the fatty

acid pool available for cholesterol esterification and/or the level and specificity of the enzymes associated with the formation of the cholesterol esters. Heterogeneity in the composition of the cholesterol esters synthesized by the liver would be consistent with the findings on the role of the liver in lipoprotein cholesterol ester metabolism. Perfusion studies (Roheim et al 1963) with rat liver indicate that both high and low density serum lipoprotein cholesterol esters can arise from the liver. The low density lipoproteins were shown to contain a high proportion of saturated and monounsaturated cholesterol esters, while the high density lipoproteins contain a preponderance of polyunsaturated cholesterol esters. The rate of synthesis and composition of the liver cholesterol esters would be determined by type and amount of each lipoprotein formed by the liver.

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