Effect of Retinol on Liver Lipid Metabolism of Rats

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Effect of feeding 4.23, 16.94 and 27.53 mg of retinol daily for 10 days on the liver lipids of adult rats has been studied. Feeding of different amounts of retinol produced dose dependent toxicity symptoms in rats. Retinol feeding resulted in significant elevations of liver total lipids, total fatty acids, and glycerides. The amounts of liver esterified cholesterol were significantly raised in rats fed different amounts of retinol. Acetate-1-¹⁴C incorporation was increased in liver total cholesterol of rats fed 27.53 mg retinol and in free cholesterol of all retinol fed rats. Total ¹⁴C activity of hepatic triglycerides of retinol fed rats was the same as that of control, but their specific activity was decreased. Significant alterations were noted in phosphatidyl serine, lysophosphatidyl choline, lysophosphatidyl ethanolamine, sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine and phosphatidic acid and polyglycerophosphate fractions in liver rats fed different amounts of retinol.

In a previous report the effect of intramuscular administration of 100,000 I.U. of retinol on liver lipids of rats has been described.¹⁾ The rats given retinol showed retinol toxicity symptoms, but did not lose body weights, had enlarged livers, and showed increased levels of liver lipids, hyperlipidemia and hyperphospholipidemia as compared to controls. Since differences in retinol toxicity between rats orally fed and parenterally injected with retinol have been reported, this work was undertaken to study the effects of different amounts of orally fed retinol on liver lipids of rats.

MATERIALS AND METHODS

Twenty four male rats (Wistar strain) from our Institute maintained colony, weighing 160~170 g were divided into four groups of six each. The maintenance and diet of the rats was the same as published earlier.²⁾ To three groups of rats 4.23, 16.94 and 27.53 mg respectively of retinol (Prepalin, a vitamin A alcohol concentrate, 29.41 mg/ml, Glaxo Private Ltd., India) were given orally daily for 10 days. The fourth group was given groundnut oil daily for the same period and served as the control. Daily records of their food consumption and tri-weekly records of their body weights were maintained. On the 10th day rats in all groups were injected intraperitoneally (10 μ Ci/100 g body weight) sodium acetate-1-¹⁴C (12.6 mCi/mmmole obtained from Bhabha Atomic

Research Centre, Bombay) and were killed after three hours by cervical dislocation. The animals were cut open quickly and their livers removed, immersed in a volume of chloroform:methanol (2:1, v/v) solvent mixture, and processed for lipid extraction and purification according to the methods published earlier.^{1,3)} The final solutions of lipids were made in a known volume of chloroform and stored in stoppered tubes at -15°C until used for analysis.

Analysis. Procedures for the analysis of lipids, cholesterol, phospholipid phosphorus, glyceride-glycerol, fatty acids, vitamin A and quantitative thin layer chromatography for neutral lipids and phospholipids were the same as published earlier.^{1,3)} For measurements of radioactivity in non-saponifiable, and triglyceride fractions from thin layer chromatoplates, measured aliquots of the samples were pipetted into stainless steel planchets, dried under heat lamp and counted in Tracer-lab gas flow counter. The samples had infinite thickness and counts were corrected for back-ground.

Solvents. All solvents used in this investigation were freshly distilled before use.

Statistical analysis. The results have been analyzed statistically and the significance of results between control and retinol fed rats has been determined by students 't' test.

RESULTS

Liver weight

The liver weights of rats fed different am-

TABLE I. EFFECT OF FEEDING RETINOL BY MOUTH ON BODY WEIGHTS, DAILY FOOD CONSUMPTION AND LIVER WEIGHTS OF ADULT RATS

Values are mean $\pm$ S.E. from six animals in each group. Values significantly different from control are indicated by: ^{a)} $p < 0.05$; ^{b)} $p < 0.01$; ^{c)} $p < 0.001$.

	mg of retinol fed daily for 10 days			
	None	4.23	16.94	27.53
Body weight (g)				
Start	166 $\pm$ 8.53	168.6 $\pm$ 7.35	166 $\pm$ 3.42	170.8 $\pm$ 9.91
End	172 $\pm$ 9.99	172.2 $\pm$ 5.87	161 $\pm$ 3.83	145.0 $\pm$ 4.15 ^{a)}
Daily food consumption (g/rat/day)	14.2 $\pm$ 0.69	13.3 $\pm$ 0.78	11.4 $\pm$ 1.00 ^{a)}	6.7 $\pm$ 0.68 ^{c)}
Liver weight (g)	4.27 $\pm$ 0.235	4.42 $\pm$ 0.208	4.43 $\pm$ 0.093	4.85 $\pm$ 0.250
Liver weight/100 g body weight	2.47 $\pm$ 0.112	2.54 $\pm$ 0.143	2.67 $\pm$ 0.183	3.57 $\pm$ 0.145 ^{c)}

TABLE II. EFFECT OF FEEDING RETINOL BY MOUTH ON LIVER, LIPID CHOLESTEROL, PHOSPHOLIPIDS, GLYCERIDES AND FATTY ACIDS

Values are mean $\pm$ S.E. from six animals in each group and are expressed as mg/g wet tissue. Values significantly different from control are indicated: ^{a)} $p < 0.05$; ^{b)} $p < 0.02$; ^{c)} $p < 0.01$; ^{d)} $p < 0.001$.

	mg of retinol fed daily for 10 days			
	None	4.23	16.94	27.53
			Liver	
Total lipids	54.74 $\pm$ 1.84	75.10 $\pm$ 5.89 ^{c)}	72.82 $\pm$ 3.60 ^{c)}	118.46 $\pm$ 8.57 ^{d)}
Total cholesterol	2.55 $\pm$ 0.22	2.76 $\pm$ 0.38	2.92 $\pm$ 0.05	3.87 $\pm$ 0.15 ^{b)}
Free cholesterol	2.12 $\pm$ 0.20	1.87 $\pm$ 0.06	1.93 $\pm$ 0.02 ^{d)}	1.76 $\pm$ 0.09
Esterified cholesterol	0.43 $\pm$ 0.07	0.83 $\pm$ 0.18 ^{a)}	0.97 $\pm$ 0.03 ^{d)}	0.78 $\pm$ 0.09 ^{b)}
Total phospholipid phosphorus	1.27 $\pm$ 0.05	1.42 $\pm$ 0.08	1.61 $\pm$ 0.01	1.16 $\pm$ 0.05
Total glycerides	20.53 $\pm$ 0.68	41.37 $\pm$ 4.23	40.28 $\pm$ 3.59	86.52 $\pm$ 8.64
Monoglycerides	1.10 $\pm$ 0.51	1.68 $\pm$ 0.51	2.13 $\pm$ 0.41	1.59 $\pm$ 0.96
Diglycerides	1.63 $\pm$ 0.53	5.21 $\pm$ 0.35 ^{d)}	4.34 $\pm$ 0.64 ^{c)}	3.37 $\pm$ 0.42 ^{a)}
Triglycerides	17.82 $\pm$ 0.87	34.49 $\pm$ 3.92 ^{c)}	33.78 $\pm$ 4.14	82.87 $\pm$ 10.04
Total fatty acid	31.80 $\pm$ 1.88	38.61 $\pm$ 5.43	40.40 $\pm$ 1.99 ^{d)}	71.19 $\pm$ 6.04 ^{d)}
Retinol	0.076 $\pm$ 0.021	0.258 $\pm$ 0.035 ^{d)}	0.14 $\pm$ 0.08	0.668 $\pm$ 0.119

counts of retinol were not significantly different from the control (Table I). However, on expressing liver weight per 100 g body weight a significant elevation in liver weight of rats fed 27.53 mg of retinol was noted (Table I).

Liver lipids

Feeding of different amounts of retinol to rats resulted in significant elevations in the concentration of liver total lipids as compared to the control group (Tables II). Concentration of liver total and free cholesterol (mg/g wet tissue) were not significantly affected in any of the retinol fed groups as compared to the control group (Table II). The con-

centration of esterified cholesterol was significantly increased in all the retinol fed groups as compared to control (Table II). The concentration of liver total fatty acids of rats fed 4.23 mg retinol was not significantly different from controls, but significant elevations in liver fatty acids were observed in rats fed 16.94 and 27.53 mg of retinol respectively (Table II). Feeding of different amounts of retinol to rats resulted in significant elevations in the concentration (mg/g wet tissue) of liver total glycerides of all the retinol fed rats as compared to the control (Table II). The concentration (mg/g wet tissue) of monoglycerides was not significantly affected by retinol

TABLE III. EFFECT OF ORAL FEEDING OF DIFFERENT AMOUNTS OF RETINOL ON ACETATE-1-¹⁴C INCORPORATION INTO LIVER CHOLESTEROL AND TRIGLYCERIDES OF ADULT RATS

Values are mean $\pm$ S.E. from six animals in each group. Values significantly different from control are indicated by: ^{a)} $p < 0.05$; ^{b)} $p < 0.02$; ^{c)} $p < 0.01$; ^{d)} $p < 0.001$.

	mg of retinol fed daily for 10 days			
	None	4.23	16.94	27.53
	Total cholesterol			
Counts/min/mg cholesterol	98 $\pm$ 12	117 $\pm$ 7	113 $\pm$ 31	210 $\pm$ 33 ^{b)}
	Free cholesterol			
Counts/min/mg cholesterol	19.5 $\pm$ 5	76 $\pm$ 17 ^{b)}	77 $\pm$ 29 ^{a)}	168 $\pm$ 40 ^{c)}
	Triglycerides			
Total ¹⁴ C-activity of triglycerides (counts/min/total triglycerides)	18639 $\pm$ 4452	7037 $\pm$ 1427 ^{a)}	12546 $\pm$ 1301	18615 $\pm$ 2049
¹⁴ C-activity of triglycerides/100 g body weight	10210 $\pm$ 2684	4468 $\pm$ 435 ^{a)}	8117 $\pm$ 992	13905 $\pm$ 2958
Specific activity of triglycerides (counts/min/mg triglycerides)	232 $\pm$ 61	51 $\pm$ 5 ^{b)}	90 $\pm$ 11 ^{b)}	47 $\pm$ 10 ^{b)}

TABLE IV. EFFECT OF FEEDING RETINOL BY MOUTH ON LIVER PHOSPHOLIPIDS OF RATS

Values are mean $\pm$ S.E. from six animals in each group. Values significantly different from control are indicated by: ^{a)} $p < 0.05$; ^{b)} $p < 0.02$; ^{c)} $p < 0.01$; ^{d)} $p < 0.001$.

	mg of retinol fed daily for 10 days			
	None	4.23	16.94	27.53
	μ g PLP/g wet weight			
Phosphatidyl inositol	96.64 $\pm$ 9.79	87.52 $\pm$ 10.78	76.85 $\pm$ 12.50	77.53 $\pm$ 13.93
Phosphatidyl serine	65.95 $\pm$ 3.40	82.12 $\pm$ 12.40	50.74 $\pm$ 12.10	49.56 $\pm$ 9.66
Lysophosphatidyl choline	142.89 $\pm$ 6.70	76.90 $\pm$ 14.55 ^{c)}	66.56 $\pm$ 15.31	50.46 $\pm$ 6.77 ^{d)}
Lysophosphatidyl ethanolamine	70.55 $\pm$ 7.07	86.39 $\pm$ 21.14	87.12 $\pm$ 20.03	121.01 $\pm$ 3.06 ^{d)}
Sphingomyelin	84.37 $\pm$ 4.91	60.61 $\pm$ 8.08	58.38 $\pm$ 5.29 ^{c)}	73.39 $\pm$ 12.00
Phosphatidyl choline	421.49 $\pm$ 23.63	605.54 $\pm$ 36.40 ^{a)}	494.50 $\pm$ 20.48 ^{a)}	469.23 $\pm$ 39.55
Phosphatidyl ethanolamine	286.03 $\pm$ 16.65	304.87 $\pm$ 24.60	219.82 $\pm$ 10.52 ^{c)}	180.70 $\pm$ 14.15 ^{d)}
Phosphatidic acid + polyglycerophosphatide	88.03 $\pm$ 8.04	116.72 $\pm$ 13.44	107.42 $\pm$ 8.24	124.81 $\pm$ 9.83 ^{b)}
	Phospholipid ratios			
PC/PE	1.47 $\pm$ 0.04	2.04 $\pm$ 0.18 ^{d)}	2.26 $\pm$ 0.14 ^{c)}	2.64 $\pm$ 0.28 ^{c)}
PC/LPC	2.95 $\pm$ 0.13	7.87 $\pm$ 0.81 ^{c)}	10.08 $\pm$ 3.08 ^{c)}	9.91 $\pm$ 1.46 ^{c)}
PE/LPE	4.23 $\pm$ 0.44	4.81 $\pm$ 1.14	2.52 $\pm$ 1.30	1.48 $\pm$ 0.11 ^{d)}

feeding (Table II). Significant elevations in the concentrations of diglycerides and triglycerides were noted in rats fed 4.23, 16.94 and 27.53 mg of retinol as compared to the controls (Table II).

Incorporation of acetate-1-¹⁴C into liver lipids

The incorporation of acetate-1-¹⁴C radioactivity into liver total cholesterol of retinol fed rats was not significantly different from control

rats except in rats fed 27.53 mg of retinol where it was significantly higher than the control (Table III). Incorporation of acetate-1-¹⁴C radioactivity into liver free cholesterol was significantly higher in all the retinol fed rats as compared to controls (Table III). The ¹⁴C specific activity (counts/min/mg triglycerides) of hepatic triglycerides was significantly reduced in all retinol fed rats as compared to controls (Table III). However, the total

^{14}C -activity (specific activity $\times$ mg glycerides/g liver $\times$ liver weight) of all retinol fed rats was comparable to that of control except in rats fed 4.23 mg of retinol where it was significantly lower (Table III).

Liver phospholipids

The concentration of liver total phospholipid phosphorus (mg/g wet tissue) was not significantly affected in retinol fed rats as compared to the control (Table II). Though total liver phospholipid phosphorus (mg/g wet tissue) did not show significant alterations in rats fed different amounts of retinol, significant alterations were noted in the concentration (μg phospholipid phosphorus/g wet tissue) of several phospholipid components of retinol fed rats as compared to control (Table IV). The concentrations of phosphatidyl inositol and phosphatidyl serine were not significantly affected as compared to control. The amounts of lysophosphatidylcholine were significantly decreased in rats fed different amounts of retinol as compared to control. Sphingomyelin showed significant reduction in all the retinol fed rats except in rats fed 27.53 mg of retinol where its concentration was not affected significantly as compared to controls. The amounts of lysophosphatidyl ethanolamine were unaltered in rats fed 4.23 mg and increased in rats fed 16.94 and 27.53 mg of retinol respectively as compared to control. Phosphatidyl choline was significantly increased in all the retinol fed rats except in rats fed 27.53 mg of retinol where its concentration remained unaltered as compared to control group (Table IV). Phosphatidyl ethanolamine showed a significant decrease in rats fed 16.94 and 27.53 mg of retinol respectively. The amounts of phosphatidic acid and polyglycerol phosphatide fraction were not significantly affected in rats fed different amounts of retinol, except in rats fed 27.53 mg of retinol, where its concentration was significantly increased (Table IV).

DISCUSSION

Rats fed different amount of retinol de-

veloped dose dependent toxicity symptoms.²⁾ These symptoms were much more severe than observed in rats given retinol intramuscularly¹⁾ and are similar to those reported by Moore and Wang.⁴⁾ The percentage retention of administered retinol in liver was highest (27.4%) in rats fed 4.23 mg of retinol and decreased progressively *i.e.* 22.3, 20.2 and 11.4% respectively as the amount of retinol was increased.²⁾ Similar observations have also been reported by Lemley *et al.*⁵⁾ and this perhaps may be due to the impaired absorption of retinol from the gut due to damage to epithelial lining with increasing dosing with retinol.

Studies on the incorporation of acetate-1- ^{14}C and mevalonate-2- ^{14}C into liver cholesterol of hypo- and hypervitaminotic A rats have shown that in hypovitaminotic A rats the label in cholesterol was decreased, whereas in hyper conditions it was increased, suggesting a role of vitamin A in isoprene metabolism.^{6~8)} The results on the incorporation of acetate-1- ^{14}C into hepatic cholesterol of hypervitaminotic A rats (Table III) are in confirmation with those of Rao and Olson⁸⁾ and provide further evidence on the suggested role of vitamin A in cholesterol metabolism. The increase noted in esterified cholesterol in retinol fed rats suggests also an increased rate of esterification of cholesterol possibly due to the large increase in total fatty acids available (Tables II and III). Similar increase in esterified cholesterol has also been reported in young rats given retinol intramuscularly.¹⁾

The accumulation of lipids largely triglycerides in the liver of retinol fed rats as compared to the control group points to the formation of a fatty liver (Tables II). Similar results have been observed in rats given retinol orally⁹⁾ and intramuscularly.¹⁾ A number of factors such as decreased oxidation of fatty acids, decreased turnover of phospholipids, loss of mitochondrial integrity enhanced synthesis of triglycerides and defective triglycerides secretory mechanism in liver¹⁰⁾ have been attributed as the possible cause of lipid retention in liver. The incorporation of

acetate-1-¹⁴C activity into triglycerides of whole liver shows that the total incorporation of the isotope in triglycerides in retinol fed rats is nearly comparable to that of control (Table III), except the incorporation into triglycerides of rats fed 4.23 mg of retinol where it is significantly lower. Acetate-1-¹⁴C will be incorporated into triglycerides as fatty acids. This involves firstly *de novo* synthesis of fatty acids from acetate and secondly the esterification of fatty acids to α -glycerophosphate to form triglycerides. Thus, the incorporation of acetate-1-¹⁴C into liver triglycerides in rats fed 4.23 mg retinol indicates reduced lipogenesis. However, the amount of acetate-1-¹⁴C radioactivity incorporated into liver triglycerides of rats fed 16.94 mg and 27.53 mg retinol was nearly equal to the control values (Table IV). These results necessarily do not indicate enhanced lipogenesis. On the other hand, the lipogenesis in these groups of rats may still be depressed, but the rate of esterification of fatty acids to α -glycerophosphate may be considerably enhanced as compared to rats fed 4.23 mg retinol giving rise to the amount of acetate-1-¹⁴C incorporated into triglycerides equal to control. In fact a 3–4 fold increase in the rate of esterification of palmitate-1-¹⁴C has been observed in rats fed retinol orally (H. C. Ahuja and U. K. Misra, unpublished data). The lower specific activities of hepatic triglycerides observed in retinol fed rats are due to the isotopic dilution due to the increased pool sizes. The enhanced lipolysis in adipose tissue and the flux of fatty acids from adipose tissue to liver *via* plasma in vitamin A fed rats will result in enhanced esterification in liver. The lipolysis in adipose tissue appears to be dependent on the amount of retinol fed, being low in rats fed lower amounts and increasing as the amount of retinol fed is increased. This would result in a much larger supply of fatty acids to liver for enhanced esterification in rats fed larger amounts of retinol as compared to rats fed lower amount of retinol and the control. This would overshadow the reduced lipogenesis in rats fed 16.94 mg and 27.58 mg retinol respec-

tively, which is observed in rats fed 4.23 mg retinol. Phospholipids have been reported to remain unaltered in fatty livers induced by feeding rats choline deficient diets.¹¹⁾ Decreased turnover of liver phospholipids has been attributed as one of the factors causing fatty livers.¹⁰⁾ The possible significance and the cause of the alterations of liver phospholipids components on retinol feeding is not clear. Dingle *et al.*¹²⁾ and Lucy *et al.*¹³⁾ have reported that retinol administration results in the swelling of liver mitochondria and this perhaps may result in the altered metabolism of phospholipids in retinol fed rats. The alterations noted in liver phospholipids components of retinol fed rats as compared to control (Table IV) may have resulted from either increased rate of synthesis or decreased utilization or both. The ratios of phosphatidyl choline and lysophosphatidyl choline, which increased significantly in rats fed different amounts of retinol as compared to control (Table IV) indicate perhaps a decreased breakdown and increased synthesis of phosphatidyl choline in retinol fed rats. A significant decrease in the ratio of phosphatidyl ethanolamine/lysophosphatidyl ethanolamine in rats fed 27.53 mg of retinol indicates an increased breakdown of phosphatidyl ethanolamine, perhaps by phospholipid splitting enzymes. The differences observed in some liver phospholipid constituents of rats fed orally vitamin A and those given vitamin A intramuscularly could be due to differences in the mode of administration of vitamin A, which may produce varying degree of toxicity.²⁾ A rapid *in vitro* release of hydrolytic lysosomal enzymes has been reported by vitamin A alcohol.¹⁴⁾ Recently phospholipases have been shown to be present in lysosomes¹⁵⁾ and it is likely that feeding of massive amounts of retinol releases lysosomal phospholipase A, causing an increase in lysophosphatidyl ethanolamine.

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