2. Since increases in T_3 and T_4 were not found with time, DIT might be an end product of the iodination reaction. Therefore, the increase in DIT/MIT with time could be an expression of the accumulation of the end product rather than of a decrease in iodinating enzyme activity.

The failure to demonstrate larger amounts of T_3 and T_4 in our studies suggests that most of the enzymes necessary for formation of these compounds lies within the colloid. Another reason for this failure might be that trypsinization of the thyroid cells may have damaged the necessary enzymes of the coupling process.

Summary. Normal isolated human thyroid cells were incubated in tissue culture medium containing I^{-131} for 24 to 48 hours. The nature and distribution of labeled compounds were determined. Labeled MIT, DIT, T_3 and T_4 were present following protein hydrolysis of the incubated cells. The MIT/DIT ratios ranged from 1.3 to 6.7 in the tissue cultures incubated with I^{-131} for 24 hours, while that for tissue cultures incubated for 48 hours ranged from 0.3 to 0.9. These results indicate that isolated normal human thyroid cells are able to synthesize iodotyrosines and iodothyronines in the absence of intact follicles and colloid.

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Triglyceride Accumulation and Release in the Rare-Earth Fatty Liver.* (29400)

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Our previous work(1,2) has demonstrated that the acute infiltration of hepatic cells by triglyceride after intravenous rare earths is influenced by hormones, a fact which led us to investigate lipid mobilization in animals exposed to cerium. The elevation of free fatty acids in plasma from cerium-treated rats strengthened the idea that increased mobilization of lipid from extrahepatic sites(3) and a depressed oxidation of fatty acids in the liver(4) were the likely explanations for their massive accumulation in liver. Recknagel *et al*(5) recently determined the release of triglycerides in the CCl_4-fatty liver by using Triton (p-iso-octyl polyoxyethylene phenol polymer). This detergent blocks the exit of triglyceride from plasma, and Byers and Friedman(6) estimated that after Triton is given the bulk of the triglycerides found

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TABLE I. Effect of Cerium and Ethionine on Release of Esterified Fatty Acids from Liver.

Treatment	Plasma neutral lipids, μEq esterified fatty acids per 100 ml	Liver total lipids, % of wet tissue
	Without Triton	
Control	138 (35)	5.6, 6.5
2-hr cerium	115 (3)	—
4-hr "	144 (3)	—
6-hr "	131 (4)	—
	2-hr post-Triton	
Control	1838 (3), 2120 (3)*	6.3, 6.6
6-hr cerium	2500 (3),* 2520 (3)* 2000 (3)*	6.7, 6.7
24-hr "	2125 (8)	10.8, 7.8
48-hr "	930 (3)	16.1, 13.3
72-hr "	1719 (6)	15.1, 12.9, 15.9
24-hr ethionine	576 (4)	15.1, 16.9
	6-hr post-Triton	
Control	6108 (6)	7.4, 7.7, 6.2
24-hr cerium	5256 (6)	9.4, 15.4, 12.8
48-hr "	3167 (5)	12.5, 15.4, 14.4
72-hr "	4444 (5)	9.2, 14.0, 18.1
24-hr ethionine	1547 (4)	—

Values in parentheses represent number of rats used to obtain a pooled sample for analysis. Liver total lipid values were obtained from individual rats in each group.

* These rats were not fasted; all other groups were fasted 24 hr before sacrifice.

in the plasma compartment is derived from the liver. The effect of CCl_4(5) was to inhibit this release from the liver measured by the reduction of the hyperlipemia caused by Triton. Using the same technique(5) the present report demonstrates the effect that cerium has upon the hepatic release of esterified fatty acids. Our data also show the fatty acid composition of triglycerides and total lipids of liver during various stages of their accumulation in cerium-treated rats.

Experimental. Female rats of the Carworth-Farms Nelson strain weighing 150 to 200 g were used in all experiments. The method of inducing fatty livers with cerium (2 mg/kg), the dietary regimen, and the extraction of lipids have been reported(2). Triton-induced hyperlipemia was produced according to the technique described by Byers and Friedman(6). The time sequence between cerium and Triton (WR-1339, Winthrop Laboratories) administration is described in Table I. Plasma lipids were extracted with chloroform:methanol (2:1), and the neutral and phospholipids of the plasma were separated on silicic acid(7). Ester linkages of the neutral fraction were determined with the hydroxaminolysis reaction(8). Liver and adipose tissue lipids were extracted and purified according to the procedure of Folch (9). Triglycerides were isolated by thin-layer chromatography using Silica Gel G as adsorbent and petroleum ether, diethyl ether, and acetic acid (90:10:1) as the solvent system(10).

Methyl esters of the fatty acids of total lipids and triglycerides were prepared for gas-liquid chromatography (GLC) according to the method of Nelson and Freeman(11) after validating this procedure with fatty acids and triglycerides of known purity (Hormel Foundation). The methyl esters were applied to columns of ethylene glycol succinate polyester 16% on Chromosorb W (100-140 mesh) packed in an 8-ft copper coiled tubing. The packing material was obtained from Applied Science Laboratories. The gas chromatograph used in these studies was a Beckman GC-2A.

Results and discussion. Normally at 24 hours after an intravenous cerium injection, less than 10% of the liver weight is fat(1); however, the cerium-injected rats (6-hr post-Triton) for this period had a fatty liver of essentially the same magnitude as at 2 and 3 days after a single injection of cerium (Table I). We attribute the speed-up in fatty infiltration by cerium in the presence of Triton to be due to the mass-action effect of the plasma triglycerides that build up under these conditions. The data obtained for the 2-hr post-Triton rats indicate that Triton here has little effect on the speed-up of fatty infiltration by cerium and also that cerium has no effect on the release of esterified fatty acids at 6 and 24 hours after the cerium injection. At 6 hours (Fig. 1), the appearance of the triglyceride change in the cerium liver is easily detectable by GLC of the total fatty acids of liver, whereas the triglyceride release was not altered. On the other hand, the release of the esterified liver fatty acids is decreased in the cerium-treated rats at the peak of fatty infiltration. In the ethionine fatty liver, which has approximately

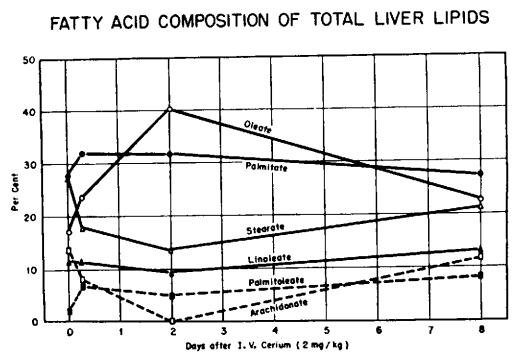

FIG. 1. Distribution of major fatty acids in liver lipid extracts obtained from rats injected with cerium (2 mg/kg), with 100% based on only the fatty acids represented on graph.

the same amount of fat as the cerium fatty liver, a more pronounced decrease in the release of fatty acid esters occurs. Our results (Table I) obtained for the glyceride release when *only cerium* is injected (in the absence of Triton) are similar to those obtained by Lombardi and Recknagel(12) but differ from their interpretation of the cerium-triton response. This is probably accounted for by the fact that their data(12) were obtained for only one time period, with animals that already had fatty livers; if a block in the release of glycerides is responsible for the fatty infiltration induced by rare earths, then it should be observed before the gross appearance of fat in the liver. The CCl_4 fatty liver exhibits a significant decrease in liver triglyceride release in both the presence and absence of Triton(5). The data in this paper support the conclusion that a block in the release of glycerides by liver is not important as a causative factor in the development of fatty liver induced by cerium as it is with CCl_4(5); it does, however, merit consideration in interpretation of the recovery phase since the high fat content of the cerium liver does to some extent impair the release of esterified fatty acids as measured in the Triton experiments.

Fig. 1 summarizes the alteration in liver total fatty acid composition that accompanies the gross increase in glycerides following intravenous injection of cerium (2 mg/kg). The most striking change is in the liver ratio of stearic to oleic acid (stearic > oleic in control rats; stearic < oleic in cerium rats, Figs. 1 and 2). The accumulated triglycerides in the cerium liver account for this change because the content of oleic acid in the triglycerides of these rats exceeds that of stearic acid (Fig. 3). Since adipose tissue is composed largely of triglycerides, the stearic/oleic ratio in total adipose lipids is essentially equal to that of triglycerides. The data show no major differences in the relative proportions of the C-16 and C-18 fatty acids of triglycerides found in normal rat liver and adipose tissue (Fig. 3). The absence of any qualitative alterations in triglyceride composition indicates that cerium does not selectively alter esterification of

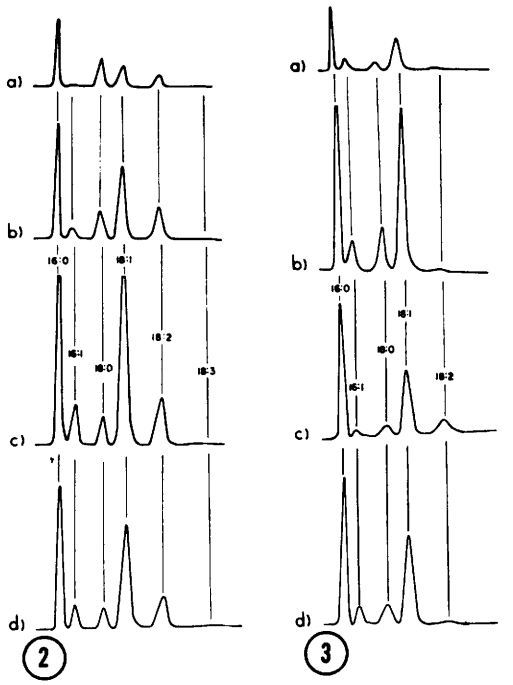

FIG. 2. Relative distribution of C-16 and C-18 fatty acids in *total lipids* from liver and adipose tissue of control and cerium-treated rats. a) control liver, b) cerium liver, c) control perirenal fat, d) cerium perirenal fat. The fatty acids are designated as: palmitic acid (16:0); palmitoleic (16:1); stearic (18:0); oleic (18:1); linoleic (18:2); linolenic (18:3).

FIG. 3. Relative distribution of C-16 and C-18 fatty acids in *triglycerides* from liver and adipose tissue of control and cerium-treated rats. a) control liver, b) cerium liver, c) control perirenal fat, d) cerium perirenal fat. The fatty acids are designated as: palmitic acid (16:0); palmitoleic (16:1); stearic (18:0); oleic (18:1); linoleic (18:2); linolenic (18:3).

fatty acids in triglyceride synthesis (excluding position). Accordingly, these results do not define the specific source of the excess triglycerides that accumulate in the cerium liver, but they do show that gas-liquid chromatography of total liver lipids can reveal this hepatic accumulation of triglyceride within a few hours after intravenous cerium (Fig. 1) and long before its gross occurrence(1,2).

Summary. It has been demonstrated that the reduction in the release of esterified fatty acids from the liver is an insignificant factor in the mechanism of the rare-earth fatty liver. Intravenous cerium in rats caused the fatty acid composition of total liver lipids to become similar to that of adipose tissue even before the detection of the massive accumulation of fat in hepatic cells. This change is explained on the basis of the fatty acid composition of the triglycerides that accumulate in the liver.

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Biosynthesis of Liver Phospholipids During the Development of a Fatty Liver.* (29401)

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The lipotropic effect of lecithins appears to act on the metabolism of fatty acids in the liver, rather than enhancing their mobilization in the form of plasma phospholipids(1). Lecithins are probably an integral part of an enzyme system necessary for oxidation of fat and fatty acids since this is the only phospholipid which is lipotropic for neutral fat or fatty acids. In the liver of the dog (2) or rat(3) maintained on a choline-deficient diet there is a progressive decrease in concentration of the lecithins and a diminished ability to synthesize these phospholipids. Diets low in protein reduce the level of total liver phospholipids(4,5) and lecithin P and cephalin P in liver mitochondria(6). In this paper are reported studies on incorporation of P_i^{32} into mitochondria phospholipids and the concentration of these lipids during the development of a fatty liver. Evidence is presented that these phospholipids are involved in this process.

Materials and methods. Male albino rats of Sprague-Dawley strain weighing 100-150 g were maintained 1, 5, 7, 9 and 21 weeks on a 5% casein-5% fat diet(7). Controls were fed a 25% casein-5% fat diet(8). At the end of the dietary regime the rats were injected with 200 μc of $NaH_2P^{32}O_4$. Six hours later the animals were killed by decapitation, livers removed, weighed and divided into 2 fractions. The acid soluble phosphorus was removed from one fraction, 0.5-1.0 g of liver, by extracting with 10% trichloroacetic acid (TCA). Radioactivity(9) and phosphorus(10) were determined on the

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