Incorporation of 1-^{14}C-Palmitate into Liver and Serum Triglycerides of Choline Deficient Rats.* (28057)

BENITO LOMBARDI AND MICHAEL C. SCHOTZ (Introduced by E. Farber)
Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, Pa., and Radioisotope Service, Veterans Administration Center and Department of Physiological Chemistry, University of California Medical Center, Los Angeles

It was recently reported that a block in the transfer of triglyceride (TG) from liver to plasma is responsible for pathological fat accumulation in the liver of rats poisoned with various toxic agents(1,2). The key experimental finding was the failure of rats with toxic fatty liver to develop a hypertriglyceridemia following intravenous administration of the non-ionic detergent Triton. However, rats with fatty livers induced by feeding a choline deficient diet did exhibit a marked post-Triton hypertriglyceridemia, implying that failure of hepatic secretion of TG could not be held accountable for hepatic fat accumulation in choline deficiency(2). Since it was felt that this point deserved further investigation, experiments were undertaken in which the incorporation of 1-^{14}C-palmitate into liver and serum TG of choline deficient rats was studied *in vivo*. By monitoring the rate of appearance of radioactivity in plasma triglyceride fraction the present experiments provide a method for determining the functional capacity of the liver to transfer TG to the plasma which does not depend on the use of Triton.

Materials and methods. Following an initial 24 hour fast, male Sprague-Dawley rats, weighing from 60 to 80 g were offered 20 g of a choline deficient diet(3) from 4:00 p.m. to 9:30 a.m. After a second 24 hour fast, 1.25 microcuries of 1-^{14}C-palmitate, as an albumin complex, were injected into the tail vein under light ether anesthesia. Two control groups of animals, fed either a choline supplemented diet or ground purina chow, were similarly treated. All animals were group pair fed and water was allowed *ad libitum* throughout the experiment. At varying intervals of time following the injection of 1-^{14}C-palmitate the rats were anesthetized with ether and blood and liver samples were collected. Total lipids were extracted immediately from aliquots of serum and liver(4) and separated by thin layer silicic acid chromatography employing a solvent system of 16% ether, 1% acetic acid and 83% hexane. The triglyceride fraction was quantitatively eluted from the plates with chloroform(5) and aliquots of the eluate were used for triglyceride analysis(6,7) and determination of ^{14}C activity in a Packard liquid scintillation counter.

Results. The concentration of liver triglycerides in rats fed purina chow or the choline supplemented diet was 7.58 $\pm$ 1.7 and 9.94 $\pm$ 3.4 mg/whole liver/100 g b.w. respectively, and rose to 34.4 $\pm$ 5.0 mg in the rats fed the choline deficient diet. No significant difference was found in the levels of serum triglycerides which were, in the 3 groups, 25.7 $\pm$ 1.1, 25.0 $\pm$ 1.5 and 28.6 $\pm$ 1.3 mg/100 ml serum respectively. Each figure is the mean of 21 rats $\pm$ standard error. No significant differences were found in the level of hepatic and serum TG between the groups sacrificed at different time intervals after 1-^{14}C-palmitate injection.

The specific activity (S.A.) of liver triglycerides was highest 10 minutes after injection of 1-^{14}C-palmitate, and decreased gradually thereafter (Table I). In the choline deficient rats, the initial S.A. of liver TG was only from 1/3 to 1/2 that of the 2 control groups, reflecting the higher initial level of liver TG in the choline deficient rats. Serum triglyceride S.A., on the other hand, was at all times after the injection of 1-^{14}C-palmitate very similar in the 3 groups of rats. The highest S.A. for plasma TG occurred at 20 minutes for all 3 groups. These results imply that movement of TG from liver to

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TABLE I.

Time (min)	Choline deficient*	Choline supplemented*	Purina chow*
Liver triglycerides			
10	3268	9154	7242
20	1595	3740	7553
30	1549	3324	3316
40	497	2710	2941
60	595	1346	2148
90	370	510	189
150	246	278	334
Serum triglycerides			
10	323	79	187
20	7150	7012	8799
30	5876	6192	5136
40	1605	3839	3414
60	1261	1999	2854
90	598	756	932
150	409	412	699

* Specific activity (c.p.m./mg/100 g b.w.) of liver and serum triglycerides at varying time intervals after injection of 1-^{14}C-palmitate to rats fed a choline deficient diet, a choline supplemented diet or ground purina chow. Each value represents the mean of results from 3 rats.

plasma occurred at substantially the same rate in all 3 groups.

Comments. In a similar experiment carried out previously in rats with fatty liver due to carbon tetrachloride poisoning(8), hepatic triglycerides became labeled very rapidly, but their S.A. decreased at a rate much slower than that of the respective controls. Concomitantly only traces of injected 1-^{14}C-palmitate appeared in the serum triglyceride fraction of the carbon tetrachloride fed rats, even after 150 minutes.

These results fully confirm the study carried out previously with Triton(2). From the results of the Triton experiments, and from the results of the present work, it appears that the accumulation of hepatic triglycerides which occurs in choline deficient rats has a different mechanism from that operating in carbon tetrachloride poisoned rats, and is not due to a block in the transfer of hepatic triglycerides to the plasma.

These data are now being analyzed according to a 3 pool model which has been designed to give an estimate of the rate of TG exchange between liver and plasma, rate of TG utilization by the liver, and rate of exit of TG from the plasma compartment(9). It is expected that such an analysis will confirm the interpretation presently given to the results, and it may in addition afford new clues concerning the mechanism underlying the fatty liver of choline deficiency.

Summary. The *in vivo* incorporation of C^{14}-palmitate into liver and plasma triglycerides of rats fed a choline deficient diet has been studied. The results show that the accumulation of triglycerides occurring in the liver of these rats, unlike that which follows administration of carbon tetrachloride, ethionine and other hepatotoxic agents, is not due to an impairment of the movement of triglycerides from the liver to the plasma. Choline-deficiency fatty liver has thus a pathogenetic mechanism different from that of fatty livers due to toxic agents.

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