number of properties examined.

1. Nelson, J. B., *J. Exp. Med.,* 1943, v78, 231.

2. Downie, A. W., *Virus and Rickettsial Diseases of Man,* 3rd edition, edited by Rivers, T. M., and Horsfall, F. L., Jr., J. B. Lippincott, Philadelphia, 1959, 673.

3. Smadel, J. E., *ibid.,* 2nd edition, edited by Rivers, T. M., J. B. Lippincott, Philadelphia, 1952, 414.

4. Rabinovitz, E., Bernkopf, H., *Bull. Research Council Israel,* 1952, v2, 207.

5. Kempe, C. H., *Diagnostic Procedures for Viral and Rickettsial Diseases,* 2nd edition, A. P. H. A., 1956, 341.

6. Helbert, D., *Variola and Alastrim,* Thesis for M. D. Degree, Univ. of Liverpool, England, 1956.

7. Cairns, H. J. F., *Nature,* 1950, v166, 910.

8. Youngner, J. S., *J. Immunol.,* 1956, v76, 288.

9. Maitland, H. B., Postlethwaite, R., *Symposium Virus Growth and Variation,* Soc. Gen. Microbiol., Cambridge Univ. Press, Cambridge, England, 1959, 185.

10. Pirsch, J. B., Mika, L. A., *Bact. Proc.,* 1960, v60, 125.

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A New Insight into Pathogenesis of Carbon Tetrachloride Fat Infiltration.* (25923)

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Recent investigations in a number of laboratories have made possible the formulation of a simple hypothesis regarding the underlying functional derangement responsible for accumulation of fat in the liver following carbon tetrachloride poisoning. This hypothesis is as follows: The liver is constantly secreting large quantities of triglycerides into the plasma. Carbon tetrachloride poisons the secretory mechanism and as a result triglycerides accumulate in the liver. Key observations which led to formulation and testing of this hypothesis were the following: It was observed(1) that following intubation of carbon tetrachloride into the stomach of rats, the hydrocarbon reached its peak concentration in the liver within 1 to 2 hours. In another study(2) it was shown that liver triglycerides were elevated 34% within one hour and 195% within 3 hours following carbon tetrachloride poisoning. Furthermore, we have shown that mitochondrial oxidation of octanoate, and mitochondrial respiratory control, are completely unaffected 2 hours after carbon tetrachloride poisoning at which time liver triglycerides are elevated 70% (Recknagel, R. O., and B. Lombardi, unpublished). These observations in conjunction with our earlier studies(3,4) of mitochondrial function following carbon tetrachloride poisoning, as well as related work of others(5) appeared to us to demonstrate convincingly that failure of fat oxidation, or uncoupling of oxidative phosphorylation, could not account for the early rise in liver triglyceride content. It was further observed(6) that 4 hours after carbon tetrachloride poisoning, hepatic glucose-6-phosphatase, a microsomal enzyme, was depressed to 59% of control levels. Subsequently, (Recknagel, R. O. and B. Lombardi, unpublished) it was shown that this enzyme was depressed 30% 2 hours after carbon tetrachloride intoxication. Neubert and Maibauer (7) had previously noted that the hepatic microsomal enzyme system involved in aminopyrine detoxication was destroyed in carbon tetrachloride poisoned rats at a time when no derangement in mitochondrial oxidative phosphorylation was evident. These observa-

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tions pointed to the membranous component of the endoplasmic reticulum as the most probable locus for a hitherto unknown mechanism intimately concerned with hepatic lipid metabolism. Consideration of the role of liver in transport of lipids in the body led to the hypothesis that an hepatic lipid secreting mechanism was probably involved. The recent reviews by Fredrickson and Gordon(8) and by Olson(9) may be consulted for the historical roots of this hypothesis. More recently, Byers and Friedman(10), by preventing exit of triglycerides from the plasma by means of Triton injection, demonstrated convincingly that the liver is capable of secreting large amounts of triglycerides into the plasma. It appeared to us to be highly likely that this hepatic triglyceride secretory mechanism was the locus of a functional derangement in the carbon tetrachloride poisoned liver. This communication presents data in support of this hypothesis.

Methods. Sixteen male rats of the Sprague-Dawley strain, from 208 to 274 g body weight, were used. The rats were without food overnight for 17 hours. The experiment which was carried out twice was designed for 8 rats divided into 4 equal groups. Four rats were force-fed mineral oil (0.25 ml/100 g body weight), and 4 rats were force-fed a 1:1 carbon tetrachloride-mineral oil mixture (0.5 ml of the mixture/100 g body weight). Force-feeding was done through a stomach tube under light ether anesthesia. After 2 hours, 2 of the mineral oil fed rats were injected with 0.9% NaCl and 2 were injected with Triton (Winthrop Labs, WR-1339). Similarly, carbon tetrachloride fed rats were injected with either 0.9% NaCl or Triton. The dose of Triton was 100 mg/rat, dissolved in 0.5 ml of 0.9% NaCl, injected into a saphenous vein under light ether anesthesia. Ninety minutes after Triton (or 0.9% NaCl) injection, 5 ml of blood were withdrawn from the abdominal aorta. The liver was removed and weighed, and a liver sample taken for triglyceride analysis. Blood and liver samples were taken under sodium pentobarbital anesthesia (5 mg/100 g body weight, intraperitoneally). Plasma triglycerides were estimated according to Van Handel and Zilversmit(11). Total liver lipid was extracted according to Folch *et al.*(12), redissolved in chloroform and passed over a silicic acid column. The chloroform eluate of the silicic acid column was analyzed for triglycerides according to Van Handel and Zilversmit(11).

TABLE I. Effect of Carbon Tetrachloride Poisoning on Plasma Triglyceride Content in Triton Treated and Non-Triton Treated Rats.

Treatment of animals	No. of rats	Plasma triglycerides, mg/100 ml plasma	Liver triglycerides, mg†
Control	4	24.4 ± 2.65	20.5 ± 2.93
CCl_4 fed	4	6.4 ± .86	60.9 ± 8.84
Triton (no CCl_4)	4	286 ±21.6	19.3 ± 3.68
CCl_4 fed + Triton	3*	69 ±10.3	63.0 ± 4.33

* One rat lost under nembutal anesthesia.

† All data given as mean ± stand. error. Liver triglycerides given as mg/whole liver/100 g body wt.

Results. Ninety minutes after Triton administration to normal rats, plasma triglyceride content was elevated 12-fold (Table I). If the rats had been poisoned with carbon-tetrachloride 2 hours previously, this increase in plasma triglycerides was markedly depressed. Plasma triglyceride concentrations in carbon tetrachloride poisoned rats not given Triton were also markedly lower than corresponding controls. Data of Table I also indicate that 3.5 hours post carbon tetrachloride feeding, liver triglyceride content has been elevated 3-fold. Triton administration did not alter hepatic triglyceride concentration in normal rats, nor did it affect hepatic triglyceride accumulation due to carbon tetrachloride. This suggests that insofar as lipid transport mechanisms are concerned, Triton selectively inhibits exit of triglycerides from plasma whereas carbon tetrachloride selectively inhibits exit of triglycerides from liver. Plasma triglyceride values formed 4 distinct groups with no overlapping values. Liver triglyceride values formed 2 distinct groups (CCl_4-fed *vs.* non-CCl_4-fed) with no overlapping values.

Discussion. The data presented suggest that with respect to the hepatic triglyceride secretory mechanism, carbon tetrachloride has, in effect, produced a functional hepatectomy. This is evident in the non-Tritonized

animals, but the effect is markedly exaggerated following Triton administration. Failure of the triglyceride secretory mechanism appears to be almost complete 2 hours after intoxication, which corresponds in time with attainment of the peak concentration of carbon tetrachloride in the liver(1). Failure of the hepatic secretory mechanism results in a dramatic fall in plasma triglycerides and a corresponding retention of triglycerides within the liver. The small rise of plasma triglycerides in the carbon tetrachloride poisoned rats injected with Triton could be due in part to a contribution from the gut(10).

The hypothesis in its present form has been restricted to the pathology of carbon tetrachloride poisoning. It has been tested only in regard to the action of this hepatotoxin. However, it appears self-evident to us that the hepatic triglyceride secretory mechanism must be complex and that it could fail as a result of a variety of pathological disturbances of the liver cells. It will therefore be most interesting to determine whether ethionine administration, choline deficiency, phosphorus poisoning, etc. likewise produce hepatic fat accumulation by destruction of or interference with the normal function of the hepatic triglyceride secretory mechanism. The elegant demonstration of this hepatic function by Byers and Friedman(10) has provided a simple method for determining the extent to which the hypothesis here presented is in fact a general one and not peculiar to carbon tetrachloride poisoning alone.

Summary. Intravenous administration of Triton to rats causes a marked elevation of plasma triglycerides. If the rats have been poisoned with carbon tetrachloride 2 hours previously, accumulation of triglycerides in the plasma following Triton administration is markedly reduced. The existence of an hepatic triglyceride secretory mechanism is inferred from the work of others, and it is postulated that carbon tetrachloride poisons this triglyceride secretory mechanism. Triglycerides, which would normally be secreted into plasma, are retained in the liver.

1. Recknagel, R. O., Litteria, M., *Am. J. Pathol.*, 1960, v36, 521.
2. Schotz, M., Recknagel, R. O., *Biochim. et Biophys. Acta*, 1960, v41, 151.
3. Recknagel, R. O., Anthony, D. D., *J. Biol. Chem.*, 1959, v234, 1052.
4. Share, L., Recknagel, R. O., *Am. J. Physiol.*, 1959, v197, 121.
5. Calvert, D. N., Brody, T. M., *J. Pharm. and Exp. Therap.*, 1958, v124, 273.
6. Recknagel, R. O., *Fed. Proc.*, 1960, v19, 137.
7. Neubert, D., Maibauer, D., *Arch. exp. Path. u Pharmakol.*, 1959, v235, 291.
8. Fredrickson, D. S., Gordon, R. S., Jr., *Physiol. Rev.*, 1958, v38, 585.
9. Olsen, R. E., *Perspect. Biol. and Med.*, 1958, v2, 84.
10. Byers, S. O., Friedman, M., *Am. J. Physiol.*, 1960, v198, 629.
11. Van Handel, E., Zilversmit, D. B., *J. Lab. and Clin. Med.*, 1957, v50, 152.
12. Folch, J., Lees, M., Sloane-Stanley, G. H., *Fed. Proc.*, 1954, v13, 209.

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Adrenal Ascorbic Acid and Corticosteroidogenesis Following Unilateral Adrenalectomy in the Rat.* (25924)

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In spite of widespread use of adrenal ascorbic acid concentration as parameter of adrenal cortical activity, little is known regarding its true relationship to corticosteroidogenesis. Since a sensitive method for deter-

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