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Received Dec. 20, 1967. P.S.E.B.M., 1968, Vol. 128.

Impaired Secretion of Triglycerides by the Liver; A Cause of Tetracycline-Induced Fatty Liver* (32964)

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Tetracycline in large doses induces a fatty liver in experimental animals and has been incriminated as a cause of fatty liver and hepatic dysfunction in man (1–4). Most of the experimentally induced rise in hepatic lipids has been accounted for by an increase in liver triglycerides (2), but the mechanism by which tetracycline induces a fatty liver is unknown.

Inasmuch as tetracyclines inhibit protein synthesis *in vitro* (5) and *in vivo* (6), and triglycerides are normally bound to hepatic lipid acceptor protein and are transported from liver to plasma as lipoproteins (7), it seemed possible that tetracycline, in high concentrations, might inhibit hepatic lipid acceptor protein synthesis and thus impair the normal removal of triglycerides from the liver. Such a mechanism has been implicated in other types of fatty liver (8,9). To test this hypothesis, tetracycline-injected and control fasted rats were given Triton, a nonionic detergent, which blocks the exit of triglycerides from the plasma compartment so that triglycerides secreted by the liver accumulate in the plasma (8,10). A decrease of post-Triton hypertriglyceridemia in fasted tetracycline–injected rats, when accompanied by a fatty liver, was taken as evidence of an impaired secretion of triglycerides into plasma.

Material and Methods. Female Sprague-Dawley rats, weighing 200–250 gm and fasted for 24 or 48 hours were used. Tetracycline hydrochloride (Achromycin), supplied in vials containing 500 mg of tetracycline and buffered with 1250 mg of ascorbic acid, was freshly dissolved in isotonic saline to a concentration of 10 mg of tetracycline/ml. This solution, at pH 2.45, was infused via a femoral vein in a dose of 100 mg of tetracycline/kg of body weight, at the rate of 50 mg/kg per min, into rats briefly maintained under ethyl ether. This dose of tetracycline has been shown previously (1,2) and in preliminary experiments in this study, to induce a fatty liver in rats. Control animals received a comparable weight-adjusted volume of isotonic saline without ascorbate (pH 6.10), or in five instances, containing ascorbate in an amount equivalent to that present in the tetracycline solution, and adjusted to pH 2.45 with dilute HCl. At various times thereafter (see Table I), both tetracycline-injected and control groups were infused with Triton, prepared as described previously (8), at a dose of 50 mg/100 gm of body weight. Plasma was obtained for triglyceride assay at 0.75–3 hours after Triton. Liver triglycerides were meas-

* This investigation was supported in part by Research Grants NB-05841 and HE 03439 from the United States Public Health Service.

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TABLE I. Plasma Triglyceride Concentration in Triton-Treated Control and Tetracycline-Injected Rats.

Duration (hours)[a]		Increase in plasma triglycerides after Triton					
		(mg/100 ml)		(mg/100 ml of plasma/hour)[d]		Decrease from control (%)	p value
Tetracycline	Triton	Saline control	Tetracycline	Saline control	Tetracycline		
1.25	0.75	224.6 ± 33.4[b](5)	129.2 ± 14.6 (5)	300.8 ± 43.9	172.3 ± 19.5	42.5	<0.03
2	1.5	726.8 ± 29.5 (5)	385.2 ± 28.4 (5)	484.5 ± 19.7	256.8 ± 19.0	47.0	<0.001
3.5[e]	3	1208.8 ± 38.8 (5)	893.8 ± 98.0 (5)	400.0 ± 15.1	297.9 ± 32.7	26.1	<0.02
7[f]	3	1151.7 ± 73.6 (5)	515.8 ± 26.3 (6)	383.9 ± 24.5	171.9 ± 8.8	55.2	<0.001
7[e]	3	959.4 ± 44.6(14)	668.6 ± 33.6(10)	319.8 ± 14.9	234.6 ± 15.3	30.3	<0.001
21[e]	3	1032.3 ±132.7 (7)	972.5 ± 21.6 (6)	345.8 ± 45.5	330.8 ± 9.0	5.8	$=0.5$

[a] Duration of tetracycline and Triton refers to the time between injection of tetracycline or Triton and sacrifice of the animals. Subtraction of the Triton from tetracycline time gives the duration of exposure to tetracycline alone prior to Triton infusion.

[b] Mean ± SE of number of animals shown in parentheses. The plasma triglycerides in two groups of 10 rats each injected with tetracycline or saline 4 hours previously, but not given Triton, were 31.2 ± 4.1 (SE) and 23.1 ± 0.9 mg/100 ml ($p = 0.08$). To accurately compare hepatic triglyceride secretion in the tetracycline and control animals, these baseline mean plasma triglyceride values were subtracted from the post-Triton data and the final results are given above.

[c] One injection of tetracycline/day for 3 days. All other groups refer to a single injection of tetracycline.

[d] These data are expressed as a rate and are derived by dividing the absolute increase in plasma triglyceride after Triton by the duration of the post-Triton interval. This is considered justified in view of the findings of Otway and Robinson (10) that plasma triglycerides increase linearly for at least 3 hours after Triton administration.

[e,f] Data from these experiments used for calculation given in "Appendix" (Tables II and III).

ured in five rats each given tetracycline or saline 7 hours previously but no Triton. It has been documented previously that Triton does not alter hepatic triglyceride content (8). In other groups of rats given only tetracycline, serial plasma and liver tetracycline concentrations were measured. Triglycerides were determined in triplicate by the technique of Van Handel and Zilversmit (11). Tetracycline concentrations were measured in duplicate by the fluorometric method of Ibsen (12).

Results Plasma tetracycline concentrations at 0.5, 2, 4, 6, 8, and 14 hours after infusion of tetracycline were respectively 99.4 ± 1.7 (SE), 50.3 ± 4.0, 32.4 ± 2.9, 18.9 ± 2.0, 12.9 ± 3.5, and 2.2 ± 0.2 μg/ml; while tetracycline levels in liver were respectively 354.2 ± 31.7, 459.8 ± 26.2, 362.2 ± 38.2, 272.2 ± 44.7, 130.2 ± 55.8, and 8.3 ± 0.1 μg/gm of wet weight.[3] As shown in Table I during the 7 hours after tetracycline administration, when hepatic tetracycline concentrations were high, the increments in plasma triglycerides after Triton were less by 42.5 ($p<0.03$), 47.0 ($p<0.001$), 26.1 ($p<0.02$), and 55.2% ($p<0.001$), respectively at 1.25, 2, 3.5, and 7 hours than in the saline-injected controls. At 21 hours after tetracycline, when hepatic tetracycline concentration was low, the increments in plasma triglycerides after Triton were comparable in the tetracycline-injected and control animals ($p=0.5$) (Table I). The pH-adjusted ascorbic–saline solution alone did not affect the rise in plasma triglycerides at 7 hours. Hepatic triglycerides, 26.6 ± 8.5 mg/gm of wet weight 7 hours after tetracycline injection, were significantly higher than the control values of 14.0 ± 3.6 mg/gm ($p<0.03$). This increment in hepatic triglyceride after administration of tetracycline closely parallels the degree of fatty liver found by us (2) and others (1) previously in a larger number of animals when this dose of antibiotic was administered for a longer period of time.

[3] Cited with the permission of The American Journal of Digestive Diseases, 12:429, 1967.

TABLE II. TC-Induced Decrease in Hepatic TG Secretion.

(hours)	Decrease in hourly rate of TG secretion after TC: Control rate (mg/100 ml per hour)	× Fractional decrease	× Time (hours)	× Plasma volume (14)/100 gm of rat as fraction of 100 ml	= Decrease in TG delivery into plasma (mg/100 gm of body wt.)
0–3.5[a]	400	0.261	3.5	0.04	14.6
3.5–7[b]	384	0.552	3.5	0.04	29.7
0–7 Total					44.3 mg

[a] Data of footnote *e*, Table I are assumed to represent rate of TG secretion for entire 0–3.5-hour period.

[b] Data of footnote *f*, Table I are assumed to represent rate of TG secretion for entire 3.5–7-hour period.

TABLE III. TC-Induced Accumulation of TG in Liver in 7 Hours.

	TG concentration (mg/gm of wet wt.)	× Liver wt. (gm/100 gm of body wt.) (2)	= Hepatic TG (mg/100 gm of body wt.)
Control	14.0	3.54	49.6
TC	26.6	3.54	94.2
Increment in hepatic TG after TC			44.6 mg

Discussion. These data clearly indicate that tetracycline significantly depresses the rise in triglyceride concentration in plasma observed after administration of Triton. Inasmuch as Triton is believed to interfere primarily with the egress of triglycerides from the plasma compartment (10), and since the extent of rise of plasma triglycerides in fasted animals for at least 3 hours after Triton administration is believed to reflect the rate of hepatic triglyceride secretion (10), the present observations indicate that tetracycline interferes with this secretory mechanism. This conclusion is based on the assumption that tetracycline does not render Triton a less effective inhibitor of peripheral utilization of plasma triglycerides. Furthermore, impairment of hepatic secretion of triglycerides appears to be the major factor in tetracycline-induced fatty liver since most of the triglyceride accumulated in the liver can be accounted for by the decrease in hepatic triglyceride output (see "Appendix"). The role of other factors, such as augmented influx of free fatty acids and enhanced synthesis of triglycerides in the liver was not examined, however, and cannot be excluded.

The manner in which tetracycline interferes with hepatic triglyceride output into plasma was not specifically investigated. However, there is good evidence that the antibiotic inhibits protein synthesis (13). Since triglycerides mobilized from liver are attached to hepatic acceptor protein, one can speculate that tetracycline also impairs the hepatic synthesis of this protein. The mechanism of this inhibitory effect of tetracycline on protein formation is felt to involve the prevention of attachment of amino acids in the form of amino acyl–sRNA to ribosomes already containing messenger RNA, probably by competing with amino acyl–sRNA for available binding sites (13). The observations that triglyceride output from liver was depressed very rapidly, at a time when hepatic tetracycline concentration was very high, and that this effect persisted throughout the period of high hepatic tetracycline concentration but disappeared when liver tetracycline values became very low, all support this possibility. While this interpretation appears to be a good working hypothesis, it needs to be experimentally confirmed. Other possible mechanism which need to be examined include an effect

of tetracycline on the assembly of hepatic lipoproteins and their release into the space of Disse.

Summary. The increments in plasma triglyceride levels observed after administration of Triton were consistently and significantly lower in tetracycline-injected rats, when hepatic tetracycline concentration was high, than in Triton–injected controls. These data are interpreted as indicating impaired release of hepatic triglyceride in tetracycline-injected animals. Hepatic triglyceride increased in tetracycline-injected rats and most of this could be accounted for by impaired release of liver triglyceride. This mechanism, possibly due to impaired synthesis of hepatic lipid acceptor protein, appears to be the major cause of tetracycline-induced fatty liver.

Appendix. The following calculation provides a rough measure of the decrease in triglyceride (TG) delivery into plasma during the first 7 hours after tetracycline (TC) injection and compares this with hepatic TG increase. The TC-induced decrease in hepatic TG secretion was somewhat variable in different experiments (Table I) and hepatic TG and rates of TG secretion were not measured in the same animals, hence the calculations in Tables II and III are an approximation.

We are indebted to Mrs. Mary Naylor for technical assistance.

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Received Dec. 21, 1967. P.S.E.B.M., 1968, Vol. 128.

In Vitro Release of Histamine from Human Peripheral Leukocytes with Compound 48/80* (32965)

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Many substances have been studied for their effect on the release of histamine from tissues. Some of the more effective histamine releasers include D-tubocurarine (1), opium alkaloids (2), primary amines (3), polyvinylpyrollidone (4), and dextran (5). One of the most extensively studied releasers of histamine is compound 48/80, a polymeric condensation product of *p*-methoxyphenethylmethylamine and formaldehyde.

While the study of compound 48/80 has included its histamine releasing effect on many different tissues from a variety of animal species, very little work has been done on the effect of compound 48/80 in man. Paton (6) demonstrated whealing and flaring on intradermal injection of compound 48/80 in man. Others (7) have confirmed and extended this *in vivo* observation.

* Aided by USPHS Research Support Grant No. 5-SO1-FR 05388.