Hyperlipidemia, fatty liver, and bromsulfophthalein retention in rabbits injected intravenously with bacterial endotoxins

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SUMMARY Endotoxin derived from *Serratia marcescens* (Shear's polysaccharide) or *Escherichia coli* was injected intravenously into rabbits. A transitory rise in plasma free fatty acids (FFA) occurred from 1 to 3 hr after the injection; the bromsulfophthalein excretion (BSP) test was abnormal from 2 to 24 hr after the injection. In 24 hr there was a 4- to 9-fold increase in the average plasma triglyceride concentration, with a 2- to 3-fold rise in the average serum cholesterol and phospholipid levels. The plasma FFA concentration rose again after a second injection; serum triglycerides remained elevated or increased still further. Fatty livers were found in 25% of animals given two doses of Shear's polysaccharide and in all animals given two doses of *E. coli* endotoxin. No direct correlation could be established between the serum lipid levels and the extent and distribution of intravascular thrombosis and necrosis in the lungs, liver, and spleen, or the production of the generalized Shwartzman phenomenon in the kidney. However, there appeared to be a direct correlation between the elevated serum triglyceride levels and the mortality rates of the animals.

ENDOTOXINS are complex lipopolysaccharides synthesized by several species of gram-negative bacteria. These compounds produce leucocytosis, hyperpyrexia, alterations in local blood flow, shock, and often death when injected intravenously into most mammalian species (1). Intravascular thrombi, focal necrosis, and interstitial hemorrhages are found in 40–60% of the livers, lungs, and spleens of rabbits after one or more injections. Characteristically, intraglomerular thrombi and renal cortical necrosis occur in the rabbit kidney only after two intravenous doses, spaced 6–36 hr apart (2). Renal lesions produced under these conditions are analogous to the localized skin reactions described by Shwartzman after two spaced intradermal injections of endotoxin (3); hence the renal lesions are referred to as the generalized Shwartzman reaction.

The male and nonpregnant female rat do not develop the generalized Shwartzman reaction, but the pregnant female rat develops the characteristic lesions after only a single dose of endotoxin (4). Further, this lesion can be produced in the pregnant rat without injecting endotoxin, by feeding them a diet that is low in tocopherol, but contains 5% oxidized cod liver oil (5). McKay and Kaunitz have shown that pregnancy in the rat induces elevations of the plasma FFA, triglyceride, cholesterol, and phospholipid concentrations, and the total lipid content of the liver and kidney (6). Pregnancy or the feeding of the tocopherol-poor diet containing the oxidized cod liver oil to nonpregnant female rats both induce increases in palmitoleic, oleic, and longer-chain (C_{20}–C_{24}) fatty acids in the serum and the liver, along with decreases in arachidonic and stearic acids (7). These reports suggested that changes in serum and tissue lipids might play a role in the pathogenesis of the generalized Shwartzman phenomenon and other lesions induced by endotoxins. As a first step in exploring this possibility, serum and hepatic lipid concentrations were measured in rabbits injected with endotoxins.

Substantial increases in the concentration of all classes of serum lipids and hepatic triglycerides were found. There was no correlation between the changes in lipid levels and the extent or distribution of anatomic lesions, but there was a direct correlation between elevated serum triglyceride levels and mortality rate.

TABLE 1 PLASMA FFA CONCENTRATION OF YOUNG RABBITS THAT RECEIVED SHEAR'S POLYSACCHARIDE ENDOTOXIN

Time		Plasma FFA Concentration	
		μeq/liter	
Before Injection		182 ± 21* (n = 30)	
After 1st Injection	After 2nd Injection	Endotoxin	Control
hr	*hr*		
2	—	432 ± 81 (n = 11)	190 ± 30 (n = 16)
4	—	347 ± 10 (n = 5)	—
24	—	297 ± 36 (n = 14)	197 ± 16 (n = 16)
26	2	591 ± 60 (n = 8)	148 ± 25 (n = 14)
28	4	329 ± 44 (n = 6)	—
48	24	260 ± 43 (n = 10)	214 = 26 (n = 14)

* Standard error of the mean.

MATERIALS AND METHODS

The rabbits utilized were of mixed breeds and both sexes. Their exact ages were not known. Body weight was used to divide them into two age groups; animals weighing less than 2.0 kg were considered "young" rabbits, and those weighing more than 2.0 kg were designated "old" rabbits. The animals had free access to Purina Rabbit Chow and water up to and throughout the duration of the experiments. The endotoxin derived from *Serratia marcescens* (Shear's polysaccharide)[1] and the lipopolysaccharide of *Escherichia coli*[2] were used in separate experiments. Solutions of the endotoxins in a concentration of 0.1 mg/ml were made up in 0.85% saline and stored frozen until used. All blood samples were obtained from the marginal ear vein of the rabbit. Plasma FFA were measured by the method of Dole (8); serum and tissue triglycerides by the method of Van Handel and Zilversmit (9). The bromsulfophthalein (BSP) retention test was conducted by injecting Sulfobromophthalein Sodium (USP),[3] 50 mg/ml, into the marginal ear vein of one ear in a dose of 25 mg/kg body weight, and collecting blood samples from the other ear 15 min later. The concentration of BSP in the plasma was measured spectrophotometrically, and the percentage retention was calculated by the method of Rosenthal and White (10). All animals were autopsied and sections of the kidney, liver, lungs, and spleen were stained with hematoxylin and eosin and with phosphotungstic acid–hematoxylin, and examined microscopically.

Frozen sections of tissues were stained with Oil Red O.

[1] Generously supplied by Dr. Murray Shear of the National Institutes of Health, whose many kindnesses we gratefully acknowledge.

[2] Bacto-Lipopolysaccharide E. coli, purchased from Difco Laboratories, Detroit, Mich.

[3] Donated by the Vitarine Co., Inc. New York, N. Y.

RESULTS

Plasma Lipid Levels and Lesions in Young Rabbits that Received Shear's Polysaccharide

The average control plasma FFA concentration of 30 "young" rabbits (weighing between 1.2 and 2.0 kg) was 182 μeq/liter (Table 1). Fourteen of these animals each received an intravenous injection of 0.2 mg of Shear's polysaccharide per rabbit; the remaining 16 control rabbits received an equivalent volume of a 0.85% saline solution. Table 1 shows that 2 hr after the injection, the average plasma FFA concentration in the treated group was double that of the control group. In the next 2 hr the FFA level declined toward normal but rose again after the second dose of 0.2 mg of endotoxin per rabbit, given 24 hr after the first dose. Two hours after this second injection the levels were three times the preinjection control values.

More striking changes were noted in the serum triglyceride concentration of 24 rabbits similarly treated. No change occurred in the first 6 hr, but 24 hr after the first injection the average serum triglyceride concentration had increased 3-fold over control values. The range of values was large—from 98 to 1020 mg/100 ml—with half the values in excess of 300 mg/100 ml. Those sera with the highest concentration of lipid were lactescent. In the 24 hr following the second dose of endotoxin the triglyceride levels remained elevated or increased to even higher levels. Serum cholesterol and phospholipid levels of the treated group were twice those of the control group. These data are contained in Table 2.

TABLE 3 RELATIONSHIP BETWEEN PLASMA TRIGLYCERIDE CONCENTRATION, DISTRIBUTION OF LESIONS, AND DEATH IN YOUNG RABBITS THAT RECEIVED SHEAR'S POLYSACCHARIDE ENDOTOXIN

No. of Animals	Plasma Triglycerides 24 hr after Injection	No. of Animals with Lesions in				Deaths, 24–48 hr
		Liver	Kidney	Lungs	Spleen	
	mg/100 ml					
12	66–275	8	4	9	4	1
12	337–1020	6	5	7	5	7

Anatomic studies of the liver, kidney, lung, and spleen were carried out on the 24 treated animals, and lesions similar to those reported by others were found (2). Lesions in the liver consisted of small, round thrombi in the sinusoids and central veins, or small areas of necrosis in the mid-portion of the lobules; often both lesions were seen together. The kidneys contained thrombi in the glomerular capillaries, usually associated with broad areas of tubular necrosis. Lungs and spleen also contained small thrombi, alone, or less frequently associated with acute passive hyperemia or hemorrhagic infarcts.

The distribution of lesions between these four organs was compared with the serum triglyceride concentration

TABLE 2 PLASMA TRIGLYCERIDE, SERUM CHOLESTEROL, AND SERUM PHOSPHOLIPID CONCENTRATIONS IN YOUNG RABBITS THAT RECEIVED SHEAR'S POLYSACCHARIDE

Time		Triglyceride		Cholesterol		Phospholipid	
		mg/100 ml		*mg/100 ml*		*mg/100 ml*	
Before Injection		108 ± 10* (n = 30)		62 ± 5 (n = 26)		130 ± 8 (n = 25)	
After 1st Injection	After 2nd Injection	Endotoxin	Control	Endotoxin	Control	Endotoxin	Control
hr	*hr*						
24	—	368 ± 76 (n = 14)	113 ± 16 (n = 16)	133 ± 18 (n = 11)	53 ± 6 (n = 13)	223 ± 33 (n = 11)	112 ± 7 (n = 10)
48	24	416 ± 93 (n = 10)	148 ± 34 (n = 14)	114 ± 13 (n = 7)	60 ± 6 (n = 11)	260 ± 32 (n = 7)	140 ± 13 (n = 9)

* Standard error of the mean.

24 hr after injection of endotoxin in order to learn whether or not the changes in lipid concentration were correlated with the presence or absence of lesions in a particular organ. The data are contained in Table 3. The 24 animals were divided into two equal groups, 12 with the lower serum triglyceride levels and 12 with the higher values. It can be seen from the table that approximately the same number of lesions in each of the four organs were found in each group, indicating that there was no correlation between triglyceride concentration and the presence or absence of lesions. However, the last column in Table 3 indicates a striking correlation between the height of the serum triglyceride concentration and the mortality rate. Eight animals died in the 24 hr period following the second injection. Of these eight, seven were in the group with the higher triglyceride levels, while only one was in the lower group. This latter animal had a serum triglyceride level of 180 mg/100 ml at 24 hr, but this value had risen to 385 mg/100 ml at 48 hr, just prior to death. This association between serum triglyceride concentration and mortality rate suggested that in individual animals, the increase in serum triglyceride might be related to the severity of the effects of the endotoxin. To test this possibility, the number of lesions per animal, a rough guide to the severity of the effect of the endotoxin, was compared with the serum triglyceride concentration. The four animals with no discernible anatomic lesions had an average triglyceride concentration of 420 mg/100 ml (range 109–722); for the five animals with one lesion each the average concentration was 377 mg/100 ml (range 66–1020); for the four animals with two lesions each it was 362 mg/100 ml (range 252–580); for the eight animals with three lesions each it was 353 mg/100 ml (range 146–623), and for the three animals with lesions in all four organs it was 372 mg/100 ml (range 180–788).

Plasma Lipids and Lesions in Older Rabbits that Received Shear's Polysaccharide

The high incidence of lesions and the large percentage of animals with elevated serum lipid levels in the previous experiment might have obscured any correlation between them. The incidence of the generalized Shwartzman phenomenon—intraglomerular thrombosis and renal cortical necrosis—can be sharply reduced to less than 10% by using rabbits 10–12 weeks old weighing 2–3 kg instead of rabbits 4–7 weeks old weighing 0.5–1.0 kg (11). The incidence of lesions in the lungs, liver, and spleen remains the same. The reasons for this difference in the incidence of the renal lesion is unknown. A group of older animals was injected with endotoxin, in order to learn whether or not the low incidence of renal lesions would be accompanied by less marked changes in serum lipid levels.

Eleven rabbits, each weighing between 3.0 and 4.0 kg, were injected with two doses of Shear's polysaccharide given 24 hr apart; each dose was 0.15 mg/kg body weight. The incidence of renal lesions in this group of animals was only 9%, while the incidence of lesions in the other organs was approximately the same as in the prior series. Four of the eleven animals died between 3 and 24 hr after the second injection, a mortality rate of 36%. There was a slight rise in the average plasma FFA concentration 2–3 hr after the first injection of endotoxin, but the values were not significantly different from those of the control animals (Table 4). The average serum triglyceride concentration rose from 39 mg/100 ml before injection to 368 mg/100 ml 24 hr after the first injection. The second injection produced a further rise in six of the seven animals that survived the duration of the experiment. In spite of the low incidence of renal lesions in these older animals, the mortality rate and the changes in serum FFA and triglyceride concentrations were approximately the same as those in the younger group.

Plasma Lipids and Lesions in Rabbits that Received the Lipopolysaccharide Endotoxin of E. coli

The lipopolysaccharide endotoxin derived from *E. coli* was administered to a third group of rabbits in order to

TABLE 4 PLASMA FFA AND TRIGLYCERIDE CONCENTRATIONS IN OLD RABBITS THAT RECEIVED SHEAR'S POLYSACCHARIDE ENDOTOXIN

Time	Plasma FFA	
	μeq/liter	
Before Injection	122 ± 19* (n = 16)	
After 1st Injection	Endotoxin (n = 11)	Control (n = 5)
min		
15–45	184 ± 61	122 ± 17
90–180	292 ± 51	127 ± 44

Time		Plasma Triglycerides	
		mg/100 ml	
Before Injection		39 ± 8 (n = 16)	
After 1st Injection	After 2nd Injection	Endotoxin (n = 7–10)	Control (n = 5)
hr	*hr*		
24	—	368 ± 161	61 ± 11
48	24	314 ± 114	68 ± 23

* Standard error of the mean.

learn whether the serum lipid changes noted after injection of Shear's polysaccharide are brought about by that endotoxin alone, or whether other endotoxins can produce similar effects. Seventeen rabbits, each weighing between 2.1 and 3.5 kg, were utilized. Each rabbit received 0.2 mg of endotoxin per kg of body weight at the start of the experiment, and the survivors received a similar dose 24 hr later. Plasma FFA concentrations

TABLE 5 PLASMA FFA AND TRIGLYCERIDE CONCENTRATION IN OLD RABBITS THAT RECEIVED *E. coli* LIPOPOLYSACCHARIDE ENDOTOXIN

Time	Plasma FFA	
	μeq/liter	
Before Injection	155 ± 15* (n = 26)	
After 1st Injection	Endotoxin (n = 11–12)	Control (n = 6)
15–45 min	269 ± 47	203 ± 49
1½–2 hr	271 ± 30	173 ± 12
3–5 hr	373 ± 33	119 ± 18

Time		Plasma Triglyceride	
		mg/100 ml	
Before Injection		59 ± 9 (n = 25)	
After 1st Injection	After 2nd Injection	Endotoxin	Control
hr	*hr*		
24	—	1002 ± 197 (n = 9)	62 ± 13 (n = 8)
48	24	751 ± 134 (n = 3)	41 ± 6 (n = 5)

* Standard error of the mean.

again showed a tendency to rise in the period from 2 to 5 hr after each injection. The serum triglyceride concentrations in these animals were the highest of the three groups studied; five of the eight triglyceride values exceeded 1000 mg/100 ml (Table 5). The mortality rate was 82%, considerably higher than in the two groups given Shear's polysaccharide. The distribution of lesions among the organs was similar to that seen in the group of older rabbits given Shear's endotoxin.

Effect of Endotoxin on Hepatic Triglyceride Content and Bromsulfophthalein (BSP) Retention Test

The triglyceride content of the livers of representative animals in the control group, and in the two groups of older rabbits are listed in Table 6. There was a 4-fold increase in the triglyceride content of the livers of two of the eight animals that received Shear's polysaccharide; all six of the animals that received the *E. coli* endotoxin developed a 2- to 3-fold rise. Frozen sections of livers that contained an increased amount of triglycerides had small sudanophilic droplets dispersed throughout the cytoplasm of the hepatic cord cells. There was no stainable lipid in the parenchyma of the kidneys or spleens of these animals.

BSP was administered to eight uninjected control animals, and to five groups of rabbits immediately after, or

TABLE 6 LIVER TRIGLYCERIDE CONCENTRATION IN OLD RABBITS GIVEN ENDOTOXIN

Rabbit No.	Plasma Triglyceride	Liver Triglyceride
	mg/100 ml	*g per 100 g wet wt*
	Controls	
1	9	0.64
2	47	0.60
3	56	0.54
4	77	0.18
5	150	0.61
	Shear's Polysaccharide*	
6	99	1.21
7	139	0.46
8	154	0.74
9	167	0.57
10	193	0.97
11	529	0.66
12	917	4.31
13	—	4.51
	E. coli Lipopolysaccharide*	
14	584	3.14
15	633	1.64
16	652	1.25
17	752	2.58
18	1328	1.79
19	1576	1.25

* Each rabbit in these groups received 2 injections of endotoxin spaced 24 hr apart, and died or was sacrificed 3–24 hr after the 2nd injection.

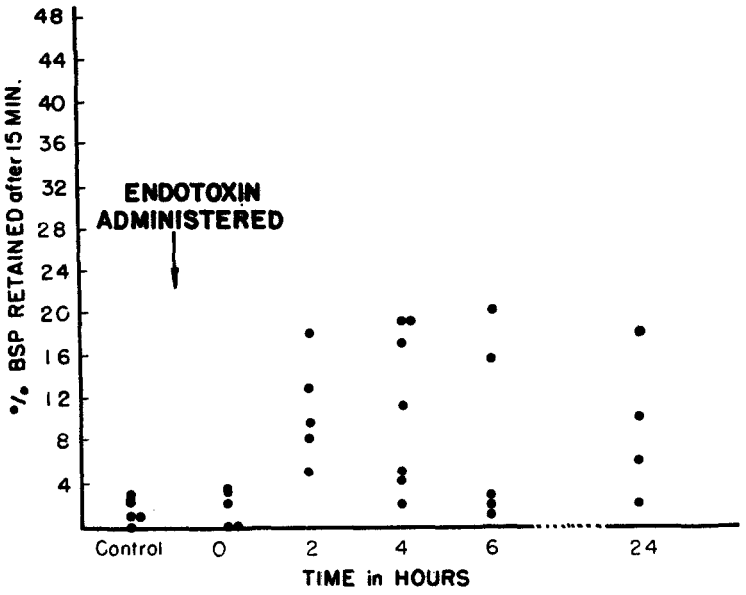

FIG. 1. Retention of bromsulfophthalein (BSP) by rabbits given Shear's polysaccharide endotoxin intravenously. Each point represents an individual animal.

2, 4, 6, or 24 hr following a single injection (0.2 mg per rabbit) of Shear's polysaccharide. All rabbits in this experiment weighed between 1.2 and 2.2 kg. BSP retention in the control animals was 0–3% of the test dose in 15 min. Animals tested immediately after injection of the endotoxin did not show an increase in BSP retention. However, retention of BSP in the range of 5 to 22% of the injected dose was found in the majority of animals tested 2, 4, 6, and 24 hr after administration of endotoxin. These data are summarized in Fig. 1.

DISCUSSION

These studies demonstrate a small rise in plasma FFA concentration and a moderate retention of BSP beginning 2 hr after the intravenous injection of endotoxin into rabbits. The rise in plasma FFA concentration is transitory, but the BSP retention persists for at least 24 hr. Twenty-four hr after the injection, most of the animals have fatty livers and are hyperlipidemic. A second injection is followed by a second small rise in plasma FFA, and the serum triglyceride concentration rises further. These changes in serum and tissue lipid concentration occur following the injection of either Shear's polysaccharide or the endotoxin of *E. coli*, which suggests that such changes may be produced by endotoxins in general.

The mechanisms responsible for the development of fatty livers and hyperlidemia after the injection of endotoxin are not established. It is unlikely that these changes are due solely to increased mobilization of FFA from adipose tissue to the liver. In order to produce hyperlipidemia by the mobilization of FFA from adipose tissue the plasma concentration of FFA must be sustained at high levels for 6–8 hr. Hyperlipidemia does develop in rabbits in this way following a single injection of pituitary Fraction H. A single injection of ACTH produces plasma FFA levels comparable to those obtained with Fraction H, but these increases last only 1–3 hr and are not followed by hyperlipidemia (12). However, hyperlipidemia can be produced with ACTH if it is given as a continuous infusion which maintains high plasma FFA levels for 14 hr or more (13). The elevation of plasma FFA produced by endotoxins is neither high enough nor sustained long enough to account for the other lipid changes. Thus it seems necessary to postulate that other mechanisms are involved.

The retention of 5–22% of the test dose of BSP 2–24 hr after administration of endotoxins may be due to reduction in hepatic blood flow (14) or some direct influence on hepatic cord cells. In either event, the finding suggests that endotoxins may also interfere with certain aspects of hepatic lipid metabolism, such as inhibition of the synthesis or release of lipoproteins, or decreased rate of utilization of lipids by the liver. Any of these effects could produce a fatty liver but none of them can account for the subsequent hyperlipidemia. However, if the effects leading to fatty liver are short-lived and reversible, the hyperlipidemia could be the result of subsequent release of lipids from the liver into the blood.

The plasma lipid changes also could be the result of an increased rate of synthesis of lipids induced by endotoxins, or a decreased rate of clearance of lipids from the blood. The reduced rate of blood flow previously mentioned could conceivably reduce the direct removal of chylo-

micra from the blood. Alternatively, clearance of lipids from the blood could be inhibited if endotoxins inhibit the action of lipoprotein lipase. Additional data are necessary to establish which of these various mechanisms are responsible for the lipid changes induced by endotoxins.

The comparison between changes in serum lipid levels and the distribution of anatomic lesions was carried out in an attempt to establish a cause-and-effect relationship between them; that is, to obtain any indication that the production of lesions in one or more organs leads to hyperlipidemia, or that the establishment of a hyperlipidemia leads to lesions in one or more organs. No such correlation was found. It is possible that the two phenomena are directly related, but the relative times required for each change to become manifest are so different that a correlation cannot be established by the means employed. On the other hand, it is equally plausible that the two phenomena are separate manifestations of the effects of endotoxins and not directly related to each other. There does appear to be a direct correlation between the height of the serum triglyceride levels and death of the animals. Animals rendered hyperlipidemic by injections of crude pituitary extracts or pituitary Fraction H often sicken and die during the hyperlipidemia (15), but animals rendered hyperlipidemic by the surface-active agent Triton WR 1339 do not (16). It would be of interest to establish whether or not the sickness and death of animals given endotoxin or pituitary extracts was caused by some feature of the hyperlipidemia induced by these agents.

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