Bile Acids in Rat Serum as Indicators of Hepatotoxicity by Hornet Venom (43148)

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Abstract. **The effect of hornet venom sac extract (VSE) on hepatic function in rats was evaluated by measurement of bile acid (BA) levels. A significant dose-dependent elevation of BA in rat serum upon repeated VSE administration was found. Rats envenomed for 2 weeks showed a significant rise of serum BA compared with the controls or with rats envenomed for 6 days. In rats envenomed for 1 week and killed after another week without envenomation, an improvement of the biochemical status was observed. These observations demonstrated that BA levels reflect the VSE-induced hepatic injury. They suggest that the cholestatic effects of VSE may be due to an impairment of bile acid excretion, but the elevations could also reflect parenchymal hepatic injury.** [P.S.E.B.M. 1990, Vol 195]

Clinical and experimental observations have clearly shown the cholestasis induced by hornet venom sac extract (VSE) (1). In Israel, Glaser (2) has reported that patients with multiple hornet stings have a clinical cholestasis and a parenchymal injury, i.e., a centrolobular necrosis and a mild portal lymphocytic infiltrate. Borochowitz and Hardoff (3) also reported cholestasis in a multiply stung child, and Barr-Nea *et al.* (4) have provided histologic, histochemical, and electron microscopic evidence for VSE-induced cholestasis. Neuman *et al.* (1, 5) have presented enzymatic evidence for this phenomenon in mammalian serum and tissue. Likewise, changes in bile flow and in total bile acids (BA) have been observed to follow VSE envenomation of the isolated perfused rat liver (6). The reports of cholestasis have triggered our interest in examining serum BA levels in conjunction with repeated rat envenomations. That is the subject of this investigation.

Materials and Methods

Animals and Design of the Experiment. Forty-eight male Wistar rats weighing 200–250 g were used as test animals. These rats received daily intraperitoneal injections of 5 mg of VSE/kg body wt in 0.5 ml of saline, as shown in Table I. Each day, 1 hr after this treatment, six rats were killed. The last surviving 12 animals were divided into two groups, Group A consisting of six rats receiving six consecutive (daily) VSE injections followed by a rest period of 8 days and Group B consisting of six rats subjected to 14 consecutive (daily) injections. Another 54 control rats received only daily intraperitoneal injections of 0.5 ml of saline and were then killed (Table I).

The animals were born and raised in the "Glasberg" Tower for Medical Research of the Sackler Faculty of Medicine, where they were maintained on a commercial chow diet and water, given *ad libitum*. All rats were kept in groups of six per cage. The envenomations were performed each day between 9 AM and 11 AM to avoid possible variation due to circadian rhythm. Blood was obtained by cardiac puncture. Samples of the serum were kept at −20°C until analyzed.

Fresh VSE prepared as described previously (5) was used in the present study.

Biochemical Methods. Total BA levels were measured spectrophotometrically using the enzymatic procedure of Fausa and Gjone (7), as modified by Brungsaard *et al.* (8), which is based on the enzyme 3α-hydroxysteroid dehydrogenase (EC 1.1.1 50). A summary of the procedure is given by the designers of the product Sterognost 3 alpha R Pho (Nyegaard & Co. A/S, Oslo). Briefly, the absorption of the sample is read against a blank at 340 nm in a Kontron 805 spectro-

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Table I. Chronic Repeated Envenomation: Experimental Design

	Day							
	1	2	3	4	5	6	14	
							A	B
No. of injections	1	2	3	4	5	6	6	14
No. of animals treated daily	48	42	36	30	24	18	6	6
Controls	24	21	18	15	12	9	3	3
Rats killed daily								
Treated	6	6	6	6	6	6	6	6
Control	7	7	6	6	7	7	7	7

Table II. Serum BA Concentration in Rats Treated Repeatedly with VSE

	Day							
	1	2	3	4	5	6	14	
							A	B
No. of treatments	1	2	3	4	5	6	6	14
BA (μM)								
Treated	4.0 ± 1.0	4.2 ± 0.6	4.2 ± 0.6	4.8 ± 0.8	5.6 ± 0.6	8.0 ± 0.5	5.4 ± 0.6	14.0 ± 1
Control	3.8 ± 0.6	4.0 ± 0.5	3.8 ± 0.8	4.0 ± 0.5	4.0 ± 0.5	4.2 ± 0.8	3.8 ± 0.8	4.2 ± 1
Significance								
vs control	NS[a]	NS	NS	NS	NS	$P < 0.05$	NS	$P < 0.001$
vs previous treatment	NS	NS	NS	NS	NS	NS	$P < 0.05$[b]	$P < 0.05$

[a] NS, not significant.
[b] Significance of the decreasing values versus previous treatment.

photometer. This enzymatic method measures the total BA without discriminating between them. Neither are the three conjugates determined by this assay.

Statistical Methods. Statistical analysis was based on Student's t test for assessing significance (9).

Results

Results are presented in Table II. As can be seen, for the first 5 consecutive days the levels of BA in treated rats were not significantly increased compared with those in control rats. It was only after the sixth day of treatment that a marked elevation of the BA was noted in test animals that was statistically significant compared with the control.

In rats receiving six daily treatments but killed only 6 days later (Subgroup A), the serum BA showed significant diminution, albeit not reaching the basal values. However, in rats of Subgroup B, who were exposed to VSE for 14 consecutive days, there was a further rise in BA levels, which was significant not only compared with the level in controls but also compared with the level in rats receiving six consecutive treatments. This latter finding suggests a dose dependency of the BA levels. Incidentally, the body weights of control rats increased by about 10% during the 14-day period, whereas those of the treated rats increased by only 7%, but the difference here was not statistically significant.

The liver weight/body weight ratio of the 48 treated rats (4.90 ± 0.3) was 30% higher than that of 54 control rats (3.7 ± 0.4), indicating that the livers had increased in size significantly ($P < 0.05$).

Discussion

"Cholestasis" implies an obstruction to the flow of bile and may be brought about by diverse mechanisms. The etiology and possible mechanisms of cholestasis have been discussed by Popper and Schaffner (10, 11), Plaa and Priestly (12), Phillips *et al.* (13), Popper (14), and Strange (15). The present investigation set out to explore the possibility that VSE also induces cholestasis. Barr-Nea *et al.* (4), in a similar experiment, presented histochemical evidence that VSE induces cholestasis, by demonstrating that alkaline phosphatase stain accumulates increasingly around the bile canaliculi of treated rats as compared with the controls. The histologic pattern of liver injury induced by VSE reveals bile ducts with an eosinophilic infiltration similar to that obtained following chlorpromazine administration (16). The cholestatic liver damage produced by VSE and revealed by electronmicrographs entails not only changes in the biliary canaliculi, such as dilation and

loss or stunting of the microvilli, but also the occurrence of swollen abnormal mitochondria with peculiarly shaped cristae. In a previous study (6), we have shown that VSE increases the amount of BA in the bile of perfused rat livers. Further evidence for cholestasis in rats repeatedly exposed to VSE was the observed increase in serum and tissue alkaline phosphatase activity (1).

The data presented herein provide another facet to previous evidence that VSE can lead to hepatic injury, as measured by biochemical and morphologic parameters that are dose dependent. They also clearly are consistent with the view that an important component of the injury is cholestatic. The present study throws no light on the mechanism(s) whereby BA concentration is augmented in the serum following VSE administration. Our previous morphologic data (4) argue in support of an excretory defect. Also, it was shown that increased intracellular BA concentration could be injurious to hepatocytes (17, 18). Conversely, the elevated BA levels may be secondary to VSE-induced hepatic injury.

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