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# Changes in Plasma Levels of Lysosomal and Non Lysosomal Enzymes During Hemorrhagic Hypotension[1] (38950)

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It is well established that plasma levels of lysosomal enzymes increase during hemorrhagic hypotension (1–3). Additionally, there appears to be a good positive correlation between the circulating levels of these enzymes and the severity of the shock state (2, 3). These enzymes are probably released into the blood as a result of tissue damage in the splanchnic region and indeed, increases in plasma lysosomal hydrolase levels during shock have been related to changes in lysosomal stability in liver (3), intestinal mucosa (4) and pancreas (5).

Since there is considerable tissue damage it is not surprising that the plasma levels of other tissue enzymes are also increased during hemorrhagic hypotension. It has been shown, e.g., that the plasma levels of ornithine carbamyltransferase, a liver specific enzyme, and the intestinal fraction of alkaline phosphatase increase during hemorrhagic shock (6, 7).

The present study was carried out to determine the time course of changes in the plasma levels of lysosomal and nonlysosomal enzymes during hemorrhagic hypotension in order to determine which of the enzymes might be the more sensitive indicators of the severity of the shock state.

*Methods.* Male dogs weighing 18–28 kg were anesthetized with iv sodium pentobarbital (32 mg/kg). The animals were heparinized and hemorrhaged via an arterial cannula into a pressurized reservoir at a bleeding rate not in excess of 50 ml/min. The mean arterial blood pressure was held at 35 mmHg for 4 hr; sham shock animals were similarly prepared and followed for 4 hr but without bleeding.

Arterial blood samples were taken for plasma enzyme and lactate determination. Acid phosphatase (AP) was determined using the method of Smith and Filkins (8); the method for cathepsin (CATH) determination was similar to that described by Gianetto and de Duve (9) except that 0.2 ml of plasma was incubated with the substrate for 2 hr at pH 3.6. Analysis of ornithine carbamyltransferase (OCT) was carried out according to the method of Ceriotti and Gazzangia (10). The intestinal fraction of plasma alkaline phosphatase (IAkP) activity was determined using the procedure described by Bounous and Hugon (7) which is based on the inhibition of IAkP activity by L-phenylalanine (11). Plasma levels of aspartate aminotransferase (AAT) and creatine phosphokinase (CPK) were determined using the methods of Babson and Philips (12) and Rosalki (13), respectively. Blood lactate levels were determined using the method of Hohorst (14). Determinations of AAT, CPK, and lactate were carried out using commercially available reagent kits.

*Results.* The reservoir model of hemorrhagic hypotension as used in the present investigation permits the estimation of the severity of the shock state on the basis of relative amount of the shed blood taken back spontaneously by the animal. The mean time to initial uptake of shed blood by the animals was 1.5 hr and mean time to uptake of 30% of the maximal shed volume was 3.5 hr. Previous studies using this model have indicated that an uptake of more than 30% of the shed volume represents an irreversible stage of hemorrhagic shock (15). Thus, after 2 hr of hypotension the animals were just beginning the refractory stage and by 4 hr were generally in the irreversible state of shock.

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TABLE I. CHANGES IN PLASMA ENZYME AND BLOOD LACTATE LEVELS DURING HEMORRHAGIC HYPOTENSION IN THE DOG.[d]

| | | n | Control | 1 hr | 2 hr | 3 hr | 4 hr |
|---|---|---|---|---|---|---|---|
| CATH | Sham | 8 | 1.8±0.3[a] | | 1.4±0.1 | | 2.0±0.2 |
| | Hypotension | 12 | 1.4±0.1 | 2.9±0.4[c] | 14.5±3.5[b, c] | 30.5±4.3[c] | 37.6±4.0[b, c] |
| AP | Sham | 8 | 0.3±0.1 | | 0.3±0.1 | | 0.38±0.10 |
| | Hypotension | 11 | 0.3±0.1 | 0.4±0.1 | 1.0±0.2[b, c] | 1.83±0.42[c] | 2.43±0.46[b, c] |
| OCT | Sham | 7 | 10.0±3.4 | | 14.5±3.6 | | 14.8±3.2 |
| | Hypotension | 12 | 14.3±2.5 | 16.7±2.8 | 19.4±2.6 | 46.8±12.6[c] | 82.7±19.4[b, c] |
| IAkP | Sham | 8 | 1.5±0.1 | | 1.5±0.1 | | 1.4±0.1 |
| | Hypotension | 12 | 0.95±0.1[b] | 0.95±0.1 | 1.5±0.1[c] | 2.3±0.2[c] | 2.9±0.3[b, c] |
| AAT | Sham | 8 | 62.4±6.8 | | 69.3±8.5 | | 74.4±10.1 |
| | Hypotension | 12 | 59.2±3.3 | 59.8±3.2 | 87.7±12.2[c] | 254.5±57[c] | 678.0±149[b, c] |
| CPK | Sham | 7 | 125±17 | | 179±31 | | 244±48[c] |
| | Hypotension | 12 | 112±7.2 | 116±10 | 193±43 | 429±86[c] | 658±76[b, c] |
| Lactate | Sham | 7 | 3.2±0.2 | | 3.5±0.2 | | 3.5±0.3 |
| | Hypotension | 11 | 3.4±0.2 | 16.8±1.4[c] | 27.8±1.7[b, c] | 32.9±1.8[c] | 31.9±1.8[b, c] |

CATH = cathepsin. AP = acid phosphatase. OCT = ornithine carbamyltransferase. IAkP = plasma alkaline phosphatase. AAT = aspartate aminotransferase. CPK = creatine phosphokinase.

[a] Mean ± SE of mean.

[b] $P < 0.05$ compared to sham control at comparable time.

[c] $P < 0.05$ compared to respective prehemorrhage control level.

[d] CATH expressed in mg/tyrosine/100 ml serum/hr; AP expressed in mg $PO_4$/100 ml plasma/hr. OCT, IAkP, AAT, and CPK expressed in Int units/ml and lactate as mg/100 ml blood.

The mean plasma levels of the enzymes and the blood lactate levels are shown in Table I. The lysosomal enzymes (CATH and AP) showed increases at 2 hr of hypotension and progressive increases to the end of the experiments. CATH was the only enzyme that increased after 1 hr of hypotension. At 4 hr, plasma CATH had increased 27-fold and AP 8-fold above control activity. IAkP increased at 2 hr but this value was not different from the corresponding value of the sham group; after 4 hr IAkP showed only a 3-fold increase over prehemorrhage levels. AAT showed a pattern similar to that of IAkP but after 4 hr showed an 11-fold increase. OCT and CPK did not increase until 3 hr of hypotension and at 4 hr both enzymes had increased to about six times the prehemorrhage levels. Only CPK showed a significant change in the sham animals. Blood lactate levels increased progressively, especially during the first 3 hr of hypotension.

*Discussion.* The animals progressed through the stages of hemorrhagic shock from an initial "reversibility" (at 1–1.5 hr) to an intermediate stage of expected "partial irreversibility" (2–3 hr) and finally to a stage of "irreversibility" at 4 hr of hypotension (15). At 1 hr, only CATH and lactate had increased but in the later stages (3–4 hr) all of the enzymes had shown relatively significant increases.

Of the enzymes studied, CATH showed the largest increase at 2 hr and the largest increase by far at 4 hr (27 × control level); AP and AAT showed pronounced but lesser increases above the control levels and IAkP had the smallest total increase. CPK and OCT showed intermediate elevations. All of the nonlysosomal enzymes showed lesser increases at 2 hr than the lysosomal enzymes (CATH and AP) indicating that in this hemorrhagic shock model, the lysosomal enzymes and specifically plasma CATH, seemed to be the most sensitive indicator of shock severity.

It is usually considered that the primary site of release of the lysosomal enzymes during shock is the splanchnic region, probably because of the intense vasoconstriction and ultimate tissue damage in this area. The increase in plasma CPK levels indi-

cates that there is some enzyme release from muscle during shock since this enzyme is present only in muscle and nervous tissue (16). The tissue damage that occurs in the liver and intestinal mucosa during hemorrhagic hypotension is indicated by the increase in the plasma levels of liver specific OCT (17) and the intestinal specific IAkP (7, 11). The earlier increase in plasma IAkP may suggest that intestinal cellular damage may occur before liver cellular damage with this hypotensive model.

Since it is well-known that the functional state of the reticuloendothelial system (RES) and other tissues deteriorates during hypotension and that the degree of RES failure is closely related to the severity of the shock (18, 19), it seems likely that the increased plasma enzyme levels are associated with an increased rate of release from damaged tissue. Current evidence also suggests that many enzymes, including lysosomal hydrolases, are cleared from the circulation by the RES (20–22) indicating that decreased clearance may also be a factor in the elevated blood levels, particularly in the case of CATH which is rather rapidly removed by the RES (21).

*Summary.* In a study of 4-hr hemorrhagic hypotension in dogs, the plasma levels of the lysosomal enzymes, cathepsin (CATH) and acid phosphatase (AP) showed early and progressive increases in activity. The plasma levels of the intestinal fraction of alkaline phosphatase (IAkP) and aspartate aminotransferase (AAT) were increased after 2 hr of hypotension and the liver specific enzyme, ornithine carbamyltransferase (OCT), and creatine phosphokinase (CPK), after 3 hr. All of the enzymes showed large increases after 4 hr of hypotension. The plasma levels of CATH showed the earliest and largest relative increase indicating that with the shock model used, this enzyme was the most sensitive indicator of shock severity. The increase in plasma enzyme levels was probably the result of tissue damage in the splanchnic region but the elevation of plasma CPK, a muscle specific enzyme, indicates some muscle cell damage as well. While the increase in the plasma enzyme activity is probably due, in large part, to cellular damage, it is likely that a decreased enzyme removal rate—resulting from a hypofunctional RES—also contributes to the elevated plasma enzyme levels during hemorrhagic hypotension.

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