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Correlation of Plasma Lysosomal Enzyme Levels with Hepatic Reticuloendothelial Function after Trauma[1] (39323)

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Reticuloendothelial system (R.E.S.) phagocytic failure has been implicated as an important event in the pathogenesis of irreversibility following various forms of experimental shock. The role of the R.E.S. in shock survival is based primarily on four lines of evidence: (a) R.E.S. function deteriorates during shock (1-5); (b) survival following shock has been directly related to the degree of postshock R.E.S. recovery (2, 4); (c) survival is reduced when R.E.S. phagocytic activity is depressed prior to shock (1, 4, 5); (d) survival is increased when R.E.S. phagocytic activity is stimulated prior to shock (1, 4, 5). While the exact mechanism by which the R.E.S. exerts this protective defense role is not known, emphasis has been placed on the ability of the R.E.S. to remove and prevent the accumulation of particulate and toxic material in the circulation. Such potentially deleterious agents appear to include microthrombi, bacteria, endotoxin, damaged platelets, and lysosomal enzymes (5-10). Thus, it has been postulated that there is a failure in the mechanisms which clear toxic material from the blood during shock at a time when the elaboration of such material is greatly accelerated.

The present study was carried out to evaluate this concept by determining if a relationship exists between the plasma accumulation of one of these potentially toxic agents, i.e., lysosomal enzymes, and hepatic Kupffer cell phagocytic capacity following traumatic shock.

Methods. Male Sprague-Dawley rats (200-300 g) anesthetized with sodium pentobarbital (20 mg/kg) were subjected to Noble-Collip drum trauma (11) at 40 rpm. This trauma model allows the application of a highly standardized insult. On the basis of 24-hr survival it was determined that 300 revolutions (rev) resulted in less than 5% mortality (defined as sublethal), while 500 and 700 rev resulted in approx 50 and 95% mortality, respectively.

The test colloid employed to evaluate *in vivo* hepatic Kupffer cell clearance capacity was the gelatinized ^{131}I-"R.E.-test-lipid emulsion" as previously described (12). The emulsion was injected iv in both normal and post-trauma rats at a dose of 50 mg/100 g and hepatic Kupffer cell uptake evaluated 15 min following injection. The liver was rapidly excised and rinsed in chilled isotonic saline. The total organ was weighed and random aliquots removed for quantification of ^{131}I colloid activity as previously described (12). Results were expressed as milligrams of test colloid phagocytized per gram (mg/g) and per total organ (mg/TO).

Plasma levels of the lysosomal enzymes, cathepsin, and acid phosphatase, were determined using the methods described by Gianetto and DeDuve (13) and Smith and Filkins (14), respectively. For cathepsin determinations a 2% solution of denatured hemoglobin was used as the substrate and the reaction was carried out at pH 3.6. The aromatic degradation products were measured by the Lowry protein method using a tyrosine standard. The acid phosphatase assay was carried out at pH 5.0 with determination of the amount of inorganic phosphorus removed from the beta-glycerophosphate substrate.

Quantification of radioactivity was achieved with a Searle Analytic deep well autogamma scintillation counter. All samples were counted in duplicate with independent isotopic standards prepared for each experiment. Data were analyzed with a PDP/12 digital computer by the unpaired Student's *t* test placing the confidence level at 95%. Determination of correlation coef-

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ficients, and line placement by the method of least squares was also done with computer assistance. Data are presented as means ± standard error.

Results. The temporal changes in plasma cathepsin and acid phosphatase levels after sublethal trauma (300 rev) is presented in Fig. 1. Both enzymes followed similar patterns of change with an initial increase at 1/2 hr, and a return to control levels by 24 hr after trauma. Plasma cathepsin reached a maximum level equivalent to 144% of control at 3 hr while a maximum 59% elevation in acid phosphatase was observed at 1 hr after trauma.

Plasma levels of cathepsin and acid phosphatase at 60-min post-trauma increased progressively with increments in trauma intensity (Fig. 2). Plasma cathepsin showed a comparatively greater increase following trauma than acid phosphatase. Thus, 1 hr after 500 rev (LD_{50}) cathepsin increased 177% while acid phosphatase increased

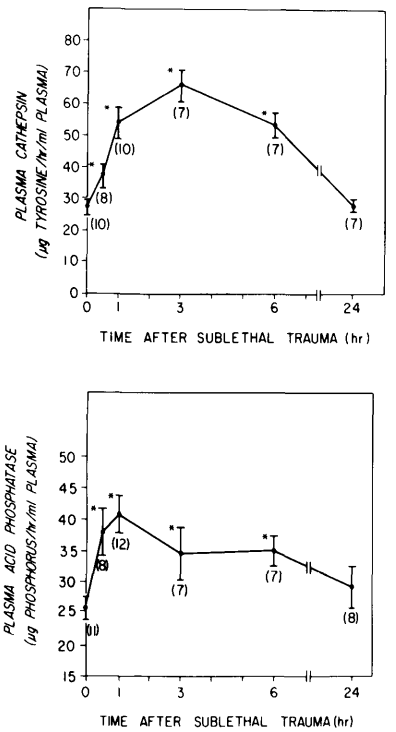

FIG. 1. Temporal alterations in plasma cathepsin and acid phosphatase following sublethal trauma (300 rev). Data are expressed as the mean ± standard error with the number of animals indicated in parentheses. Asterisk (*) indicates significant ($P < 0.05$) difference from pretrauma control levels.

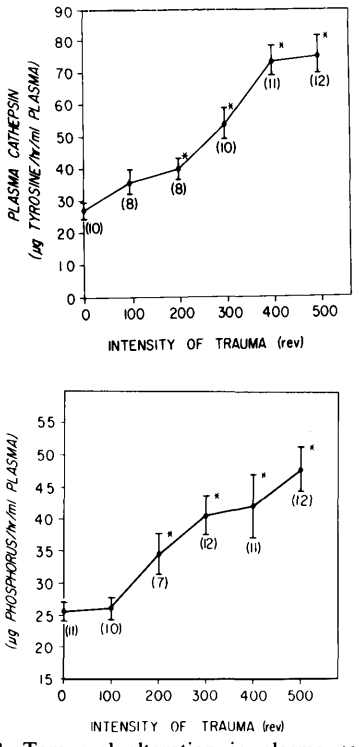

FIG. 2. Temporal alteration in plasma cathepsin and acid phosphatase at 60-min post-trauma with various intensities of trauma (100–500 rev). Data are expressed as mean ± standard error with the number of animals indicated in parentheses. Asterisk (*) indicates significant ($P < 0.05$) difference from pretrauma control levels.

92%. The difference in the magnitude of change of these two enzymes is consistent with previous findings during hemorrhagic hypotension in the dog (9). In an attempt to correlate these enzyme alterations with the functional state of the hepatic Kupffer cell clearance mechanism, the functional state of the hepatic R.E.S. was evaluated over a similar time course.

Hepatic phagocytosis after sublethal trauma showed a maximum depression at 1 hr with recovery to pretrauma levels by 24 hr (Table I). With increasing trauma intensity there was a progressive decrease in hepatic phagocytosis 60-min post-trauma on both a per gram and total organ basis (Table I). Previous evaluation of other R.E. organs (spleen, bone marrow, and lung) during a period of Kupffer cell depression revealed increases of colloid localization in these extrahepatic sites as the half-time for colloid clearance from the circulation increased (12, 15–18).

TABLE I. HEPATIC KUPFFER CELL PHAGOCYTIC ACTIVITY FOLLOWING TRAUMATIC SHOCK AS A FUNCTION OF POST-TRAUMATIC INTERVAL AND DEGREE OF INJURY.[a,b]

	Number of animals	Kupffer cell phagocytosis[c]			
		mg/g	%C	mg/TO	%C
Post-Trauma interval					
Pretrauma controls	7	3.35 ± 0.19	100	46.39 ± 3.37	100
0.5 hr	7	1.89 ± 0.21[d]	56	27.17 ± 3.43[d]	59
1 hr	6	1.49 ± 0.07[d]	45	20.26 ± 0.83[d]	44
3 hr	7	1.77 ± 0.12[d]	53	24.54 ± 2.33[d]	53
6 hr	4	2.19 ± 0.30[d]	65	28.01 ± 3.71[d]	60
24 hr	8	3.40 ± 0.22	102	43.87 ± 2.40	95
Trauma intensity					
Pretrauma controls	5	4.69 ± 0.18	100	57.72 ± 7.64	100
100 rev	5	3.67 ± 0.50	78	41.68 ± 6.14	72
200 rev	5	4.30 ± 0.46	92	53.64 ± 7.13	93
300 rev	5	2.90 ± 0.53[d]	62	30.35 ± 4.96[d]	53
400 rev	5	2.97 ± 0.31[d]	63	40.89 ± 4.56[d]	71
500 rev	5	2.32 ± 0.17[d]	50	27.82 ± 4.01[d]	48

[a] In the time course study (0–24 hr), rats were subjected to 300 rev of NCD trauma and evaluated with a 50 mg/100 g test dose of the colloid at times indicated.

[b] In the trauma intensity series (0–500 rev), rats were evaluated at 60-min post-trauma with the test colloid.

[c] Data is presented as means ± SE of the mean. Values are presented as milligrams test emulsion phagocytized per gram of liver (mg/g) and per total organ (mg/TO) on a wet weight basis. %C is the percentage of pretrauma control values.

[d] Significantly different from control group ($P < 0.05$).

Numerous studies have indicated that increased plasma lysosomal enzyme activity and decreased phagocytic capacity of the R.E.S. are related to shock severity (1–5, 8–10); however, a direct relationship between these two parameters has not been previously demonstrated. Figure 3 documents this relationship and reveals a significant correlation between post-trauma hepatic phagocytosis and the plasma levels of both cathepsin ($P < 0.05, r = -0.604$) and acid phosphatase ($P < 0.05, r = -0.634$). This inverse relationship between hepatic phagocytosis and plasma lysosomal enzyme levels was observed with the time course changes after sublethal trauma as well as with the changes 1 hour after various intensities of trauma.

Discussion. The observed changes in hepatic R.E. function following trauma are consistent with previous data which demonstrated an initial fall in R.E.S. function followed by a recovery or even hyperactivity within 24 hr (2–4, 16, 17). Similarly, the changes in plasma lysosomal enzyme levels observed were qualitatively similar to previous findings (8–10). The present study extends previous investigations in terms of quantifying the time course of changes following traumatic shock while further documenting the relationship between the degree of trauma and both the stability of the R.E.S. as well as the plasma lysosomal enzyme levels.

There are at least four possible interpretations of the present observation of the correlation of plasma lysosomal enzyme levels with R.E.S. function after trauma. These include: (a) Kupffer cell function decreases and plasma lysosomal enzyme levels increase independently as a function of the degree of tissue injury with no cause and effect relationship; (b) lysosomal enzymes are cleared from the blood by the R.E.S., and Kupffer cell clearance depression allows enzyme accumulation in the blood following trauma; (c) high plasma lysosomal enzyme levels in the post-trauma state depress R.E.S. phagocytic function; (d) enzymes released into the plasma following trauma are released primarily from injured R.E. cells and thus reflect the stability of this cellular system.

The possible clearance of lysosomal enzymes by the R.E.S. is supported by the finding that exclusion of the liver from the circulation via a porta-caval shunt preparation results in a greatly prolonged retention

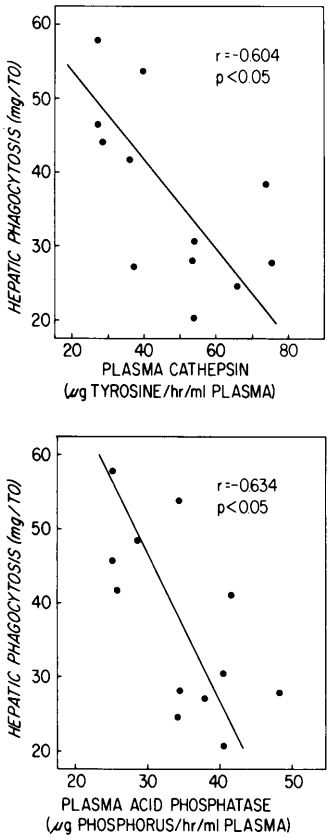

FIG. 3. Correlation between hepatic Kupffer cell phagocytosis and plasma cathepsin (upper) and plasma acid phosphatase (lower) after trauma. Plotted values are means of data obtained at various times (1/2–24 hr) after sublethal trauma (300 rev) and 1 hr after various levels of trauma (100–500 rev).

of lysosomal enzymes in the blood (19, 20). Depression of R.E.S. function may therefore result in a greater accumulation of these hydrolase enzymes in the plasma as a result of decreased clearance. Trauma may induce alterations in the splanchnic microcirculation (21, 22) which could result in: (a) R.E.S. failure by limiting delivery of particulate material to phagocytic cells; (b) ischemic injury of hepatic Kupffer cells; and (c) release of lysosomal enzymes from ischemic splanchnic tissue. Additionally, high circulating lysosomal enzyme levels have been shown to decrease splanchnic blood flow (19).

R.E. function and lysosomal enzyme levels may change independently of each other or in a related manner as a result of tissue injury. Lysosomal hydrolases may be released as a direct consequence of cellular injury while R.E. depression may result from opsonin depletion (17, 18). Alternatively, since lysosomal enzymes are released from macrophages during phagocytosis (23), and since R.E. depression is observed following phagocytosis (12) these phenomenon may occur secondarily to an accumulation of phagocytizable debris subsequent to trauma. While R.E. cells do contain a high concentration of lysosomal enzymes (24), the magnitude of the enzyme release coupled with the size of the R.E. cell population cast doubt that all the enzyme activity is released from ischemic or injured R.E. cells. Indeed, the flux of such enzymes from the intestine and pancreas, organs with minimal R.E. activity, would further negate this possibility (25). Lysosomal hydrolases may be released from injured R.E. cells, but histological evidence indicates that Kupffer cell damage is only present with irreversible shock (1).

Thus, the present study has shown that there is a correlation between hepatic Kupffer cell function and the accumulation of lysosomal enzymes in the circulation following traumatic injury. Since previous studies have demonstrated that R.E.S. function correlates well with shock survival (2, 4), the observed relationship between circulating lysosomal enzyme levels and R.E.S. function warrants further investigation.

Summary. Plasma lysosomal enzyme levels and hepatic phagocytosis were determined following Noble–Collip drum trauma in the rat. Circulating cathepsin and acid phosphatase activity increased after sublethal trauma (300 rev), reaching maximal levels at 1–3 hr and returning to pretrauma levels at 24 hr after trauma. Hepatic phagocytosis was decreased maximally at 1 hr and recovered to control levels at 24 hr after sublethal trauma. Increasing trauma intensity (100–500 rev) resulted in a progressive failure in hepatic Kupffer cell phagocytosis and a progressive increase in plasma lysosomal enzyme levels when tested at 60-min post-trauma. A significant inverse correlation was found between the plasma lysosomal enzyme levels and Kupffer cell phagocytosis after trauma. The functional significance of the relationship between these two

parameters and its importance in shock survival remain to be determined.

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