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Circulating Reticuloendothelial Depressing Substance following Thermal Injury and Intestinal Ischemia (41100)[1]

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Abstract. This study was carried out to determine if a reticuloendothelial (RE) depressing substance is present in the circulation following thermal injury and intestinal ischemia. Plasma extracts were prepared from blood collected from animals at 3 hr after thermal injury or after 3 hr of intestinal ischemia. RE depressing activity was assayed by measuring the colloidal carbon clearance rate in rats or mice following the injection of plasma extracts. Control animals never demonstrated the presence of RE depressing activity. Thermal injury in dogs and rats produced consistently detectable circulating levels of RE depressing activity. Acid hydrolysis of plasma extracts from thermally injured dogs destroyed the RE depressing activity. Intestinal ischemia in dogs resulted in detectable RE depressing activity in portal vein blood. These findings indicate that an RE depressing substance may contribute to the RE depression which occurs following thermal injury and intestinal ischemia.

The studies of Blattberg and Levy first demonstrated that a reticuloendothelial (RE) depressing substance was present in the circulation following experimental hemorrhagic shock and intestinal ischemia (1, 2). Further studies demonstrated that this substance is a small peptide (molecular weight of about 700) which would depress the reticuloendothelial system (RES) in a dose-related manner (3). Other investigators have confirmed that a RE depressing peptide is present in the circulation following hemorrhagic shock (4, 5). It was also shown that steroid treatment prevented the formation of RE depressing substance during hemorrhagic shock, suggesting a role for lysosomal enzymes in the elaboration of this substance (5). The injection of RE depressing substance was found to increase susceptibility to endotoxin and traumatic shock (6). Thus, it was concluded that this RE depressing substance contributed to the depression of RES phagocytic function and impairment of host defense during shock.

The present study demonstrated that an RE depressing substance is present in the systemic circulation following thermal injury in dogs and rats. RE depressing activity was also found following intestinal ischemia. These data indicate that an RE depressing substance may contribute to the RES depression which occurs following thermal injury (7, 8) and intestinal ischemia (9).

Methods. *Thermal injury.* Male dogs weighing 15–25 kg were anesthetized with sodium pentobarbital (30 mg/kg) and arterial blood pressure was monitored via a femoral artery. The fur was clipped and the dorsum was immersed in 90° water for 30 sec. The burn involved an estimated 30% of the body surface area. At 3 hr after thermal injury heparin was injected (400 units/kg) and blood was collected from the portal vein. In two animals a 50-ml arterial blood sample was collected before the burn and at 3 hr after the burn.

Male Sprague–Dawley rats were anesthetized with ether and the dorsum was immersed in 90° water for 30 sec. This technique produces thermal injury to 26–28% of the body surface area (10). Three hours after the injury the animals were heparinized and blood was collected from the interior vena cava. Blood from 20 injured and 10 control rats was required to give the necessary volume of plasma extract.

Intestinal ischemia. Male dogs were anesthetized with 25 mg/kg sodium pentobarbital and arterial blood pressure was

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monitored. A midline abdominal incision was made, the spleen was contracted with epinephrine, and the splenic blood supply was permanently occluded. The celiac artery, superior mesenteric artery, and the inferior mesenteric artery were clamped for 3 hr. In two animals only the superior mesenteric artery was clamped for 3 hr. Heparin (400 units/kg) was injected 5 min before releasing the arterial clamps. Immediately before releasing the arterial clamps, the portal vein was ligated close to the liver. Following release of the arterial clamps as much blood as possible was collected from the portal vein. In four animals 50–100 ml of blood was also collected from a femoral artery. Control animals were treated in the same manner except that the mesenteric arteries were not clamped.

Preparation of plasma extracts. The blood was centrifuged and the plasma proteins were precipitated with 2 vol of cold 15% trichloroacetic acid (TCA). Following centrifugation, the clear supernatant was extracted three times with an equal volume of cold ethyl ether to remove the TCA. Following lyophilization, the dry material was dissolved in a volume of water 12 times less than the volume of plasma and the pH was adjusted to 7.8 with NaOH. Ninhydrin-reactive nitrogen content of the extracts was determined using the method described by Rosen using tyrosine standards (11). Sodium content of the plasma extracts was determined with flame photometry.

Portal vein plasma extracts from four dogs subjected to thermal injury were combined and an aliquot was hydrolyzed. The hydrolysis was carried out in 6 *N* HCl at 105° for 16 hr under nitrogen. The HCl was removed by lyophilization and the hydrolyzed material was assayed for RE depressant activity.

Assay of RE depressing substance. RE depressing substance activity was determined by comparing the colloidal carbon clearance rates in groups of rats or mice injected with either saline or plasma extracts. This assay was originally developed for use in rats by Blattberg and Levy (3). Male Sprague–Dawley rats weighing 200–250 g or Swiss–Webster male mice weighing 20–30 g were used as assay animals. Rats were anesthetized with sodium pentobarbital (30 mg/kg, iv) and heparinized (100 units/100 g), mice were lightly anesthetized with ether and similarly heparinized. Plasma extracts or saline were slowly injected (over 2–3 min) iv via the dorsal penis vein at a dose of 0.7 ml/100 g. At 15 min after injection of the extracts, the clearance rate of colloidal carbon was determined. Colloidal carbon (C11/1431a, Pelikan, Accurate Chemical Corp., Hicksville, N.Y.) without added gelatin, was injected iv (4 mg/100 g in rats, and 8 mg/100 g in mice) and small blood samples (0.1 ml in rats and 0.02 ml in mice) were taken from the tail at 2-min intervals for 10 min. The samples were lysed in 0.1% sodium carbonate and optical density was measured at 650 nm against a blank containing blood taken prior to the injection of the colloidal carbon. The clearance half-time was determined from semilogarithmic plots of optical density against time. Phagocytic index (K) was calculated from the expression: $K = 0.301/\text{half-time}$, where 0.301 is the $\log_{10}$ of 2 and half-time is in minutes. Phagocytic index values were expressed as the mean ± SE and compared using the Student's t test with the confidence level set at 95%. A significant difference in phagocytic index between a group of assay animals injected with saline and a group injected with plasma extract was taken as an indication of the presence of RE depressing substance activity in the plasma extract. In this way the assay provides a qualitative measure of RE depressing activity. Preliminary studies indicated that rats and mice exhibit similar sensitivity in the detection of RE depressing activity.

Results. RE depressing activity could not be detected in portal vein plasma extracts obtained from control animals (Table I and Table II). Thermal injury produced detectable RE depressing activity in portal vein plasma extracts in each of four dogs (Table I). In two dogs preburn arterial plasma extracts had no activity while postburn arterial plasma extracts contained activity. Thermal injury in rats was also associated with RE depressing activity in plasma extracts, while no activity was

TABLE I. RE DEPRESSING ACTIVITY FOLLOWING THERMAL INJURY[a]

Control					
(Portal vein plasma extracts)					
Control 1	Saline	0.0532 ± 0.0050[b,c]			
	Extract	0.0502 ± 0.0052			
Control 2	Saline	0.0836 ± 0.0108[d]			
	Extract	0.0891 ± 0.0111			
Control 3	Saline	0.0662 ± 0.0040[d]			
	Extract	0.0569 ± 0.0073			
Thermal injury in dogs					
(Portal vein plasma extracts)					
Experimental 1	Saline	0.0438 ± 0.0025[c]			
	Extract	0.0324 ± 0.0024	$P < 0.05$		
Experimental 2	Saline	0.0752 ± 0.0055[c]			
	Extract	0.0552 ± 0.0037	$P < 0.05$		
Experimental 3	Saline	0.0500 ± 0.0016[c]			
	Extract	0.0277 ± 0.0017	$P < 0.05$		
Experimental 4	Saline	0.0752 ± 0.0055[c]			
	Extract	0.0459 ± 0.0028	$P < 0.05$		
(Arterial plasma extracts)					
		Preburn		Postburn	
Experimental 5	Saline	0.0488 ± 0.0033[d]	Saline	0.0769 ± 0.0123[d]	
	Extract	0.0533 ± 0.0098	Extract	0.0448 ± 0.0039	$P < 0.05$
Experimental 6	Saline	0.0599 ± 0.0073[d]	Saline	0.0499 ± 0.0073[d]	
	Extract	0.0513 ± 0.0130	Extract	0.0392 ± 0.0059	$P < 0.05$
Thermal injury in rats					
(Inferior vena cava plasma extracts)					
		Control		Burn	
	Saline	0.0663 ± 0.0032[d]	Saline	0.0663 ± 0.0032[d]	
	Extract	0.0691 ± 0.0067	Extract	0.0391 ± 0.0070	$P < 0.05$

[a] Thermal injury consisted of a 30% body surface area scald (30 sec in 90° water). At 3 hr after injury blood was collected from the femoral artery or portal vein in dogs and from the inferior vena cava in rats. Plasma extracts were prepared from blood samples from individual dogs and from pooled blood samples from 10 control rats and from 20 burn rats. Plasma extracts were concentrated to 12 times plasma concentration. Controls were followed for 3 hr without thermal injury.

[b] Values are phagocytic index determined from the clearance rate of colloid carbon in assay rats or mice following the injection of saline or plasma extracts. Data are expressed as the mean ± SE with five to seven assay animals per group.

[c] Rats used as assay animals.

[d] Mice used as assay animals.

present in control rats (Table I). Acid hydrolysis of portal vein plasma extracts from dogs subjected to thermal injury was effective in destroying the RE depressing activity (Fig. 1). Arterial blood pressure did not fall below the preburn level during the 3-hr observation period following thermal injury in dogs.

Intestinal ischemia consisting of clamping only the superior mesenteric artery for 3 hr did not result in detectable levels of RE depressing activity (Table II). However, clamping the celiac, superior mesenteric, and inferior mesenteric arteries for 3 hr did produce detectable activity in portal vein plasma extracts. To date, 12 assays on portal vein plasma extracts from 24 animals have consistently contained detectable RE depressing activity. Table II shows data on the analysis of extracts from a group of 3 dogs and a group of 7 dogs. Data are also presented on the analysis of plasma extracts of arterial blood and portal vein samples taken after 3 hr of intestinal ischemia. Portal vein plasma extracts from each of the 4 dogs contained activity while two of the four arterial plasma extracts contained activity (Table II). Arterial blood pressure did

TABLE II. RE DEPRESSING ACTIVITY FOLLOWING INTESTINAL ISCHEMIA[a]

Control				
(Portal vein plasma extracts)				
Control 1	Saline	0.0591 ± 0.0038[b,c]		
	Extract	0.0631 ± 0.0133		
Control 2	Saline	0.0679 ± 0.0071[d]		
	Extract	0.0608 ± 0.0027		
Control 3	Saline	0.0679 ± 0.0071[d]		
	Extract	0.0611 ± 0.0049		
Superior mesenteric artery clamped				
(Portal vein plasma extracts)				
Experimental 1	Saline	0.0445 ± 0.0041[c]		
	Extract	0.0425 ± 0.0038		
Experimental 2	Saline	0.0692 ± 0.0147[d]		
	Extract	0.0614 ± 0.0096		
Superior mesenteric, inferior mesenteric, and celiac arteries clamped				
(Portal vein plasma extracts combined from three animals)				
	Saline	0.0556 ± 0.0065[c]		
	Extract	0.0323 ± 0.0023	$P < 0.05$	
(Portal vein plasma extracts combined from seven animals)				
	Saline	0.0619 ± 0.0147[d]		
	Extract	0.0279 ± 0.0026	$P < 0.05$	
	Arterial plasma extracts		Portal vein plasma extracts	
Experimental 1	Saline	0.0945 ± 0.0127[d]	Saline	0.0945 ± 0.0127[d]
	Extract	0.0648 ± 0.0084 ($P < 0.05$)	Extract	0.0528 ± 0.0059 ($P < 0.05$)
Experimental 2	Saline	0.1152 ± 0.0017[d]	Saline	0.1152 ± 0.0017[d]
	Extract	0.0642 ± 0.0107 ($P < 0.05$)	Extract	0.0545 ± 0.0116 ($P < 0.05$)
Experimental 3	Saline	0.0513 ± 0.0060[d]	Saline	0.0513 ± 0.0060[d]
	Extract	0.0546 ± 0.0087	Extract	0.0395 ± 0.0028 ($P < 0.05$)
Experimental 4	Saline	0.0584 ± 0.0080[d]	Saline	0.0584 ± 0.0080[d]
	Extract	0.0461 ± 0.0056	Extract	0.0370 ± 0.0040 ($P < 0.05$)

[a] Intestinal ischemia consisted of clamping the indicated arteries for 3 hr. Immediately following release of the clamps blood was collected from the portal vein and the femoral artery. Plasma extracts were concentrated to 12 times plasma concentration. Control animals were followed for 3 hr without intestinal ischemia.

[b] Values are phagocytic index, as determined from the clearance rate of colloidal carbon in assay rats or mice following the injection of saline or plasma extracts. Data are expressed as the mean ± SE with five to seven assay animals per group.

[c] Rats used as assay animals.

[d] Mice used as assay animals.

not decrease during the period of intestinal ischemia.

Analysis of the plasma extracts for ninhydrin-reactive material revealed that extracts from intestinal ischemia and thermal injury animals contained twice as much of this material as plasma extracts from control animals (Table III). However, the sodium concentration in plasma extracts from intestinal ischemia and thermal injury animals was not different from the sodium concentration in plasma extracts from controls.

Discussion. Thermal injury consistently produced detectable circulating RE depressing activity. While other investigators have reported that RE depressing substance is formed during hemorrhagic shock and intestinal ischemia (1–5), thermal injury has not been studied by others in this regard. This finding indicates that RE depressing substance may contribute to the

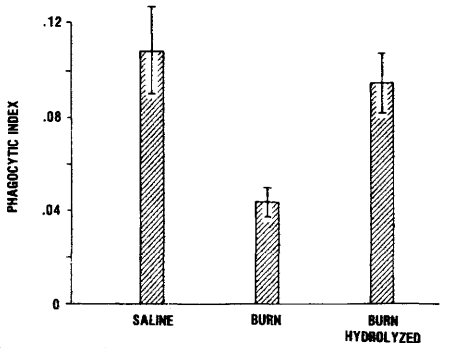

FIG. 1. Analysis of a plasma extract combined from four dogs subjected to thermal injury for RE depressing activity before and after acid hydrolysis. Assay mice were injected with either saline, plasma extract, or hydrolyzed plasma extract 15 min prior to measurement of phagocytic index using colloidal carbon. The plasma extract was at 12 times plasma concentration. There is a significant difference ($P < 0.05$) between the groups injected with saline and the plasma extract from burn animals. Data are presented as the mean ± SE with six assay mice per group.

depression of RES phagocytic function following thermal injury (7, 8).

It has been postulated that intestinal ischemia is necessary for the formation of RE depressing substance (1, 2, 12, 13) and that lysosomal enzymes play a role in the elaboration of this peptide (5). Turner *et al.* have shown that dogs subjected to the degree of thermal injury employed in the present study are able to maintain normal arterial blood pressures even though cardiac output decreased 40% suggesting that some degree of intestinal ischemia was produced due to vasoconstriction (14). While the degree of intestinal ischemia produced by arterial occlusion is probably more intense than that produced by vasoconstriction, the factors responsible for the elaboration of this substance following thermal injury remain to be fully elucidated.

The detection of RE depressing activity in portal vein plasma following intestinal ischemia is consistent with the findings of Blattberg and Levy (1–3). Occlusion of the celiac, superior mesenteric and inferior mesenteric arteries was found to be necessary for the formation of detectable activity. The presence of activity in arterial blood in some of the animals suggests the presence of variable degrees of collateral circulation.

The question of chemical similarity between the RE depressing substances formed following thermal injury and intestinal ischemia remains to be resolved. The RE depressing substance formed with intestinal ischemia has been characterized as a peptide with a molecular weight of about 700 (3). With thermal injury the active component does not precipitate with TCA and activity is destroyed with acid hydrolysis.

The use of plasma extracts at 12 times plasma concentration and at a dose of 0.7 ml/100 g would produce plasma levels in assay animals about 2.0 times that present in the original plasma providing that the RE depressing substance remained only in the vascular compartment. With a molecular weight less than 1000 this peptide would rapidly equilibrate with the interstitial space and reduce the plasma concentration below that present in the original plasma. Results from this laboratory have shown an inability to consistently detect RE depressing activity using plasma extracts at 8 times plasma concentration, however, Blattberg and Levy were able to detect ac-

TABLE III. SODIUM AND NINHYDRIN-REACTIVE MATERIAL CONCENTRATION OF PLASMA EXTRACTS

	Sodium (mEq/L)	Ninhydrin-reactive material (μM/ml tyrosine)	Number of determinations
Control[a]	1372 ± 67	26.1 ± 2.3	3
Thermal injury[b]	1650 ± 150	56.1 ± 9.1	2
Intestinal ischemia[c]	1350 ± 58	54.7 ± 2.3	4

[a] Analysis of plasma extracts from the three control animals shown in Table I.
[b] Analysis of the plasma extracts combined from burn dogs 1–4 and combined from burn dogs 5 and 6 shown in Table I.
[c] Analysis of the four individual experimental animals in which three mesenteric arteries were clamped shown in Table II.

tivity using extracts at 2 or 3 times plasma concentration (2). Unfortunately, these studies may not be comparable due to differences in the assays used to detect RE depressing activity. Similarly, it is difficult to compare this work with those studies which failed to find RE depressing activity because of differences in the methods employed (15–17).

Several investigators have reported the presence of circulating toxins following thermal injury. A large-molecular-weight protein is formed in heated skin which is capable of increasing susceptibility to infection, depressing myocardial function, and impairing cellular metabolism (18–20). The large molecular weight of this protein makes it unlikely that it contributes to the RE depressing activity in the plasma extracts prepared for the present study. Constantian has reported that an immunosuppressive peptide with a molecular weight of less than 1000 is present in the serum of burn patients (21). This peptide can be isolated from normal plasma proteins, and is capable of suppressing phytohemagglutinin-induced proliferation of lymphocytes *in vitro* and the *in vivo* induction of splenic plaque-forming cells in mice (22). Myocardial depressing factor (MDF) has also been shown to be present in the circulation following thermal injury (23). While MDF and RE depressing substance are chemically similar (24), the full identity of these peptides is unknown (3, 25).

The mechanisms of action of RE depressing substance on the clearance of particulates from the circulation is presently unknown. However, it may affect RES function by (a) depressing phagocytic cell function, (b) interfering with the opsonization of material to be phagocytized, (c) reducing hepatic blood flow. Blattberg and Levy were unable to demonstrate that this substance interfered with *in vitro* leukocyte phagocytosis of colloidal carbon or bacteria (1).

The depression of RES phagocytic function which accompanies several forms of shock and injury is considered to contribute to the deterioration of the organism during shock (26–28). The present study indicates that an RE depressing substance may contribute to this RES depression. The relationship of RE depressing substance to other mechanisms such as depressed phagocytic cell function (29), impaired opsonization (28), or inadequate hepatic blood flow (30), remain to be determined. The role of RE depressing substance in the high mortality rate associated with clinical cases of intestinal ischemia (31–33) and in the impaired immune function in burn patients (34, 35) is an interesting area for further investigation.

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