Hydrocortisone and Lysosomal Enzymes in Tourniquet Shock.* (32446)

KENNETH D. SERKES, WILLIAM BERMAN, AND STANLEY LANG
(Introduced by M. D. Pareira)
Department of Surgery, Jewish Hospital of St. Louis and Departments of Surgery and Physiology, Washington University School of Medicine, St. Louis, Mo.

A standardized tourniquet trauma, lethal to approximately 96% of male rats, has been previously reported from this laboratory(1,2). Various fluids in controlled amounts, infused into the traumatized animals over a period of several hours, resuscitated the animals from this hypovolemic shock. Of all of the infusion solutions used, Cohn Fraction II (gamma globulins) of human plasma, suspended in a saline-bicarbonate vehicle was the most effective. One possible explanation for the protective action of such infusions might be the reestablishment of a coordinated, functional relation between pre- and post-capillary resistance, which Lillehei(3) and others believe is disrupted during shock from various etiologies(4).

Other investigators have indicated that hydrolytic enzymes released from lysosomes during injurious processes may also be involved in the development of shock(5,6). Sambhi *et al*(7) and Weissmann and Thomas (8) have published studies in which corticosteroids were used to "stabilize" the lysosomal membrane, thereby preventing or decreasing the release of contained enzymes. Weissmann and Thomas(8) tested the protective effect of corticosteroids by giving varying doses of prednisolone to animals in endotoxic shock. In their studies, sublethal doses of corticosteroids increased survival in the groups of shocked animals (rats and mice); in fact, percentage survival increased as the dosage increased up to 240 mg/kg of body weight. Janoff(6) demonstrated a rapid increase of serum lysosomal acid hydrolases shortly after the onset of shock. He also showed that pre- or post-treatment with corticosteroids lowered the serum titers of these substances. Survival data were not included but it was implied that lysosomal stabilization might prevent "ante-mortem autolysis" from occurring during the early stages of shock, thereby improving survival. Microcirculatory resistive elements represent one of many possible sites where such "autolysis" could have important deleterious effects. Aside from where or how lysosomal enzymes act, there is evidence which suggests that release of these enzymes may play a role in the mechanism of progressive shock.

In the experiments reported here, a correlation was sought between survival after tourniquet trauma and the serum level of two enzymes which have been shown to be of lysosomal origin, namely acid phosphatase and beta-glucuronidase. The effect of hydrocortisone on survival and enzyme levels was also determined.

Methods. Groups of male rats were anesthetized and subjected to tourniquet trauma as standardized in this laboratory(1,2). Upon tourniquet release, each animal had a small polyethylene catheter inserted into its external jugular vein. Infusion fluids of differing compositions were run into these catheters, at a constant rate, for 3 hours. After the catheters were removed, the animals were grouped according to type of infusion; part of each group was observed for 48-hour survival, while another part was bled for serum acid phosphatase and beta-glucuronidase determinations.

Three experiments were performed: 1) The serum enzyme levels and survival were compared in 2 groups of traumatized animals; one infused with saline, the other with hydrocortisone. 2) The second contrasted enzyme levels and survival between a traumatized, saline-infused group and a traumatized, human plasma fraction II infused group. 3) Control enzyme studies were performed on a non-traumatized, non-infused group and on non-traumatized saline, hydrocortisone and fraction II infused groups. Volumes infused were either 3 or 3.5 ml/100 g of body weight for all experiments. Hydro-

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cortisone was given at 3 different dose levels: 7.5, 15 and 30 mg/kg of body weight (Table I).

Of the animals which comprised any infusion group, most were returned to their cages for observation of 48-hour survival, while some were bled immediately from the common carotid artery or by cardiac puncture and the serum separated for enzyme analysis. Assays for betaglucuronidase and acid phosphatase were the methods of Fishman, Springer and Brunetti(9) and Bessey, Lowry and Brock(10) respectively.

Results. The serum acid phosphatase levels showed no relation to hydrocortisone dosage. Instead, the serum level of this enzyme was closely related to the degree of hemolysis in the blood sample. Presumably, erythrocytic acid phosphatase was detected by this assay. Beta-glucuronidase activity was therefore used as the sole indicator of the degree of presumed lysosomal breakdown. Plaice(11) has demonstrated no beta-glucuronidase activity present in the red cell, and therefore, no significant differences in assay level of the enzyme can be shown before and after hemolysis. Beta-glucuronidase serum levels, however, are widely variable from ostensibly similar animals (Table I).

As shown in Table I, hydrocortisone in all dosages reduced the level of the beta-glucuronidase in the blood of traumatized animals. Survival of the hydrocortisone-infused groups, however, was significantly less than that of the saline-infused groups. Further, the level of the enzyme in the serum of fraction II infused groups was not statistically different from that of the saline infused groups (Table I), although fraction II infusion is known to produce statistically superior survival rates (Table III). The dif- any difference between the enzyme levels of non-traumatized, saline-infused and non-infused controls is not significant, nor is there any difference between the enzyme levels of non-traumatized animals infused with saline, hydrocortisone or a fraction II solution. Statistical analysis also indicates no differences existing between the two saline and the fraction II infusion groups of the traumatized animals or animals not infused and whose tourniquets were not removed.

There are statistical differences, however, between the non-traumatized groups, regardless of infusion fluids used, and the traumatized animals receiving either saline or fraction II. The traumatized-hydrocortisone groups infused at 3.5 ml/100 g of body weight

TABLE I. Serum Beta-Glucuronidase Levels.

Condition of animals		Vol of infusion ml/100 g body wt	Infusion fluid	β-Glucuronidase activity (units bacterial enzyme standard)		
				Mean	S.D.*	p†
Non-traumatized	(4)‡	0	None	476.6	± 224.0	
"	(3)	3.0	Saline	294.4	138.4	= .40
"	(3)	3.0	Fraction II	389.9	125.7	> .50
"	(6)	3.5	15 MG F §	319.9	80.1	> .30
4 hr. tourniquets	(13)	3.5	Saline	589.6	298.0	
"	(6)	3.0	"	745.7	270.0	> .40
"	(6)	3.0	Fraction II	720.4	355.8	> .50
"	(3)	3.0	15 mg F	343.5	183.3	= .30
"	(6)	3.5	7.5 mg F	206.6	50.5	= .02
"	(4)	3.5	15 "	153.3	68.5	= .02
"	(4)	3.5	30 "	49.0	43.5	< .01
Tourniquets not released	(5)	0	None	613.3	148.7	= .90

* = Standard deviation.

† = Probability from student t distribution. Comparisons in non-traumatized groups were made to the non-infused control. Comparisons in the tourniquet traumatized groups were made to the saline infused control which was infused at the rate of 3.5 ml/100 g of body wt.

‡ No. of animals.

§ = Hydrocortisone (Upjohn's Solu-cortef).

TABLE II. Statistical Analysis.*

Comparisons		Mean	S.D.†	Probability
Pooled non-traumatized groups	(10)‡	395.9 ± 143.0		
VS				
Pooled traumatized groups except hydrocortisone infusions	(30)	650.9	333.0	=.50
Non-traumatized—3.5 ml/100 g body wt containing 15 mg hydrocortisone	(6)	319.9	80.1	
VS				
4 HRμ tourniquet trauma—3.5 ml/100 g body wt containing:				
7.5 mg hydrocortisone	(6)	206.6	50.5	.1 <p <.05
15.0 "	(4)	153.3	68.5	.05<p <.02
30.0 "	(4)	49.0	43.5	p <.005

* See text for further explanation.
† = Standard deviation.
‡ = No. of animals.

are statistically different from any other traumatized groups and also different from their own control (15 mg hydrocortisone infusion at 3.5 ml/100 g of body weight in non-traumatized animals) at dose levels of 15 and 30 mg of hydrocortisone. These evaluations are summarized in Table II.

Finally, combined survival for the saline-infused groups after the tourniquet trauma was about 50% (Table III), which provided a good base line for comparison of the therapeutic infusions.

Discussion. Hydrocortisone did effectively lower the level of beta-glucuronidase in the blood, presumably by stabilizing lysosomal membranes whose fragility increased with the onset of shock(5,6). However, traumatized rats infused with a hydrocortisone solution showed a lesser survival rate than did rats infused with normal saline solutions. Lillehei *et al* in their comprehensive review on *The Nature of Shock*(3), state that corticosteroids combat hypovolemic shock by virtue of their vasodilating action. However, endotoxic shock was the experimental shock used to demonstrate steroid protection. It has been established that in tourniquet trauma, acute hypovolemia is the primary cause of shock.

TABLE III. 48-Hour Combined Survival Data.

Infusion	Lived	Died	% Survival
Saline	19	21	47.5
Hydrocortisone	6	29	17.2
Fraction II	9	3	75.0

The vasodilating action of corticosteroids might be expected to increase the effects of hypovolemia by causing pooling and decreased venous return. Conversely, if hypovolemia has not occurred on a large scale, as in endotoxic shock, vasodilation, along with fluid replacement would lead to improved tissue perfusion and, in turn, to improved recovery. After a lethal tourniquet trauma saline replacement alone, in amount of 7% of the body weight, resuscitates a very high percentage of animals. Such effective results from fluid replacement alone suggest that hypovolemia, rather than irreversible autolysis, determines survival after tourniquet shock. The vasodilation produced by hydrocortisone, in sublethal doses, may contribute to the decreased survival of such animals in tourniquet shock.

It is possible to postulate the converse of the main thesis, namely that high levels of lysosomal enzymes in the blood favor survival from tourniquet shock. This seems unlikely in view of the finding that serum levels of beta-glucuronidase in the fraction II infused animals were statistically the same as those in the saline-infused groups. Fraction II infusion, known to increase survival from tourniquet shock, did not affect enzyme levels in the serum. There was no relation between 48-hour survival and the serum enzyme levels.

Finally, the time of administration of corticosteroids in resuscitative experiments is obviously fixed. The corticosteroids can be administered only after the trauma. Weissmann(5), however, has shown that lysosomal

enzyme levels increase rapidly after the onset of shock. It is possible that corticosteroid application would be most effective when used prior to insult as, indeed, Weissman and others(8) have demonstrated in other shock-producing traumas.

Summary. Hydrocortisone administered to tourniquet traumatized rats, in infusion fluids, was effective in lowering the levels of beta-glucuronidase in the serum. However, survival in hydrocortisone-infused animals was less than in saline-infused animals. Moreover, an effective infusate containing Cohn Fraction II of lyophilized, reconstituted, human plasma did not significantly alter the level of beta-glucuronidase in the blood. Hydrocortisone, as given in these experiments, proved an ineffective agent for resuscitation from tourniquet shock. Serum levels of beta-glucuronidase, presumed to reflect lysosomal enzyme release, did not correlate with survival.

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Rate of Initial Entry of Ca^{47} and Sr^{85} from the Intestine into the Vascular Space.* (32447)

HIRAM HART AND HERTA SPENCER
Department of Physics, City College of The City University of New York, and Medical Physics Laboratory, Division of Neoplastic Medicine, Montefiore Hospital, New York City
Metabolic Research Unit, Veterans Administration Hospital, Hines, Ill.

Extensive studies have been previously performed in this Research Unit on the absorption and excretion of both radioactive calcium and radioactive strontium in man (1-4). The net absorption as well as the true absorption, taking into consideration the endogenous fecal excretion, has been determined from plasma levels, from cummulative fecal and urinary excretions, and from a comparison of the plasma levels following a single oral and a single intravenous dose of the radioisotope in the same person(5). However, the dynamics of the rate of entry of the radioisotopes from the intestinal tract into the blood stream have not been completely clarified. In this study the rate of the initial entry of both radiocalcium and radiostrontium has been determined. The initial entry rate must be differentiated from the integrated steady state rate of absorption along the entire gastrointestinal tract. The initial entry rate denotes entry of the radioisotope from one compartment into another compartment without consideration of recirculation or feedback and reflects the gradual passage of the remaining unabsorbed dose through different portions of the intestinal tract exhibiting different transport activity. It is calculated by adapting an integral equation approach for use with a digital computer. A short review of the history of this approach has been published recently (6).

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Material and methods. Three male patients with mild osteoporosis ranging in age from 42-