Lymph Lysosomal Enzyme Acid Phosphatase in Hemorrhagic Shock

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THE ROLE of the lymphatic system in hemorrhagic shock has been re-emphasized in the last decade as both a protein and a volume restorer.[1,9,23,27] More recently it has been implicated in the carriage of splanchnic metabolic products and lysosomal enzymes released during shock to the general circulation.[2,7,10,16,36] The lethality and tissue toxicity of shock has been related to the release of such factors.[7] Implicated have been lysosomal enzymes,[13,36] vasoactive compounds,[22] adenine nucleotides,[36] bacterial endotoxins,[3,26] and more recently, myocardial depressant factor.[26,38,39] However, the release of a measurable quantity of lysosomal enzymes and presumably other products, as well as their effect on the shock animal has been questioned.[31]

This study was undertaken to investigate the quantitative release of the lysosomal enzyme, acid phosphatase, into the serum and thoracic duct lymph during carefully monitored standard lethal canine hemorrhage shock of two and one quarter hours' and four hours' duration. The two shock models are contrasted to determine the influence of time and nature of shock on enzyme release. In addition, the cumulative effect of collected lymph serial infused into normal recipient animals was assessed by monitoring for signs of hemodynamic, metabolic or tissue abnormalities indicating of a toxic effect of shock lymph.

Methods

Fasted, splenectomized, 15–25 Kg. mongrel dogs were anesthetized with intravenous pentobarbital (25 mg./Kg.) and anticoagulated with 250 units/Kg. of intravenous aqueous heparin. By means of a Harvard pump respirator, a Po_2 above 80 mm. Hg and a Pco_2 above 35 mm. Hg was maintained to minimize the effects of hyperventilation upon lactate determinations. A heating blanket kept the temperature at 37° C. monitored by an esophageal temperature probe. Continuous intravenous normal saline was administered at a rate equal to the blood removed for sampling. Femoral arterial, portal and central venous pressures were monitored via #16 polyethylene catheters connected to pressure transducers and amplified on a Sanborn polygraph recorder. In one half the animals the cervical thoracic duct was exposed and catheterized. Arterial acid phosphatase, hemoglobin, lactate, pyruvate, pH and Po_2 values were determined during the control period and at pre-determined intervals in all groups.

Liver and ileal biopsies were performed upon opening the abdomen, during shock and again 1 hour after retransfusion of blood. Procaine penicillin 600,000 U. and streptomycin 500 mg. I.M. were administered at the conclusion of the acute experiment and the convalescences of the animals followed daily until day 7 or death.

The Animals Were Divided into Two Groups

Group I—Standard 2¼ Hour Shock

Dogs were bled to a mean arterial blood pressure of 50 mm. Hg via inferior vena caval catheters into ACD vacuum bottles at a rate not exceeding 50 cc. per minute. Pressure was maintained at this level for 90 minutes, then reduced to 30 mm. Hg by additional bleeding and maintained at this level for 45 minutes. Retransfusion of shed blood buffered with sodium bicarbonate to pH 7.3–7.4 and warmed to 37° C., was then accomplished in 15

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minutes. Five ml. of calcium gluconate was administered with each unit of blood. In six animals the cervical thoracic lymph duct was cannulated in the others and cervical duct only exposed. Lymph was collected by gravity drainage into graduated tubes and flow expressed in ml./Kg./15 minutes. Lymph acid phosphatase was determined and expressed as total content per unit time.

Lymph collected from each thoracic duct fistula animal was infused into a normal recipient partner dog. The recipient animal was lightly anesthetized and catheters placed in the femoral artery, inferior vena cava, and portal vein for pressure monitoring. Heart rate and respiration were followed. Control and interval arterial bloods were drawn for acid phosphatase hemoglobin, lactate, pyruvate, pH and Po_2 determinations. An aliquot of accumulated lymph was saved for analysis and residual lymph rapidly infused into the inferior vena caval catheters of recipient animals at the end of each 15 minutes collecting period. The data were collected and parameters monitored during the control, shock, and retransfusion periods. In addition, liver biopsies were performed pre and post lymph infusion, and repeated on day 1, 3, and 5 post infusion.

Group II—Standard 4 Hour Shock

Twelve dogs were bled to a mean arterial blood pressure of 40 mm. Hg via an inferior vena caval catheter into ACD vacuum bottles at a rate not exceeding 50 cc. per minute. They were maintained at this pressure for 4 hours, when retransfused of their then buffered warmed shed blood was accomplished over a 15-minute period. Five ml. of calcium gluconate were administered with each unit of blood retransfused. One half the animals were prepared with a cervical thoracic duct lymph fistula and the other half had a sham procedure.

Collected lymph was infused into normal recipient animals prepared as in Group I and similar studies carried out.

Plasma Acid Phosphatase Assay

The acid phosphatase of lymph and plasma was assayed according to the colorimetric method of C. F. Boehringer and Sons. Aliquots (0.2 ml.) were incubated with 0.0055M p-nitrophenylphosphate in 0.05M citrate buffer, pH 4.8 at 37° C. for 30 minutes. Values are expressed in milliunits per ml. One milliunit corresponds to 0.06 millimole units according to Bessey-Lowry. Normal values in human serum of total acid phosphatase are up to 11 milliunits per ml. Normal values in dogs have not been defined.

Acid Phosphatase Correction Factor

Hemoglobin assays were performed according to the rationale and protocol of Sutherland.[36] Hemoglobins were determined according to the method of Drabkin using the Coleman junior spectrophotometer at a wave length 540 um., and corrections applied according to a preconstructed hemoglobin enzyme calibration curve.

Determinations of pH and portal pressures of oxygen were made using an IL blood gas analyzer (Model 113).

Lactate and pyruvate determinations were performed by ultraviolet methods using commercially available kits (C. F. Boehringer and Sons) and results were recorded as mg. per 100 ml. of whole blood.

Results

Group I—Standard 2¼ Hour Shock

Mean control thoracic duct lymph flow was 0.59 ml./Kg./15 minutes (Fig. 1). This progressively fell 46% to 0.32 ml./Kg./15 minutes by 135 minutes. Retransfusion produced a peak flow of 1.06 ml./Kg./15 minutes, or, an 80% rise over control. Total control lymph acid phosphatase content (APC) was 66 Mr. in 78 cc. (Table 1). Shock lymph contained 6.42 Mr. in 172 cc., while the value for retransfusion lymph was 1,434 Mu. in 306 cc. These figures represent a 345 rise at 135 minutes and an 1,155% rise at 1 hour after retransfusion.

Serum arterial and venous acid phosphatase concentrations reflected the effect of lymph fistula (Table 1). Control, 135 minutes and 1 hour after retransfusion serum acid phosphatase values in the non-drained animals averaged 2.54, 10.61, and 12.47 Mu./ml., respectively, while the lymph fistula dogs averaged 2.36, 5.52, and 7.88 Mu./ml. This represented a 391% versus an 234% maximum rise. The arterial-venous difference is most pro-

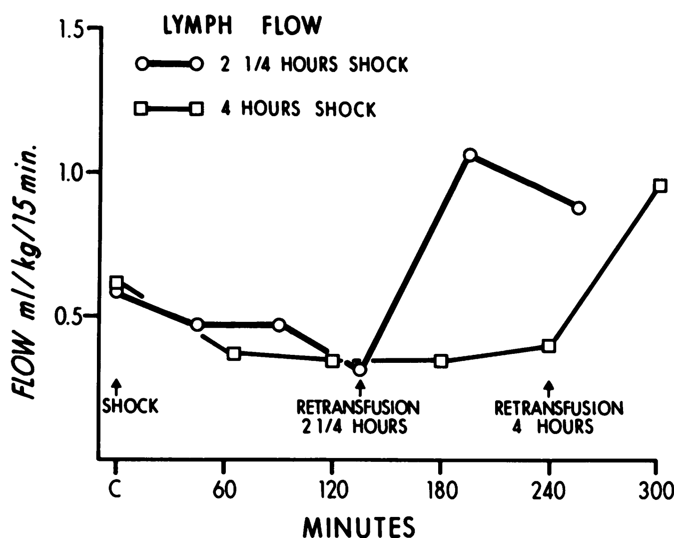

FIG. 1. Lymph Flow fell to 46% of control by 2¼ hours of shock and increased 80% above control one hour after retransfusion. In the 4 hour animals flow dropped to 33% of control and was augmented to 119% above control with return of shed blood.

TABLE 1. *Acid Phosphatase*

	Control Group		Lymph Fistula Group			Lymph Infusion Group
Time	Arterial	Venous	Arterial	Venous	Lymph	Arterial
			Group I (2 1/4 hr. Shock)			
Control	2.54	2.72	2.36	2.52	11	2.32
45 min.	4.11	4.07	2.59	3.83	20	2.02
90 min.	7.71	8.34	4.11	6.06	38	2.19
135 min.	10.61	11.17	5.52	8.18	49	2.12
1 hr.*	12.47	13.25	7.88	10.08	138	3.00
2 hr.*	10.08	12.75	6.70	8.76	101	2.83
			Group II (4 hr. Shock)			
Control	2.12	2.84	2.32	2.65	15	1.65
1 hr.	3.43	4.16	3.63	3.15	15	1.71
2 hr.	5.62	5.82	3.63	4.24	27	2.02
3 hr.	8.32	12.83	5.25	9.50	30	2.29
4 hr.	10.45	18.98	6.82	13.13	94	2.76
1 hr.*	15.35	25.75	10.40	17.14	252	2.57

* Post-retransfusion.

nounced after 2 hours of shock and following retransfusion.

Arterial lactates, pyruvates, pH's and P_{O_2}'s were very similar in absolute values and time courses in both the nondrained and lymph fistula dogs during the control period (Table 2). Lactate values rose from 12.08 mg./100 ml. maximally to 31.62% at 135 minutes of shock, then rapidly fell toward normal levels after retransfusion. Pyruvate levels of 1.08 mg./100 ml. during the control period rose maximally to 1.88 mg./100 ml. by 1 hour after retransfusion. The linear decrease in arterial pH reflected the duration of shock and returned to the 7.30 level by 30 minutes after retransfusion. Arterial P_{O_2} decreased progressively as the duration of shock increased, then with retransfusion rapidly returned to normal.

The infusion of control and shock lymph had no effect on central venous pressure, portal venous pressure, pulse or respiration of recipient animals (Table 4). Likewise, arterial acid phosphatase (Table 1), lactate, pyruvate, pH, P_{O_2} (Table 2) were essentially unchanged by lymph infusion. Post retransfusion lymph, however, caused transient changes in the recipients (Table 4). There was a 20–40 mm. Hg decrease in mean arterial blood pressure for 30–60 seconds (Fig. 2), a slight sustained rise in portal venous pressure of 1–2 mm. Hg for 1 hour, a 15% rise in heart rate for 5–10 minutes, and an increase in respiration of 4 breaths per minute for 5–10 minutes. Serum acid phosphatase rose slightly (Table 1), and then returned toward normal. No change was noted in the recipient lactate, pyruvate, pH and P_{O_2} (Table 2).

Serial liver biopsies in shock animals by H & E stain showed slight cytoplasmic vacuolization progressing to centilobular edema and some vascular congestion after retransfusion. Structural architecture was preserved.

After 2¼ hours of shock ilial biopsies showed apical villus cell vacuolization with some areas of necrosis and slough. Retransfusion resulted in villus core edema and congestion as well as additional apical necrosis.

Serial liver biopsies immediately after lymph infusion, and on days 1, 3, and 5 post-infusion showed no change from control pre-infusion biopsy. All recipients survived and were healthy from their first day of convalescence while all shock dogs died within 48 hours.

TABLE 2. *Metabolic Parameters (2 1/4 Hour Shock)*

Time	P_{O_2}	pH	Lactate	Pyruvate
		Control Group		
Control	111	7.38	11.59	1.13
45 min.	108	7.39	15.16	1.16
90 min.	106	7.20	22.40	1.47
135 min.	103	7.08	37.68	1.67
1 hr.*	115	7.20	22.32	1.90
2 hr.*	112	7.24	16.33	1.33
		Lymph Fistula Group		
Control	101	7.41	12.08	1.08
45 min.	106	7.35	14.72	1.27
90 min.	103	7.24	20.87	1.55
135 min.	108	7.10	31.62	1.74
1 hr.*	118	7.29	24.49	1.88
2 hr.*	112	7.32	17.34	1.74
		Lymph Infusion Group		
Control	108	7.43	9.16	0.92
45 min.	111	7.39	11.24	0.89
90 min.	109	7.38	12.02	0.94
135 min.	108	7.39	12.94	1.14
1 hr.*	110	7.37	11.96	1.24
2 hr.*	114	7.35	12.11	1.35

* Post-retransfusion.

Group II—Standard 4 Hour Shock

Mean control thoracic duct lymph flow was 0.62 ml./Kg./15 minutes (Fig. 1). This progressively fell 48% to 0.32 ml./Kg./15 minutes by 2 hours, down 33% to 0.41 ml./Kg./15 minutes by 4 hours, and then peaked at 1.37 ml./Kg./15 minutes one hour after retransfusion. Total lymph APC in the collected infusions were 90 Mu. in 78 cc. of control lymph, 986 Mu. in 242 cc. of shock

lymph and 2.494 Mu. in 332 cc. of retransfusion lymph (Table 1). These figures represent total elevations of 433% at 2 hours, 1,773% at 4 hours, and 4,947% at 1 hour after retransfusion.

Arterial and venous serum acid phosphatase concentration in lymph fistula dogs averaged 2.32, 3.63, 6.82, and 10.40 Mu./ml. at control, 2 hours of shock, 4 hours of shock, and 1 hour after retransfusion (Table 1). In contrast, non drained dogs averaged 2.12, 5.62, 10.45 and 15.35 Mu./ml. at the same time periods (Table 1).

Arterial lactates, pyruvates, pH and Po_2 were similar in absolute levels and time courses in both the lymph fistula and non-drained dogs (Table 3). Lactate levels rose to a maximum at 1 hour after retransfusion, then gradually fell toward normal. Pyruvate values behaved similarly. The linear decrease in arterial pH, reflected the duration of shock, then slowly rose after retransfusion. Arterial Po_2 decreased slowly during the shock period, then returned to normal with retransfusion.

Similar to 2¼ hours shock animals, infusion of collected control and shock lymph resulted in no change in arterial blood pressure, central venous pressure, portal venous pressure, pulse or respiration (Table 4). Likewise, acid phosphatase (Table 1) and metabolic parameters did not change (Table 3). Post retransfusion lymph infusion did produce transient changes in recipient dogs similar to those seen in 2¼ hour shock group (Table 4). In addition, little change was noted in serum acid phosphatase values (Table 1) and metabolic parameters (Table 3). Again, serial liver biopsies were normal and all infusion animals survival while all shock animals succumbed.

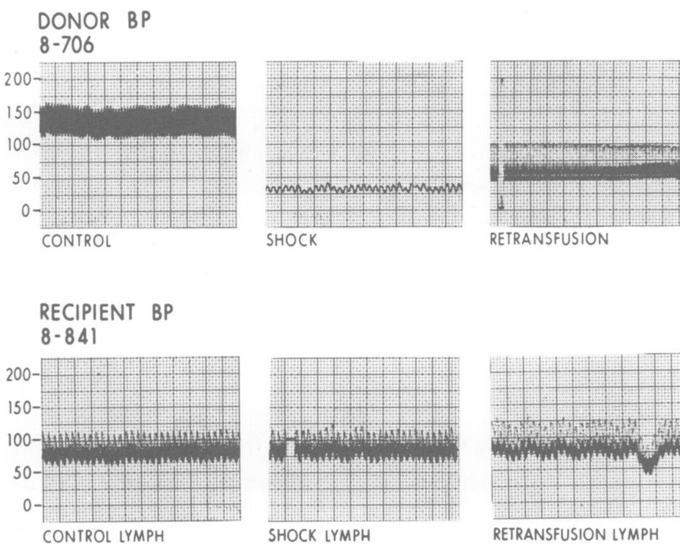

FIG. 2. Blood pressure of a 4-hour shock animal is contrasted to a recipient animal who received serial lymph infusions during the study periods. The only change occurred with infusion of retransfusion lymph where a 30 mm. Hg drop in pressure occur. Maximum depression was noted at 30 seconds and recovery from onset was 70 seconds.

TABLE 3. *Metabolic Parameters (4-Hour Shock)*

Time	P_{O_2}	pH	Lactate	Pyruvate
Control Group				
Control	115	7.39	11.14	1.21
1 hr.	109	7.25	18.40	1.16
2 hr.	110	7.18	25.25	1.69
3 hr.	106	7.01	31.68	1.94
4 hr.	108	6.88	40.12	2.12
1 hr.*	116	7.13	48.60	2.16
Lymph Fistula Group				
Control	119	7.46	10.63	1.38
1 hr.	117	7.35	15.75	1.26
2 hr.	107	7.26	19.50	1.81
3 hr.	105	7.08	24.09	1.88
4 hr.	101	6.98	35.33	2.24
1 hr.*	122	7.10	46.29	2.11
Lymph Infusion Group				
Control	113	7.44	10.14	1.00
1 hr.	110	7.42	9.74	0.91
2 hr.	115	7.39	8.86	0.98
3 hr.	109	7.40	12.01	1.12
4 hr.	112	7.38	11.59	1.31
1 hr.*	113	7.38	12.58	1.28

* Post-retransfusion.

Routine H & E stains of serial liver biopsies in shock animals showed some cytoplasmic vacuolization at 2 hours which increased by 4 hours and after retransfusion. Centrilobular edema and vascular congestion were apparent in the 4-hour and retransfusion specimens. At no time was architectural structure disrupted.

Serial ileal biopsies showed vacuolication of apical villus cells at 2 hours of shock which progressed to frank hemorrhagic necrosis and sloughing at the 4-hour period. With retransfusion, edema and capillary hemorrhage within the villus core is apparent, with progressive apical necrosis.

Discussion

Lysosomes, known to contain a large variety of hydrolytic enzymes, have been found in every cell type studied to date.[10,12] Normally, these enzymes are repressed within a single unit lipoprotein membrane and participate in the digestive process of cellular renewal

TABLE 4. *Lymph Infusion into Recipient Normal Dogs*

Period	Parameters: Blood Pressure	Arterial Venous Pressure	Portal Venous Pressure	Pulse	Respiration
Control	NC	NC	NC	NC	NC
Shock	NC	NC	NC	NC	NC
Retransfusions	C*	NC	C**	C#	C##

NC = No Change.
C = Change.
* Decreased 20 mm. Hg. mean arterial pressure for 30–60 seconds.
** Increased 1–2 mm. Hg for 1 hour.
# Increased 15% for 10 minutes.
Increased 4/m for 10 minutes.

and phagocytosis or to cause autolysis at the time of death.[12] Inappropriate disruption of this membrane can occur with acidosis, decreased osmotic pressure, increased temperature, excess vitamin A, endotoxin, ultraviolet light exposure, and streptolysis O & S *in vitro* stimuli.[12] Severe ischemia has been shown to release approximately 80% of the contained lysosomal enzymes by 3–4 hours.[11] Severe acidosis alone can approximate this figure.[11] Added to this is the fact that the activity of several released hydrolases is optimal at pH 5, a condition approximating that found in ischemic shock tissues.[11,20] If these enzymes are deposited into the cytoplasm, allowing them to act upon essential phosphates and nucleotides as well as degrading and altering biologically active compounds such as proteins, lipids and polysaccharides, the result could lead to: a loss of structural integrity; stimulation of circulating autoantibodies to the altered proteins; the appearance in the general circulation of partially degraded peptide chains which may possess toxic properties to other tissues; and ultimate cellular autolysis.[11] This process in turn, may be propagated to other tissues by release of these enzymes and products into the extracellular fluid with lymphatic and venous effluent carriage to the general circulation.[16,24,27]

This lysosomal theory became attractive to the investigators in the field of shock for three reasons. First, the reduced tissue perfusion, consequent cellular hypoxia and subsequent metabolic acidosis provided a natural medium for the release and activation of lysosomal enzymes. Secondly, the hypoxia leads to a metabolic block at the pyruvate-acetyl CoA level preventing the entrance of substrate into the Krebs cycle for adenosine triphosphase production.[7,30] This inhibition or block may occur at the oxygen dependent respiratory enzyme chain level.[32] It has been shown that just 6 hours of ischema can reduce the cytochrome oxidase system activity 90%.[11] Consequent to the reduced ATP production[6,19,28] the ATP dependent hydrolyzing enzyme (a Na-K ATPase) which is intimately involved with the active transport pump mechanism of the cell and in maintaining an adequate intracellular concentration of potassium,[34] can no longer function efficiently, thus allowing sodium and water into the cells.[18,33] This reduced osmolality of the intracellular medium may be enough to render the lysosomal membranes permeable. The third and final reason is that shock tissues observed by electron microscopy show a gradual increase in numbers of lysosomes as shock progresses.[4,8,19] These organcells advance to terminal phases, characterized by residual bodies and swollen autophagic vacuoles, which evenually dominate the intracellular picture by 4½ hours of shock.[19]

The rise in specific lysosomal enzymes during shock has been demonstrated by three distince methods: the release of specific enzymes into organ effluent blood or lymph[2,10]; histochemical staining technics for specific lysosomal enzymes in liver and intestinal mucosal cells[36]; and increased enzyme activities of tissue homogenates during shock.[20,21] The tissue rise has been contested as an artifact of the procedure used for analysis of tissue lysosomes.[31] Also, species difference in enzyme release and organ susceptibility has questioned the role of lysosomes in shock.

The role of the intestine, despite these criticisms, has been continually emphasized in the pathogenesis of experimental shock as a source of hydrolytic enzymes and lethal factors in irreversible shock.[5,7,14,15,25,35] Since 65–70% of the thoracic duct lymph comes from the intestine and since an analysis of this modality should accurately reflect the metabolic state of the tissue source,[24] it seemed appropriate that this system should be studied during the shock state.[2] Although the lymphatic system has been thought of as a volume and protein restorer,[1,9,23] it is certainly possible that the very conduit of natural compensation against the pathology of shock could also deliver factors responsible for the creation not only of extra-intestinal lesions, but of the experimental irreparable state.[37]

The experimental data indicated that the release of acid pohsphatase and presumably all other lysosomal hydrolases during shock is time related. This is especially well illustrated in the 4-hour shock preparation where two distinct slopes are identified. During the first 2 hours of shock, serum acid phosphatase rose at the rate of 1.5 Mu./ml./hour, while from 2–4 hours, the slope increased to 6.5 Mu./ml./hour. This represents a phase of increasing lysosomal permeability. In addition, the histopathologic findings of hemorrhagic necrosis and sloughing of apical villus cells in the ileum after 2 hours of shock serves as a demonstrable source of these enzymes. It appears from histochemical staining technics[36] that the process of lysosomal permeability begins in these apical cells and progresses down the villus toward the base with time.

The enzymes released are thought to be absorbed into the lacteals as well as into the vast capillary bed of the villi. This is supported by the fact that serum acid phosphatase in the lymph drained dogs rose 259% of control by 4 hours, while the non-drained dogs experienced a 796% rise. This indicates that a majority of the lysosomal enzymes are absorbed via the lacteals. Furthermore, with retransfusion of shed blood, a maximum 20,700% rise in lymph APC occurred, while serum acid phosphatase rose only 1,101%. Of additional interest is the venous acid phosphatase concentration remained higher than arterial during shock which could reflect clearing of some enzyme by either the lung or heart.

There is evidence that lysosomal hydrolytic enzymes released during shock may act both locally and at dis-

tant sites as well. In the intestine a marked decrease is noted in the mucin coat [36] as a result of shock and a secretion of these enzymes in the gastric juice has been associated with an increased production of superficial gastric ulcers.[5,17] In addition, a secretion of these enzymes into the intestinal chyme, along with other tryptic digestive enzymes, results in hemorrhagic necrosis of the intestinal mucosa.[6] Animals surviving standard shock have been noted to develop hemorrhagic necrotic lesions in heart, lung, kidney, and other organs which are thought to be due to the toxic effect of circulating lysosomal enzyme.[7,36]

Since shock lymph is so rich in lysosomal enzymes and presumably other shock products it was anticipated that recipient animals would suffer marked deleterious effects from shock lymph infusion. However, recipient dogs showed only transient, acute and no chronic change in metabolism or hemodynamics and no change in histologic appearance of tissues studied. This speaks strongly against lysosomal enzymes initiating cellular injury in normal tissues in a non-acidotic, normotensive and normovolemic dog. Certainly cells made more permeable by damage to the active transport mechanims or in an acidotic milieu may be less resistant to the effects of the lytic enzymes.[37] The data also evoke skepticism concerning pharmacologic activity of other so-called toxic factors in shock particularly since most are low molecular weight particles which should be contained in the splanchnic lymph drainage.

Finally serum lactate and pyruvate levels were not altered by lymph drainage. This corroborates the findings of previous investigators, that, during shock serum lactate rise is from the skeletal muscle mass [29] entering directly into the circulatory system.

Conclusions

1. Standard canine hemorrhagic shock models of 2¼ and 4 hours were contrasted to determine the influence of time and the nature of shock on the release of the lysosomal enzyme, acid phosphatase, into serum and thoracic duct lymph. Metabolic and hemodynamic parameters monitored the degree of shock. Collected control, shock, and retransfusion period lymph was serially infused into normal recipient animals to evaluate the hemodynamic, metabolic and tissue toxic effect.

2. During the first 2 hours of shock serum acid phosphatase rose an average of 1.5 Mu./ml./hour while from 2 to 4 hours the rate increased to 6.5 Mu./ml./hour. Lymph drainage reduced serum acid phosphatase accumulation from 796% above control at 4 hours to 259%. Lymph flow decreased to 33% of control at 4 hours while lymph acid phosphatase content rose 1,773%. Retransfusion augmented lymph flow above control and increased acid phosphatase content to 4,947% in 4 hours of shock animals.

3. Control and shock lymph infused into normal recipient animals had no effect on metabolic or hemodynamic parameters. Retransfusion lymph infusion decreased mean arterial pressure 20 mm. Hg for up to 30 seconds. Metabolic and tissue changes were not observed.

4. During shock the majority of lysosomal enzymes released from the intestine into the lacteals only after 2 hours of shock and gains access to the general circulation via the thoracic duct lymph.

5. Acid phosphatase contained in lymph during shock and retransfusion was not deleterious to a normal dog hemodynamically, metabolicly or histiologically.

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