Plasma Lactic Dehydrogenase Activity in Experimental Hemorrhagic Shock.* (25046)

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Recent reports describe increased serum lactic dehydrogenase (LDH) activity in several disease states which affect primarily individual tissues(1,2,3). This study was undertaken to determine whether widespread damage produced by hemorrhagic shock would result in elevations of plasma LDH activity.

Materials and methods. A. *Hemorrhagic shock in dogs.* Ten healthy mongrel dogs (average weight 20 kg), premedicated with morphine† (1-2 mg/kg intramuscularly), were bled from femoral artery into an elevated reservoir in parallel with a mercury manometer (4). The artery was cannulated under local anesthesia. Heparin, 40 mg, was administered to prevent clotting Height of reservoir was adjusted so that arterial pressure was 30 mm Hg in 6 dogs, and 40 to 50 mm Hg in 4 dogs. The animals were maintained at these pressures until death. Four ml of blood for LDH assay was obtained at intervals of 1 to 2 hours by needle puncture from uncannulated femoral artery. Eight additional dogs similarly prepared were transfused with all blood in reservoir after 1 to 5 hours of hemorrhagic hypotension. Arterial pressure was maintained at 30 mm Hg until transfusion. Arterial blood was obtained at intervals for assay of plasma LDH. Arterio-venous differences in plasma LDH activity were determined in 3 dogs. Catheters were placed in hepatic and renal vein *via* jugular and femoral vein respectively. Placement was assured by laparotomy under pentobarbital anesthesia. After closure of abdomen and recovery from anesthesia animals were subjected to hemorrhagic hypotension at arterial pressure 30 mm Hg. Simultaneous samples were drawn before hemorrhage and at intervals thereafter from the hepatic, renal and femoral veins and femoral artery until death. Post-mortem examination revealed that catheters had remained in place during experiments. B. *Hemorrhagic shock in rabbits.* Six adult albino rabbits (average weight 2 kg) were subjected to hemorrhagic shock by same technic. Arterial pressure was kept at 50 mm Hg. Arterial samples for LDH assay were taken before shock and at intervals until death. C. *Lactic dehydrogenase assay.* Blood specimens were centrifuged immediately. Plasma was kept at 4°C for less than 24 hours, then analyzed spectrophotometrically for LDH activity by the method of Wróblewski and LaDue (1). One unit of activity was defined as a decrease in optical density of 0.001/ml of plasma on scale of Beckman DU spectrophotometer using 1 cm path length at wave length of 340 mu. Since red cells exhibit much greater LDH activity than plasma(1), the extent of hemolysis was determined. Plasma hemoglobin concentrations of dogs in shock, determined by modified dibenzidine method (5), indicated no significant contribution of hemolysis to the observed elevations in LDH activity. Electrophoretic separation of dog plasma obtained before and during shock was performed on starch supporting medium and the eluates of half inch starch segments were assayed for LDH activity(6). Protein was determined by modification of Folin-Ciocalteau procedure(6). A water flow experiment revealed no differential absorption of LDH activity by starch. D. *Other enzymes in shock.* Simultaneous measurements of activity of leucine aminopeptidase(7), alpha-glucosidase(8), and beta-glucuronidase(9) were made in plasma of 2 dogs during hemorrhagic shock. Ceruloplasmin(10) and glutamic oxalacetic transaminase (GO-T)(11) were assayed in 3 dogs, and malic dehydrogenase (MDH)(3) and alkaline phosphatase(12) in three.

* Aided by grant from Nat. Heart Inst., Bethesda, Md. and Office of Surgeon General, U. S. Army.

† This dose of morphine does not produce increases in plasma LDH activity.

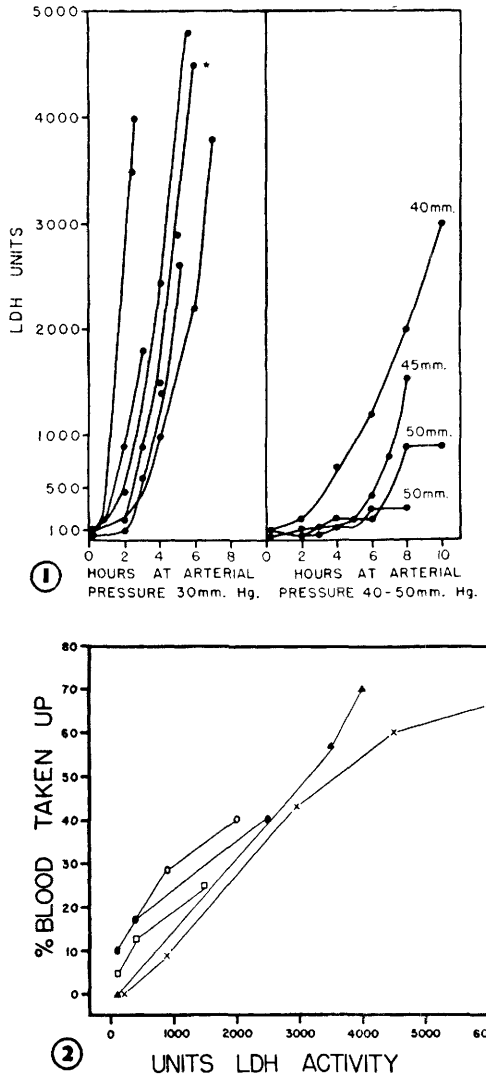

FIG. 1. Plasma LDH activity of 10 dogs during sustained hemorrhagic hypotension. Curve marked with asterisk went on to 16,000 units before death at 9 hr.

FIG. 2. Relationship between spontaneous withdrawal of blood from the reservoir ("taking up") and plasma LDH activity in 5 dogs maintained at arterial pressure 30 mm Hg.

Results. A. *Hemorrhagic shock in dogs.* Plasma LDH activity of 6 dogs maintained at arterial pressure of 30 mm Hg showed initial lag period of 1 to 3 hours followed by sharp rise (Fig. 1). The activity rose from a mean of 75 units before shock to 1000 to 4000 units in all animals at this pressure for more than 2 hours.

In 4 dogs maintained at arterial pressure 40 to 50 mm Hg the lag period was longer, 4 to 6 hours, and elevations immediately thereafter were more gradual. As these animals deteriorated, LDH activity increased markedly so that slope of curve terminally resembled that of dogs bled to arterial pressure 30 mm Hg.

Animals maintained at constant hypotensive levels by elevated reservoir technic eventually exhibit spontaneous return of blood from reservoir, a response called "taking up" (13). Fig. 2 reveals a direct linear relationship between plasma LDH activity and rate of "taking up." To determine whether LDH activity was affected by blood returning from reservoir, samples of reservoir blood throughout hemorrhagic shock were assayed and no change in plasma LDH activity occurred. Furthermore, when a liter of blood permitted to stand *in vitro* for 8 hours was infused by single exchange transfusion into a normal dog, no alteration in LDH activity of recipient occurred.

Of 8 additional dogs bled to an arterial pressure of 30 mm Hg for 1 to 5 hours and then transfused with all blood remaining in reservoir, the 3 animals that died showed progressive increases in plasma LDH activity after transfusion, reaching 90 to 200 times initial values (Fig. 3). These animals exhibited the course and post-mortem findings characteristic of irreversible hemorrhagic

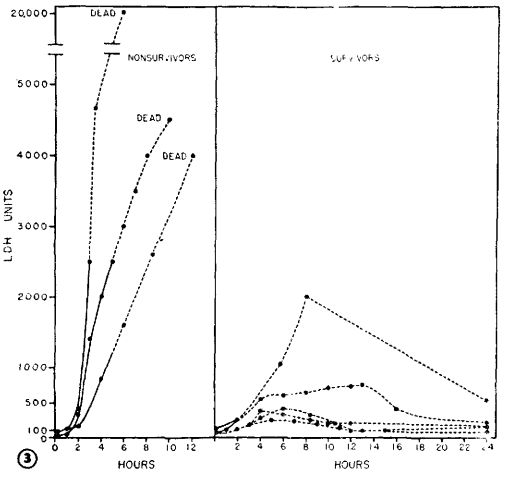

FIG. 3. Plasma LDH activity in 8 dogs bled to arterial pressure 30 mm Hg for 1, 2, 3, and 5 hr and then given replacement transfusion. Solid lines show activities during hemorrhagic hypotension, broken lines after transfusion.

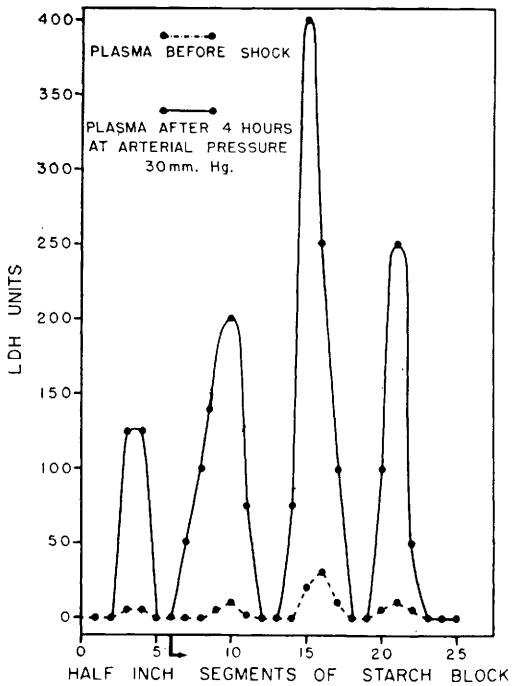

FIG. 4. Electrophoretic pattern of LDH activity in dog plasma before and during hemorrhagic hypotension. Four peaks of LDH activity are present. From the left these are located in gamma globulin, beta globulin, alpha-2 globulin and between alpha-1 globulin and albumin. All are elevated in shock. The origin is at the arrow.

shock. The 5 survivors, bled 1 to 2 hours, showed a post-transfusion rise of 3 to 25 times the initial value. These elevations were sustained for 6 to 12 hours and receded toward normal within 24 hours.

Plasmas from 2 dogs in hemorrhagic shock were fractionated electrophoretically. Fig. 4 shows distribution of plasma LDH activity before and during shock in one animal. Four peaks are present and located in the gamma globulin, beta globulin, alpha-2 globulin, and between the alpha-1 globulin and albumin. All peaks are elevated in shock plasma. Findings in a second experiment were similar.

In all 3 experiments in which the A-V difference in LDH activity was measured, plasma LDH activity in hepatic, renal and femoral venous blood attained consistently higher levels than that of arterial blood. Maximum differences were 200 units in renal and hepatic venous blood and 100 units in femoral venous blood.

B. *Hemorrhagic shock in rabbits.* In 6 experiments plasma LDH activity increased from initial values below 150 units to 1800 to 3200 units within 10 hours. An initial lag period of 2 to 4 hours was observed.

C. *Other enzymes in shock.* Elevations of 3 other enzymes, a dehydrogenase (malic), a transaminase (glutamic oxalacetic) and a phosphatase (alkaline) were observed during hemorrhagic hypotension at 30 mm Hg. In 3 experiments simultaneous determinations of LDH, MDH and glutamic oxalacetic transaminase (GO-T) revealed that the pattern of alterations of these 3 enzyme activities was very similar. Elevations of 20 to 40 times prehemorrhagic values appeared within 8 hours. Another experiment showed elevations of alkaline phosphatase activity which reached 10 times prehemorrhagic values within 7 hours. In all cases a lag phase of 2 hours preceded the elevations.

A number of enzymes assayed simultaneously during sustained hemorrhagic hypotension did not show significant alteration in activity. These include leucine aminopeptidase, alpha-glucosidase, beta-glucuronidase, and ceruloplasmin.

Discussion. These experiments reveal a marked elevation of LDH activity in plasma of dogs and rabbits subjected to hemorrhagic shock. The significance of alterations of this enzyme in shock lies in the relationship these alterations bear to survival or death of an animal. Duration of the lag phase of the activity curve is about equal to duration of hemorrhagic hypotension which most animals will survive if given replacement transfusion.‡ In animals that do not survive hemorrhagic hypotension, whether transfused or not, LDH activity invariably continues to rise until death. In animals that do survive hemorrhagic hypotension, LDH activity continues to rise for several hours after replacement transfusion, but thereafter recedes toward normal. This suggests that a declining curve of LDH activity is a favorable prognostic sign.

Alterations in plasma LDH activity in

‡ Survival is usual if animal is transfused within 2 hours. If transfused after 4-5 hours death is the rule.

hemorrhagic shock coincide with certain other parameters of biological deterioration. Fig. 2 shows a linear relationship between LDH activity and rate and extent of "taking up," an important index of survival(13). The greater the percentage of take up, the less likely is the dog to survive. That the curve of LDH activity reflects extent of trauma imposed is indicated by longer lag period and more gradual elevation of activity in dogs maintained at arterial pressures of 40, 45, and 50 mm Hg as compared to those at 30 mm Hg (Fig. 1).

Since present methods measure LDH activity, not concentration, some factors which affect activity were considered. An inhibitor of LDH activity has been described by Greig in livers of dogs in hemorrhagic hypotension (14). When we mixed shock plasmas of high and low LDH activities, the mean values obtained suggested that an activator or inhibitor substance in plasma was not present. Nor was an activator found in reservoir blood. Alterations in pH are not a likely explanation of the rise in LDH activity, for the pH optima for LDH are alkaline(15) whereas in shock the pH falls. Nor is elevation in activity attributable to concentration of serum proteins, since these do not change significantly(16). Increase in blood pyruvate which occurs in hemorrhagic shock(16) was not permitted to influence measurement of LDH activity, for the pyruvate was removed by incubation prior to LDH assay(1).

The higher LDH activities in hepatic, renal and femoral vein blood compared to that in arterial blood indicate that many tissues may contribute to the elevation in plasma activity. These results are in harmony with a report showing appreciably reduced GO-T activities in homogenates of skeletal muscle, kidney, liver and cardiac muscle in rats after severe anoxia(17).

Starch zone electrophoresis has been used to investigate homogeneity of lactic dehydrogenase. Results indicate that LDH is electrophoretically heterogeneous(18) and that more than one enzyme with LDH activity is present in each of several mammalian tissues(19). The finding of 4 enzymes in dog plasma is in contrast to the 3 enzymes in human plasma and to the 2 in rabbit plasma(15,18). Of the 3 in human plasma, one is elevated in myocardial infarction and a different one in leukemia (15,18,20). In shock in dogs all 4 enzymes are elevated. If these enzymes are derived from different tissues, the elevation of all peaks may be taken to reflect known widespread damage in shock.

Summary. 1. Marked elevations of plasma LDH activity in experimental hemorrhagic shock are described. An initial lag period is followed by a sharp rise to 15 to 50 times the original value. 2. Alterations of plasma LDH activity in shock bear a consistent relationship to such parameters of biological deterioration as rate of spontaneous return of blood from the reservoir to the animal ("taking up") and reversibility to replacement transfusion. 3. Catheterization experiments in which consistent arterio-venous differences were demonstrated indicate that the sources of elevated plasma LDH activity are widespread and include liver, kidney and tissues of an extremity. 4. Electrophoretic separation of normal dog plasma reveals 4 enzymes with LDH activity. All 4 are elevated in plasma of dogs in shock. 5. Marked elevations of plasma GO-T, MDH and alkaline phosphatase were observed in dog plasma during hemorrhagic hypotension. No significant elevations in beta-glucuronidase, alpha-glucosidase, leucine aminopeptidase and ceruloplasmin occurred. 6. These experiments suggest that serial plasma LDH determinations may serve as useful indicators of the extent of biological deterioration in hemorrhagic shock.

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Received April 29, 1959. P.S.E.B.M., 1959, v101.

Action of Semen Inducing Rupture of Yolk Membrane in Chicken Egg. (25047)

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Pincus(1) and Yamane(2) simultaneously reported that cumulus cells which surround the eggs of rabbits were diffused by adding their semen. The latter concluded that 2nd maturation division of the ovum is dependent on penetration of spermatozoon and that it also can be artificially induced by treating with pancreatin. Recently in experiments on fertilization we found that the yolk membrane of hen's eggs was ruptured on addition of semen. These tests were made *in vitro* where artificially ovulated eggs were used. The present study was made to analyze above-mentioned phenomenon.

Methods. Newly laid eggs and ova artificially ovulated *in vitro* according to methods described by Neher, Olsen and Fraps(3) and Saeki and Katsuragi(4) were used. Semen collected from the animal was placed in contact with the yolk membrane of eggs kept in porcelain dishes and incubated at 38°C. Lengths of time required for semen to rupture the vitelline membrane and release of yolk material were recorded. Hyaluronidase activity of fowl semen was estimated by decreasing the viscosity of hyaluronic acid solutions (a modification of McClean's method) (5) and by increasing the area of spread of intradermally injected substances. Purified hyaluronidase "Sprase" of Mochida Pharma. Mfg. Co., Tokyo, was used.

Results. Effect of Semen of Different Animals on Yolk Membrane. Rupture of yolk membrane of hen's eggs is brought about by semen from cocks and from other domestic animals. Length of time required for rupturing the membrane after adding semen is shown in Table I. Vitelline membrane of eggs serving as controls where semen was not added, was kept intact during first 5 days in the following experiment (Table I). The vitelline membrane of artificially ovulated ova was ruptured within 2 hours by cock's semen. The same semen required 6.3 hours to rupture the yolk membrane of normally laid egg. It seemed strange that semen of rabbits, although unrelated to Aves, was more effective than semen from the duck, which is in the same class with the domestic fowl. Boar's semen was least effective.

Action of spermatozoa and semen serum. Spermatozoa were separated from seminal fluid by centrifuging the semen 20 minutes at 20,000 RPM, then both fractions were placed separately on the vitelline membrane of egg yolks. As shown in Table II, the destructive action of spermatozoa was higher than that of semen serum. This suggests that the former contains more effective substance(s) than the latter.