# Plasma and Cardiac Lactic Dehydrogenase Activity in Burn Shock[1] (37035)

D. K. DEETS[2,3] AND V. V. GLAVIANO[4]

*University of Health Sciences, The Chicago Medical School, Chicago, Illinois 60612*

Wróblewski and LaDue (1) showed the presence of lactic dehydrogenase (LDH) activity in human serum and in serum and tissues of the dog. They found that experimental myocardial infarction as early as 6 hr after the infarction leads to an increase in serum LDH activity. Vesell and Bearn (2) reported that plasma subjected to electrophoresis on a starch medium showed the enzyme LDH to be made up of 5 component isozymes. These isozymes were observed to change their relative proportions when an individual organ was diseased as in acute myocardial infarction or hepatitis. In earlier work, Vesell *et al.* (3, 4) reported that widespread tissue damage in hemorrhagic or endotoxin shock resulted in an increase in all isozymes of LDH.

Experiments performed on dogs in burn shock indicated that similar to hemorrhagic and endotoxin shock, a significant elevation occurred in plasma LDH. Further studies were undertaken in the burned dog to determine whether the increase in plasma activity of LDH could originate in part from a release of this enzyme from the heart.

*Methods.* For this study a total of 34 mongrel dogs ranging in weight from 10 to 20 kg were anesthetized with 30 mg/kg of sodium pentobarbital by vein. Through a midline neck incision, the trachea was cannulated and in 16 of 34 dogs the right common carotid artery was catheterized to record mean arterial blood pressure. The animal's hair was shaved from medial portions of the hind limbs and abdomen and as previously described (5), a full thickness skin burn was produced in 9 of the 16 dogs by exposing the lower half of the dog's ventral surface for 3 min in a chamber containing infrared heating lamps. Within 30 sec, heat from these lamps caused a rapid rise in skin surface temperature to 70° while temperature in subcutaneous tissues remained close to the average recorded blood temperature of 40°. The cutaneous burn covered approximately 35% of the body surface area. Before and during a 4-hr postburn period, duplicate samples of venous blood were drawn from a catheter inserted via the right jugular vein into the right ventricle of the heart. After centrifugation, plasma from these blood samples was analyzed for LDH activity using the Determatube LDH kit (Worthington Biochemical Corp.). The principle for determining LDH activity was based on the method of Wacker *et al.* (6), which involves the reduction of NAD to NADH in the conversion of lactate to pyruvate. The reaction was measured in a Beckman DU spectrophotometer using a wavelength of 340 nm. The average change in absorbancy per minute was calculated from measuring the increase at 30 sec intervals for 3 min. Activity of LDH in units per milliliter of plasma was calculated from the change in absorbancy $\times$ 1000 per ml of plasma. The measurement of LDH activity is known to be directly proportional to the quantity of plasma used in the analysis (1). The volume of plasma for all determinations was 0.2 ml, a quantity that was found to yield the maximal activa-

[1] These studies were aided by Contract Nonr 3502(01) NR 101 521 between Office of Naval Research, Dept. of Navy, and the University of Health Sciences/The Chicago Medical School.

[2] Submitted in partial fulfillment of the requirements for the M.S. degree in Physiology.

[3] St. Joseph's Hospital, Chicago, Ill.

[4] Reprint Request from: Dr. V. V. Glaviano, Prof. & Chairman, The Chicago Medical School, 2020 West Ogden Avenue, Chicago, Illinois 60612.

tion rate of the enzyme. Since thermal trauma causes hemolysis of red blood cells, the contribution of LDH from red cells was corrected in each postburn plasma sample by determining the concentration of hematin (7). In all experiments, arterial blood was drawn at predetermined intervals from the right common carotid artery for determination of the hematocrit. Before and during the 4 hr period following the injury, plasma LDH, hematocrit and mean carotid arterial pressure were measured hourly. Measurements in control dogs were made before and after 4 hr in 7 of the 16 dogs subjected to the same procedures in the absence of a burn injury.

To monitor LDH release from the heart, 9 open-chest dogs were prepared with catheterization of the coronary sinus. With the animal on a positive pressure respirator, the chest was opened in the 5th left intercostal space and the heart suspended in a pericardial cradle. Via the left jugular vein a long metal cannula was inserted into the coronary sinus with its opening fixed at the sinus ostia by a ligature. Outflow from the coronary sinus was returned to the animal by a tube inserted into the right atrium via the right jugular vein. By clamping the tube entering the right jugular vein, the coronary sinus blood flow from a T tube could periodically be measured with a graduated cylinder and a stopwatch. Coronary blood flow was expressed in ml/min/100 g of left ventricular cardiac muscle (8). The change in LDH activity attributed to the heart was calculated in units per ml of plasma/min/100 g of left ventricular cardiac muscle from the product of the arterial–coronary venous plasma difference and coronary flow. Six of the 9 dogs with coronary sinus catheterization underwent a thermal burn of 35% of the body surface area while the remaining 3 dogs served as controls. During a control period and at 30 and 60 min following the burn, the coronary sinus flow was measured and simultaneous samples of arterial and coronary sinus blood were drawn for LDH analysis.

To determine the effects of 4 hr of anesthesia in both control dogs and dogs subjected to burn injury, hemodynamic measurements were made in another group of 9 dogs anesthetized with sodium pentobarbital, with 4 of the 9 dogs serving as controls. Brachial artery pressure, cardiac output, central venous pressure, and hematocrit were measured immediately before the burn and every hour during a 4-hr postburn period. The brachial artery was catheterized from the medial surface of the right forelimb. Central venous pressure was recorded from a catheter inserted via the left external jugular vein and passed close to the opening of the superior vena cava to the right atrium. A catheter was also inserted into the right ventricle via the left external jugular vein for collecting a mixed venous blood sample for analysis of oxygen content. Oxygen consumption for determination of cardiac output by the Fick principle was measured for 6 min on a pen recording spirometer containing 100% oxygen and a $CO_2$ absorbant. Oxygen content of anaerobic blood samples taken simultaneously from the right ventricle and brachial artery were analyzed according to the method of Roughton and Scholander (9). Pressures from the brachial artery and central veins were measured with Hewlett Packard 267A pressure transducers. Recordings of these pressures were made on a direct-writing Hewlett Packard recorder. In all experiments an initial dose of 5 mg/kg of heparin sodium was given by vein to prevent the coagulation of blood; thereafter one half of the initial dose was administered every hour during the course of the experiment.

*Results.* Figure 1 compares the average plasma activity of LDH between 7 dogs serving as controls and 9 dogs subjected to a burn injury. The mean control values for plasma LDH activity were 42.4 $\pm$ 18.3 and 46.0 $\pm$ 10.3 units/ml in both groups of dogs. From the figure, it can be noted that burn injury caused a significant elevation of plasma LDH activity within 1 hr, followed by a further increase occurring at the end of the 2nd and 3rd hr. Four hours after the thermal burn, the animals attained a mean level in LDH activity of 137.0 $\pm$ 28.1 units/ml, while control dogs averaged only 44.7 $\pm$ 10.7 units/ml ($p < 0.01$) for the same period.

The output of plasma LDH in units/min/100 g of left ventricle were plotted for each

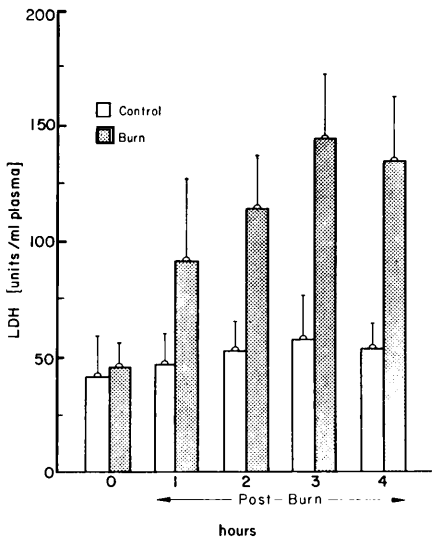


FIG. 1. A comparison between the average total plasma lactic dehydrogenase (LDH) activity found in 7 control dogs and in 9 dogs subjected to an acute cutaneous burn. The standard error of LDH activity for each period is shown on top of the bar.

of nine open-chest dogs in Fig. 2. It can be noted that despite variations in initial levels in the control and burned dogs, the burned group all showed a marked increase in LDH output from the heart within 30 min after burn injury. The release rather than uptake of plasma LDH from the heart was ascertained from the fact that coronary venous blood had a higher level of activity than that found in arterial blood entering the heart. As mentioned earlier, the uptake (or release in this case) of a substance by the heart is calculated from the product of the arterial-coronary venous blood difference of the substance and coronary flow. A negative value for uptake by the heart indicates that the venous effluent in the burned dogs had a higher level of LDH than arterial blood entering the coronary circulation. Within 60 min, 3 of the 6 burned dogs continued to release additional LDH, while the remaining dogs had diminished outputs of the myocardial enzyme. The average change in plasma LDH from the burn group as compared to their control level, was significant at 30 ($p < 0.05$) and 60 min ($p < 0.05$) following the burn injury. Within 60 min, only 1 of the 3 control dogs had a slight efflux of LDH.

The results from studying the hemodynamic effects of an experimental burn injury in the anesthetized dog (Table I) were found to be in agreement with observations previously reported by other laboratories (10–15). Despite some variation in response to burn trauma, significant hemodynamic changes occurred in 5 burned dogs 4 hr after the injury. Statistical differences were noted between the means of the control and the 4-hr post-burn period in cardiac output ($p < 0.02$), total peripheral resistance (TPR) ($p < 0.05$) and hematocrit ($p < 0.02$). Cardiac output decreased 51% from 2.7 to 1.3 liter/min, while in the 4 control animals cardiac output decreased from 2.5 to 2.0 liter/min, to 20%. Central venous pressure, mean arterial blood pressure and heart rate did not show a significant change in either group of animals. Total peripheral resistance rose in the burned dogs from 5,223 to 10,881 dynes-sec/cm$^5$, an increase which in part could be attributed to the increase in hematocrit which rose from 45 to 59%. However, in the absence of a burn injury, the control animals after surgery and 4 hr of anesthesia had a rise in hematocrit of 11% ($p < 0.02$),

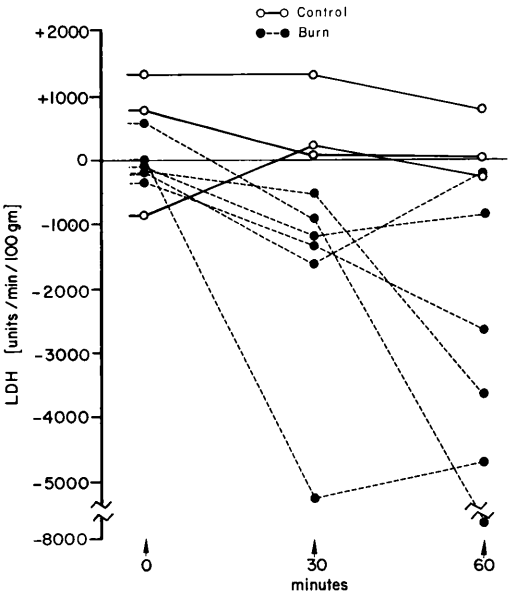


FIG. 2. A comparison between lactic dehydrogenase (LDH) activity released by the heart in 3 control dogs and in 6 dogs subjected to aucte burn trauma for 1 hr.

TABLE I. A Comparison of Hemodynamic Changes in Control Dogs and Dogs Subjected to Burn Trauma.

| | Control | | Burn | |
|---|---|---|---|---|
| | Control | 4 hr | Control | 4 hr |
| Venous Pressure (mm Hg) | −0.1 ± .5[a] | −0.9 ± .4 | −0.5 ± .5 | −2.0 ± .7 |
| Mean Arterial Pressure (mm Hg) | 147 ± 8 | 130 ± 12 | 144 ± 9 | 132 ± 3 |
| Cardiac Output (L/min) | 2.5 ± .3 | 2.0 ± .3 | 2.7 ± .6 | 1.3 ± .4[b] |
| TPR (dynes-sec/cm$^5$) | 5182 ± 873 | 5150 ± 722 | 5223 ± 1451 | 10881 ± 2698[c] |
| Heart Rate beats/min) | 168 ± 6 | 171 ± 22 | 169 ± 31 | 174 ± 33 |
| Hematocrit (%) | 47 ± 1 | 52 ± 2[b] | 45 ± 3 | 59 ± 3[b] |

[a] SEM.
[b] $p < .02$.
[c] $p < .05$.

a rise which indicates that a significant volume of fluid leaves the vascular space during long periods of anesthesia.

*Discussion.* In the present study on burned dogs, an increase in plasma LDH activity was found using the animal as its own control. This increase was significant within 1 hr ($p < 0.01$), with further increases occurring in the 2nd and 3rd hr following the burn (Fig. 1). As in hemorrhagic and endotoxin shock, the elevation in total plasma LDH activity most likely represents a widespread release of the enzyme from many tissues. In sampling coronary venous blood from the heart, a significant increase in LDH activity was observed to occur within 30 min following the injury (Fig. 2). The factors responsible for the myocardial release of LDH in burn shock cannot be delineated from the reported experiments. Possibly one of the factors associated with the release of LDH in the burned animal is an alteration in cardiac intracellular metabolism. A preliminary report from this laboratory shows that the observed lowering in myocardial extraction of pyruvate and the decrease in arterial $pO_2$ and coronary blood flow all point to cardiac hypoxia being present in burn shock (16). Loegering and Critz (17) have shown in the dog that generalized hypoxia caused by breathing gas mixtures low in $O_2$ acts as a potent stimulus to increasing total plasma LDH activity. On the other hand, toxins, or an increase in concentration of normally occurring blood substances in animals subjected to experimental burns may also play a role in the myocardial release of LDH. Using cross-circulation experiments, Baxter (18) suggested a "myocardial depressant factor" as a blood toxin present in the burned dog. Toxins released from burned skin (19, 20) or the elevation in plasma histamine (21–23) as well as the increase observed in plasma catecholamines (24) can in themselves or collectively play a role in the release of myocardial LDH.

The possibility of a neural factor causing the release of tissue LDH in the burned dog was investigated by pretreating dogs with adrenergic blocking drugs. Two dogs were treated with the beta-adrenergic blocking drug, *dl*-propranolol (1 mg/kg) while one dog was treated with the alpha-adrenergic blocking drug, phenoxybenazmine (5 mg/kg). In all 3 dogs, the rise in total plasma LDH activity in the 4-hr post-burn period was of the same magnitude as that observed in untreated burned dogs. The possibility that high blood levels of histamine may cause the rise in LDH activity was determined by infusing histamine dihydrochloride by vein in doses sufficient to simulate, in a normal dog, the post-burn levels of blood histamine (23). The elevation of plasma histamine to 54 μg/liter, failed to induce a rise in plasma LDH activity.

The results from studying the hemodynamic effects of experimental burn injuries showed that characteristic decline always occurred in the cardiac output (Table I). The fall in cardiac output does appear to be due to an inadequate venous return of blood to the heart as observed from the decrease in central venous pressure, although a statistically significant change was not found, possibly due to individual variations and the small number of experiments. On the other hand,

Gilmore (12) postulated that the decrease in cardiac output in burns was of myocardial origin, since he found little or no change in right atrial pressure. Whether the release of cardiac muscle LDH directly results from an underlying injury to the cardiac pump in burn trauma cannot be determined from the reported experiments. What can be concluded is that dogs subjected to a burn injury will undergo a significant elevation in plasma LDH activity, part of which was attributed to the release of the enzyme by the heart. However, the underlying mechanism for myocardial release of LDH or its significance in terms of cardiac function in the burned animal remains undetermined.

*Summary.* Anesthetized dogs subjected to a standardized cutaneous burn injury covering 35% of their surface area showed a marked increase in total plasma LDH activity. The increase in LDH activity was accompanied, as determined in another group of burned dogs, by a significant decrease in cardiac output and an increase in total peripheral resistance and hematocrit. In control dogs, plasma LDH activity showed little or no change during a 4-hr period, while burned dogs increased their plasma LDH activity from 44.7 to 137.0 units/ml. The negative myocardial uptake of LDH indicated that cardiac muscle in burned dogs released LDH into the circulation. In the absence of burn trauma, dogs subjected to the same experimental procedures had no significant efflux of LDH from the heart. The release of myocardial LDH in the burned animal may possibly be related to the action of a burn toxin, a state of myocardial anoxia or the presence of an underlying injury to the heart.

1. Wróblewski, F., and LaDue, J. S., Proc. Soc. Exp. Biol. Med. **90**, 210 (1955).

2. Vesell, E. S., and Bearn, A. G., J. Clin. Invest. **40**, 586 (1961).

3. Vesell, E. S., Feldman, M. P., and Frank, E. D., Proc. Soc. Exp. Biol. Med. **101**, 644 (1959).

4. Vesell, E. S., Palmerio, C. F., and Frank, E. D., Proc. Soc. Exp. Biol. Med. **104**, 403 (1960).

5. Okamoto, R., Glaviano, V. V., and Pindok, M., Proc. Soc. Exp. Biol. Med. **137**, 347 (1971).

6. Wacker, W. E., Ulmer, D. D., and Vallee, B. L., New Engl. J. Med. **255**, 449 (1956).

7. Cohen, B., and Smith, A. H., J. Biol. Chem. **39**, 489 (1919).

8. Herrman, G. R., Amer. Heart J. **1**, 213 (1925).

9. Roughton, F. J. W., and Scholander, P. F., J. Biol. Chem. **148**, 541 (1943).

10. Blalock, A., Arch. Surg. **22**, 610 (1931).

11. Noble, R. P., and Gregerson, M. L., J. Clin. Invest. **25**, 172 (1946).

12. Gilmore, J. P., and Handford, S. W., J. Appl. Physiol. **8**, 393 (1956).

13. Fozzard, H. A., Ann. Surg. **154**, 113 (1961).

14. Michie, D. D., Goldsmith, R. S., Mason, A. D., Jr., and Moncrief, J. A., J. Trauma **3**, 111 (1963).

15. Moncrief, J. A., Ann. of Surg. **164**, 723 (1966).

16. Glaviano, V. V., Okamoto, R., Kaye, M., and Deets, D., The Physiologist **15**, 147 (1972).

17. Loegering, D. J., and Critz, J. B., Amer. J. Physiol. **220**, 100 (1971).

18. Baxter, C. R., Cook, W. A., and Shires, G. T., Surg. Forum **17**, 1 (1966).

19. Allgower, M., Burri, C., Cueni, L., Engley, F., Fleisch, H., Gruber, U. F., Harder, F., and Russell, R. G. G., Ann. N.Y. Acad. Sci. **150**, 807 (1968).

20. Rosenthal, S. R., Thadhani, K. C., Crouse, G., and Spurrier, W., Ann. N.Y. Acad. Sci. **150**, 792 (1968).

21. Barsoum, G. S., and Gaddam, J. H., Clin. Sci. **2**, 357 (1935).

22. Rose, B., and Browne, J. S. L., Ann. Surg. **115**, 390 (1942).

23. Glaviano, V. V., Suzuki, A., and Cova, R., Proc. Soc. Exp. Biol. Med. **130**, 387 (1969).

24. Goodall, McC., Ann. Surg. **145**, 479 (1957).

Received July 28, 1972. P.S.E.B.M., 1973, Vol. 142.