# Effect of Endotoxemia on Myocardial Cation Concentrations[1] (37571)

ANIECE A. YUNICE, DONALD J. BAXTER, AND ROBERT D. LINDEMAN

*Medical Service, Oklahoma City Veterans Administration Hospital and Departments of Medicine, Physiology and Biophysics, University of Oklahoma College of Medicine, Oklahoma City, Oklahoma 73104*

Marked alterations in myocardial cation concentrations appear rapidly in ischemic and infarcted myocardium (1–3). Sodium and calcium concentrations increase and potassium and magnesium concentrations decrease. Although zinc concentrations decrease only slightly in intact myocardium, significant shifts in the subcellular distribution of radiozinc occur in infarcted myocardium (3).

Circulatory failure has been described in endotoxin shock by numerous investigators (4–8). Controversy still exists as to whether this is due to a fall in cardiac output secondary to pooling or trapping of blood in peripheral vascular beds or to primary pump failure.

The purpose of this study was to determine if a 15-hr period of endotoxemia in dogs would alter myocardial cation concentrations as observed in ischemic or infarcted myocardium.

*Methods.* Nineteen mongrel dogs were placed randomly into two groups, one received an estimated $LD_{50}$ dose of *E. coli* endotoxin (Difco, Detroit), the other served as an untreated control. Eight of 11 dogs (73%) given endotoxin survived the 15-hr period of endotoxemia. Based on previous observations, this dose of endotoxin produced significant metabolic abnormalities over this period of time and sufficient time would be allowed for shifts in cation concentrations to occur. Animals could be sacrificed after overnight endotoxemia and a full work day remained to prepare the subcellular fractions from fresh myocardium by ultracentrifugation. Eight animals served as controls. All persons responsible for analyses were kept blinded.

Each animal received 200 $\mu$C zinc 65 chloride intravenously at 5 PM. The endotoxin-treated animals also received 0.25 to 0.35 mg endotoxin/kg body weight. All animals were followed until 8 AM the following morning. Blood samples were obtained for hematocrit and pH determinations at the beginning and end of the study. Animals then were sacrificed with Nembutal anesthesia; hearts were rapidly removed, placed in cellophane containers and immersed in ice. Three samples of left ventricle were resected from each animal. These were used immediately or stored frozen until analyses were performed. The first sample was used to determine the concentrations of six cations (sodium, potassium, calcium, magnesium, zinc and copper), DNA and RNA. These samples were weighed wet, then dried and reweighed, and finally ashed in a low temperature asher and reweighed a third time. Since wet weight to dry weight to ash weight ratios were essentially the same in endotoxin and control animals, it made no difference which index of myocardial mass was utilized. Ash weight was the index used.

The ash was dissolved in 5 ml of 0.1 *N* perchloric acid. Strontium chloride was added to produce a final concentration of 0.15%. Addition of strontium has the dual purpose of suppressing the effect of phosphates and enhancing the absorption of alkali metals. An aliquot or further dilution of this solution was used to quantify calcium, magnesium, zinc and copper on the Perkin-Elmer 303 atomic absorption spectrophotometer using the three slot Boling burner head with acetylene as the fuel and compressed air as the oxidizer (3). After linearity was established

[1] Grant support: Veterans Administration Hospital, NHLI - HE12882.

with standard solutions prepared in the same diluent, the readings were recorded on a direct concentration readout attachment. Sodium and potassium concentrations were quantified using a flame photometer (Instrumentation Laboratories). Known amounts of calcium, magnesium, zinc and copper were added to minced myocardial tissues and the concentrations in these samples were compared against concentrations in untreated samples. Recoveries ranged between 95 and 105%.

Sample preparation for DNA and RNA analyses was according to the modified method of Maggio, Siekovitz and Palade (9). DNA was determined by the diphenylamine procedure of Burton (10) and RNA by the orcinol procedure as described by Schneider (11). Calf thymus DNA and yeast RNA (Sigma) were used as standards.

The second myocardial samples were used for radiozinc uptake and differential centrifugation studies. These samples were homogenized and filtered through a 4 × 4 in. gauze to remove the minced muscle fibers from the sample. Pellets of nuclear, mitochondrial, microsomal and soluble protein material were harvested and counted using techniques previously described (3). Electron microscopic studies were performed on pellets from two animals treated with endotoxin to compare with control pellets as previously described (3).

The third myocardial samples were processed for light microscopic studies and stained with H and E stains.

*Statistical techniques.* Means and standard errors of the means were determined using standard techniques. Students' $t$ tests for unpaired data were utilized to determine the statistical significance of observed differences between the endotoxin-treated and control groups.

*Results.* Table I compares myocardial cation, DNA and RNA concentrations in myocardium harvested from eight endotoxin-treated dogs surviving 15 hr and eight control animals. Only 3 of 11 animals expired from the $LD_{50}$ dose of endotoxin prior to 15 hr. Three additional endotoxin-treated animals had definite increases in hematocrit and/or decreases in pH levels at the termination of the study consistent with persistence of endotoxin effect. The myocardial cation concentrations were no different in these three animals than in the rest of the group. None of the control animals had comparable changes in pH or hematocrit. Nevertheless, it must be recognized that the animals studied represented a group expected to survive the endotoxin insult. No alterations in myocardial concentrations of sodium, potassium, calcium, magnesium, zinc, copper, DNA or RNA were observed. The DNA and RNA were used as additional indices of tissue mass.

Table II compares the uptake of radiozinc into the intact myocardium and each of its subcellular fractions (nuclear, mitochondrial, microsomal and soluble protein) during the 15 hr of endotoxemia in 8 dogs against that in 8 control animals. No alteration in zinc distribution intracellularly was noted in en-

TABLE I. Comparison of Myocardial Concentrations of Sodium, Potassium, Calcium, Magnesium, Zinc, Copper, DNA and RNA in Eight Dogs 15 hr Postendotoxin Administration vs Eight Control Dogs.

| | | Endotoxin[a] (mean ± SEM) | Control (mean ± SEM) |
|---|---|---|---|
| Sodium | (mg/g ash wt) | 62.4 ± 1.88 | 62.8 ± 1.36 |
| Potassium | (mg/g ash wt) | 248 ± 3.7 | 244 ± 4.8 |
| Calcium | (mg/g ash wt) | 2.87 ± 0.13 | 2.71 ± 0.11 |
| Magnesium | (mg/g ash wt) | 17.75 ± 0.22 | 17.63 ± 0.30 |
| Zinc | (μg/g ash wt) | 1231 ± 24 | 1212 ± 30 |
| Copper | (μg/g ash wt) | 273 ± 13.6 | 295 ± 7.9 |
| DNA | (μg/g wet wt) | 1023 ± 122 | 901 ± 133 |
| RNA | (μg/g wet wt) | 2646 ± 139 | 2403 ± 175 |

[a] $p > 0.05$ in all comparisons.

TABLE II. Comparison of $^{65}Z$ Uptake into Intact Myocardium and Its Subcellular Fractions in Eight Dogs 15 hr Postendotoxin Administration vs Eight Control Dogs.

| | | Endotoxin[a] (mean ± SEM) | Control (mean ± SEM) |
|---|---|---|---|
| Intact myocardium | (ncpm/g dry wt) | 8605 ± 2974 | 9616 ± 2598 |
| Nuclear fraction | (ncpm/g dry wt) | 3629 ± 1041 | 4150 ± 1050 |
| Mitochondrial fraction | (ncpm/g dry wt) | 1226 ± 326 | 1452 ± 297 |
| Microsomal fraction | (ncpm/g dry wt) | 1784 ± 366 | 1586 ± 276 |
| Soluble protein fraction | (ncpm/g dry wt) | 4126 ± 1245 | 2868 ± 729 |

[a] $p > 0.05$ in all comparisons.

dotoxin treated *vs* control animals.

No differences in electron microscopic morphology of the subcellular fractions were found in the endotoxin-treated animals compared against normal animals.

*Discussion.* The precise role that the heart plays in endotoxin shock remains unresolved. Much evidence has been presented to indicate that the circulation fails early in endotoxin shock (4–8). Ventricular contractile force has been reported to diminish after endotoxin administration even when arterial pressure is maintained (7). Lefer and associates have identified a myocardial depressor substance in the plasma of animals in endotoxin shock which reportedly precipitates irreversible failure either by depressing excitation–contraction coupling or by impairing myocardial function directly (12–14). Hinshaw *et al.* (15), on the other hand, have failed to demonstrate any circulating cardiotoxic material when myocardial performance is assessed in isolated left ventricle perfused with blood from donor endotoxin-treated animals for periods up to 9 hr.

Most investigations into the cardiovascular effects of endotoxemia have focused on the hemodynamic alterations. Little is known about the metabolic effects of endotoxemia, particularly its effect on the myocardium.

Hinshaw *et al.* (16, 17), have shown that the mean $O_2$ uptake, mean $CO_2$ production and mean respiratory quotient along with myocardial performance are unaltered in isolated hearts at varying blood pressures when perfused with blood from endotoxin-treated animals compared to untreated control animals. These studies offered no evidence of any circulating toxic or depressant substance damaging to the myocardium.

Several investigators have shown that mitochondrial energy-linked reactions are diminished in the liver after a few hours of experimental shock (18–20). Endotoxemia causes both morphologic and enzymatic alterations in liver mitochondria. The cellular etiology of the mitochondrial dysfunction remains poorly defined. Respiratory control ratios fall indicating poor coupling of the liver mitochondrial respiratory activity with generation of high energy phosphates. Lysosomal enzyme release occurs during endotoxemia (21, 22). Lysosomal enzymes added to normal liver mitochondria *in vitro* at a pH below 6.5 produce the same metabolic defects induced in liver mitochondria *in vivo* by endotoxin (22). These changes are not observed at higher pH levels. After ischemia, intracellular concentrations of sodium and calcium increase while potassium and magnesium concentrations decrease in liver, kidney and heart (3, 23); similar changes might be expected in endotoxemia. Availability of free calcium and sodium ions accentuates the inhibitory effect of lysosomal enzymes on mitochondria; potassium is protective against the damaging effects of lysosomes (22). These studies suggest that the release of lysosomal enzymes, particularly in the presence of an intracellular acidosis and shifts in electrolyte concentrations, plays an important role in the development of cellular damage and dysfunction in endotoxin shock.

The mitochondrial damage seen in the liver 4 hr after administration of endotoxin does not appear to occur in the heart (22). Respiratory control activity in heart mitochondria remains high after endotoxemia. In contrast to the falling pH observed in liver and blood in endotoxemia, myocardial pH in-

creases approaching the optimal pH for mitochondrial enzymatic and metabolic activity (22). There is no evidence of structural or functional damage to heart mitochondria in endotoxemia.

The animals in this study were probably all in a group destined to survive the endotoxin shock. Whether different results would be obtained in the three animals expiring from endotoxin was not determined. The possibility that postmortem autolysis would prejudice our findings particularly in the subcellular fractions made us decide not to utilize the hearts in these animals. In retrospect, the 15-hr period of endotoxin shock allowed may have been longer than desirable. Recent studies indicate that myocardial mitochondria exhibit a surprising capacity to regenerate and reestablish normal metabolic function (24, 25). The possibility cannot be excluded that cation shifts existed earlier in the course of the endotoxemia but sufficient time lapsed to allow complete recovery.

Nevertheless, these findings are compatible with the clinical picture in endotoxemia where cardiac output may remain high and myocardial performance normal when function in other organ systems shows a steady deterioration. Maintenance of normal cation concentrations and the subcellular distribution of radiozinc is further confirmation of intact metabolism and function in the endotoxic heart.

*Summary.* Sodium, potassium, calcium, magnesium, zinc and copper concentrations in intact myocardium and radiozinc uptake into intact myocardium and its subcellular fractions (nuclear, mitochondrial, microsomal and soluble protein) were compared in 8 dogs 15 hr after administration of an $LD_{50}$ dose of endotoxin against 8 control animals. No differences were found, thus providing further evidence of the intact metabolism and function of the heart in endotoxemia.

The authors acknowledge the technical assistance of Mrs. Eleanor Haygood, Mrs. Dee Hammarsten, Mr. Howard Guiles, Mrs. Norma Luster and Mrs. Dorothy Shirey and the surgical assistance of Mr. Carl Haygood. We also acknowledge the assistance of Dr. L. B. Hinshaw and Mrs. Linda T. Archer in producing the endotoxin shock in our study animals.

1. Shen, A. C., and Jennings, R. B., Amer. J. Pathol. **67**, 417 (1972).

2. Raab, W., Ann. N.Y. Acad. Sci. **147**, 627 (1969).

3. Lindeman, R. D., Yunice, A. A., Baxter, D. J., Miller, L. R., and Nordquist, J., J. Lab. Clin. Med. **81**, 194 (1973).

4. Weil, M. H., MacLean, L. D., Visscher, M. B., and Spink, W. W., J. Clin. Invest. **35**, 1191 (1956).

5. Gilbert, R. P., Physiol. Rev. **40**, 425 (1960).

6. Alican, F., Dalton, M. L., and Hardy, J. D., Amer. J. Surg. **103**, 702 (1962).

7. Solis, R. T., and Downing, S. E., Amer. J. Physiol. **211**, 307 (1966).

8. Seigal, H. W., and Downing, S. E., Amer. J. Physiol. **218**, 772 (1970).

9. Maggio, R., Siekevitz, P., and Polade, C. E., J. Cell Biol. **18**, 267 (1963).

10. Burton, K., Biochem. J. **62**, 315 (1956).

11. Schneider, W. C., *in* "Methods in Enzymology" (S. P. Colowick, and N. O. Kaplan, eds.), Vol. 3, p. 680. Academic Press, New York (1957).

12. Lefer, A. M., and Rovetto, M. J., Proc. Soc. Exp. Biol. Med. **134**, 269 (1970).

13. Thalinger, A. R., and Lefer, A. M., Proc. Soc. Exp. Biol. Med. **136**, 354 (1971).

14. Lefer, A. M., and Inge, T. F., Jr., Proc. Soc. Exp. Biol. Med. **142**, 429 (1973).

15. Hinshaw, L. B., Shanbour, L. L., Greenfield, L. J., and Coalson, J. J., Arch. Surg. **100**, 600 (1970).

16. Hinshaw, L. B., Archer, L. T., Greenfield, L. J., and Guenter, C. A., Amer. J. Physiol. **221**, 504 (1971).

17. Hinshaw, L. B., Greenfield, L. J., Owen, S. E., Archer, L. T., and Guenter, C. A., Amer. J. Physiol. **222**, 1047 (1972).

18. Mela, L., Bacalzo, L. V., and Miller, L. D., Amer. J. Physiol. **220**, 571 (1971).

19. Schumer, W., Das Gupta, T. R., Moss, G. S., and Nyhus, L. M., Ann. Surg. **171**, 875 (1970).

20. Sayeed, M. M., and Bane, A. E., Amer. J. Physiol. **220**, 1275 (1971).

21. Mela, L., Olofsson, K., Miller, L. D., Bacalzo, L. V., and White, R. R., Surg. Forum. **22**, 19 (1971).

22. Mela, L., Miller, L. D., and Nicholas, G. G., Surgery **72**, 102 (1972).

23. Trump, B. F., Croker, B. P., and Mergner, W. J., *in* "Cell Membranes: Biological and Pathological Aspects" (G. W. Richter and D. G. Scarpelli, eds.), p. 84. Williams and Wilkins, Baltimore (1971).

24. DeGasparis, C., Miani, A., and Donatelli, R., J. Mol. Cell. Cardiol. **1**, 169 (1970).

25. Partin, J. S, Benzing, G,, III, and Partin, J. C., J. Mol. Cell. Cardiol. **4**, 345 (1972).

Received March 26, 1973. P.S.E.B.M., 1973, Vol. 144.