Myocardial Depletion of Norepinephrine in Hemorrhagic Hypotension.* (26746)

VINCENT V. GLAVIANO AND BERNELL COLEMAN†
Loyola University, Stritch School of Medicine, Chicago, Ill.

In shock, elevated plasma levels of catecholamines(1,2) were primarily found to result from a highly augmented secretion from the adrenal medulla(3,4). These observations prompted us to explore whether changes in plasma catecholamine levels would induce an alteration in myocardial concentration of epinephrine and norepinephrine. We assumed that these observations would be significant, since recent observations have correlated myocardial catecholamines with cardiac performance(5,6).

Methods. Analysis of myocardial epinephrine and norepinephrine were conducted on 2 groups of albino rabbits ranging in weight from 1 to 3 kg. One group comprising 12 normal rabbits were killed by a blow on the head. The heart was removed and a sample of left ventricle was weighed after the muscle was washed with 0.9% NaCl and dried on filter paper. The muscle was homogenized in 10% trichloroacetic acid in a Potter-Elvehjem tissue grinder, immersed in ice and water. The supernatant liquid, after refrigerated centrifugation, was extracted with diethyl ether. The aqueous portion was diluted with an equal volume of 0.2 N sodium acetate, and the pH adjusted to 8.2 with 0.5 N Na_2CO_3. The tissue extract was passed through an alumina (Fischer) column, which had previously been washed 3 times with tripled distilled water. Eluates from columns were analyzed according to a modification of the trihydroxyindole fluorescence method(7) with epinephrine and norepinephrine standards. Reagent blanks and tissue blanks represented approximately 15% of the total fluorescence. Differentiation of epinephrine from norepinephrine was based on using Corning exciting filters of 400 mμ and 440 mμ with Wratten Filter 35. A Corning 500 mμ filter with a Wratten Filter 57 was used for emission. Because of low transmission, interference filters for greater spectral purity were not employed. The sensitivity of our instrument was arbitrarily adjusted to detect accurately 0.01 to 1.5 μg of epinephrine and norepinephrine. Ether extraction of homogenized heart tissue was found to increase fluorescence by 8%. Our recoveries from adding catecholamines to the alumina column averaged 85%. In those steps involving the analysis of eluates, recovery of added amounts to 10 samples ranged 88 to 110%. The overall recovery for all steps of the catecholamine analysis averaged 82%.

The cardiac catecholamines from the control group were compared with those found in 12 rabbits subjected to hemorrhagic hypotension for 3 hours at mean arterial blood pressures of 50 mm Hg. Under local anesthesia, the femoral artery of a hind limb was catheterized for bleeding and for maintaining mean blood pressure. Blood pressure was maintained constant by elevating a graduated cylinder containing the initial bleeding volume (av. 30 ml/kg) to a height equivalent to 50 mm Hg pressure. Near the end of the hypotensive period, 25 ml of blood was drawn from the artery for plasma catecholamines analysis in 4 of 12 rabbits. The heart was taken out for analysis after the animal was killed by rapidly removing with a large syringe, the remaining blood volume. An initial dose of 5 mg/kg of Heparin‡ was administered as an anticoagulant, ½ of the initial dose was repeated every hour. Peripheral plasma catecholamine levels were studied in 4 normal rabbits from blood drawn by cardiac puncture.

Results. A striking difference in myocardial content of catecholamines was found when normal rabbits were compared to rabbits subjected to hemorrhagic hypotension

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TABLE I. Myocardial Norepinephrine and Epinephrine in Normal and Hypotensive Rabbits.

	No. of rabbits	Control (μg/g)	Hypotension (μg/g)	% diff.	P†
Norepinephrine	12	1.05 ± .13*	.16 ± .05	−84.8	<.001
Epinephrine	12	.14 ± .06	.24 ± .06	+71.5	<.01

* Mean ± stand. error.

† Calculated by Student's "t" test.

for 3 hours. The summary of data in Table I shows that a marked depletion of norepinephrine (NE) occurred in the hypotensive group. Control levels averaging 1.05 ± .13 μg/g were reduced to 0.16 ± .05 μg/g, a reduction of 85%. This depletion of NE occurred concomitantly with a significant increase in epinephrine (E), where control levels of 0.14 ± .06 μg/g were elevated to 0.24 ± .06 μg/g, an increase of 72%. Despite the relatively high percentage increase in myocardial E, total catecholamine content (NE and E) of left ventricle was reduced to 34% of the normal rabbit heart.

In 4 normal rabbits, plasma E and NE were present in concentrations below the sensitivity of analysis (<.01 μg/15 ml). Plasma analysis in 4 of 12 rabbits with hemorrhagic hypotension averaged 1.4 μg/100 ml for E and 0.2 μg/100 ml for NE. The elevated myocardial content of epinephrine was probably due to its high concentration in circulating blood(8).

In Table II are shown myocardial NE levels in 4 additional hypotensive rabbits. These animals were treated, after a 3 hour period of hemorrhagic hypotension at 50 mm arterial pressure with varying doses of NE (Levophed base). Thirty minutes later, the hearts from these rabbits were removed for NE analysis after rapid exsanguination. The data indicate a good dose-uptake relationship between NE dose and level found in left ventricle. Levels of myocardial NE were also found to be related to the level present in plasma (Table II). The most significant point which can be made for these uptake experiments is that myocardial depletion of NE in the hemorrhagic hypotensive animal is reversible. It also follows that the extent to which the myocardium will store exogenous NE depends on the dose administered, thus indicating that its passage into the muscle cell may simply be governed by diffusion rather than an active process.

TABLE II. Myocardial Uptake of Norepinephrine in Hemorrhagic Hypotension.

Rabbit No.	Dose (μg/kg)	Heart (μg/g)	Plasma (μg/100 ml)
1	500	1.59	1.90
2	100	.93	1.10
3	50	.85	.43
4	25	.65	.21

Discussion. Current reports have focused attention on the role of NE from sympathetic postganglionic nerve endings on myocardial function. Sarnoff(6) considers the "catecholamine stimulus" on ventricular contractility equal in importance to the Frank-Starling relationship of end-diastolic pressure and fiber length. Mayer and Moran(9) reported that injected catecholamines augmented myocardial phosphorylase activity, which in turn was found related to contractile force of the heart *in situ*. Indirectly, function of the adrenergic transmitter has also been ascertained from studies involving its depletion by cardiac sympathectomy or pharmacological agents. These studies show the depleted heart to have less force of contraction, a slower heart rate, and a decrease in right atrial pressure(10,11). In view of these findings, our results on myocardial depletion of NE indicate that failure of this organ to maintain circulatory homeostasis in a progressing state of shock will be severely limited. The experimental design of the reported experiments does not permit us to explain the underlying mechanism of myocardial depression in near terminal hemorrhagic shock(12). However, our data suggest that genesis of myocardial failure in this state could be due to NE depletion.

Gilmore *et al.*(13), and Fozzard and Gilmore(14) reported that treatment of animals in hemorrhagic shock with NE will prolong survival. Lansing and Stevenson(15) found

that administration of NE in shock prevented decreases in cardiac output, which occurred in the non-treated animals with the progression of shock. Caliva *et al.*(16) using an electropolarographic technic, observed that NE in early shock will restore normal myocardial oxygen levels. These investigators proposed that the prolonged survival period of NE-treated animals was due to a cardiotonic-like action of the hormone. In accordance with our results, the increased survival time may have arisen from the uptake of administered NE by the depleted myocardium. Replenishment of the adrenergic transmitter could have sustained cardiac contractility, thus preventing further decline in cardiac output. On the other hand, some laboratories have noted no change in the steady downhill course of NE-treated animals in hemorrhagic shock(17,18). The current disagreement on the therapeutic value of NE probably arises in comparing experimental results from animals in which NE was injected at different times during the course of shock. It is highly probable that the reported ineffectiveness of this catecholamine in prolonged shock is due to the entry of other factors responsible for its fatal outcome.

Summary. Analysis of NE and E in the left ventricle of 12 normal albino rabbits averaged 1.05 ± .13 and 0.14 ± .06 μg/g respectively. In 12 rabbits subjected to hemorrhagic hypotension for 3 hours at mean arterial blood pressures of 50 mm Hg, myocardial NE declined significantly to an average of 0.16 ± .05 μg/g ($P<.001$). Epinephrine levels in this group rose to an average of 0.24 ± .06 μg/g ($P<.01$). Injection of graded doses (500 to 25 μg/kg) of NE in hypotensive rabbits caused an elevation of myocardial NE in rough proportion to the dose administered and level in plasma.

1. Watts, D. T., Bragg, A. D., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 609.

2. Manger, W. M., Bollman, J. L., Maher, F. T., Berkson, J., *Am. J. Physiol.*, 1957, v190, 310.

3. Glaviano, V. V., Bass, N., Nykiel, F., *Circulation Res.*, 1960, v8, 564.

4. Nykiel, F., Glaviano, V. V., *J. Appl. Physiol.*, 1961, v16, 348.

5. Raab, W., *Am. J. Cardiology*, 1960, v5, 571.

6. Sarnoff, S. J., *ibid.*, 1960, v5, 579.

7. Price, H. L., Price, M. L., *J. Lab. and Clin. Med.*, 1957, v50, 769.

8. Raab, W., Gigee, A. B., *Circulation Res.*, 1955, v3, 553.

9. Mayer, S. E., Moran, N. C., *J. Pharm. and Exp. Therap.*, 1960, v129, 271.

10. Ward, D. R., Kottegoda, S. R., Krayer, O., *ibid.*, 1958, v124, 340.

11. Lee, W. C., Shideman, F. E., *Science*, 1959, v129, 967.

12. Wiggers, C. J., *Am. Heart J.*, 1947, v33, 633.

13. Gilmore, J. P., Smythe, C. M., Handford, S. W., *J. Clin. Invest.*, 1954, v33, 884.

14. Fozzard, H. A., Gilmore, J. P., *Am. J. Physiol.*, 1959, v196, 1029.

15. Lansing, A. M., Stevenson, J. A. F., *ibid.*, 1958, v193, 289.

16. Caliva, F. S., Napodano, R., Zurek, R., Pombo, T., Lyons, R. H., *Am. J. Med. Sci.*, 1959, v238, 308.

17. Frank, E. D., Frank, H. A., Jacob, S., Weizel, A. E., Korman, H., Fine, J., *Am. J. Physiol.*, 1956, v186, 74.

18. Hackel, D. B., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 780.

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Antigenicity of Short Ragweed Pollen for White Mice.* (26747)

SAUL MALKIEL AND BETTY J. HARGIS

Department of Medicine, Harvard Medical School, Peter Bent Brigham Hospital and Children's Cancer Research Foundation, Boston, Mass.

In the course of our early studies on enhancement of anaphylactic sensitivity by *Bordetella pertussis* it was found that proteins differed in their ability to sensitize(1-4). Included in the group was extract of

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