7 and 12 and Coxsackie A9 viruses was destroyed by snake venom phosphodiesterase (SVP) whereas the infectivity of the corresponding intact viruses was unaffected by this enzyme. At 25° using ECHO 7 RNA and 2 μg SVP per ml, rate of inactivation was constant through approximately 20 minutes, then progressively decreased.

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Received February 10, 1964. P.S.E.B.M., 1964, v116.

Effects of Norepinephrine on Myocardial K, Na, Cl and H_2O in Hemorrhagic Shock.* (29182)

VINCENT V. GLAVIANO AND BERNELL COLEMAN†

Department of Physiology, Stritch School of Medicine, Loyola University, Chicago, Illinois

Clinical investigators do not generally agree on the efficacy of exogenous norepinephrine (NE) for treatment of hemorrhagic shock. In addition to not extending the survival period, administration of this sympathomimetic amine has caused hepatic, renal and cardiac damage(1,2). Treatment of shock with NE has also failed to correct the inability of the heart to utilize pyruvate or prevent the decline in myocardial efficiency(3). On the other hand, maintenance of cardiac output and an increase in survival time have been reported for NE treated animals in shock(4, 5). These beneficial effects have been attributed to the stimulating action of this catecholamine on the myocardium(6).

Dogs in irreversible hemorrhagic shock were recently reported to undergo increases in myocardial interstitial K sufficient to cause significant lowering of the resting membrane potential(7). Since NE has been observed to increase the intracellular/extracellular ratio of K in normal cardiac muscle(8,9), myocardial levels of K, Na, Cl and H_2O were compared in normal dogs with dogs in hemorrhagic shock, treated and not treated with exogenous l-NE.

Methods. Thirty mongrel dogs anesthetized with 35 mg/kg of sodium pentobarbital were divided equally into 3 groups. One group served as controls, a second group was subjected to hemorrhagic shock and a third group was treated with l-norepinephrine after inducing shock. The trachea was cannulated and the left common carotid artery was connected to a mercury manometer for monitoring mean arterial pressure. The left femoral artery and vein were cannulated for bleeding and reinfusion of blood respectively. Shock was induced by bleeding the animal (50 ml/min) until mean pressure stabilized at 40 mm Hg. Following the maintenance of this level of pressure for 4 hours, the total bled volume was reinfused. Prior to inducing shock, a control arterial blood sample was taken for analysis of plasma K, Na, Cl and H_2O. Coagulation of blood was prevented by initial administration of 5 mg/kg of hep-

* These studies were aided by contract between Office of Naval Research, Dept. of the Navy and Loyola Univ. Stritch School of Medicine.

† Predoctoral Research Fellow, Nat. Inst. of Health; present address: St. Louis Univ. School of Med., St. Louis, Mo.

arin. Thereafter, 2.5 mg/kg was given every hour throughout the experiment. In 10 of 20 dogs subjected to shock, reinfusion of blood was followed by intravenous administration of 2.5 μg/kg/min of l-norepinephrine (Levophed base)‡ for 30 minutes. A thoracotomy was performed with the animal connected to a respirator when mean pressure in the normovolemic period of treated or untreated dogs declined to 60 mm Hg. Samples of cardiac muscle from the anterior apical portion of the left ventrical were quickly excised, trimmed of fat, washed, blotted on filter paper and frozen in liquid nitrogen for subsequent storage in a deep freeze at —20°C. Analyses of plasma electrolytes and H_2O were repeated on an arterial sample taken just before excision of cardiac muscle.

Analyses for K, Na and Cl were performed on homogenates prepared from cardiac muscle in deionized water. Potassium and Na contents were read in a flame photometer from a nitric acid digest of the homogenate. Levels of Cl were determined with the amperometric method(10). Water content was measured by drying the muscle to a constant weight for 48 hours at 70°C. Neutral fat content was calculated from the loss in weight of the desiccated muscle after double extraction with diethyl ether. Concentrations of K, Na and Cl were expressed in meq/100 g fat-free dry tissue (FFDT) and H_2O was expressed in g/100 g FFDT. The intracellular-interstitial distribution of electrolytes was calculated on the assumption that they are distributed between plasma and intracellular water according to a Gibbs-Donnan equilibrium. Based on a protein concentration of 4% in cardiac lymph, a Donnan ratio of 0.98 was employed for K, Na and Cl(11). It was assumed in calculating the interstitial space that Na is largely restricted to the extracellular compartment and on the basis of this restriction intracellular Cl closely approximates the concentration of interstitial K(8). The following relationships, formulated by Benson *et al*(12) and others(13), were used to calculate the distribution of water and electrolytes in cardiac muscle.

‡ Supplied by Winthrop Laboratories, New York.

$$[K]_E = [K]_P \times 0.98$$
$$[Cl]_E = [Cl]_P/0.98$$
$$(H_2O)_E = \frac{1000\,(Cl)_T - [K]_E\,(H_2O)_T}{[Cl]_E - [K]_E}$$
$$(H_2O)_I = (H_2O)_T - (H_2O)_E$$
$$(K)_I = (K)_T - [K]_E\,(H_2O)_E/1000$$
$$[K]_I = 1000\,(K)_I/(H_2O)_I$$

Brackets in these equations refer to concentration in milliequivalents per kilogram of water, parentheses refer to milliequivalents of electrolytes or grams of water per 100 g fat-free dry tissue (FFDT). The subscripts E, I, T and P refer to extracellular phase, intracellular phase, total tissue and plasma, respectively.

Results. Analyses of plasma K, Na, Cl and H_2O are summarized in Table I. The elevation in K was the only significant change that occurred in the plasma. These data are in agreement with previously reported findings on plasma analysis of electrolytes in hemorrhagic shock(14). Administration of l-norepinephrine did not alter the plasma concentration of K, Na, Cl and H_2O. In the same Table, levels of these electrolytes in cardiac muscle are compared between NE treated and untreated dogs in hemorrhagic shock. In untreated dogs, marked increases in myocardial K ($P < .01$) and significant decreases in Na ($P < .005$) occurred with no change in Cl and H_2O content. In early normovolemia NE administration did not alter K or H_2O content; however, Na and Cl rose from 13.1 to 16.5 meq/100 g and 13.4 to 16.0 meq/100 g respectively.

In Table I the calculated interstitial and intracellular myocardial levels of K, Na and H_2O are summarized for the 3 groups of experiments. Significant changes from control levels of intracellular H_2O (275 ± 7.5 g/100 g) were not detected in untreated animals in shock (296 ± 13.3 g/100 g) nor in shocked animals given NE (279 ± 8.6 g/100 g). Similarly, no difference was found in myocardial interstitial H_2O between controls (83 ± 5.2 g/100 g) and untreated animals in shock (87 ± 7.7 g/100 g). An increase of borderline significance in interstitial H_2O did occur between treated and untreated dogs in shock with NE ($P = .05$). The calculated

TABLE I. Plasma and Myocardial K, Na, Cl and H_2O Content and Distribution in Normal Dogs and Dogs in Hemorrhagic Shock Before and After Treatment with Norepinephrine (NE).

	K (meq)	P	Na (meq)	P	Cl (meq)	P	H_2O (g)	P
			Plasma‡					
Control (10)†	4.13 ± .10*		160.5 ± 7.1		116.1 ± .6		924 ± 1.2	
		<.001		<.10		<.40		<.20
Shock (10)	6.65 ± .39		147.6 ± 2.9		113.6 ± 3.3		913 ± 2.0	
		<.20		<.20		<.30		<.25
Shock + NE (10)	6.06 ± .30		156.5 ± 3.4		116.3 ± 1.8		919 ± 1.5	
			Myocardium§					
Control (10)	40.7 ± 1.1		17.2 ± .8		11.7 ± .6		351 ± 9.0	
		<.01		<.005		>.10		>.10
Shock (10)	47.3 ± 2.1		13.1 ± .8		13.4 ± .8		377 ± 8.6	
		>.40		<.025		<.05		>.40
Shock + NE (10)	49.9 ± 1.7		16.5 ± 1.2		16.0 ± .8		390 ± 9.6	

	Myocardial Interstitial and Intracellular Water and Electrolytes											
	$[K]_E$¶	P	$[K]_I$**	P	$[Na]_E$	P	$[Na]_I$	P	$(H_2O)_E$‖	P	$(H_2O)_I$‖	P
Control (8)	4.56 ± .13		151 ± 3.8		176 ± 5.5		6.89 ± 2.08		83 ± 5.2		275 ± 7.5	
		<.005		>.4		>.05		>.05		>.5		>.2
Shock (8)	7.52 ± .48		159 ± 5.1		164.5 ± 2.0		4.02 ± 1.85		87 ± 7.7		296 ± 13.3	
		>.2		<.005		>.2		>.05		=.05		>.4
Shock + NE (8)	6.23 ± .41		183 ± 3.4		170.2 ± 5.3		2.00 ± 1.24		110 ± 8.5		279 ± 8.6	

* Mean ± S.E.

† No. of dogs.

‡ Electrolytes and water expressed in meq and g per liter of plasma, respectively.

§ Electrolytes and water expressed in meq and g per 100 g fat-free dry tissue.

( )‖ g of interstitial ( )$_E$ or intracellular ( )$_I$ water per 100 g fat-free dry tissue.

[ ]$_E$ ¶ meq per kg of interstitial water.

[ ]$_I$ ** meq per kg of intracellular water.

distribution of intracellular and interstitial K demonstrated that increases in myocardial K observed in shock (Table I) are primarily due to an elevation of this cation in the interstitial compartment (4.56 ± .13 to 7.52 ± .48 meq/kg, $P < .005$). Infusions of NE caused intracellular K to rise from 159 ± 5.1 to 183 ± 3.4 meq/kg ($P < .005$) while Na decreased by an average of almost 50% (4.02 to 2.00 meq/kg). Although average intracellular Na for this group was markedly decreased with NE treatment, 3 of 8 dogs demonstrated no significant change from levels present in untreated animals ($P > .05$).

Discussion. In the first reported study on effects of epinephrine on myocardial electrolytes, Robertson and Peyser(8) noted increases in intracellular K and decreases in intracellular Na with no change in H_2O. Waddell(9) showed K influx to be greater than efflux in quiescent and spontaneously beating rabbit atria treated with NE. Bülbring(15) suggested, from observations on catecholamines added to smooth muscle, that these substances have a dual action in causing both a change in metabolism and permeability. In heart slices and intact myocardium metabolic effects of NE chiefly involves stimulation of glycolysis through activation of phosphorylase(16,17,18). In experiments reported herein, treatment of dogs in hemorrhagic shock with NE, raised myocardial intracellular K significantly above levels present in control animals. This response implies that the action of NE on glycolysis increases the availability of energy for transport of K against an electrochemical gradient. A preliminary publication from this laboratory indicates that myocardial glycogen in hemorrhagic shock could readily become the source of energy for active transport of K, since the levels are not significantly different from controls(19).

According to the techniques employed and our interpretation of the data, the elevation in myocardial K was found to be confined to the interstitial compartment (Table I). The elevation of K in this compartment caused the ratio of intracellular to interstitial K to decrease from a control value of 33 to 21. Treatment of the animal with NE increased the ratio from 21 to 29. In the normal heart a decrease in the resting membrane potential leads to a decrease in threshold, a decrease in membrane action potential and a concurrent increase in excitability(20). Assuming the intracellular/extracellular K ratio to be a coarse indicator of the relative magnitude of the resting membrane potential, the myocardial ratio of this electrolyte in dogs infused with NE agrees with observations reported by other investigators. Although there is considerable disagreement, more recent evidence demonstrates that NE increases the RMP of isolated cardiac muscle recorded from microelectrodes(21,22,23).

The return to a normal myocardial intracellular/interstitial ratio of K in shocked animals treated with NE is regarded by the authors as a favorable result for NE therapy. On the other hand, it is unlikely that a specific cardiotonic action of NE will reverse the inevitable downhill course of the circulation since the heart has not been established as the sole cause of irreversible shock.

Summary. In a comparison of myocardial K, Na, Cl and H_2O in normal dogs and dogs in hemorrhagic shock, levels of K were found significantly elevated. Although Na concentrations declined, H_2O content did not change from controls. Administration of l-norepinephrine to dogs in normovolemic shock caused no change in K and H_2O while Na and Cl levels returned to control. Analysis of myocardial K distribution showed that intracellular/interstitial ratio of this cation declined from 33 to 21 in shock. Norepinephrine treatment of these animals raised K ratio to 29, a change that primarily reflected the increase which this pressor amine causes in intracellular K.

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Received October 28, 1963. P.S.E.B.M., 1964, v116.

Inhibition of Herpes Virus by Natural and Synthetic Acid Polysaccharides. (29183)

K. K. TAKEMOTO AND P. FABISCH (Introduced by S. Baron)
U. S. Department of HEW, P.H.S., National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Biology of Viruses, Bethesda, Md.

A synthetic sulfated polysaccharide, sodium dextran sulfate, has been reported by this laboratory to inhibit the multiplication of certain viruses (EMC, ECHO, Coxsackie A9, and herpes) while other viruses (type 1 poliovirus and variants from attenuated types 1 and 2 poliovirus) not only were resistant but their plaque-forming ability was enhanced by the presence of the acid polysaccharide in the medium(1). Later Nahmias and Kibrick(2) reported that heparin, a naturally occurring sulfated polysaccharide, inhibited several strains of herpes virus. Our original observations on the inhibition of herpes virus by dextran sulfate have been extended and the nature of the inhibition as well as its reversal by basic polymers are included here. The effects on herpes virus growth of heparin, chondroitin sulfate, and the sulfated polysaccharide from agar are also described.

Materials and methods. Cell cultures. HeLa cells were grown in Eagle's medium supplemented with 10% bovine serum. Procedures for plaque assays in these cells as well as the medium employed were the same as previously described(3).

Virus. Herpes virus was originally obtained from Dr. A. S. Kaplan who studied its growth in rabbit kidney cultures(4). Plaque counts of this virus were found to be increased 5- to 10-fold when DEAE-dextran(6) was added to the agar overlay, indicating that inhibitor-sensitive particles which were incapable of forming plaques in untreated overlay medium were present in this original virus stock. In addition, when this stock virus was plated in untreated agar overlay, plaques of 2 sizes were observed. These were plaque-purified for use in the experiments described below, and designated Lpf (large plaque former) and Mpf (minute plaque former). The Lpf virus induces polykaryocyte formation while the Mpf virus causes cell rounding and clumping; in this respect, they correspond to Roizman's Mp and mp strains(5). Since they were selected for their ability to form plaques