Effect of Hemorrhagic Shock on Contractility of Papillary Muscle.* (30426)

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Ventricular failure is an important component of hemorrhagic shock(1,2); heart size and left atrial pressure are increased and coronary blood flow, stroke volume and cardiac output are decreased(3,4). The current study explores the possibility that the ventricular failure of shock may be due to irreversibly decreased myocardial contractility.

Methods. Rats, 290-440 mg in weight, were anesthetized with pentobarbital (30 mg/kg) and subjected to irreversible hemorrhagic shock by controlled hemorrhage into a reservoir *via* a polyethylene catheter inserted in a carotid artery(5). The vertical distance between the blood level of the reservoir and the artery was adjusted to maintain systemic arterial pressure at 50 mm Hg for one hour, and then at 35 mm Hg for 2 hours. The heart of each of 7 rats in shock and of 11 pentobarbital anesthetized control animals was rapidly excised and immersed immediately in a refrigerated solution of the following ionic composition, expressed in mEq per liter: Na, 145; K, 5.32; Ca, 5.08; Mg, 1.24; Cl, 129.2; HCO_3, 25.0; PO_4, 1.81; and SO_4, 1.24. In less than 5 minutes from beginning thoracotomy the papillary muscle had been dissected free and mounted on a stainless steel anchoring electrode in the 50 ml muscle chamber. The temperature of the bath was maintained at 37°C. A mixture of 95% O_2 and 5% CO_2 aerated the bath and maintained pH at 7.4. The free end of the muscle was attached by means of a silk thread to a Grass tension transducer mounted directly above it, and isometric tension was recorded by a Grass direct writing oscillograph. Under these conditions papillary muscles have been found to perform reproducibly for 24 hours. The length of the muscle was altered by vernier adjustment of the distance of the transducer carriage above the fixed end of the muscle. Square wave stimuli, delivered from a Grass stimulator by means of the anchoring electrode and an electrode mounted near the free end of the muscle, were suprathreshold and produced an all or none response. Stimulus frequencies were one per 10 seconds (f=0.1) and 3 per second (f=3.0), except as noted below, and all stimuli were of one millisecond duration. Prior to the collection of data, each muscle was allowed to equilibrate in the muscle chamber for one hour. During this time it was driven at f=0.1 and the bathing solution was renewed several times. These rinses produced no alteration in contractility. After the equilibration period, all tension was removed from the muscle and resting length was determined by moving the transducer carriage until the minimum detectable tension (approximately 3 mg) was exerted in the quiescent muscle. Muscle length was then increased 10 to 15% and the muscle allowed to stabilize for 25 minutes at the new length, at f=0.1, before a 2-minute data recording period. This was followed by a 7-minute period at f=3.0; data were obtained during the last 30 seconds. Stimulation frequency was then returned to f=0.1 and muscle length readjusted by increments of approximately 0.5 mm (10 to 15% increase) for subsequent test runs. To make possible comparison of strips of different initial thickness and length, tension is expressed in terms of cross sectional area and as a function of per cent of resting length. Cross sectional area was calculated from the measured resting length and wet weight of the papillary muscles, on the assumption that the muscles were cylindrical and that the tissue had a specific gravity of one (Table I).

Results. The performance of muscle from animals in shock was not appreciably different from that of the control group with

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TABLE I. Characteristics of Papillary Muscles from Left Ventricle of the Rat.

Length (mm)	Cross section area (cm^2)	Slope of length-tension plot (g/cm^2/% change in rest length) f=.1	f=3.0	% of max tension after 10 sec rest
Control papillary muscles:				
4.0	.00672	2.85	1.31	
4.0	.00607	3.82	1.05	
4.0	.00732	4.12	1.42	
4.3	.00474	3.93	1.54	87
5.3	.01067	6.85	1.23	
4.7	.00948	3.51	.67	82
4.8	.01510	1.04	.38	93
5.7	.01107	1.93	.62	
6.0	.01271	2.79	1.08	91
4.0	.01100	1.08	.52	
6.5	.01358	3.36	1.11	90
Papillary muscles from animals in shock:				
4.0	.00662	5.76	1.62	
4.0	.01045	2.73	1.12	
5.5	.01454	1.38	.61	
7.0	.00928	6.77	1.81	83
7.0	.01004	4.13	1.49	88
5.5	.01534	2.66	.75	93
5.0	.01240	1.84	.42	87

respect to the 2 parameters studied, tension development and negative treppe.

Tension development increased linearly as length was increased from 100% to 160% of rest length, and at any given length tension development was greater at the frequency of one stimulus/10 sec than it was at 3/sec. At 150% of rest length tension developed by muscle from control and shocked animals at f=0.1 was 128.8 ± 17.3, and 162.7 ± 15.8 g/cm^2, respectively; and at f=3.0, 43.3 ± 4.4 and 54.7 ± 4.3 g/cm^2, respectively. Comparison of the slopes of the calculated lines of best fit for the linear portions of the curves relating tension development to muscle lengths (Table I), reveals no significant difference between the control and shock groups.

After a contraction, a comparatively long period of time is required before rat myocardium regains full contractility. This phenomenon is known as negative treppe and is the reverse of Bowditch staircase phenomenon. The effect of shock on negative treppe was studied by comparing the effect of quiescence of 5, 10, 15, 20, 30, 45, 60, 120, and 180 seconds, on the magnitude of the first contraction after the rest period. Five control and 4 shock papillary muscles were stimulated at a frequency of one stimulus/sec for 60 seconds. The muscle length used in this study was one intermediate on the linear portion of the length-tension curve. The largest increments in tension development occurred after rests of 5, 10, and 15 seconds, rising from 65 to 85% of the peak tension developed after 5 seconds, to 84 to 98% after 15 seconds (Table I). Maximum tension developed after a rest of 30 to 60 seconds. There was a slight decrement of tension after rests of 120 and 180 seconds. Papillary muscles from control and shock animals behaved alike.

Discussion. The fundamental question pertaining to the irreversibility of hemorrhagic shock is: Why do animals which experience sufficient blood loss to produce sustained hypotension proceed relentlessly to death even though their blood volume be restored, whereas correction of similar blood volume deficit earlier in the course of hemorrhagic hypotension permits survival? Our evidence indicates that the irreversibility of hemorrhagic shock is not due to irreversible alteration of contractility of heart muscle. Papillary muscles removed from control rats and from rats in shock, after they had equilibrated with the bath, were indistinguishable with respect to their ability to develop active tension at various initial lengths and in their display of negative treppe. Equilibration with the bathing solution may, of course, have obscured differences, either by causing restoration of contractility to muscles from the shock group or by causing deterioration of muscles of the control group. Also, some subtle alteration of contractility may have escaped detection in the relatively small number of experiments performed, even though the heart failure which occurs in shock is profound.

If the myocardial contractile elements of the animal in irreversible hemorrhagic shock are capable of performing indistinguishably from those of control animals, then the cardiac failure associated with shock, and therefore shock itself, may be reversible. This possibility shifts the emphasis of the mechanism of irreversibility away from an intrinsic

derangement of the myocardial contractile process, to such extrinsic problems as cardiotoxins, the electrolyte environment, or neurogenic control. Although the data are not conclusive, they are meaningful because they imply that the myogenic failure of profound shock is reversible.

Summary. Left ventricular papillary muscle from rats in profound hemorrhagic shock and from control animals were compared with respect to tension development and negative treppe. In both muscles tension development increased as resting fibre length increased and as frequency of stimulation decreased. No difference in the performance of muscles from the two types of animals was detected; the contractile element of cardiac muscle appears not to be irreversibly altered by hemorrhagic hypotension.

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Regulation of Cholesterol Catabolism by Bile Salts and Glycyrrhetic Acid *in vivo*. (30427)

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Bergström and Danielsson(1) found that continuous intraduodenal infusion of sodium taurochenodeoxycholate (5 to 10 mg/hr) into rats reduced the biliary excretion of taurocholate by these animals compared with that in bile-fistula rats not receiving such an infusion. They concluded that the concentration of bile salts supplied to the liver *via* the portal blood influenced the rate of hepatic synthesis of bile salts from cholesterol. Further evidence for this apparent negative feedback control of cholesterol catabolism was provided by observations that added bile salts depressed the ATP-dependent oxidation of cholesterol by fortified rat-liver mitochondrial preparations *in vitro*(2,3). However, different bile salts inhibited this *in vitro* cholesterol catabolism to different degrees, taurocholate being much less potent than taurochenodeoxycholate at the same concentration. Furthermore, bile salts have been observed to inhibit ATP biosynthesis in liver mitochondria by uncoupling oxidative phosphorylation and by inhibiting NADH oxidation(4). In both respects taurochenodeoxycholate is a more potent inhibitor than taurocholate. It was, therefore, important to discover whether or not taurocholate and taurochenodeoxycholate differed in their effects on cholesterol catabolism *in vivo* in rats. Since they are both normal constituents of rat bile and present in portal blood (due to the enterohepatic circulation), they might both be expected to regulate cholesterol catabolism to a similar degree if a "true" negative feedback control is operative. This was not found to be so.

Methods. Bile ducts of female albino rats (200 to 300 g) were ligated at the mid-point under anesthesia (intravenous hexobarbital). A plastic cannula was inserted in the upper half of the duct to receive bile, and a second cannula was inserted into the lower half for intraduodenal infusion. Animals were kept in restraining cages with access to Oxoid rat diet and drinking water containing 5% glucose, 0.05% KCl and 1% NaCl. Ten μc of

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