Studies of Myocardial Actomyosin and Myosin After Shock, Acute Hemorrhage, Acute Hypoxia, and Cardiopulmonary Bypass

By Young W. Cho, M.D., M.S., Pierre M. Galletti, M.D., Ph.D., and Leonard Nelson, Ph.D.

A DECREASE in cardiac efficiency is observed in dogs submitted to endotoxic shock, acute hemorrhage, and after heart-lung bypass. This may result from diminished coronary blood flow, tissue hypoxia, or lower oxygen tension of arterial blood in the cases of endotoxic shock and acute hemorrhage. The etiology of a shocklike syndrome following cardiopulmonary bypass has been studied by Brom and Nauta¹ and has been attributed to metabolic alterations; however, the precise mechanism of decrease in the myocardial function is unknown.

Since the myocardial mechanical efficiency greatly depends upon the various chemical activities which lead to muscular contraction,² such as the energy conversion processes, physiochemical properties of the contractile proteins, or the coupling processes of the wave of excitation at the cell membrane with mechanical contraction of the myocardium,³ it seemed possible that interference with these processes at any point might lead to inefficiency of myocardial contraction. Specifically, since the energy utilization process within the myocardium is directly related to the physiochemical properties of cardiac contractile protein, and since the actomyosin-ATP (adenosinetriphosphatase) system, together with certain ions, is the simplest form of contractile system that can be obtained from the cardiac muscle,⁴ actomyosin was extracted from the cardiac muscle in dogs subjected to conditions of acute hemorrhage, endotoxic shock, and cardiopulmonary bypass. In order to determine whether lower oxygen tension of the arterial blood had any effect upon the contractile system, acute hypoxia was induced in a group of dogs and the actomyosin was studied.

Cardiac myosin was also extracted from these animals and its ATPase (adenosinetriphosphatase) activity was measured.

Material and Methods

A total of 32 apparently healthy, adult mongrel dogs of both sexes, weighing 12 to 15 Kg. each, was anesthetized with pentobarbital sodium (25 mg./Kg.) or chloralose (80 mg./Kg.) plus phenobarbital (20 mg./Kg.) prior to the experimental procedures.

Acute Hemorrhage

Six dogs were bled at a rate which lowered mean arterial pressure to 50 mm. Hg within 10 minutes. After 10 minutes of observation, they were bled further until the arterial pressure dropped to 10 or 15 mm. Hg.⁵ After an additional 20 minutes, the dogs were sacrificed. The oxygen tension of the arterial blood (pO₂), measured continuously during the procedure, dropped from the control values of 98 to 100 mm. Hg to 50 to 55 mm. Hg within 10 minutes.

Acute Hypoxia

Six dogs were anesthetized with chloralose plus phenobarbital, and a gaseous mixture (92 per cent nitrogen and 8 per cent oxygen) was given through a tracheal tube for five hours. The pO₂, pH of the arterial blood, cardiac rates, arterial blood pressure, and respiratory rates were recorded. The pO₂ of the arterial blood was dropped from the control values of 98 to 100 mm. Hg to 50 to 58 mm. Hg at the end of five hours. The blood pH

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measurements indicated that the animals were in the condition of respiratory alkalosis without any evidence of tissue hypoxia.

**Acute Endotoxic Shock**

Large doses of *Escherichia coli* endotoxin (average, 6 mg./Kg.) were given intravenously to six dogs and the mesenteric peripheral circulation was studied. After 60 minutes of endotoxic shock, the heart was excised.

**Complete Heart-Lung Bypass**

Four animals were subjected to complete right-sided heart-lung bypass by means of a disk oxygenator and two roller pumps. Polyethylene tubes inserted into both femoral arteries served as the arterial inflow, and tubes inserted into both venae cavae served as a venous return by gravity. The pulmonary artery was cannulated, a polyethylene tube was inserted into the right ventricle, and the right ventricular output was measured at various flow rates. Both venae cavae were tied proximally, the pulmonary artery was ligated distal to the catheter, and the dogs were placed on total right heart-lung bypass. The body and the blood temperatures were kept at 37 to 38 C. The flow rates were gradually increased from 20 ml./Kg./min. to 100 ml./Kg./min. and the coronary sinus blood return rates (right ventricular output rates) were measured. At the end of two hours of complete heart-lung bypass, the beating heart was excised.

**Control Group**

Ten dogs were anesthetized with pentobarbital sodium and observed for at least 30 minutes before the beating heart was excised.

**Extraction of Actomyosin**

Thirty Gm. of freshly ground cardiac muscle was treated with 5 volumes of ice-cold, Guba-Straub solution (0.3 M KCl and 0.15 M phosphate buffer, pH 6.7) for 20 to 24 hours. The extract was diluted with 5 volumes of water, the sediment was centrifuged, and the precipitate was redissolved with one-third of its volume of 2 M KCl solution to yield an ionic strength of 0.5 (pH 6.7 to 6.8). The viscosity of the actomyosin solution was measured 10 times before and 10 times after the addition of ATP (0.5 to 0.7 ml. of 10-1 M ATP per 5 ml. of solution) at four-minute intervals, at 26 ± 0.1 C. The relative viscosity, specific viscosity, intrinsic viscosity, ATP activity, ATP sensitivity, and the rate of ATP hydrolysis (k) were calculated from the viscosity measurement data.

**Extraction of Myosin**

Thirty Gm. of cardiac muscle was extracted with 5 volumes of Guba-Straub solution for 15 minutes; the suspension was diluted with 7 volumes of water, the sediment was centrifuged, and the precipitate was dissolved with 6 ml. of 2 M KCl and 3 ml. of 0.25 M phosphate buffer solution. Water was added to give an ionic strength of 0.5. The solution was centrifuged and the supernatant fluid was brought to an ionic strength of 0.28 with water. Actomyosin was removed by 30 minutes of centrifugation, and the supernatant fluid was diluted with water to an ionic strength of 0.05 with gentle stirring in the cold. The sediment was centrifuged and the precipitate was redissolved with buffered 0.5 M KCl. The ATPase activity of this myosin solution was determined by the Fiske-Subbarow method. Tris buffer solution at pH 7.85 and glyeine buffer solution at pH 9.0, with CaCl2 10-2 M as an ionic activator, were used in this ATPase-activity measurement. The liberated inorganic phosphorus (Pi) was expressed in m. P_{i}/mg. protein/min. (and μg. P_{i}/mg. protein/10 min.).

The protein concentration of the actomyosin and myosin solutions was measured by Lowry's method.

**Results**

In figures 1 and 2, the means of intrinsic viscosity data of control, acute hypoxia, acute hemorrhage, endotoxic shock, and cardiopulmonary bypass are illustrated graphically. ATP activities, ATP sensitivities, and the rates of ATP hydrolysis were calculated from these figures and are given in table 1. It may clearly be seen that the controls had the highest intrinsic viscosity, while the intrinsic viscosities of the actomyosin prepared from acute hemorrhage and acute endotoxic shock animals were lower. High intrinsic viscosities of the actomyosin in control and acute-hypoxia groups suggest that the actin in these groups is present in the form of long polymer.

With the addition of ATP, the intrinsic viscosity...
viscosity dropped in all cases as the actomyosin dissociated into actin and myosin. When the rate of drop of viscosity is expressed in terms of ATP activity and ATP sensitivity, the controls show an ATP sensitivity of 72.8 per cent and an ATP activity of 113.5 per cent. The actomyosin prepared from the cardiac muscles in the experimental studies, except hypoxia, showed lower ATP activities and ATP sensitivities (see Table 1).

As the myosin hydrolyzes the ATP, the actin and myosin recombine to form actomyosin in vitro, and the intrinsic viscosity approaches the original level. When the intrinsic viscosity is plotted on the semilogarithmic scale against time in minutes, the recovery phase of viscosity appears to be linear (figs. 1 and 2). The slope $k$ calculated from this line is a measure of the rate of ATP hydrolysis. The $k$ of the control group was calculated to be 0.024. The $k$ values in the experimental groups were lower than in the controls (see Table 1).

The measured myosin-ATPase activity showed that the control was 92.5 ($\mu$M/mg. protein/min. $\times 10^{-2}$) at pH 7.85 (tris buffer) and 88.3 at pH 9.0 (glycine buffer). The acute-hemorrhage group had an ATPase activity of 23.5 at pH 7.85 and 30.0 at pH 9.0, while the acute-hypoxia group had an activity of 25.1 at pH 7.85 and 27.8 at pH 9.0. The ATPase activity of the endotoxic-shock group was 24.2 at pH 7.85 and 25.5 at pH 9.0. The ATPase activity of the heart-lung-bypass myosin was not measured. ATPase activity of myosin solutions are illustrated graphically in figure 3 (in the unit of $\gamma$ P$_i$/mg. protein/10 min.).

### Table 1

**Viscosity Data of the Actomyosin Solution (Mean ± S.D.)*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acute hemorrhage</th>
<th>Endotoxic shock</th>
<th>Heart-lung bypass</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of experiments</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>ATP sensitivity</td>
<td>72.5 ± 20.5</td>
<td>41.3 ± 10.2</td>
<td>63.4 ± 4.7</td>
<td>48.4 ± 16.2</td>
<td>80.7 ± 20.3</td>
</tr>
<tr>
<td>ATP activity</td>
<td>113.5 ± 19.9</td>
<td>62.4 ± 13.9</td>
<td>90.7 ± 5.6</td>
<td>86.6 ± 11.8</td>
<td>120.6 ± 20.7</td>
</tr>
<tr>
<td>Intrinsic viscosity</td>
<td>0.282 ± 0.002</td>
<td>0.183 ± 0.008</td>
<td>0.208 ± 0.007</td>
<td>0.254 ± 0.004</td>
<td>0.241 ± 0.004</td>
</tr>
<tr>
<td>Intrinsic viscosity after ATP</td>
<td>0.129 ± 0.001</td>
<td>0.115 ± 0.008</td>
<td>0.108 ± 0.008</td>
<td>0.135 ± 0.004</td>
<td>0.110 ± 0.003</td>
</tr>
<tr>
<td>$k$</td>
<td>0.024</td>
<td>0.011</td>
<td>0.004</td>
<td>0.009</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*S.D. = standard deviation.

Discussion

The precise mechanism of muscle contraction is not known, although several theories have been proposed, such as Szent-Györgyi's folding model of actomyosin, the sliding model of H. E. Huxley and J. Hanson and A. F. Huxley, and Podolsky's "folding" filament model. All of the theories, however, are predicated on some kind of interaction between actin and myosin.

Short extraction of cardiac muscle in Guba-Straub solution yields myosin, while prolonged extraction yields actomyosin. In the presence of ATP, the actomyosin dissociates into actin and myosin in vitro; therefore, the viscosity decreases. Portzehl et al. termed this viscosity response to the addition of ATP an "ATP sensitivity," and Straub has called it "ATP activity." The higher the actomyosin content or the greater the proportion of actin in the "active" (or polymerized) form, the larger is the drop in the viscosity. Assuming the same phenomena to occur with cardiac actomyosin, the decreases in ATP activity and ATP sensitivity of the actomyosin obtained from the experimental cardiac muscles (except that from the acute-hypoxia group) indicate either that the actin is "inactivated" (or in the depolymerized form) or that extractability of the actin is reduced.
under these experimental conditions. Moreover, since no changes occurred in the ATP activity or ATP sensitivity of actomyosin obtained from the acute-hypoxia group, low oxygen alone apparently cannot account for the alterations in the actin under our experimental conditions.

In this study, the enzymatic activity of the myosin-ATPase was measured directly and also expressed as the calculated value, k. Both values decreased in the experimental groups, suggesting that the myosin-ATPase activity is affected under conditions of acute hemorrhage, acute hypoxia, endotoxic shock, and after bypass procedures.

Since cardiac inefficiency may result from diminished coronary blood flow, tissue anoxia, or a combination of both in the cases of acute hemorrhage and endotoxic shock, it is conceivable that these conditions may adversely affect the contractile proteins and result in inefficient energy utilization and decreases in the contractile force.

In the acute-hypoxia group, the blood pH measurements indicated that the animals remained in respiratory alkalosis; moreover, since there were no indications of tissue anoxia (no metabolic acidosis was detected), it may be assumed that oxygenation of the myocardium was probably adequate. It has been reported that the coronary circulation and cardiac output increase during the hypoxia, and possibly these compensating mechanisms may have protected the myocardium from anoxia. The present study suggests that, of the parameters studied, only the myosin-ATPase activity was affected by hypoxia.

A. Senning has reported that under ideal conditions of cardiopulmonary bypass, no postperfusion hypotension develops; nevertheless, under the present conditions, the animals developed a shocklike syndrome immediately after the bypass procedures. This may have occurred because of insufficient coronary perfusion, metabolic alteration, difficulties in the blood-handling procedures, or some other unknown mechanisms. Perhaps with the present technique, the animals on bypass have suffered blood loss, overperfusion (loaded), inadequate oxygenation of the perfusing blood, or any combination of these, and the actomyosin and myosin of the myocardium were thereby altered in similar fashion to that observed in the acute-hemorrhage and endotoxic-shock groups. These findings indicate that physicochemical changes in cardiac actin and myosin may affect the energy-utilization process within the myocardium, which appears to cause the shocklike syndrome following our bypass procedures.

The next step will be to study the cardiac
contractile proteins under "ideal" conditions of bypass, and also to study the possible reversibility of these protein alterations after the recovery of animals from the bypass procedures.

Summary
Decreases in cardiac efficiency have been observed in experimental endotoxic shock, acute hemorrhage, and cardiopulmonary bypass. Diminished coronary blood flow, tissue anoxia, or metabolic alteration may affect the contractile protein which, in turn, causes cardiac inefficiency. To test this hypothesis, cardiac actomyosin and myosin were extracted with Guba-Straub solution, and intrinsic viscosity, ATP (adenosinetriphosphate) activity, ATP sensitivity, the rate of ATP hydrolysis, and myosin-ATPase activity were determined. Under these experimental conditions, both myosin and actin were altered.

In order to exclude the possible effect of hypoxia of the arterial blood upon the cardiac contractile system, acute hypoxia was produced in dogs and the physicochemical properties of actomyosin and myosin were studied. During acute hypoxia, the myosin-ATPase was affected, but actin appeared to remain unaltered.

These changes of actomyosin and myosin may cause insufficient energy utilization by the contractile system and thereby affect the cardiac efficiency.

References
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