Contractility and Extractability of Heart Actomyosin After Death

By L. Dettli, M.D. and R. J. Bing, M.D.

The contractility and extractability of actomyosin and proteins prepared from dogs' heart immediately, and one hour after death were compared. The yield of total and soluble proteins and of actomyosin was found to be identical. In addition, there was no difference between the after-loaded contractions of actomyosin bands of fresh preparation and those obtained one hour after death.

Studies on patients suffering from congestive failure have shown no abnormality in the extraction and utilization of glucose, pyruvate, lactate, fatty acids, amino acids and ketone bodies by the human heart. This finding has suggested that the basic defect in the heart muscle in myocardial failure is primarily in mechanisms concerned with energy utilization. Similar conclusions were reached by Benson who, by producing experimental chronic failure in dogs, was able to show that actomyosin from failing hearts differed from that of normal hearts. To the present, no experiments have been published which deal with changes of actomyosin of failing human heart muscle. Such investigations could be carried out by comparing actomyosin obtained from heart muscle of patients who died of congestive failure with actomyosin obtained from normal hearts. Since these studies are based on the premise that characteristic properties of actomyosin do not change for a brief period after death, an investigation was begun which deals with a comparison of properties of actomyosin prepared from dogs' hearts immediately after death and one hour later.

Methods

Eleven adult dogs were anesthetized by the intravenous injection of sodium pentobarbital (30 mg./Kg. weight). The chest was then entered and approximately 15 Gm. of left ventricular muscle were excised. The dead animal was then left on the table at room temperature. One hour later another portion of left ventricular muscle was excised.

In both instances actomyosin (myosin-B) was extracted from the specimen, using a modification of the method reported by Benson, Holloway and Freier. All procedures were carried out in the cold room at a temperature of $1^\circ$C. The muscle was minced with iris scissors and 10 Gm. were homogenized in a Waring blender for 3 min., with a fourteen-fold volume of Weber-Edsall solution. The muscle was then extracted for 24 hours at $6^\circ$C. in a twenty-fold volume of Weber-Edsall solution to which the disodium salt of ATP was added to make a total concentration of 0.015 per cent ATP. After extraction, the homogenate was mixed gently by shaking it and a duplicate sample of 2 ml. was withdrawn for determination of total proteins. ATP was again added to give a total concentration of 0.015 per cent and the homogenate was centrifuged and filtered through gauze. At this point, a duplicate sample (aliquot 2 ml.) was withdrawn for soluble protein determination and another duplicate sample (2 ml.) for actomyosin determination. The filtrate was then diluted with a twelvefold volume of cold distilled water. Tenth-normal acetic acid was added until the pH of the solution was 6.9. After 2 to 3 hours, the actomyosin had precipitated and the supernatant fluid was discarded. The precipitate was washed twice in the centrifuge with cold distilled water. Potassium chloride was then added, resulting in a KCl concentration of 0.6 M/L. The dissolved actomyosin was then stored in the refrigerator at $0^\circ$C. For actomyosin precipitation the samples were brought to pH 6.8 with 2 ml. tenth-molar barbital buffer and diluted to a twelvefold volume with distilled water. The precipitated actomyosin was washed 5 times in the centrifuge, dried to constant weight at $110^\circ$C and determined by weight.

The contractile properties of actomyosin were studied according to the method previously described. This method has been devised for comparative experimental studies. With this procedure, contractile bands of actomyosin are formed by compression of surface precipitated films of the pro-
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teins. Afterloaded isotonic contractions of these bands were used in these experiments. The weights lifted by the bands were 0, 5, 10 and 15 nig. respectively. The solution in the contraction chamber was as follows: .005 M ATP, .05 M KCl and .001 M MgCl₂/L. The solution was buffered at pH 7.5 with 0.05 M barbital buffer. Temperature in the contraction chamber was kept at 24 C. Following contraction, the nitrogen content of the band was determined using the micro Kjeldahl method. All contraction experiments were performed within 2 days following protein extraction.

The relative shortening in per cent of resting length of the bands was measured 30 min. after the addition of ATP to the bath. By that time, contraction had been practically completed. Shortening was expressed as a function of the load per "Specific Nitrogen content" of the bands. According to definition, "Specific Nitrogen content" represents the amount of nitrogen in μg/1 mm band in resting length (1 μg. nitrogen/mm = 1 U N).

The regression equation for the results was calculated by the method of least squares.

RESULTS AND DISCUSSION

Table 1 represents the results obtained from 119 protein determinations. It may be seen that there was little difference between fresh heart and hearts extracted one hour after death in the yield of total and soluble protein and actomyosin per 100 Gr. heart weight. The p values of the differences are not significant (p > .2).

The "Specific Nitrogen content" of the bands varied between 1 and 20 U N. The light symbols (series I, fig. 1) represent the results obtained from 24 actomyosin bands which had been prepared from heart muscle immediately after death. The standard error of estimate of these data was S_y = 2.9 per cent, with a correlation coefficient of r = -.88. The heavy symbols (series II, fig. 1) represent the data obtained from 18 bands which were prepared from actomyosin from hearts one hour after death of the animal. Here, S_y = 2.5 per cent and r = -.86. Since in the whole investigated range (0 to 4.5 mg./U N) the differences between the regression lines of the 2 series do not exceed half a standard error of estimate, there is no significant difference between the two sets of data obtained.

The opinions concerning the postmortem stability of actomyosin in vivo and in vitro differ markedly. Weber and Meyer⁶ found that the decrease in solubility of muscle proteins after death was restricted to the myosin fraction. For this reason, they suggest that myosin be extracted immediately after the death of the animal. Minsky⁷ on the other hand, demonstrated that the postmortem decrease in solubility of myosin was not the result of true denaturation of this protein. Erdoes⁸ Banga and Szent-Györgyi⁹ and Engelhardt and Ljubimova¹⁰ have shown that the postmortem changes of actomyosin are, to a large extent, the result in diminution in ATP and are reversible by the addition of this compound. Mommaerts and Seraiderian⁴ also stated that the lability of actomyosin has been generally overrated. Gelotte¹¹ found that actomyosin prepared from hearts was less stable than that

![Fig. 1. Relationship between mg. load/U N of actomyosin bands (abscissa) and their percentage of shortening (ordinate). Light symbols (series I), results obtained from actomyosin bands prepared from hearts immediately after death. Heavy symbols (series II), data obtained from actomyosin bands extracted from hearts one hour after death. No significant difference was found.](http://circres.ahajournals.org/)

**Table 1.**

<table>
<thead>
<tr>
<th>Protein in Gr./100 Gr. Myocardium (Wet Weight)</th>
<th>Extracted immediately after death</th>
<th>Extracted 1 hour after death</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>14.9 ± 1.6</td>
<td>15.2 ± 1.3</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>10.9 ± 1.0</td>
<td>10.8 ± 1.2</td>
<td>&gt;.3</td>
</tr>
<tr>
<td>Actomyosin</td>
<td>5.0 ± .6</td>
<td>5.1 ± .6</td>
<td>&gt;.2</td>
</tr>
</tbody>
</table>
from skeletal muscle. Using the methods of extraction described above, extractability of heart muscle removed one hour after death of the animal is identical with extractability of fresh heart muscle. This is apparent from table 1, which shows no significant differences in the yield of total and soluble protein and actomyosin. However, the yield of actomyosin per unit of heart weight was slightly less than that obtained by Benson, Hallaway and Freier. The small difference is probably the result of different techniques of homogenization of muscle.

The results reported in this paper dealing with the contractility of actomyosin reveal no essential difference between the fresh preparation and that obtained from hearts one hour after death. This finding is not surprising since postmortem stability of the contractile elements in the isolated muscle in vitro has been the basis for widely used pharmacological tests. The insult to the protein during its extraction is probably more severe than the denaturation that occurs during the first hour after death. Furthermore, in order to form actomyosin threads which can produce tension, the protein is subjected to procedures which usually have a highly denaturing action. Thus one method consists in partial drying of the threads, and in another, the threads are formed after spreading the actomyosin as a film on the surface of a solution. Despite these vigorous treatments the contractile properties of the protein are preserved to a great extent.

A solution to the question of whether or not experiments on models such as those reported in this paper bear any relationship to the behavior of protein in the intact heart has been sought by many investigators. Mommaerts considers them as reconstructions of the contractile system. Weber, in comparing models and living systems, finds a close similarity between the two. For example, the maximum tension developed by the models is of the same extent as the maximum tension reached by the corresponding living muscle during contraction. Furthermore, when both living muscle or model, contracting isometrically, are released so that they can shorten freely, the tension does not drop to the isometric maximum value of the new length, but is completely abolished.

In addition, according to Stroebele muscles and fiber models of warm-blooded animals, when uncontracted, show the same intrinsic form and total birefringence. Working with threads compressed from surface precipitated films of actomyosin, Robb and Mallov found a definite effect of ouabain on the contractility of these models. This would hardly seem possible, if the model had lost all similarity to the protein of the intact heart.

Summary

A comparison was made of the contractility of actomyosin and extractability of proteins prepared from dog hearts immediately after death of the animal and one hour after. No significant difference in the yield of total and soluble proteins and of actomyosin was found. Studies of the afterloaded contractions of actomyosin bands revealed no difference between the fresh preparation and that obtained one hour after death.

These studies may open the way for an investigation of the properties of actomyosin obtained from failing human hearts.

Summario in Interlingua

Esseva executate un comparation del contractilitate de actomyosina e del extractibilitate de proteinas de cordes canin, preparate immediamente post le morte del animal e un hora plus tarde. Nulle significative differentia del rendimento de total e de solubile proteinas e de actomyosina esseva trovate.

Studios del post-cargate contractiones de bandas de actomyosina revelava nulle differentia inter preparatos fresc e preparatos obtenite un hora post le morte.

Iste studios va possibilemente preparar le via de investigations del qualitates de actomyosina obtenite ab disfalliente cordes human.

References

Closure of the Ductus Arteriosus After Birth

Many hypotheses have been proposed as to the mechanisms which operate to close the ductus arteriosus after birth. Since 1900 it has been the consensus that closure occurs in two stages: an immediate functional closure, followed by gradual anatomical closure by a process resembling endarteritis obliterans. The immediate closure has been attributed to (a) mechanical compression, constriction or kinking by external forces, (b) closure of a valvulike flap at the junction of the ductus and aorta by the aortic-pulmonary artery pressure difference, and (c) constriction of the muscular fibers in the media of the ductus arteriosus (Virchow 1856).

Recently Dawes, Mott and Widdecombe (1955) reported that a narrowing rather than complete obliteration occurs in the newborn lamb. Flow through the constricted lumen gives rise to a characteristic systolic murmur.

Continuing their studies the investigators at the Nuffield Institute of Medicine at Oxford, England, offer a possible clue as to the nature of the stimulus causing physiologic constriction of the ductus arteriosus. To do this two rather complicated preparations were used. In one, the placenta of one lamb was replaced by the lungs of a twin lamb. In a second an isolated heart-ductus-artificial lung circuit was made.

On the basis of various experimental tests, the conclusion was reached "that either a large increase in arterial O₂ tension, or the release of sympathetic amines by asphyxia, may cause constriction of the ductus arteriosus at birth."

Since constriction in their preparations was not sufficient to cause obliteration but was accompanied by a characteristic systolic murmur and since such murmurs are uncommon in newborn babies, various reasons for the differences are discerned. These do not include the possibility that conditions incident to their complicated experimental technique might not have reenacted actual conditions during normal birth.

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Circ Res. 1956;4:519-522
doi: 10.1161/01.RES.4.5.519

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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