Contractile Proteins of Heart Muscle in Man

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This report deals with the contractile proteins of human muscle in congestive failure, and with the role played by the contractile proteins and by biochemical processes in the regulation of the mechanical function of the heart. The contractility of actomyosin bands prepared from heart muscle of patients who had died in congestive failure was diminished as compared to those prepared from normal hearts. This may have been the result of defective protein synthesis. The increase in heart rate was correlated with the activity of phosphorylase a in heart muscle and with changes in carbohydrate intermediates (lactate, glucose-6-phosphate [G-6-P] and glycogen). The heart rates over 300 per minute were associated with a transient increase, followed by a decrease, in phosphorylase a activity; glycogen diminished, while lactate and G-6-P increased. The oxidation-reduction potential in heart muscle became more negative. In the absence of myocardial anoxia, the increased rate of stimulation of the heart produced no alterations in either the concentration of carbohydrate intermediates or the phosphorylase a activity. Alterations in function of the heart that come into play upon rapid changes of cardiac activity are the result of the integration of several diverse biochemical cellular reactions. The contractile proteins are but following the lead of the cellular elements concerned with the production of energy.

CONTRACTILE PROTEINS of Heart Muscle in Man" is the topic assigned. Strict adherence to this title would limit the field to human heart muscle alone and would make this primarily a technical discussion. Therefore, little opportunity would be afforded for dealing with problems of broader physiologic significance. Consequently, although the title of this presentation will be adhered to in principle, it will also be used as a starting point to contrast the role played by the contractile elements and by biochemical processes in the regulation of the mechanical function of the heart.

Contractile Proteins and Regulation of Cardiac Function

When one considers the properties of actomyosin in solution, it appears that this protein is most susceptible to the influence of adenosine triphosphate (ATP) and ions. In the presence of ATP, at low salt concentra-

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such types as beriberi or thyrotoxic heart disease, the disturbance may be in the contractile proteins. If one could prepare actomyosin models from failing human hearts, one might supplement the studies of Olson, Davis, and Benson, who had attacked this problem by means of biophysical and physical-chemical techinics.

The success of this work depended on 2 unknowns: (1) are available actomyosin models suitable for this work, and (2) since the studies were to be based on the contractility of actomyosin bands obtained from hearts after the death of the patient, it would have to be shown first that characteristic properties of actomyosin do not change for a brief period after death. Dettli explored both these problems; in answer to the first question, he found that actomyosin threads, produced by the compression of surface spread fibers of the proteins, possess certain definitive disadvantages. In a thick thread, such as the one prepared by Hayashi, most of the molecules within the thread are not exposed to immediate contact with ATP but must be reached by unequal diffusion of ATP from the bath. In addition, the thickness of the thread varies a great deal, making it difficult to obtain uniform results. Dettli overcame these difficulties by compressing actomyosin, spread on the surface of a solution, into bands not into threads. This afforded a more constant diffusion of ATP into the preparations and made them a useful tool in comparative experimental studies (fig. 2). Dettli also devised an apparatus for the measurement and recording of afterloaded isotonic contractions; in this system, refined by Kako, it is not necessary to handle the sticky actomyosin band directly; instead, the band can be loaded by moving the weighing spring of a torsion balance by the desired number of milligrams (fig. 3). Since the band contracts on the addition of ATP, the arm of the torsion balance follows the con-

This shows the isotonic contractions of extracted heart muscle after the addition 0.8 per cent ATP. The work of the isotonically contracting muscle strip calculated per unit fiber (millimeter length per millimeter diameter) is related to the tension exerted on this preparation. The work performance increases with rising tension up to an optimal value and decreases when the tension becomes excessive. (From Taeschler and Bing.)

opened and the work, and between the speed of contraction and tension? Dr. Taeschler, using the “fiber model” preparation of Szent-Györgyi (the glycerinated heart muscle fiber) found that the work of the extracted heart muscle increases with rising tension up to a maximal value and then decreases as this load is exceeded (fig. 1*). In this respect, extracted heart muscle reacts similar to fresh heart or skeletal muscle or, for that matter, to the whole heart.

This observation, which was interpreted as evidence that the molecular orientation of the contractile proteins of heart muscle during stretch determines the work performance, prompted us to extend our studies to the contractile elements of the human heart. If our conclusions were correct, then similar techinics should enable us to accumulate evidence of an altered state of contractile proteins in heart muscle of patients who had died in congestive failure. From our metabolic studies, we had already reached the tentative conclusion that in heart failure, excluding

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*Figure 1 reproduced from Taeschler and Bing: Circulation Research 1: 129, 1953. By permission of the American Heart Association, Inc.

traction. This initiates an electronic servo feedback mechanism, which moves the trough in a direction opposite to that of the contraction. Thus, the arm of the torsion balance is always kept in the equilibrium position and a counter force is produced that equalizes the tension of the thread.\(^4\) The movements of the trough are recorded, and, without appreciable friction, it is possible to register shortening of the band at a magnification of 23 diameters.

The second problem, possible changes in the contractility of actomyosin after death, was studied by Dettli for the dog's heart and by Kako in actomyosin bands prepared from human hearts.\(^4\)\(^9\) Kako found that the contractility of actomyosin bands remained undiminished for at least 6 hours after the death of the patient.\(^4\)

The way now appeared open for a comparison of the contractility of actomyosin bands prepared from the left ventricles of normal and of failing human hearts. The actomyosin bands prepared from heart muscle of patients who died in congestive failure were found to possess diminished contractility (fig. 4). It is not within the scope of this paper to dwell on the reasons for this diminished contractility on a molecular level, but the actomyosin bands have an advantage over actomyosin solutions in that studies on the essential property of the contractile proteins, their contractility, can be carried out. The results on actomyosin bands prepared from human hearts are in agreement with those of Benson, who, using glycerinated heart muscle strips from failing myocardium of dogs, found that these fibers did less work than fiber bundles from normal hearts under equivalent conditions of length, temperature, pH, and ATP concentration.\(^10\)

We have asked ourselves repeatedly about what factors could cause a change in contractile elements of heart muscle leading to diminished contractility. We favored the hypothesis of stretch as playing an important role, but this has never been conclusively demonstrated. A change in orientation of actomyosin is also unlikely, since Olson observed changes in myosin molecules of failing heart muscle.\(^6\)

Recently, a very interesting observation on failing hearts has been published by Meerson and Zayats.\(^11\) Their report is of particular
interest, since it touches on the previous discussion, while leading into the regulation of cardiac function by biochemical processes. These authors measured the rate of protein synthesis in hearts of rabbits with experimentally produced aortic stenosis.\textsuperscript{11} Protein synthesis was determined by the rate of uptake of \textsuperscript{35}S-labeled methionine into the heart muscle. The changes occurring in protein synthesis during the development of failure are illustrated in figure 5. Immediately after the production of aortic stenosis, a period called the "state of sudden overload," the heart dilates and its weight increases. The rate of protein synthesis doubles and microscopic changes in heart muscle are noticeable. Muscle glycogen and creatine phosphate diminish, while lactic acid concentration in heart muscle rises. During the second stage, that of stable hyperfunction, the heart weight first increases, then remains constant, and the rate of protein synthesis returns to normal. There is hypertrophy of the muscle fibers. The myocardial concentration of phosphocreatine and glycogen is normal, but the lactic acid concentration remains elevated. During the third stage, that of cardiac decompensation, the heart weight remains stable, but there is dilatation and protein synthesis decreases.

(From Kako and Bing,\textsuperscript{4})

**Figure 4**
The contractility of actomyosin bands prepared from failing human hearts and the effect of digoxin and of calcium and digoxin combined. The regression line and standard deviations obtained from normal data are represented. Many of the points obtained from failing hearts are below the standard deviation of the normal. Digoxin does not influence the percentage shortening (the group mean is still below the deviation of normal data). The combination of digoxin and calcium results in marked improvement of contractility, indicated by the fact that the group mean is now on the normal regression line.

\[ \text{Control: } b = -0.879, \ p < 0.01; \quad \text{failure, control: } b = -0.019, \ p > 0.9; \quad \Delta \text{digoxin: } b = 0.295, \ p > 0.1; \quad \text{digoxin + Ca: } b = 0.404, \ p > 0.5 \]

(From Kako and Bing,\textsuperscript{4})

**Figure 5**
Data from Meerson and Zayats\textsuperscript{11} are graphically illustrated in this figure. The rate of incorporation of \textsuperscript{35}S methionine into myocardial protein increases immediately after the production of aortic insufficiency and then decreases during the state of stable hyperfunction; it reaches its lowest level during myocardial failure.

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The Role Played by Biochemical Processes in the Regulation of Cardiac Function

The challenging concept of Meerson and Zayats relating possible alterations in contractile elements to protein synthesis leads us into the second portion of the discussion, a consideration of biochemical events concerned with the functional regulation of the heart muscle. It is not difficult to predict the influence of these biochemical factors. They are likely to be responsible for a greater speed of contraction and should play a predominant role in the adaptation of the heart muscle to rapid changes in internal environment. Actomyosin bands, gels, superprecipitations have with few exceptions no ways of grading their responses except in an all-or-none fashion. Superprecipitation is an example. As mentioned before, in the presence of ATP, a slight increase in KCl causes an intense precipitation, provided one starts at low potassium chloride concentrations. If 2 consecutive test tubes differ by no more than 0.02 molar potassium chloride, dissolution is found in 1, superprecipitation in the other (fig. 6). In all likelihood, it is the presence of the cell membrane that is responsible for a smooth regulation of the intracellular ionic concentration. In addition to the presence of a membrane or membranes, the organelles of energy production, the mitochondria regulate the speed of contraction in vivo. Their ubiquitous presence makes ATP accessible to every portion of the fibril, while in artificial models the contraction depends on the penetration of ATP from the bath solution.

Therefore, although actomyosin bands may present true models of the contractile elements, they are stereotyped and devoid of the ability to respond quickly and to adapt themselves independently to alterations in environment.

The relationship between enzymatic activity at the cellular level of organization and the functional activity of the heart muscle may be investigated by integrating changes in either the rhythm or the force of con-

Figure 6

At very low potassium chloride concentrations and in the presence of ATP, actomyosin is dissociated. As the potassium chloride concentration is increased, superprecipitation occurs. As the ionic strength is further increased, dissociation and dissolution again take place. (After Szent-Györgyi.)

Figure 7

The effect of ventricular fibrillation on active phosphorylase activity of the left ventricle is shown. Active phosphorylase activity first increases, then diminishes, as ventricular fibrillation proceeds. Mean value of 4 experiments.

Figure 8

The rise in heart rate results in an increase in lactate concentration (μM/100 Gm. muscle) of left ventricular muscle. The highest concentrations are found in ventricular fibrillation.
traction of the heart with well-defined biochemical reactions. In 1943, the Coris discovered the enzyme phosphorylase, which catalyzes the reaction glycogen + inorganic phosphate = glucose-1-phosphate. The enzyme exists in an active and in an inactive form; enzymes in muscle can convert the active into the inactive form or can catalyze the reverse reaction. Phosphorylase appears, therefore, as an important enzyme in determining the rate of glycogenolysis in skeletal muscle. Cori, summarizing the effect of stimulation on phosphorylase a content of skeletal muscle, stated that increasing the speed, as well as the total number of contractions, causes a progressively greater increase in the amount of active phosphorylase; however, during tetanic contractions, the ratio of phosphorylase a to total phosphorylase diminished.

An increased rate of stimulation of skeletal muscle also results in major changes in the carbohydrate intermediaries of the Embden-Meyerhof cycle. Thus, in anaerobic muscle, glycogen disappears, while lactate and hexosephosphate accumulates; apparently, phosphofructokinase becomes the rate-limiting step during anaerobic contraction. If the response of the heart muscle and of skeletal muscle is similar, then the increased heart rate should result in glycogenolysis, with accumulation of glucose-6-phosphate (G-6-P) and lactic acid; it should also lead to an increase in the relative concentration of phosphorylase a to total phosphorylase. This supposition is correct but applies only to the anoxic heart muscle.

Figure 7 illustrates that ventricular tachycardia, with beats over 300 per minute, and ventricular fibrillation first cause an increase and then a diminution in phosphorylase a activity. Similar results are obtained in atrial muscle during atrial fibrillation. The increase followed by the fall in active phosphorylase can be explained by assuming that, during the first seconds of tachycardia, the heart muscle is still alkaline, favoring the enzyme that synthesizes phosphorylase a.

The increased heart rate is also accompanied by definite changes in carbohydrate intermediates. As in skeletal muscle, the increased rate of contraction leads to an increase in lactate and G-6-P concentration and to a decline in glycogen (figs. 8, 9, and 10). This suggests that, as in skeletal muscle, the enzyme phosphofructokinase is the rate-limiting enzyme under these conditions. Undoubtedly, anoxia as initiated by a decline in coronary blood flow is present under these conditions.
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The shift to an anaerobic metabolism and to glycogenolysis and to the transient increase in phosphorylase activity are the results of quick adaptive reactions in energy production; the actomyosin band or thread lacks this ability; it is the fly-wheel of the steam engine without the steam generator.

In the absence of myocardial anoxia, an increased rate of stimulation of the heart produces no alterations in either the concentration of carbohydrate intermediates or in the phosphorylase a activity (table 1). These results were obtained in hearts in situ in which coronary arteries were perfused from a donor animal. Apparently, the increased rate of stimulation, in the absence of anoxia, fails to evoke the metabolic pattern described as typical for muscular contraction. An increase in heart rate alone is not sufficient to stimulate the activated enzyme.

Likewise, the increased force of contraction, for example, as initiated by angiotensin, is without effect on phosphorylase a activity. This enzyme therefore appears to be activated only under the influence of anoxia or catecholamines, which has been shown by Meyer and Moran and by Kukovetz and others.

The conclusions that may be drawn from this discussion are of a specific and of a general nature. In the first place, whatever the underlying physical reasons may be, actomyosin prepared from failing human heart possesses diminished contractility. This adds some weight to the argument that the fundamental defect in heart failure lies in the organs of energy utilization. It is intriguing to consider a causal relationship between defective protein synthesis and diminished contractility.

In general, alterations in function of the heart that come into play upon rapid changes in demand are the result of the integration of several diverse biochemical reactions in the

## Table 1

Percent Changes in Phosphorylase A as a Result of Atrial Fibrillation and of Ventricular Fibrillation after Perfusion of the Coronary Arteries

<table>
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<tr>
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<th>Active phosphorylase % of total</th>
<th>Active phosphorylase % of total</th>
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<tr>
<td></td>
<td>Exp.</td>
<td>Exp.</td>
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<tr>
<td>Time</td>
<td>Control*</td>
<td>Control before perfusion</td>
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<td>5 min. after perfusion</td>
<td>3 min. after perfusion</td>
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<tr>
<td></td>
<td>0.5 min.</td>
<td>Ventricular fibrillation 0.5 min.</td>
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<th></th>
<th>Mean ± S.E.</th>
<th>Mean ± S.E.</th>
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<td>Left atrial appendage</td>
<td>Right atrial appendage</td>
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<tr>
<td></td>
<td>± 5.7 ± 5.9</td>
<td>± 4.0 ± 4.5 ± 4.3</td>
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*Left atrial appendage.
†Right atrial appendage.

cell, which are regulated by the cell membrane and the substrucutres of the cell concerned with energy production. The contractile proteins are but following the lead and command of those cellular elements concerned with energy production and those endowed with the regulation of ionic transfer into, and out of, the cell.

References


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