Mechanochemistry of Cardiac Muscle

IV. UTILIZATION OF HIGH-ENERGY PHOSPHATES IN EXPERIMENTAL HEART FAILURE IN CATS

By Peter E. Pool, M.D., Brian M. Chandler, M.D., James F. Spann, Jr., M.D., Edmund H. Sonnenblick, M.D., and Eugene Braunwald, M.D.

ABSTRACT

This investigation was designed to determine whether a defect in energy utilization exists in heart failure. Accordingly, the direct conversion of chemical energy to mechanical work was studied in right ventricular papillary muscles from normal cats and cats with experimental right ventricular failure secondary to pulmonary artery constriction. Energy production was inhibited by iodoacetic acid and \( \text{N}_2 \). After resting or performing variable amounts of internal contractile element work under isometric conditions, muscles were instantly frozen, and the total amount of chemical energy (\( \sim P = \text{creatine phosphate} + \text{ATP} \)) used was correlated with work performed and the number of contractions. The contractile properties of papillary muscles from cats with heart failure were severely depressed. There was a significant depression in initial \( \sim P \) stores in muscles from cats with heart failure, but there was no significant change in the resting rate of \( \sim P \) utilization. Although the muscles from cats with heart failure performed, on the average, 13% as much work and were activated 64% as many times, the average amount of energy used was only 7% of that used by normal muscles. It is concluded that in this form of experimentally produced heart failure the utilization of \( \sim P \) is reduced but only in relation to the reduction in contractile element work and that the direct conversion of chemical energy to mechanical work is not an inefficient process in this state.

ADDITIONAL KEY WORDS

creatine phosphate papillary muscle adenosine triphosphate energetics

Elucidation of the biochemical basis for congestive heart failure has been of interest to investigators for several decades. There is now evidence that substrate extraction (1), oxygen uptake (1, 2), and oxidative phosphorylation (3) are normal in the presence of heart failure resulting from mechanical overload. It also appears that the physical properties of the contractile proteins are normal (4, 5). A reduction of high-energy phosphate stores of modest degree may be found in the failing myocardium in vivo (6-8), but there is no support for the view that these reduced high-energy phosphate stores are the cause per se of the depression of contractile function. A defect may exist in the utilization of energy in the contractile process, i.e., that there is a reduction in the efficiency with which chemical energy is converted into mechanical work (9-12). The specific aim of the present study was to examine this possibility and to determine whether the efficiency of the conversion of chemical energy to mechanical work is altered in the state of heart failure.

Adenosine triphosphate (ATP) and creatine phosphate appear to be the immediate sources of energy for muscle contraction,
and it has recently been demonstrated that the utilization of these high-energy phosphates by isolated heart muscle preparations can be directly correlated with their mechanical activity (13, 14). This has been accomplished by inhibiting energy production by blockade of both oxidative phosphorylation and anaerobic glycolysis, and subsequently estimating the utilization of energy stores both at rest and during activity. Such studies were carried out on the myocardium of normal cats and cats with experimental heart failure, and this approach provided a means of determining whether heart failure alters the conversion of chemical energy to mechanical work.

**Methods**

**PREPARATION**

Right ventricular papillary muscles were obtained from normal adult mongrel cats (2.0 to 3.2 kg) and cats in which the pulmonary artery had been constricted by a clip 2.8 mm in diameter 25 to 35 days earlier, as previously described (15). This reduced the lumen to 10% of control and resulted in right ventricular failure, as shown previously (15) and documented in the present study. Impaired muscle function, marked right ventricular hypertrophy, and chronic passive congestion were present in all animals in the failure group.

All cats were anesthetized with sodium pentobarbital, 25 mg/kg ip; the papillary muscle was then removed and placed in a myograph containing Krebs solution (13). The base of each muscle was held by a spring-loaded clip, and its length-active tension curve was then determined (12) and with a voltage 10% above threshold. The muscle was held by a spring-loaded clip, and its length-active tension curve was then determined (12) and with a voltage 10% above threshold. The muscle bath was kept at a constant temperature of 28°C. Muscles were stimulated directly by platinum wires on the inner surfaces of the attachment clip with square-wave pulses of 3-msec duration at a frequency of 12/min and with a voltage 10% above threshold.

Glycolysis was inhibited in all muscles by exposure to 5 x 10^-4 M iodoacetic acid for 30 minutes. The adequacy of this blockade has been demonstrated previously (13). Inhibition of aerobic metabolism was achieved by exposing the muscles to Krebs solution previously equilibrated with 95% N_2-5% CO_2. At the completion of each experiment the muscle was rapidly frozen by quickly replacing the muscle bath with a beaker of 2-methylbutane (isopentane), previously cooled in liquid nitrogen to -150 to -160°C. This procedure froze the muscle without further contraction.

**EXPERIMENTAL DESIGN**

The first part of the experiment was similar for all muscles in the study. Each muscle was stimulated to contract isometrically for 30 minutes with a resting tension of less than 0.1 g. Its length-active tension curve was then determined, and the muscle was stimulated to contract at the apex of this curve for an additional 30 minutes. Stimulation was then terminated and iodoacetic acid added to the bath to a final concentration of 5 x 10^-4 M. After the muscle had remained in this solution for 30 minutes without tension and without being stimulated, the bath was abruptly drained, flushed with 95% N_2-5% CO_2, and refilled with Krebs solution previously equilibrated with this gas mixture.

To determine the effects of experimentally produced heart failure on the energy utilization of resting muscles, 76 muscles from normal cats and 35 muscles from cats with experimentally produced heart failure were not stimulated and were frozen either immediately after the change to a nitrogenated solution or after 3, 7, or 10 minutes of nitrogenation. To determine the effects of experimentally produced heart failure on the energy costs of contraction, 63 muscles from normal cats and 41 muscles from cats with heart failure were studied. After 3 minutes of rest in the nitrogenated solution, muscles were stretched to that resting tension which had been found at the apex of their previously determined length-active tension curves and were stimulated to contract isometrically (12/min) a varying number of times before freezing.

**CHEMICAL ANALYSIS**

Detailed descriptions of the methods used for the chemical determinations of total creatine, creatine phosphate, ATP, and inorganic phosphate (13, 14), calculations of total energy utilization (14), and statistical methods (14, 16) have been published.

The initial stores of creatine phosphate and ATP in each muscle were predicted from the ratios of creatine phosphate to total creatine and ATP to total creatine as previously described (14, 16). These ratios in 20 control muscles resting 3 minutes in N_2 were 0.54 ± 0.02 and 0.34 ± 0.01, respectively; in 9 muscles from animals with heart failure these ratios were 0.55 ± 0.04 and 0.59 ± 0.04, respectively. Because the concentration of myocardial creatine phosphate depends to a large extent on the total concentration...
creatinine content of the heart, the creatine phosphate to creatine ratio is similar in papillary muscles from normal cats and those with heart failure. The higher ATP to creatine ratio found in papillary muscles from cats with heart failure reflected the lower total creatine concentration in the hearts of these animals rather than significant alterations of ATP concentration.

**Calculations**

Calculations of contractile element work (CEW) were made from the formula CEW = F/k, where F equals the summed total active tension (g/mm²) generated by each muscle during the experimental period and k is the series elastic constant previously derived (17) from the relation of the modulus of elasticity of the series elastic (dF/dl) to load, F:

\[
dF/dl = k \cdot F.
\]

It has been previously demonstrated that the series elastic constant is similar in papillary muscles obtained from normal cats and from cats with heart failure (18).

**Assumptions and Limitations of Methods**

In evaluating the results of this investigation, the following assumptions and limitations should be kept in mind: (1) Only experimental heart failure produced by ventricular overload was studied. This stress was sufficiently severe that approximately 50% of the animals operated upon survived while the others died of acute congestive heart failure. (2) Each papillary muscle studied was stimulated to contract a variable number of times and thus performed a variable amount of work and used a variable amount of chemical energy. The variation in number of stimuli delivered made it impossible to make direct statistical comparisons between groups and precluded the determination of standard errors for these quantities. (3) There are large differences in the mechanical performance of normal papillary muscles and muscles from cats with heart failure (15). The low amount of work produced by muscles from animals with heart failure made it more difficult to determine the regression axis of energy utilization in this group than in the normal group and thus statistical comparisons based on this regression were less reliable. (4) Actual concentrations of ATP and creatine phosphate could be directly assessed only at the end of the experiment. Initial energy stores were predicted as described above. Although the error of this prediction was shown to be small, the quantities thus derived cannot be compared statistically.

**Results**

Marked right ventricular hypertrophy and chronic passive congestion were present in all 52 cats included in the failure group. The right ventricle to left ventricle weight ratio averaged 0.62 ± 0.02 (sx) in this group, compared to 0.24 ± 0.01 in normal animals (P < 0.001). Severe depression of in-vitro mechanical function was seen in the papillary muscles from cats in the failure group as reflected by a profound decrease in the maximum force, in the maximum rate of force development (dF/dt) at the apex of the length-active tension curve, and in the average work performed per contraction. In papillary muscles from normal animals, peak dF/dt in the presence of metabolic blockade averaged 20.4 ± 5.0 g/sec/mm²; this was 2.4 ± 0.3 g/sec/mm² in papillary muscles from cats with heart failure (P < 0.001). Average tension was 2.2 ± 0.1 in muscles from normal cats and 0.6 ± 0.1 g/mm² in muscles from cats with heart failure.

**Resting Energy Utilization**

Resting energy utilization was evaluated by determining total energy stores (\(-P = \text{creatine phosphate} + \text{ATP}\)) at the onset and after 3, 7, and 10 minutes of nitrogenation. The resting rate of energy utilization was determined by a linear regression analysis on time of the data from 76 muscles obtained from normal cats and 35 muscles from animals with failure. The average initial store of energy (\(-P\)) in the muscles from normal cats was 16.7 ± 0.5 μmoles/g. In the muscles from cats with failure this store was significantly reduced to 10.9 ± 0.5 μmoles/g (P < 0.001) (Fig. 1). Although the resting rate of energy utilization was slightly reduced from an average of 0.77 ± 0.07 μmoles/g/min in muscles from normal cats to 0.60 ± 0.09 in those from animals with failure (Fig. 1), this difference was not significant (0.20 > P > 0.15).

**Effect of Heart Failure on Energy Utilization of Isometric Contractions**

Sixty-three muscles from normal cats and 42 muscles from cats with heart failure were stimulated to contract a varying number of
times, and consequently they performed varying amounts of mechanical work. The amount of energy used per muscle for activation and the performance of work was obtained by subtracting resting energy from the total energy used. The resting energy was calculated from the average resting energy utilization in the regression analysis. This resting energy utilization in muscles from normal animals was not significantly different from that from animals with failure. On an average the muscles from cats with heart failure performed 13% as much work as normal, and they were activated 64% as many times. However, the average amount of energy used was only 7% of that used in normal muscles (Table 1).

To test the significance of these differences, a multiple regression analysis of the data was performed using total energy utilization ($\overline{E}$) as the dependent variable and the number of activations and contractile element work as the independent variables. This provided one time-related and one tension-related variable. The regression model both for muscles from normal cats and from those with experimentally produced heart failure was highly significant, as demonstrated by the multiple correlation coefficient ($r$) and the analysis of variance of the regression for each group (Table 2).

The beta weights of individual regression coefficients of the muscles from normal cats and those with heart failure were compared, and no significant differences either for activation or work were found ($P > 0.5$). The

\[\text{FIGURE 1} \]

Resting energy utilization in papillary muscles from normal cats and from cats with heart failure as determined by regression analysis. $\overline{E} = \text{sum of creatine phosphate and ATP.} T = \text{minutes of nitrogenation.}$ Mean $\overline{E} \pm SE$ is given at 0, 3, 7, and 10 minutes of nitrogenation. Number of muscles evaluated at each time period was in normals 17, 20, 18, and 21, and in heart failure, 9, 9, 9, and 8.

\[\text{TABLE 1} \]

Energy utilization in muscles from normal cats and from those with heart failure.

\[\text{TABLE 2} \]

Multiple regression analysis of energy utilization.

---

2For a table giving the summary of data from these animals, order document NAPS 00264 from ASIS National Auxiliary Publications Service, c/o CCM Information Sciences, Inc., 22 West 34th Street, New York, New York 10001; remitting $1.00 for microfiche or $3.00 for photocopies.
actual value of the contractile element work coefficient of the muscles from cats with heart failure was 0.000 ± 0.007 μmoles/g-cm, a value in itself not significant, and which reflected both the extremely small total amount of work performed by these muscles and the greater variability of the results in this group. In addition, an analysis of the homogeneity of regressions also failed to detect a significant difference between muscles from normal animals and those with heart failure in the slope of their regression planes (P > 0.5). Thus, although average values for the energy utilization and work performed might indicate that muscles from cats with heart failure performed work with a smaller expenditure of energy than did muscles from normal animals (Table 1), the differences between these values were not statistically significant.

Because the amount of work performed per contraction by the muscles from normal cats was greater than that of the muscles from cats with heart failure, the possibility arose that this difference, in itself, might affect the comparability of the groups. In addition, fewer contractions were performed by the muscles from cats with heart failure, and this may have affected comparability. To test the possibility that these muscles might have been less efficient than normal muscles if these variables had been controlled, muscles were arbitrarily selected from the two experimental groups to form separate smaller groups in which average values for these variables would be similar. First, the 12 muscles from cats with heart failure with the greatest average work (or tension) output per contraction were compared to the 12 normal muscles with the least work output per beat. In this case, it was necessary to select only the extremes of the work-output spectrum in each group. Under these conditions, work output per contraction was similar (3.6 g-cm/g/contraction in muscles from cats with heart failure compared to 3.8 in normal muscles). The average energy used, work performed, and number of contractions was calculated for the two groups. Although these muscles from cats with heart failure contracted 94% as many times and performed 83% as much work as normal muscles, they used only 16% as much energy.

Next, the 27 muscles from cats with heart failure which contracted the greatest
ber of times were compared with the 37 normal muscles which contracted the least number of times. These two new groups contracted a similar number of times (25.4 in normals and 25.7 in the others). Under this condition of a similar number of contractions, muscles from cats with heart failure performed 22% as much work as normal muscles and used 26% as much energy.

Discussion

The aim of this study was to determine whether the failing heart is inefficient in converting chemical energy into mechanical work. Since the experiment was purposefully designed to measure only energy utilization in the absence of energy production, any alterations in intermediary metabolism which might occur in heart failure were eliminated. The results indicate that the basal rate of energy utilization in muscles from cats with experimental right ventricular failure is not greater than normal (Fig. 1). In fact, a slightly higher basal rate of energy utilization was found in the normal group, although this difference was not statistically significant (0.20 > P > 0.15).

Although a large number of isometrically contracting muscles from animals with heart failure were studied, statistical evaluation of these muscles was complicated by several factors. The estimation of their initial energy stores was subject to almost twice the variability found in normal muscles. Furthermore, the total amount of work performed by the muscles from cats with failure was extremely small, thus making the regression axis of energy utilization and work performance subject to considerably greater variation. The pattern of work performance by each group of muscles was similar, but because muscles from animals with heart failure had lower initial stores of energy, they were stimulated fewer times to prevent excessive lowering of total energy stores which might lead to the development of rigor. In this way, extra energy utilization which would be associated with rigor development was avoided. Nevertheless, although these muscles performed 13% as much mechanical work and were activated 64% as many times, they used only 7% as much energy as did those muscles from normal cats. Thus, these results clearly indicate that the failing heart is not inefficient in the conversion of chemical energy to mechanical work. This observation lends no support to the view that there is inefficiency in the utilization of high-energy phosphates by the contractile elements in heart failure and is consonant with findings in the intact heart, recently reported from this laboratory, that in the face of acute pharmacologic depression of cardiac contractility, myocardial oxygen consumption (MVO₂) falls concomitantly and overall efficiency increases modestly (19).

In evaluating these results certain limitations inherent in the analysis of the data should be appreciated. In the initial design of this investigation, it was considered important to attempt to delineate the energy cost of activation in addition to the energy cost of performing work. For this purpose it was necessary in each experiment to allow both the number of contractions and amount of work performed to vary. As a result, the data presented in Table 1 have no meaningful error terms, and thus it is not possible to establish the limits of confidence for differences between the groups. The only treatment of these data which is amenable to statistical analysis is that found in Table 2. In this case no significant differences between the groups were discovered. However, because of the large errors involved, it is difficult to evaluate an inability to find significant differences. Thus, the conclusions from these results may be said to be derived from the average data shown in Table 1 rather than from the results of statistically demonstrable significant differences.

The effects of congestive heart failure on overall efficiency have been a matter of controversy. Although it is generally recognized that the useful minute work of the left ventricle is reduced in heart failure, Blain et al. (1), Levine and Wagman (20), and Messer and Neill (21) showed MVO₂ per 100 g of

Circulation Research, Vol. XXIV, March 1969
heart to be unchanged. Since minute work is generally reduced, one might conclude that efficiency was reduced. However, the present study has demonstrated that the efficiency of the conversion of chemical energy into mechanical work is not reduced, at least in the specific form of experimental heart failure produced. Since both activation and work utilize energy, a normal efficiency of energy utilization would be preserved if both the number of activations and the work performed were reduced in heart failure proportional to the reduction in energy utilization. A tendency toward a proportionally greater reduction in the amount of energy used for these processes, as found in the present investigation, implies an improved efficiency of energy utilization.

The intrinsic speed of muscle contraction ($V_{\text{max}}$) has been related to the rate and extent of energy release in both skeletal and cardiac muscle (21-24). Since $V_{\text{max}}$ is known to be decreased in the presence of heart failure (15), one might anticipate that the amount of energy released per unit of work performed by cardiac muscle might be decreased in heart failure, and thus the overall calculated efficiency of the failing heart might actually be greater than that in the normal heart. It is possible that the lower calculated mechanical efficiency observed by other investigators in patients with heart failure (1, 20, 21) reflects the energy costs of increased myocardial wall tension resulting from ventricular dilatation. The "inefficiency" of cardiac function then might be considered to be in the fraction of contractile element work which is expressed as useful external work, rather than in the conversion of chemical energy to contractile element work.

It has recently been observed that the ATPase activity of myofibrils prepared from failing hearts similar to those used in the present study is significantly decreased and that this depression is correlated with the depression of contractility found in these hearts (25). This decrease in ATPase activity may reflect a decrease in the rate of energy release in the failing heart. These findings are in accord with the results of the present investigation, and when taken together, these two studies suggest the failing heart may utilize energy with a normal or slightly increased efficiency but at a decreased rate.

Acknowledgments
The expert technical assistance of Nancy S. Ditemore, Robert M. Lewis, and Richard McGill is gratefully acknowledged. John P. Mullooly, Ph.D., of the Biometrics Research Branch, National Heart Institute, provided valuable advice in mathematical analysis. Advice in computer programming and computation was provided by Caynell E. Jayson and Edward Kronson of the Computer Data Processing Branch, Division of Computer Research, National Institutes of Health.

References
8. Fox, A. C, Wikler, N. S., and Reed, G. E.: High-energy phosphate compounds in the myocardium during experimental congestive heart failure: Purine and pyrimidine nucle-


Mechanochemistry of Cardiac Muscle: IV. Utilization of High-Energy Phosphates in Experimental Heart Failure in Cats

PETER E. POOL, BRIAN M. CHANDLER, JAMES F. SPANN, Jr., EDMUND H. SONNENBLICK and EUGENE BRAUNWALD

Circ Res. 1969;24:313-320
doi: 10.1161/01.RES.24.3.313

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1969 American Heart Association, Inc. All rights reserved.
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:
http://circres.ahajournals.org/content/24/3/313