Effect of Ouabain on the Energy Output of Rabbit Cardiac Muscle

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ABSTRACT

The effect of ouabain, 0.3 \(\mu g/ml\), on the energy output of rabbit papillary muscles has been examined by a myothermic technique. The experiments were conducted at two temperature ranges, 19.2°C to 22.8°C and 29.0°C to 32.2°C, and both isometric and afterloaded isotonic contractions were studied. Temperature differences alone caused pronounced physiological changes, the higher temperature being associated with lower tension-independent heat and markedly higher active efficiency, external work/(external work + active heat production). The heat versus tension curve was rectilinear at higher temperatures but showed upward curvature at lower temperatures. At 19.2°C to 22.8°C, ouabain increased the tension-independent heat by 23%, maximum tension development by 23%, and mean work output by 39%. Ouabain did not significantly alter the slope of the heat versus tension curve and increased mean efficiency only slightly. At 29°C to 32.2°C, ouabain did not cause any significant change in the slope of the heat versus tension curve or in mean muscle efficiency. Ouabain produced significant increases in maximum tension development, mean work output, and the tension-independent heat. The effects of ouabain at the higher temperature were examined at two different calcium levels, 2.5 and 1.25 \(mM\). In the isometric studies the effects of ouabain were independent of the calcium level, and the calcium level itself had no significant effect on the heat-tension relationship. In the isotonic studies, ouabain increased work output but more so at the 2.5 \(mM\) calcium level. Ouabain did not affect mechanical efficiency at either calcium level but muscle efficiency was higher at the 2.5 \(mM\) calcium level. It is concluded that any effects of the cardiac glycosides on energy expenditure are consequences of their inotropic actions and do not represent changes in the energy cost of contraction.

ADDITIONAL KEY WORDS

myothermic technique papillary muscle
temperature calcium tension-independent heat efficiency
heat-tension relationship

The inotropic actions of the cardiac glycosides are well established but there is still considerable controversy over their metabolic actions (1-6). The myothermic technique has therefore been used to investigate the effects of a cardiac glycoside on cardiac energy production.

The magnitude of the inotropic response due to a cardiac glycoside depends upon several factors including temperature, calcium concentration in the medium, and number of stimuli applied during the drug equilibration period (7-13). In the experiments described here, the last of these was kept constant, but the effects of two different temperatures and two different calcium levels were studied.

Both isometric and isotonic experiments were carried out. The isometric experiments performed at different muscle lengths permitted the determination of the relationship between developed tension and cardiac heat production. It was also possible to measure the tension-independent or activation heat at muscle lengths where development of active isometric tension was zero. Afterloaded isotonic contractions were studied to determine the external work done plus the total energy output of the preparations over a wide range.
of loads. These two measurements allowed calculation of the active mechanical efficiency of the muscles before and after exposure to ouabain.

**Methods**

Papillary muscles were obtained from the right ventricles of rabbits. They had a mean weight of 7.2 mg and a mean length of 7.1 mm under a resting tension of 0.5 g. Their mean cross-sectional area was 1.01 mm² and ranged between 0.56 and 1.43 mm². The muscles were suspended vertically in a chamber containing 55 ml of Krebs-Henseleit solution which was aerated with 95% O₂-5% CO₂ and had a pH of 7.4. The first series of experiments was conducted at room temperature which ranged from 19.2° to 22.8°C, and the second series was run at temperatures ranging from 29° to 32.2°C. After dissection, the muscles were stimulated to contract isotonically under a 0.5-g load. This period of preloaded isotonic contractions normally lasted at least 2 hours, during which time there was generally a continual improvement in contractility with most of the improvement occurring in the first hour. Eventually a stable state was reached which could be maintained for 4 to 8 hours. In the changeover experiments the experimental protocol was a long one, and for this reason the equilibration period was reduced to 1 hour and in such experiments there was a time-of-day effect (see Results).

**Mechanical Measurements.**—Each preparation was fixed at both ends with braided non-capillary silk, and the silk at the ventricular end of the preparation was clamped 2 mm from the muscle. The other end of the muscle was connected by a stainless steel tube to either an isometric transducer (Sanborn FTA-100) or to an isotonic transducer (a modified Brush Metripak). When operating isometrically, the total compliance of the transducer, stainless steel tube, and cotton ties was 1.2 × 10⁻⁵ cm/g wt. The isotonic lever had a lever ratio of 20:1, and the equivalent mass of the lever plus stainless steel tube was 560 mg.

**Stimulation.**—The preparation was stimulated by two flexible platinum electrodes cantilevered from the thermopile frame. The stimulus voltage was adjusted to be 10% above threshold (range 3 to 6 v), and the stimulus duration was 0.5 msec at room temperature and 0.3 msec at temperatures close to 30°C.

**Heat Measurements.**—The thermopile used had an output of 3.45 mv/C and was used in conjunction with an Astrodatal Model 120-nv amplifier whose frequency response was reduced to 20 Hz by a filter network. The heat loss from the muscle thermopile system was practically exponential. It averaged 11.3%/sec and was corrected for electrically. The calibration procedure and the corrections to be made for the stimulus artifact have been described previously (14, 15). For the experiments at 29° to 32.2°C, a control vessel (Haake Model N. B. S. Thermostat) was used to circulate fluid to the experimental water bath containing the muscle chamber. With this system, baseline stability was not a problem and closely approached the stability found at room temperature. No attempt has been made to correct the time course of the heat records for conduction delays. Because of the relatively high heat capacity of the thermopiles and the unknown distribution of adhering fluid, any such analysis would not be warranted.

Comparative studies of the rate of heat production made under the same physical conditions are still valid but the heat records cannot be directly compared with the mechanical records; the former are undoubtedly too slow. The heat associated with the contractile response has been called the fast-phase heat, and there is evidence that this heat corresponds to the initial heat of skeletal muscle (16). To measure the total heat (i.e., initial plus recovery heats) with reasonable accuracy, it is necessary to measure the total heat liberated in a train of contractions and then to divide the summed heat by the number of stimuli in that train. The experiments can be either isometric or isotonic ones. The average total heat value per contraction can then be plotted against the average developed tension or the average work done. In the graphs and tables presented in this paper the measured heat (or energy) is always the average total heat per contraction. It should be noted that in afterloaded isotonic contractions the load is allowed to stretch the muscle out to its original length. This work done by the load upon the muscle appears as heat, and hence the total heat measurement alone gives the total energy output of the muscle, i.e., external work and active heat production.

**Efficiency.**—The problems of interpreting efficiency measurements as they relate to muscle contraction have been discussed elsewhere (17-19). We have used the following definition:

\[
\text{active efficiency} = \frac{\text{external work}}{\text{external work} + \text{active heat}}
\]

\[
= \frac{\text{external work}}{\text{total isotonic heat}}.
\]

The reason why the latter formula can be used is given in the preceding section.

**Isometric Contractions.**—In experiments involving isometric contractions, tension was altered by changing muscle length. The relationships between developed tension (g/cm²) and heat...
production (mcal/g muscle weight) for different muscles under different conditions were expressed as polynomials. The polynomials were fitted using the criterion of least squares, tension being treated as the independent variable and heat production as the dependent variable (20). Analysis of variance was used to find components of variability attributable to the regression and due to deviation from regression and to determine whether inclusion of the higher order terms reduced the error variance appreciably (21, chapter 8). In all cases, polynomials of the first or second degree were considered most suitable.

Isotonic Contractions.—Isotonic contractions were afterloaded ones. The muscle length under a resting tension of 0.5 g was taken to be $l_o$, and the tension generated at this length was called $P_o$. In this paper the loads are expressed as fractions of $P_o$ found for the prevailing conditions. To simplify the analysis and presentation of data, we always took values for work, total energy, and efficiency which corresponded to certain load values (i.e., 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 $P_o$). Since the loads actually used were generally not exact fractions of $P_o$, we plotted graphs of the observed data and read the appropriate values (e.g., at 0.2 $P_o$) from the graphs. There were always at least six points on each graph and frequently double this number.

Experimental Design.—There are now several reports that the positive inotropic effect of the cardiac glycosides in mammalian cardiac muscle is chiefly dependent upon the number of contractions during the exposure period (13). For this reason care was taken to standardize the exposure period to ouabain. In all experiments, therefore, the muscles were stimulated at a rate of 15/min for 45 minutes before the standard experiments were commenced. Higher stimulus rates would probably have raised the percentage increase in contractility in the presence of ouabain, but as we were often carrying out experiments lasting 6 to 8 hours we decided it was more important not to risk any deterioration effects caused by depletion of the energy reserves of the preparations.

Room Temperature.—Results were first obtained without ouabain, the total energy output being measured in a train of 15 contractions at a stimulus rate of 15/min. The muscle was then exposed to Krebs solution containing ouabain, 0.3 μg/ml, for 45 minutes and the total energy output measured again as before.

Data were handled using analysis of variance as for a randomized complete-block design (21, chapter 8). Each muscle preparation was considered as a block, to which each of the two treatments was applied once. Thus the variance attributable to differences between muscle preparations was separated from the variance due to experimental error.

Temperature 29°C to 32.2°C.—These experiments were divided into two groups. In the first group the same experiments were performed as at the lower temperature except that the stimulus rate was increased to one every 2 seconds, and the total number of contractions in each experimental train was 20. The drug exposure period remained at 45 minutes and during this time the preparation was stimulated at a rate of 15/min.

In the second group of experiments the design was changed in two ways. Firstly, the effects of two different calcium levels (2.5 mM and 1.25 mM) on the action of ouabain were studied, i.e., a 2 x 2 factorial arrangement of treatments was used (21, chapter 11). Secondly, the treatments were applied in turn to each muscle preparation in such a way as to eliminate any confounding of treatment effects with effects due to temporal changes in the properties of the preparation, and to minimize the likelihood that carryover effects of the drugs would affect the estimates of treatment effects. A changeover design was used in which the four treatments were applied to each of the 8 muscle preparations in a planned sequence, so that variance due to temporal changes in the muscles, as well as variance due to differences between muscles, could be isolated (22). Thus the experimental material was arranged in two 4 x 4 latin squares, each having 4 columns (i.e., muscle preparations) and 4 rows (i.e., positions in the sequence of application of treatments). A 45-minute stabilization period was allowed before each treatment.

The data obtained in the first group were pooled with corresponding data (i.e., data obtained after 2.5 mM calcium) from the second group. Analysis of variance was performed as for a randomized complete-block design.

Presentation of Data.—Significant differences due to ouabain have been indicated by footnotes associated with the mean values in the tables. The $F$ values were determined using analysis of variance. Due to limitations of space the detailed analyses have not been presented, but have been left on deposit.1

Results

Room Temperature

Pilot experiments suggested that a concentration of ouabain of 0.3 μg/ml produced a reasonable inotropic response over a 45-

1For detailed analyses, order document NAPS-00422 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.
FIGURE 1

Heat (top traces) and tension (bottom traces) responses to a single stimulus (a and b) and to a train of stimuli (c and d). Records a and c were obtained before and records b and d after treatment with ouabain. In a and b, the upper heat traces show the tension-independent heat and the lower traces show the fast phase of active heat production. The horizontal lines indicate the heat produced by the stimulus. Time calibration = 1.0 second (a and b) and 20 seconds (c and d).

minute exposure time. Higher doses were tried (e.g., 1.0 μg/ml), but although the inotropic response was more evident initially, it could not be maintained and the preparations would not last satisfactorily over the time required. The magnitude of the inotropic response at the 0.3 μg/ml level varied considerably from preparation to preparation. Whether this variability was due to different threshold sensitivities to the drug has not been investigated. In general, if the tension increment at 1, after treatment was slight, then there was relatively little effect of ouabain on the contractile duration. The classical mechanical effect of ouabain can be seen in Figure 1. The upper traces in a and b show the mechanical response and the heat output per isometric contraction. This action of ouabain can be compared with that of epinephrine (see Fig. 2 of ref. 23). The inserts in the heat traces of a and b show the tension-independent heat liberated with the muscle shortened to a length at which no tension was developed. These traces can be compared with Figure 4 of reference 23. A difficulty about comparisons of single contractions can be seen by examining traces c and d, where the total heat in a block of contractions is shown. It has always been the practice to initiate a single contraction at some fixed time after a preceding train of contractions. This has been done in an endeavor to keep the stimulus history of the muscle as uniform as possible. Notice, however, that ouabain completely alters the usual staircase pattern (see Fig. 1). Following treatment with ouabain, the first contraction after a rest interval is nearly always a maximal response, whereas under normal conditions, a maximal response would only occur after several beats in a train of stimuli. This effect of ouabain was extremely reproducible at all temperatures and necessarily complicates any single beat comparisons. In Figure 2 the time course of the tension-independent heat in response to three stimuli is shown before and after exposure of a preparation to ouabain. The increment is not as striking as that shown in Figure 1, but it is still of the order of 40%.

Heat-Tension Relationship.—The method of obtaining a heat versus tension curve has been set forth elsewhere (14), but in this paper the results have been expressed in terms...
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FIGURE 2

Tension-independent heat responses to three stimuli given under normal conditions (left) and after ouabain (right). The horizontal lines indicate the amount of heat produced by the stimulus itself. Heat calibration = 0.35 mcal/g; time calibration = 1.0 second. Muscle weighed 8.2 mg and was shortened from 7.1 to 5.8 mm to prevent tension development.

\[ h = 0.413 + 0.00390T + 0.00000507T^2 \]

FIGURE 3

The relationship at room temperature between heat production and tension per cross-sectional area. The curves represent the regression equations obtained from the pooled data of 16 muscles. The thin line is the relationship obtained before and the thick line that obtained after exposure to ouabain.

The coefficients of the fitted second-degree polynomials were treated as observations to be handled by three separate analyses of vari-

\[ h = 0.509 + 0.00341T + 0.00000514T^2 \]
Tension, work and heat production before (top traces) and after exposure to ouabain (bottom traces). Records a and f show isometric tension development; tension calibration = 1.0 g. Other records show distance shortened under loads of 2.0 g (b and g), 1.5 g (c and h), 1.0 g (d and i), and 0.5 g (e and j); length calibration = 0.2 mm. Heat calibration on all records = 2.24 g-cm. Muscle weight = 3.1 mg; muscle length = 5.5 mm; temperature = 29°C.

Analysis, one for each of the three coefficients needed to describe the curves. By simply taking the mean value for each coefficient (data from 16 muscles), the following equations (shown graphically in Fig. 3) were used to describe the heat-tension relationship: No ouabain, \( h = 0.413 + 0.00390T + 0.00000507T^2 \); ouabain (0.3 \( \mu \)g/ml), \( h = 0.509 + 0.00341T + 0.00000514T^2 \), where \( h \) is the heat produced (mcal/g) and \( T \) is the tension developed (g/cm²) in a single contraction.

Analysis of variance showed that only the zero-order coefficient (intercept of the curve) was affected by ouabain (\( P < 0.05 \)). That is, apart from the 23% increase in the tension-independent or activation heat component, ouabain did not significantly alter the cost of tension development.

Analysis of the observations made when tension development was zero showed that ouabain increased (\( P < 0.001 \)) the tension-independent heat from 0.45 to 0.56 mcal/g.
TABLE 1

Effect of Ouabain on External Work Done, Total Energy Expenditure, and Mechanical Efficiency at Room Temperature with Varying Loads (Expressed as Fractions of \( P_o \))

<table>
<thead>
<tr>
<th>Drug</th>
<th>( 0.0 )</th>
<th>( 0.2 )</th>
<th>( 0.4 )</th>
<th>( 0.6 )</th>
<th>( 0.8 )</th>
<th>( 1.0 )</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.2</td>
<td>0.4</td>
<td>0.5</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Ouabain, 0.3 ( \mu g/ml )</td>
<td>0.246</td>
<td>0.354</td>
<td>0.329</td>
<td>0.204</td>
<td>0.329</td>
<td>0.204</td>
<td>0.203*</td>
</tr>
<tr>
<td>None</td>
<td>0.45</td>
<td>1.24</td>
<td>1.77</td>
<td>2.11</td>
<td>2.33</td>
<td>2.39</td>
<td>1.71</td>
</tr>
<tr>
<td>Ouabain, 0.3 ( \mu g/ml )</td>
<td>0.56</td>
<td>1.62</td>
<td>2.24</td>
<td>2.59</td>
<td>2.74</td>
<td>2.81</td>
<td>2.09*</td>
</tr>
</tbody>
</table>

Division of table values for external work by corresponding values for total energy does not yield precisely the efficiency values shown in the tables. This is because the efficiency values for individual muscle preparations have been used to obtain the mean efficiencies shown in all the tables, and for the analysis of variance.

Means for 16 preparations are given. * Differs significantly (\( P < 0.001 \)) from the value obtained in the absence of ouabain.

**Isotonic Contractions.**—In the isotonic studies, the external work performed, the total energy liberated, and the mechanical efficiency were measured or calculated (see Discussion). Typical experimental traces are shown in Figure 4. Results for the external work, total energy liberated, and mechanical efficiency are given in Table 1, and are shown graphically in Figure 5. The load has been expressed as a fraction of the peak isometric tension at \( P_o \). This value (\( P_o \)) differed (\( P < 0.001 \)) according to whether ouabain was present or not. Ouabain increased the mean value of \( P_o \) from 3.4 to 4.2 g/cm². Differences between muscle preparations (blocks) accounted for a large part of the variation in all three measured parameters. Ouabain increased (\( P < 0.001 \)) the average external work done by 39%. The heat output per contraction also rose so that the total energy output rose (\( P < 0.001 \)) by 22%. There was a small (11%) increase (\( P < 0.001 \)) in efficiency. These increases occurred at all loads (the load \( \times \) ouabain interaction was not significant).

**TEMPERATURE 29°C TO 32.2°C**

**Heat-Tension Relationship.**—It has been shown that raising the environmental temperature decreases the tension-independent heat. In Figure 6 a typical heat versus tension relationship is shown. Notice that the heat

TABLE 2

Effect of Ouabain on Coefficients of Fitted Polynomials Relating Heat Production to Tension Developed during a Single Contraction at 29° to 32.2°C

<table>
<thead>
<tr>
<th></th>
<th>Straight lines</th>
<th>Second-degree polynomials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero-order coefficient (intercept)</td>
<td>First-order coefficient</td>
</tr>
<tr>
<td>No ouabain</td>
<td>0.311</td>
<td>0.00433</td>
</tr>
<tr>
<td>Ouabain, 0.3 ( \mu g/ml )</td>
<td>0.337</td>
<td>0.00466</td>
</tr>
</tbody>
</table>

Means for 13 muscle preparations are given. * Differs significantly (\( P < 0.05 \)) from the value obtained in the absence of ouabain.
versus tension plots obtained at high temperature are more rectilinear. Indeed at higher temperatures second-degree polynomials gave significantly ($P < 0.05$) better fits than a straight line in only 6 out of 26 cases, and in two of these the second-degree coefficient was negative. The data for heat-tension relationships at $29^\circ$ to $32.2^\circ$C are shown in Table 2, the coefficients both for fitted straight lines and second-degree polynomials being given. A 12% increase ($P < 0.05$) in the tension-independent heat due to ouabain was detectable only if second-degree polynomials were fitted. Ouabain caused no significant difference in the fitted straight lines and no consistent change in slope or curvature of the second-degree polynomials.

However, analysis restricted to the observations made with zero tension development showed that ouabain increased ($P < 0.01$) the tension-independent heat from 0.28 to 0.32 mcal/g.

**Isotonic Contractions.**—The peak isometric tension at $l_0$ ($P_o$) was increased ($P < 0.001$) from 2.79 to 3.51 g/cm² by ouabain, and the average work output rose ($P < 0.001$) by 27%. Total energy output rose ($P < 0.001$) by a similar amount overall, but the increase was more marked at greater loads than at smaller ones (ouabain × load (linear) interaction; $P < 0.05$). Thus, in contrast to the results obtained at room temperature, efficiency was not affected by ouabain at a temperature of
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TABLE 3
Effect of Ouabain on External Work Done, Total Energy Expenditure, and Mechanical Efficiency at 29°C to 32.2°C with Varying Loads (Expressed as Fractions of $P_0$)

<table>
<thead>
<tr>
<th>Drug</th>
<th>$P/P_0$</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>External Work (mcal/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.164</td>
<td>0.236</td>
</tr>
<tr>
<td>Ouabain, 0.3 µg/ml</td>
<td>0.213</td>
<td>0.279</td>
</tr>
<tr>
<td>Total Energy (mcal/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.282</td>
<td>0.732</td>
</tr>
<tr>
<td>Ouabain, 0.3 µg/ml</td>
<td>0.322</td>
<td>0.883</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19.8</td>
<td>20.7</td>
</tr>
<tr>
<td>Ouabain, 0.3 µg/ml</td>
<td>22.2</td>
<td>21.1</td>
</tr>
</tbody>
</table>

Means for 13 preparations are given. * Differs significantly ($P < 0.001$) from the value obtained in the absence of ouabain.

Data for fitted straight lines and second-degree polynomials are shown in Table 4. Ouabain increased ($P < 0.05$) the intercept by 12% but did not change the slope or curvature of second-degree polynomials. The effect of ouabain did not appear as a difference in intercept if straight lines were fitted, but rather as an increase ($P < 0.01$) in the slope of the lines. However, analysis restricted to the observations made when tension development was zero showed that ouabain increased ($P < 0.01$) the tension-independent heat from 0.34 to 0.39 mcal/g.

29°C to 32.2°C (Table 3). It is important to note however, that the increase in temperature (and possibly rate) has caused mechanical efficiency to increase markedly above its room temperature value.

EFFECT OF CALCIUM LEVEL AND OUABAIN AT 29°C TO 32.2°C

Table 3
effects new on

The factorial arrangement of treatments allowed us to test for any interaction between the calcium level and ouabain, as well as for their independent effects. The allocation of these treatments to the different muscle preparations in a changeover pattern (22) allowed isolation and measurement of any changes in the contractile state of the muscles with time of day.

Heat-Tension Relationship.—Second-degree polynomials provided significantly ($P < 0.05$) better fits to the observed data than did straight lines in only 6 out of 32 cases. Second-degree coefficients were negative in 13 out of 32 cases (including 2 of the 6 mentioned above), in contrast to the almost invariant presence of upward curvature seen in the room temperature data.

It should be noted that data reported in this section are not independent of those reported above because results obtained from these preparations when 2.5 mM calcium was present were pooled with similar data from 5 other preparations to give the figures shown in Tables 2 and 3.

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The four treatments were applied to each muscle preparation in a planned way so that effects due to order of application (row effects) could be isolated (see text). Means for 8 muscle preparations are given. Means significantly affected by ouabain are indicated as follows: ^P < 0.01, †P < 0.05.

Effect of Calcium Level and Ouabain on External Work Done, Total Energy Expenditure, and Mechanical Efficiency at 29°C to 32°C with Varying Loads (Expressed as Fractions of P_0)
Results at high temperature. Work, total energy, and efficiency plotted against $P/P_o$ as in Figure 5. Results obtained with a calcium level of 1.25 mM are shown in column A and results with a 2.5 mM calcium level are shown in column B. Each point is the mean of 8 determinations. These results are shown in Table 5. The bottom curves indicate normal conditions and the top curves the presence of ouabain.

Ouabain did not affect mechanical efficiency, but the high $Ca^{2+}$ level did result in higher efficiency, especially when the load was small (calcium $\times$ load [linear] interaction; $P < 0.05$).

A feature of major interest was that all three parameters did vary with time of day (row effect; $P < 0.01$), all being low during the first period of the day. The probable reason for this result has already been outlined in the Methods. It is encouraging to find that in the subsequent periods the mechanical response remained relatively uniform even over experimental times as long as 8 hours.

**Discussion**

**Physiological Implications**

Before examining the effects of ouabain treatment it is necessary to discuss in some detail the physiological results reported in this
paper. The statistical analysis used has allowed us to document for the first time the relationship that exists between tension development and production of active cardiac heat. Interestingly, this relationship which is normally curvilinear at room temperature becomes rectilinear as the temperature is raised so that it resembles the relationship between oxygen consumption and tension development in whole-heart experiments at 37°C (24-27).

The reason for this change with temperature is not readily apparent, but it is not because we have examined different ranges of tension development. The tension range was kept approximately constant by increasing the stimulus frequency at the higher temperature. One possible explanation for the linear plot, obtained at the higher temperature, relates to the shortening of the duration of the active state that occurs under these conditions. For skeletal muscle, Hill (28) has already postulated the existence of a weak positive feedback whereby tension prolongs activity and activity prolongs tension with the consequent liberation of extra energy. It might be expected that such a feedback system would be more noticeable in terms of energy output at temperatures where the duration of the active state is normally much prolonged. Physiological procedures so far analyzed at 37°C have failed to show that alterations in duration of the active state can influence oxygen consumption, and it has been suggested that this is because the cardiac active state is so brief at this temperature (27). Alternatively, one might consider whether tension development is the primary parameter to which energy expenditure is related. It may be important that the same experimental data that give a curvilinear heat versus tension plot at room temperature can produce a rectilinear heat versus tension-time integral plot (unpublished results). In the present series of experiments we did plot for several muscles curves of heat versus tension and heat versus the tension-time integral. The results obtained with and without ouabain are shown for a typical muscle in Figure 8. This is not to suggest that the tension-time integral is a better index of cardiac oxygen consumption than peak tension development or contractile element work but, as Sandberg and Carlson (29) have pointed out, there are theoretical reasons why the amount of ATP split in a contraction should be proportional to the time integral of force.

This problem of determining which mechanical variable correlates best with energy output has stopped us from attempting to analyze our data in greater detail. Shortening heat for example has been measured elsewhere by subtracting the external work from a total energy plot and comparing the residual with the energy expected on the basis of a heat versus tension plot (14). If other variables such as intensity or duration of the active state or the tension-time integral are determinants.
of cardiac oxygen consumption, then the previous analysis may not be valid. Indeed preliminary experiments when analyzed using the tension-time integral as an index of energy output almost eliminate a shortening heat component.

The tension-independent heat component or activation heat has been examined in some detail recently. The results reported here confirm that the magnitude of this component decreases with temperature. It is about 0.25 mcal/g/contraction at 37°C, and this value is close to that estimated on the basis of oxygen consumption studies of whole hearts (26, 30).

In our experiments, a temperature rise has apparently produced an increase in active efficiency from about 14% at room temperature to about 21% at 30°C. It is possible to see why this has occurred if we examine the work done with, for example, a load equal to 0.4Pₒ. Now if Pₒ = 3.4 g (20°C) and Pₒ = 2.8 g (30°C) and if the respective work outputs with loads equal to 0.4Pₒ are 0.252 and 0.236 mcal/g, respectively (see Tables 1 and 3), and if we let Pₒ = 3.4 g be equivalent to a cross-sectional tension of 500 g/cm², then, using the experimentally determined relationship between heat and tension:

\[
h = 0.413 + 0.00390T + 0.00000507T^2 \quad (20°C) \\
h = 0.277 + 0.00399T + 0.00000201T^2 \quad (30°C),
\]

it can be calculated that at room temperature the mechanical efficiency would be 15.1% \((0.252/(0.252 + 1.421) \times 100)\) whereas at 30°C mechanical efficiency is 19.2% \((0.236/(0.236 + 0.990) \times 100)\).

Now the above calculation is very approximate, but it can be seen that the efficiency increases because (1) roughly the same amount of external work is done even though Pₒ (in spite of the rate increase) is lower at the higher temperature, (2) there has been a decrease in the size of the tension-independent heat, and (3) the slope of the heat versus tension curve is more steep at low temperatures (see Fig. 6). The increase in efficiency at high temperature that we have found appears to be at variance with data reported by Reisman and Van Citters (31). These authors reported an efficiency increase when the temperature was dropped from 37°C to 27°C. We cannot explain this discrepancy at present but it may relate to the different temperature ranges used, to the different preparations, or to the inclusion of the resting oxygen consumption in the whole animal efficiency studies.

**EFFECTS OF OUABAIN**

There is no doubt that ouabain increased both the magnitude and the rate of production of the tension-independent heat. The increase in magnitude was more obvious when a separate analysis was made of this component. It has already been shown that an increase in the external calcium level or the action of an inotropic agent such as epinephrine increases both the rate of production and the magnitude of this component (32). These effects are always associated with an increase in the maximum shortening velocity of the contractile element. Our results show that there is an interaction between calcium and ouabain supporting many previous reports in the literature. Several authors have postulated the existence of a pool of tissue calcium that becomes exchangeable during the contractile response, and they suggest that the positive inotropic action of the cardiac glycosides is associated with increased calcium release from such an area (11, 33). Langer has shown that development of active tension is proportional to the calcium content of a region within the myocardium represented by a specific, kinetically defined phase of calcium exchange and that a fall in temperature from 30°C to 20°C results in an increase in the calcium content of phase 2 accompanied by a positive inotropic response (34, 35). There is evidence that phase 2 is probably the sarcotubular region of the muscle which functions to release calcium. Langer (36) has argued that a "sodium pump" lag leads to the increase in phase-2 calcium, and there is already abundant evidence that cardiac glycosides can cause inhibition of the sodium pump (37-39). Thus the effect of ouabain upon the tension-independent heat component indirectly supports the idea that at least one of the actions
of the cardiac glycosides is upon the excitation-contraction coupling mechanism.

It is quite evident that ouabain has not had a large effect upon the heat versus tension relationship except the effect on tension-independent heat. This immediately poses a question. Why is tension generation so much more costly in the presence of epinephrine which produces almost identical mechanical effects to those of ouabain? Unfortunately the heat versus tension curve in the presence of epinephrine is difficult to measure accurately because it is continually changing (32). Even so, it would appear that epinephrine increased the magnitude of the tension-independent heat much more than ouabain did. In part this may be due to differences in drug concentrations but even in some experiments with ouabain at a concentration of 1.0 μg/ml the increment was never as high as that seen in the presence of epinephrine (see Fig. 7 of ref. 32). A major metabolic difference between these two agents would be the ability of epinephrine to increase the formation of cyclic 3'5'-adenosine monophosphate, and it has already been suggested that the level of cyclic AMP may be one of the factors controlling the magnitude of the tension-independent heat component (23).

Until recently it has been customary to state that cardiac glycosides increase cardiac efficiency (4, 40-42). The experimental basis for this belief has been that an increased work output can be obtained from failing hearts without much change in oxygen consumption. Studies on intact animals or with whole-heart preparations are complicated by several factors, and in particular, we would like to draw attention to the resting oxygen consumption. A clinical symptom of cardiac failure is an increased end-diastolic volume of the ventricles due to an increased end-diastolic pressure. One of the consequences of cardiac glycoside therapy is a fall in this pressure so that the dilated heart returns towards its normal size. Now several authors have reported that the resting metabolism of the heart is sensitive to diastolic fiber length (14, 43-45). These results are not universally accepted (46) but the evidence is such that experiments on whole hearts should be designed to determine whether any increase in oxygen consumption, consequent upon the inotropic action of ouabain, is masked by a decrease in the resting oxygen consumption level. In the present investigations we have not examined the effects of ouabain upon resting heat production. The main reason for this omission is that there is a continuous fall in the resting heat rate during the day. This fall occurs even though there is little or no change in cardiac contractility over this same period of time. We can state, however, that for the concentration of ouabain that we employed there was never any evidence that ouabain altered resting heat production. Lee et al. (4) reported that in the presence of ouabain the active oxygen consumption of isolated papillary muscles rose by 150%, while there was a 600% rise in contractility which occurred some time before the change in oxygen consumption. We feel that the heat-tension relationship that we observed may help explain this increase in mechanical efficiency. To achieve such an increment in tension, one must at the outset have a preparation that is developing a low cross-sectional tension, let us say some 50 g/cm². If we examine a heat versus tension curve (see Fig. 3), it can be seen that a sixfold increase in tension would lead only to about 150% rise in energy expenditure, whether ouabain was present or not, i.e., there is normally an increase in efficiency whenever tension development increases in the sense that the tension-independent heat becomes a smaller fraction of the active heat production. The possibility that the increase in contractility precedes the increase in energy expenditure was not examined in our experiments but we would like to point out that in the experiments of Lee et al. (4) the oxygen electrode was some distance from the papillary muscle, and we think that the possibility must exist that the time separation between the inotropic and oxygen consumption effects might be due to chamber geometry. This conclusion is supported by the results of Coleman (47) and Klaus and Krebs (48) who found no time lag.
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between the inotropic effect and the increase in oxygen consumption.

There have been two recent studies (47, 49), one on the intact canine heart and the other on isolated cat papillary muscle, that have shown that cardiac glycosides decrease cardiac efficiency. In the canine heart experiments, heart rate, stroke volume, and mean aortic pressure were held constant but oxygen consumption rose by about one third. In experiments with cat papillary muscle it was shown that oxygen consumption rose by about 30% in isotonic and isometric contractions and that if the muscles were arranged to develop the same tension, by altering muscle length, tension generation was more costly in the presence of acetylstrophanthidin. In both papers the increased oxygen consumption was attributed to the increase in the velocity of myocardial shortening produced by the glycosides (50). There is not necessarily a serious discrepancy between the results reported by those authors and results presented in this paper. If the increase in the magnitude of the tension-independent heat component can be much greater than we found, then the results would be quite comparable. The magnitude of any such increase might depend upon several factors, such as dose level, animal species, temperature, or stimulus pattern. It is quite possible that the magnitude of the tension-independent heat component may be related to velocity of fiber shortening, but whether this is a cause-and-effect relationship remains to be established.

We conclude therefore that under our experimental conditions the cardiac glycosides do not produce major changes in the efficiency of cardiac muscle and that any increase in oxygen consumption is associated with and is probably due to the action of ouabain on the ability of cardiac muscles to manifest tension or to lift a load; this latter action is exerted predominantly on the excitation-contraction coupling mechanism.

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Effect of Ouabain on the Energy Output of Rabbit Cardiac Muscle
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