Surface Layer ATP-related Contraction in Isolated, Superfused Canine Ventricular Papillary Muscle: An Isotachophoretic Analysis

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Abstract The relationship among contractile tension, ATP content and concentration was examined in isolated, superfused ventricular papillary muscle under normoxic (P_o₂ = 300 mmHg) and hypoxic (P_o₂ = 100 mmHg) conditions, using capillary isotachophoresis. The muscle preparations were exposed to each condition for 30 min, and the contractile tension was recorded with a strain gauge. Immediately after the recordings, the preparations were homogenized and the metabolites (ATP, ADP, AMP, creatine phosphate, inosine monophosphate, NAD, NADH, glucose-6-phosphate, pyruvate, and inorganic phosphate) were extracted in 50% methanol-1.25 mm EDTA solution at -20°C for 4 days. The supernatant of the extract was used for the isotachophoretic analysis. Hypoxia markedly depressed ATP content and concentration in the tissue. Under conditions of normoxia, but not hypoxia, the developed tension positively correlated with the ATP content and concentration. Under normoxic conditions, the tension tended to be proportional to the estimated surface area of the preparation, while in hypoxia it tended to be inversely proportional thereto. The ATP concentration appeared to be inversely proportional to the muscle weight, thereby suggesting that the outer layer of the preparation contains more ATP than the inner. In fact, an isotachophoretic analysis of the tissue revealed significantly higher ATP concentrations in the outer layer. Our findings indicate that there is a central anoxic core in the isolated canine papillary muscle superfused with oxygenated Tyrode solution and that surface layer ATP probably plays a pivotal role in the initiation of contraction.

Key Words: isotachophoresis, ventricular papillary muscle, ATP, contraction.

The mechanical activity of an isolated, superfused papillary muscle would be an ideal model for investigations of cardiac muscle in vivo. However, there
are many reports indicating that the peak stress (force per cross-sectional area) developed by isolated papillary muscle declines with an increase in the muscle size and that such may not occur in the papillary muscle in vivo (Kelly and Hoffman, 1960; Bing et al., 1971; Frezza and Bing, 1976; Loisele, 1979; Loiselle and Gibbs, 1979; Delbridge and Loiselle, 1981).

Cranefield and Greenspan (1960) demonstrated that the decline in mechanical performance with the increase in size of the muscle is due to an inadequacy of diffusion of oxygen into the central core of the preparation. Hill (1928) also predicted the existence of an anoxic core in the isolated, superfused papillary muscle and used peak tension or stress as an index of myocardial oxygen uptake (Evans and Hill, 1914; Hill, 1925). Recently, Delbridge and Loiselle (1981) examined the hypothesis that the negative correlation of stress to the cross-sectional area (decrease of the stress with increasing cross-sectional area) is the result of insufficient distribution of mitochondria among the contractile matrix. However, they found no difference in the proportion of these two cytoplasmic components between small and large papillary muscle groups, thus implying that sufficient ATP could be supplied to the contractile machinery, even in the large muscle group.

Therefore, we considered it of interest to investigate whether there actually exists a central anoxic core in papillary muscles of different sizes. We examined the relationships among the contractile tension and the content and concentration of various metabolites, using canine papillary muscles of different sizes exposed to either normoxic or hypoxic conditions. For determination of metabolite concentrations, we used capillary isotachophoresis which enabled simultaneous measurement of many metabolites (including adenine nucleotides) contained in a single muscle preparation (Aomine et al., 1982). Among these metabolites, special attention was directed to the relation between ATP levels and contractile tension, in attempts to demonstrate the existence or absence of the anoxic core. We obtained data showing that the level of ATP in the surface layer of the isolated muscle preparation is considerably higher than that in the central core zone, thus indicating the existence of an anoxic core in the large papillary muscle. A preliminary report of this work has been presented elsewhere (Aomine et al., 1981).

**MATERIALS AND METHODS**

Preparation. The papillary muscle was obtained from the right ventricle of mongrel dogs (either sex, 6.2±1.0 kg, n=37) which had been anesthetized with sodium secobarbital, 30 mg/kg i.p. The heart was rapidly removed and immersed in oxygenated Tyrode solution warmed to a temperature of 37°C, and the right ventricular wall was opened. A silk thread was tied at the tendinous end of the papillary muscle, and the muscle was carefully dissected from the right ventricle.
to avoid mechanical injury.

Solution. The composition of the Tyrode solution used was as follows (in mm): NaCl 137, KCl 5.4, NaHCO₃ 11.9, MgSO₄ 1.05, NaH₂PO₄ 0.42, CaCl₂ 1.8, and glucose 10. The pH was 7.4 after bubbling with a gas mixture of 95% O₂ and 5% CO₂. The P₀₂ within the muscle bath was about 300 mmHg, as measured with an oxygen analyzer (Beckman 0260). Hypoxia was produced by changing the composition of the gas mixture to 95% N₂ and 5% CO₂. The P₀₂ was reduced to about 100 mmHg within a few minutes. The muscle bath of about 1.3 ml volume was perfused with the solutions at a constant rate of 2.35 ml/min with the use of a peristaltic pump (Tokyo Rikakikai MP-3).

Measurement of isometric tension. The preparation was mounted horizontally on the rubber floor of a muscle bath continuously perfused with Tyrode solution (37±0.5°C). The base of the preparation was pinned on the floor and the other tendinous end was connected with a thread to a force transducer (Nihon Kohden TB-612T). The output of the transducer was amplified and displayed on an oscilloscope (Nihon Kohden VC-9) and on a chart recorder (Nihon Kohden RJG-4004). The muscle was slightly stretched to give a minimum resting tension and was stimulated by isolated, rectangular pulses of 1 Hz and 5 msec duration with the intensity of 3-fold threshold voltage. The stimulating electrode consisted of a pair of silver wires insulated except at their tips, and which were attached beneath the center of the preparation. After initiation of stimulation, the muscle was gradually stretched to the length where the maximum contraction could be obtained (optimal length, Lₘₐₓ). All experiments were performed with the muscle length set at the Lₘₐₓ. The time required for the adjustment to obtain Lₘₐₓ was about 10 min. The Lₘₐₓ, as determined on a microscopic scale, ranged from 2 to 7 mm (4.8±1.5 mm, mean±S.D., n=19) in the preparations used for normoxic experiments and 3 to 7 mm (4.5±1.4 mm, n=18) for the hypoxic experiments. There was no statistically significant difference between these two groups of preparations. The muscle size can be expressed in three different ways: the diameter, the cross-sectional area or the weight. In the present experiments, the diameter measured at the half-way point of the total muscle length ranged from 1 to 4 mm (2.1±0.8 mm, n=19) for normoxic experiments and from 0.5 to 5 mm (1.9±1.0 mm, n=18) for hypoxic experiments. Again there was no significant difference between these two groups of preparations. The wet weights of the muscles were determined after blotting with filter paper, and were 21.2±15.9 mg (n=22) for preparations under normoxic conditions and 18.2±19.6 mg (n=16) for those under hypoxic conditions. There was no significant difference between these two values. Immediately after termination of the tension recording, the preparations were removed from the muscle bath and transferred to the solution for homogenization, after which an isotachophoretic analysis of the various metabolites was made.

Assay of metabolites by isotachophoresis. The concentrations of 11 metab-
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Metabolites contained in the ventricular papillary muscle were determined by isotachophoretic analysis. These metabolites were: adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), β-nicotinamide adenine dinucleotide (NAD), β-nicotinamide adenine dinucleotide, reduced form (NADH), pyruvate, inorganic phosphate (P_i), lactate, creatine phosphate (CP), and glucose-6-phosphate (G6P). The precise methods of analysis were as reported previously (Aomine et al., 1982), except for the homogenization-related procedures. The muscle was homogenized in a 50% methanol-1.25 mm EDTA solution for about 5 min using an ice-chilled Teflon hand-homogenizer, instead of a pestle in liquid N_2 used in the previous study (Aomine et al., 1982). We found that a greater number of metabolites could be extracted from the muscle using hand-homogenization.

A typical isotachophoretic record from canine papillary muscle is shown in Fig. 12 and in the previous paper (Aomine et al., 1982; Fig. 5).

Statistical analysis. Data were presented as mean±standard deviation (S.D.). The difference was analyzed by Student’s t-test and was considered significant at values of P<0.05. The correlation coefficient and the first order linear least square regression line were also calculated using a computer (Sharp, Elsi Mate PC-1300S).

RESULTS

Effect of hypoxia on force development

Figure 1 demonstrates typical records of contractile tensions before (normoxic) and after 10, 20, and 30 min of superfusion with hypoxic solution. The rapid decline in the developed tension occurred within a few minutes of initiation of the hypoxic perfusion and was accompanied by a slow development of an increase in the resting tension. During the progression of the hypoxia, the developed tension decreased while the resting tension increased gradually. The developed tension at 30-min hypoxia declined to about 28% of the control value.

Fig. 1. Changes of contractile tension before (control, $P_{O_2}=300$ mmHg) and after 10, 20, and 30 min of hypoxic perfusion ($P_{O_2}=100$ mmHg) in canine papillary muscle. The muscle was stimulated at 1 Hz throughout. The broken line indicates the level of resting tension in the control record. Note that the decline in developed tension was accompanied by a gradual rise in the resting tension during hypoxia.

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In 20 preparations, the magnitude of the developed tension under conditions of normoxia was $202.7 \pm 113.4$ mg. At 10, 20, and 30 min after the hypoxic perfusion, the magnitude decreased to $121.8 \pm 90.5$, $85.5 \pm 61.9$, and $57.1 \pm 54.1$ mg, respectively. The resting tension was increased by $69.7 \pm 180.0$, $108.8 \pm 228.0$, and $213.1 \pm 277.8$ mg at 10, 20, and 30 min after hypoxia, respectively. Neither the duration of contraction nor the time to peak tension were

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**Fig. 2.** A: levels of adenine nucleotides (ATP, ADP, and AMP) in canine papillary muscles measured in the 30-min oxygenated condition (open column) and the 30-min hypoxic condition (stippled column). The muscles were stimulated at 1 Hz, in both conditions. The data were obtained from 22 preparations (in normoxia) and 20 preparations (in hypoxia). Vertical bars indicate S.D. * marks significant difference ($P < 0.001$) between normoxia and hypoxia. B: comparison of the levels of total adenine nucleotides (ATP+ADP+AMP) in canine papillary muscle (stimulated at 1 Hz) in the 30-min oxygenated condition and the 30-min hypoxic condition,

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significantly changed from values in the controls. The decrease in the developed tension which occurred in the hypoxic tissues was primarily due to a decrease in the maximum rate of rise of the developed tension.

**Effect of hypoxia on levels of tissue metabolites**

In dog isolated, perfused heart, hypoxia may produce a variety of functional, biochemical, and morphological changes in the muscle. Among these are rapid depletion of tissue ATP, CP, and glycogen accompanied by a rapid decline in the developed tension and an increase in the resting tension (Gudbjarnason et al., 1969; Hearse et al., 1973; Nayler et al., 1976; Reibel and Rovetto, 1978; Gauduel et al., 1979).

Figure 2A shows the effect of 30-min normoxic or hypoxic perfusion on the levels of ATP, ADP, and AMP in muscle stimulated at 1 Hz. After 30 min of hypoxic perfusion, the levels of ATP, ADP, and AMP decreased to 14.9, 47.9, and 14.6% of the control value (30-min normoxic perfusion). As shown in Fig. 2B, the total amount of adenine nucleotides markedly decreased after 30-min hypoxic perfusion. The high-energy potential of the myocardial cells can be expressed as the energy charge ([ATP] + 1/2[ADP])/([ATP] + [ADP] + [AMP]) (Atkinson, 1968). The energy charge of the papillary muscle during normoxic
perfusion amounted to 0.78 while that after hypoxic perfusion decreased to 0.68. Changes in the levels of the other 8 metabolites are summarized in Fig. 3. During hypoxia, the levels of CP, NADH, NAD, pyruvate, and G6P decreased significantly, as compared to the control condition (normoxia). The decrease in CP level seemed less marked than that reported by Reibel and Rovetto (1978) and Gauduel et al. (1979), while it was comparable to the decrease reported in the case of the rabbit heart perfused using the Langendorff technique (Guarnieri et al., 1978). The levels of IMP, P1, and lactate remained unchanged during the hypoxia.

![Figure 4A](image1.png)

**Fig. 4.** A: the relationship between the wet weight and the ATP content in canine papillary muscle exposed to normoxia (○) and hypoxia (●) for 30 min. Solid line (normoxia) and broken line (hypoxia) were drawn by using the linear squares regression analysis.

![Figure 4B](image2.png)

B: the relationship between the wet weight and the ATP concentration in canine papillary muscle exposed to normoxia (○) and hypoxia (●) for 30 min. Solid line (normoxia) and broken line (hypoxia) were drawn by using linear least squares regression analysis.

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Relationship among muscle weight, ATP content, and concentration

After 30-min exposure to normoxic solution, the ATP content of the papillary muscle was $52.0 \pm 38.5$ nmol ($n=20$), while after hypoxia, the content was $5.4 \pm 5.5$ nmol ($n=16$). Figure 4A shows the relationship between the wet weight of the preparations and the ATP content measured under the condition of normoxia or hypoxia. In cases of normoxia, the ATP content increased significantly with increasing muscle weight, thereby indicating that large preparations contain much more ATP. However, under hypoxic conditions, the ATP content markedly decreased, irrespective of the muscle weight.

We then examined the relationship between the muscle weight and the ATP concentration, under both conditions (Fig. 4B). The ATP concentration tended to be inversely proportional to the muscle weight, under both normoxic and hypoxic conditions. This is in sharp contrast to the relationship between the weight and the ATP content shown in Fig. 4A and suggests a non-homogeneous distribution of ATP in dog papillary muscle.

Relationship among contractile tension, ATP content, and concentration

Figure 5 illustrates the relationship between the magnitudes of the developed tension and the ATP content, under conditions of normoxia and hypoxia. In normoxia, the developed tension significantly correlated with the ATP content,

Fig. 5. The relationship between the contractile tension and ATP content in canine papillary muscle exposed to normoxia (○) and hypoxia (●) for 30 min. The magnitude of the tension was measured at 30 min, after exposure to either condition. Solid line (normoxia) and broken line (hypoxia) were drawn by using linear least squares regression analysis.

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thereby indicating that the higher the ATP content, the larger the developed tension. On the other hand, the tension tended to be inversely proportional to the ATP content under conditions of hypoxia.

Figure 6 shows the relationship between the magnitude of the developed tension and ATP concentration, in normoxia and hypoxia. In the former but not the latter, the most significant positive correlation was evident between the magnitude of the tension and the ATP concentration. These findings suggest that under conditions of normoxia, the ATP concentration (and not the content) is the most significant determinant of tension which develops in the papillary muscle.

**Relationship among the surface area, ATP content, and concentration**

By assuming that the canine papillary muscle is of cylinder shape with a uniform cross-sectional area and that the diameter at the "cylinder" is the diameter at the half-way point of the total muscle length, we were able to make a rough estimation of the surface area of the preparation. The estimated surface area was 40.6±23.3 mm² (n=20) for normoxic preparations and 36.6±30.5 mm² (n=19) for hypoxic ones. There was no significant difference between these values. Figure 7 shows the relationship between the surface area and ATP content for both conditions. In normoxic muscles, there was a positive correlation between the surface area and the ATP content. In hypoxic tissues, the slope of the relation became steeper, indicating that under conditions of hypoxia, reduc-
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Fig. 7. The relationship between surface area and ATP content in canine papillary muscle exposed to normoxia (○) and hypoxia (●) for 30 min. The surface area of the muscle was estimated under the assumption that the preparation was of cylinder shape and that the diameter represented half of the total muscle length. Solid line (normoxia) and broken line (hypoxia) were drawn by using linear squares regression analysis.

Fig. 7. The relationship between surface area and ATP content in canine papillary muscle exposed to normoxia (○) and hypoxia (●) for 30 min. The surface area of the muscle was estimated under the assumption that the preparation was of cylinder shape and that the diameter represented half of the total muscle length. Solid line (normoxia) and broken line (hypoxia) were drawn by using linear squares regression analysis.

The relationship between surface area and ATP content is rather marked in the presence of a wide surface area. We then examined the relation between the surface area and the developed tension, under normoxic and hypoxic conditions (Fig. 8). In normoxia, the surface area of the preparation tended to be proportional to the magnitude of the developed tension, while in hypoxia the surface area tended to be inversely proportional to the developed tension. That is, under normoxic conditions, the larger the surface area, the larger was the developed tension. Conversely, in hypoxic conditions, the larger the surface area, the smaller was the developed tension. These results lend support to the concept that the surface area is the most important factor for generation of contractile tension in isolated, superfused papillary muscle. The preparation with a large surface area may be more susceptible to hypoxia, and result in low oxygen tension in the cell interior, thereby producing potential loss of various intracellular enzymes and high energy compounds, which will ultimately lead to a marked reduction in contractile tension.

Since we noted that the surface area played a direct role in maintaining the ATP content at high levels (Fig. 7), we assessed the effects of the surface area with regard to the other metabolite contents, under conditions of normoxia and hypoxia (Fig. 9). As shown in the upper part of Fig. 9, the relation between these parameters can be expressed by the equation, \( y=ax+b \), where the surface
Fig. 8. The relationship between the surface area and the contractile tension in canine papillary muscle exposed to normoxia (○) and hypoxia (●) for 30 min. Solid line (normoxia) and broken line (hypoxia) were drawn by using linear least squares regression analysis. Note that under conditions of hypoxia, the contractile tension was inversely proportional to the surface area.

Fig. 9. Illustration of “hypoxia-sensitive index.” Upper panel: the relationship of the metabolite content to the surface area \(y=ax+b\) and its modification by hypoxia (broken line). Lower panel: the ratios of slope factor, “hypoxia-sensitive index” (“a” in hypoxia divided by “a” in normoxia) calculated for 11 metabolites.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>(\frac{a \text{ in Hypoxia}}{a \text{ in Normoxia}})</th>
</tr>
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<tbody>
<tr>
<td>ATP</td>
<td>5.17</td>
</tr>
<tr>
<td>AMP</td>
<td>3.80</td>
</tr>
<tr>
<td>NADH</td>
<td>2.09</td>
</tr>
<tr>
<td>ADP</td>
<td>2.07</td>
</tr>
<tr>
<td>NAD</td>
<td>1.93</td>
</tr>
<tr>
<td>G6P</td>
<td>1.41</td>
</tr>
<tr>
<td>IMP</td>
<td>1.30</td>
</tr>
<tr>
<td>pyruvate</td>
<td>1.28</td>
</tr>
<tr>
<td>CP</td>
<td>1.07</td>
</tr>
<tr>
<td>Pi</td>
<td>0.99</td>
</tr>
<tr>
<td>lactate</td>
<td>0.75</td>
</tr>
</tbody>
</table>

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area is on the y-axis, and the metabolite content, on the x-axis. The value of "a" increased if the metabolite content decreased, while the value decreased if the content increased. The ratios of "a" in the hypoxic condition to those in the normoxic condition were calculated for 11 metabolites as shown in the lower part of Fig. 9. We call the ratio the "hypoxia-sensitive index." ATP had the highest value for this index, followed by AMP, NADH, ADP, NAD, G6P, pyruvate, and CP, in that order. Pi and lactate had values lower than unity, in-

![Graph showing metabolite content and hypoxia-sensitive index](image)

Fig. 10. Typical isotachophoretic records obtained from outer (left) and inner (right) layers of a canine papillary muscle. Note the difference of the peak height of the ATP component, in both records.

![Bar chart showing ATP levels](image)

Fig. 11. Comparison of the levels of ATP in the outer and in the inner layers of canine papillary muscle. Open column, outer layer; hatched column, inner layer. The data were obtained from 5 preparations each. Vertical bars indicate S.D. Significant difference (P<0.025) was noted between the outer and inner layers.

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indicating that the contents of these two metabolites increased during the hypoxia. ATP was most susceptible to the hypoxia since the hypoxia-sensitive index was the largest among all metabolites examined.

**Direct evidence for different levels of ATP between the outer and inner layers**

The above results strongly suggest the presence of a non-homogeneity in ATP levels within the papillary muscle. As the difference may exist in between the superficial layer and the core layer of the preparation, we examined the ATP levels in each layer. The mean wet weight of the muscles used for this purpose was $34.6 \pm 19.7$ mg ($n=5$). The papillary muscle was cut with a razor blade and divided into two parts, the outer and the inner layers. The isotachophoretic records showed a clear difference between these two layers (Fig. 10); the ATP level in the outer layer was significantly high compared to that in the inner layer. The mean value of the ATP levels in the outer layer (in 5 muscles) was compared to that in the inner layer, and the results are shown in Fig. 11. The former is significantly higher than the latter, by a factor of about 2. The levels of other metabolites examined showed no significant difference between the layers.

**DISCUSSION**

*Hypoxia and contractile tension.* Nayler et al. (1979) separated the mechanical response of myocardium to hypoxia into the following three phases. Phase 1: a precipitous decline in developed tension occurs without any concomitant change in the resting tension. At this stage re-oxygenation results in a complete recovery of function. Phase 2: the developed tension continues to decline, but at a relatively slow rate, while the resting tension begins to increase. If the muscle is re-oxygenated at this stage, recovery is almost complete. Phase 3: the development of active tension ceases, and resting tension increases rapidly. Re-oxygenation at this stage may cause a small but transient increase in the resting tension, without recovery of the developed tension.

The hypoxic condition used in the present study ($P_{O_2} \approx 100$ mmHg) may belong to "moderate" hypoxia and which may be different from the "severe" hypoxia ($P_{O_2} < 25$ mmHg) described by Nayler et al. (1979). Therefore, in the present experiments, the mechanical response to the hypoxia was relatively slow and mild. Some preparations (3 out of 17) showed no increase in the resting tension. Thus, the severity of hypoxia in the present study may correspond to the end of phase 2 or the beginning of phase 3.

*Hypoxia and metabolites.* Shortage of oxygen in the cardiac muscle produces myocardial cell damage and a significant release of cytoplasmic and plasma membrane marker enzymes (Hearse et al., 1973), rapid depletion in the intracellular levels of ATP, CP, and glycogen (Hearse et al., 1976), impairment of mitochondrial function (Lochner et al., 1976), and ultrastructural alterations
The depletion of tissue ATP and CP during hypoxia has been demonstrated by many investigators (Kübler and Spieckermann, 1970; Nayler et al., 1976; Guarneri et al., 1978; Reibel and Rovetto, 1978; Gaudueil et al., 1979). They found that decrease in the total tissue ATP seemed to develop relatively slowly, but that in the tissue CP levels proceeded rather rapidly. However, in the present study, the opposite finding was obtained at least within 30 min of hypoxic perfusion (cf. Figs. 2A and 4A). The quick decline in the ATP content could be attributed to the differences in preparation and the method of perfusion. We used a small, isolated papillary muscle which was continuously superfused with Tyrode solution, while the above-mentioned workers used a Langendorff perfusion technique and isolated hearts. Forrester and Williams (1977) reported that ATP was readily released from the isolated rat heart cell preparations at a relatively rapid rate during hypoxia. The decrease in the levels of ADP and AMP concomitant with that of ATP (Fig. 2B) suggests considerable leakage of these adenine nucleotides through the cell membrane, as a result of an increased permeability which may be secondary to the hypoxia.

Evidence for the existence of anoxic core. Hill and his co-worker (1914, 1925, 1928) and Cranefield and Greenspan (1960) have suggested the existence of an anoxic core; however, this view is not without argument. Snow and Bressler (1977) concluded that the development of an anoxic core in rabbit papillary muscle is unlikely, judging from the independence of mechanical and metabolic responses of the muscles with different cross-sectional areas, to the stimulation frequency. Loiselle (1979) reached the same conclusion, determined from the temperature-independency of the slope in the relation between the stress and the cross-sectional area. Recently, Delbridge and Loiselle (1981) conducted stereological electron-microscope analyses of the tissue components, using both large and small sized papillary muscles to test the hypothesis that the inverse correlation between the stress and the cross-sectional area is caused by the difference in the relative proportions of mitochondria to the contractile matrix. Their results were not in agreement with the idea of a central anoxic core. However, the size of the rabbit papillary muscle they used was rather small and ranged from 0.3 to 6.3 mg in weight. That is to say, the weight was about one-tenth that of the preparation we used in the present study. In addition, the stereological measurements were performed only within the layer 0.2 mm from the endocardium, and such may not be a sufficient depth to determine the inadequacy of oxygen diffusion.

Mitochondria which presumably consume oxygen are not uniformly distributed throughout a cell interior, but rather, exist mostly in the periphery of the cell (Henneman and Olson, 1965). In other experiments, mitochondria were sometimes seen clustered around the capillaries (Hoppeler et al., 1981). These findings support the concept that in the isolated, superfused papillary muscle devoid of oxygen supply, the production of ATP probably depends on the pe-
riphery-located mitochondria and not on the mitochondria present around the capillaries deep in the tissue.

In conclusion, we propose that there exists a central anoxic region in isolated canine papillary muscles superfused with oxygenated Tyrode solution and that the ATP on the surface layer seems to play a pivotal role in the initiation of muscle contraction in these preparations.

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