Depression of Myocardial Contractility in Rats by Free Fatty Acids during Hypoxia

By Andrew H. Henderson, M.B., Albert S. Most, M.D., William W. Parmley, M.D., Richard Gorlin, M.D., and Edmund H. Sonnenblick, M.D.

ABSTRACT

Rat papillary muscles were used to study the influence of glucose (5 mM), linoleate (1 to 1.75 mM), octanoate (0.5 to 1.75 mM) and pent-4-enoic acid (1 to 5 mM) on mechanical performance under oxygenated, hypoxic and anoxic conditions. The buffer solution contained 0.3 mM albumin. Free fatty acids (FFA) (1.0 to 1.75 mM) did not alter mechanical performance under oxygenated conditions. During hypoxia or anoxia, FFA (0.5 to 1.75 mM) depressed contractility and increased resting force; glucose improved mechanical performance and modified the depressant effects of FFA. The depressant effect of the nonmetabolized FFA, pentenoic acid, was similar to that of other FFA. This suggests that the effect was mediated directly by FFA or acyl CoA derivatives rather than their metabolic products, and that it might be due to a detergent effect or calcium binding by FFA present in excess of intracellular FFA binding capacity at low pH. Force development during anoxia could be augmented by calcium, implying that the reduced ability of the myofilaments to contract could not be attributed entirely to a reduction of high energy stores.

ADDITIONAL KEY WORDS linoleate octanoate pent-4-enoic acid calcium binding detergent action of free fatty acids optimal oxygen requirement of rat myocardium paired stimulation

It has been demonstrated that glucose improves the mechanical performance and prolongs the survival of anoxic hearts (1-4), but the possible influence of free fatty acids (FFA) under conditions of oxygen deprivation has received little attention. High concentrations of FFA have been shown to increase myocardial oxygen consumption (MV02) in aerobically perfused rat hearts (5) and to cause arrhythmias in dogs with experimentally induced infarction (6). Furthermore, high serum levels of FFA have been correlated with mortality in patients with ischemic heart disease (7). Unbound FFA can depress enzyme activity in vitro (8) and decrease respiration of isolated mitochondrial preparations (9). It has been suggested that concentrations of FFA in excess of binding capacity may exert a depressant influence on myocardial metabolism in vivo (5, 10, 11). Accordingly, the present investigation was undertaken to explore the influence of FFA and glucose, both singly and in combination, on the mechanical performance of rat papillary muscles during hypoxic and anoxic stress.

Methods

Posterior left ventricular papillary muscles from freely fed 300-g male Sprague-Dawley rats, narcotized with 50% O2-50% CO2 gas mixture, were rapidly removed and mounted in muscle baths containing oxygenated bicarbonate buffer at 30°C as previously described (12). The isometric muscles were stimulated (104A American Electronics Laboratories Stimulator) with a current...
just above threshold, at a rate of 6/min. After allowing the muscles to stabilize for 2 hours, they were stretched by a resting force which was near the peak of their active length-tension curves (about 1 g) and were then maintained at this length throughout the study. Force, measured with an overhead Statham G1-4-250 force transducer, time to peak force development from its onset, and the peak rate of force development (dF/dt) (electronically differentiated, U-D 20, Electronic Gear Corp.) were recorded on a Hewlett-Packard 8-channel oscillograph (7858).

The muscles were bathed in modified Krebs-Ringer solution, containing 143.4 mM sodium, 5.9 mM potassium, 2.54 mM calcium, 1.2 mM magnesium, 124.5 mM chloride, 24.9 mM bicarbonate, and 0.3 mM albumin (bovine fraction V albumin, Armour, dialyzed for 24 hours at 4°C). Solutions containing linoleate or octanoate were prepared by slowly adding concentrated aqueous solutions of their sodium salts to the warmed buffer. The albumin present contained 0.15 mM calcium; the additional presence of 1.75 mM calcium did not increase the binding of calcium.

Calcium Binding

Buffer was prepared without phosphate and calcium, and 0.3 mM murexide was added (13). The difference between the absorbance at 509 nm and that at 432 nm was followed with a dual wavelength Aminco-Chance spectrophotometer to determine the concentration of ionized calcium. Increments of calcium chloride were added to provide concentrations of total calcium ranging from 0.1 to 3 mM. The curve relating the ionized calcium signal to the amount of added calcium was then compared with curves similarly obtained after cumulatively adding phosphate, albumin, and sodium octanoate or linoleate. Albumin, 0.3 mM, in buffer was found to bind 17% of 2.54 mM calcium; the additional presence of 1.75 mM linoleate resulted in the binding of only a further 1.6%. The addition of phosphate to the buffer or of 1 mM linoleate or up to 1.75 mM octanoate to buffer containing 0.3 mM albumin did not increase the binding of calcium.

Oxygenation

The buffer solutions were bubbled with 95%, 20%, or 0% O2 (each gas mixture containing 5% CO2 and the balance N2) yielding PO2 values of 620, 147 and 5 mm Hg, respectively, and providing muscle conditions defined as oxygenated, hypoxic, and anoxic. Papillary muscles depend on inward diffusion of oxygen so that high PO2 in the medium is necessary for adequate oxygenation of core fibers. Thus muscles suspended in medium at PO2 147 mm Hg will comprise a mixed population of fibers ranging from adequately oxygenated peripheral fibers to more or less anoxic core fibers, and such muscles have been defined as hypoxic. The PO2 of the buffer following any change of gas mixtures equilibrated with the gas PO2 within 5% in 3 minutes.

Comparison of Substrates

The influence of FFA under oxygenated conditions was studied by alternating buffer containing 5 mM glucose (buffer A) and buffer containing a different substrate combination (buffer B) for three further 1-hour periods (A-B-A-B) following initial stabilization. The two buffers to be compared were alternated in reverse direction (B-A-B-A) in alternate muscles. Each solution was equilibrated with the same gas mixture and at the same temperature before substitution. Measurements were made just before each change of buffer. Each change was expressed as a percent of the value obtained with glucose, and the three changes were averaged in each muscle. During hypoxia and anoxia the influence of FFA was studied by comparing the changes induced by a 30-minute period of hypoxia (or anoxia) when different substrate combinations were present.

The sodium salt of pent-4-enoic acid, a nonmetabolized FFA which also blocks the oxidation of long-chain FFA (14), was added in some experiments. The muscles were allowed to stabilize for 20 minutes following the addition of pentenoic acid. The values obtained following stabilization with pentenoic acid under oxygenated conditions were then taken as the control values with which to compare performance during hypoxia. When pentenoic acid caused some depression under oxygenated conditions (as with 5 mM but not with 1 mM), the relative depression induced by hypoxia would have appeared greater if it had been expressed as a percent of the control values obtained before addition of pentenoic acid. However, the main purpose of using 5 mM pentenoic acid was to compare the effects of linoleate and octanoate in its presence.

In addition, the inotropic effects of calcium and paired stimulation in oxygenated and anoxic muscles were compared. Measurements of the effects of calcium were made 5 minutes after increasing the external concentration to 7.5 mM. Paired stimulation, or sustained postextrasystolic potentiation, was achieved by placing a second electrical stimulus 150 msec after each regular stimulus.

Muscle length and weight were measured at the end of each experiment, and their mean cross-sectional area was calculated assuming cylindrical shape and a specific gravity of 1.051. All results
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### TABLE 1

**Influence of Free Fatty Acids on Developed Force in Oxygenated Rat Papillary Muscles**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>No. of exp</th>
<th>Developed force*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>101.2 ± 1.7</td>
</tr>
<tr>
<td>1 mM oct</td>
<td>6</td>
<td>100.4 ± 1.8</td>
</tr>
<tr>
<td>1.75 mM oct</td>
<td>4</td>
<td>100.6 ± 2.2</td>
</tr>
<tr>
<td>G + 1 mM oct</td>
<td>2</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>G + 1.75 mM oct</td>
<td>2</td>
<td>98.9 ± 4.0</td>
</tr>
<tr>
<td>1.75 mM lin</td>
<td>2</td>
<td>100.7 ± 2.0</td>
</tr>
</tbody>
</table>

Abbreviations used: G = 5 mM glucose; 0 = no added substrate; oct = octanoate; lin = linoleate.

*% of control with 5 mM glucose.

Values are means ± SE.

are expressed as mean ± standard error. Values are considered significantly different when *P* < 0.05 (Student's *t*-test).

### Results

In the oxygenated muscles the addition of FFA did not alter mechanical performance, which remained almost constant throughout the period of study (Table 1). However, the depression of mechanical performance induced by hypoxia or anoxia was significantly greater (*P* < 0.05, see Methods) in the presence of exogenous FFA (Table 2). Figure 1 illustrates the time course of developed force (A), time to peak force (B), and rise in resting force (C) in five representative groups of muscles subjected to hypoxia or anoxia. Values obtained during oxygen deprivation are related to the control values under oxygenated conditions following stabilization, since different substrates were shown not to alter the performance of oxygenated muscles, and, moreover, the control values were similar in each group of muscles used (see final paragraph of Results). Developed force diminished during oxygen deprivation at a rate which was determined by the substrate combination present (Fig. 1A).

After 30 minutes of hypoxia, muscles were significantly more depressed in the presence of glucose and 1 mM linoleate than with glucose alone, and 1 mM linoleate alone resulted in significantly greater depression than the combination of glucose and 1 mM linoleate. In muscles made completely anoxic, the addition of 1 mM linoleate to glucose led similarly to a significantly greater depression of force development. Figure 2A shows the depression of force development after 30 minutes of hypoxia or anoxia with increasing concentrations of added linoleate or octanoate in both the presence and the absence of glucose. The depressant effect of FFA was generally related to the concentration of FFA, although not significantly so. At some points, linoleate

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TABLE 2
Mechanical Performance in Hypoxic or Anoxic Rat Papillary Muscles Mounted in Buffer Containing Different Substrate Combinations, Showing Developed Force (% of Control) and Increase in Resting Force (g) after Hypoxia (30 minutes) and after Reoxygenation (30 minutes)

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Substrate</th>
<th>Developed force (% of control under oxygenated conditions)</th>
<th>Increase in resting force above control</th>
<th>Significance of differences*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>Hypoxia</td>
<td>Reoxygenation</td>
</tr>
<tr>
<td>1.</td>
<td>0</td>
<td>4</td>
<td>51 ± 4.3</td>
<td>79 ± 6.0</td>
</tr>
<tr>
<td>2.</td>
<td>1 lin</td>
<td>7</td>
<td>38 ± 4.6</td>
<td>75 ± 3.1</td>
</tr>
<tr>
<td>3.</td>
<td>1.75 lin</td>
<td>5</td>
<td>28 ± 6.2</td>
<td>69 ± 9.0</td>
</tr>
<tr>
<td>4.</td>
<td>0.5 oct</td>
<td>5</td>
<td>33 ± 3.2</td>
<td>73 ± 3.9</td>
</tr>
<tr>
<td>5.</td>
<td>1 oct</td>
<td>5</td>
<td>29 ± 3.7</td>
<td>66 ± 6.4</td>
</tr>
<tr>
<td>6.</td>
<td>1.75 oct</td>
<td>5</td>
<td>28 ± 4.7</td>
<td>54 ± 4.8</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>6</td>
<td>66 ± 2.4</td>
<td>94 ± 1.6</td>
</tr>
<tr>
<td>8.</td>
<td>G + 1 lin</td>
<td>7</td>
<td>53 ± 3.5</td>
<td>87 ± 4.0</td>
</tr>
<tr>
<td>9.</td>
<td>G + 1.75 lin</td>
<td>11</td>
<td>57 ± 3.5</td>
<td>88 ± 3.1</td>
</tr>
<tr>
<td>10.</td>
<td>G + 1 oct</td>
<td>5</td>
<td>54 ± 2.3</td>
<td>92 ± 3.8</td>
</tr>
<tr>
<td>11.</td>
<td>G + 1.75 oct</td>
<td>5</td>
<td>44 ± 3.3</td>
<td>90 ± 2.9</td>
</tr>
<tr>
<td>12.</td>
<td>G + 1 lin</td>
<td>7</td>
<td>53 ± 3.5</td>
<td>92 ± 4.2</td>
</tr>
<tr>
<td>13.</td>
<td>G + 1 lin + ins.</td>
<td>4</td>
<td>59 ± 1.2</td>
<td>88 ± 2.6</td>
</tr>
<tr>
<td>14.</td>
<td>G + 5P</td>
<td>4</td>
<td>40 ± 4.4</td>
<td>93 ± 10.4</td>
</tr>
<tr>
<td>15.</td>
<td>G + 1 lin + 5P</td>
<td>3</td>
<td>45 ± 3.8</td>
<td>92 ± 4.7</td>
</tr>
<tr>
<td>16.</td>
<td>G + 1 oct + 5P</td>
<td>4</td>
<td>44 ± 2.9</td>
<td>93 ± 5.3</td>
</tr>
<tr>
<td>17.</td>
<td>G + 1P</td>
<td>6</td>
<td>54 ± 4.0</td>
<td>93 ± 4.6</td>
</tr>
<tr>
<td>18.</td>
<td>G + 10 DG</td>
<td>4</td>
<td>54 ± 1.6</td>
<td>92 ± 4.7</td>
</tr>
<tr>
<td>19.</td>
<td>G + 10 DG + 1 lin</td>
<td>3</td>
<td>43 ± 3.5</td>
<td>93 ± 5.3</td>
</tr>
<tr>
<td>20.</td>
<td>G + 20 DG</td>
<td>5</td>
<td>30 ± 3.6</td>
<td>93 ± 5.3</td>
</tr>
<tr>
<td>21.</td>
<td>G</td>
<td>7</td>
<td>31 ± 2.2†</td>
<td>84 ± 4.7</td>
</tr>
<tr>
<td>22.</td>
<td>G + 1 lin</td>
<td>7</td>
<td>20 ± 3.0†</td>
<td>69 ± 8.7</td>
</tr>
</tbody>
</table>

In experiment 12, muscles were stabilized in 5 mM glucose. In experiment 13, insulin was added to 50 μU/ml 15 minutes before hypoxic period. Abbreviations for substrates: G = glucose, 5 mm; DG = deoxyglucose (mm); P = pent-4-enolic acid (mm); lin = linoleate (mm); oct = octanoate (mm); ins = insulin.

*Number in parentheses is experiment number with which compared.
†Anoxia.

Values are means ± SE.
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Influence of external FFA concentration, with and without glucose, on developed force (A) and rise in resting force (B) of rat papillary muscles after 30 minutes of hypoxia or anoxia.

Changes in developed force were associated with parallel changes in dF/dt and thus reflected changes in contractility (15), since the series elastic element does not become more compliant with anoxia (Tyberg, Yeatman, Parmley, Urschel and Sonnenblick, personal communication) and muscle length was held constant. The time to peak force was shortened slightly during hypoxia or anoxia in all muscle groups (Fig. 1B). Some groups of muscles developed contracture, as indicated by a rise in resting force at constant muscle length (Fig. 1C), or in separate experiments, by a shortening of muscle length at constant resting force (preload). Resting force rose more during anoxia than hypoxia and more in the presence of added FFA than in their absence (Fig. 2B). This effect too was significantly less in the presence of glucose. The modifying influence of glucose on the rise in resting force appeared to be greater than its influence on the reduction of developed force. Thus the slopes of linear regression lines relating the rise in resting force (g) to the developed force (% of control) after 30 minutes of hypoxia differed significantly in the presence and in the absence of glucose.

On reoxygenation, force development and dF/dt recovered gradually to their maximal extent, over 20 to 30 minutes, but rarely reached control levels (Table 2, Fig. 1A). The extent of the recovery in force development of previously hypoxic muscles was directly related to the severity of the preceding depression (r = 0.9) and also to the previous rise in resting force (r = 0.9). Thus muscles recovered less well in the presence of FFA than in the presence of glucose. While recovery was inversely related to the rise in resting force in both anoxic and hypoxic muscles, the anoxic muscles recovered significantly better in relation to the extent of their depression than did the hypoxic muscles. The rise in resting force was rapidly reversible on reoxygenation in all but the most severely affected muscles (Table 2, Fig. 1C). The time to peak force showed a transient prolongation on reoxygenation, which was more marked after anoxia than after hypoxia (Fig. 1B).

The same buffer was present throughout hypoxia and reoxygenation in these experiments, so that differences attributed to the presence of different substrates during the period of oxygen limitation might have been due in part to their influence during the period of equilibration. However, a further group of muscles was stabilized in buffer
containing glucose alone, and this was substituted by buffer containing 1 mM linoleate and glucose only 10 minutes before the induction of hypoxia. The relative depression induced by hypoxia in this group was the same as in muscles exposed to 1 mM linoleate and glucose throughout the experiment (Table 2). Porcine insulin, 50 μU/ml, added 15 minutes prior to hypoxia, did not affect the mechanical performance of muscles with glucose and 1 mM linoleate in the medium while they were oxygenated, and improved it during hypoxia slightly but not significantly (Table 2).

Under oxygenated conditions, the addition of 5 mM pentenoic acid depressed force development to a small but similar extent (11 ± 3%), whether the buffer contained glucose, or glucose and 1 mM linoleate, or glucose and 1 mM octanoate. The addition of 1 mM pentenoic acid did not alter mechanical performance. Muscles with 5 mM pentenoic acid in the medium were depressed by hypoxia to a similar extent in the additional presence of glucose and linoleate, or glucose and octanoate, or glucose alone (Table 2). The degree of depression was significantly greater than in comparable groups without pentenoic acid, even when the values after 30 minutes of hypoxia are related to control values obtained after the addition of pentenoic acid. Pentenoic acid, 5 mM, represents a concentration 5 to 10 times that shown to inhibit the oxidation of long chain FFA in perfused rat livers (Ruderman, Toews, Lowy, Vreeland and Shafrir, personal communication); this high concentration was used in an attempt to ensure that long chain FFA oxidation was inhibited, since no metabolic studies were carried out to confirm this. The finding that hypoxic muscles with linoleate in the medium were depressed to a greater rather a lesser extent when 5 mM pentenoic acid was also present suggested that the depressant effect of FFA was not related to their oxidative metabolism since pentenoic acid is a FFA which is not metabolized. The buffer in these experiments thus contained 5 to 6 mM FFA. A further group of muscles was therefore studied with 1 mM pentenoic acid and glucose in the medium. These muscles were found to be depressed by hypoxia to the same extent as in the presence of 1 mM octanoate or 1 mM linoleate with glucose.

In an attempt to determine whether FFA exerted their influence by blocking glycolysis, further experiments were conducted using 2-deoxyglucose, which blocks glycolysis at the glucose-6-phosphate level (2, 16). When 10 mM 2-deoxyglucose was added to 5 mM glucose, hypoxic muscles were depressed significantly more than in the presence of glucose alone (Table 2). The additional presence of 1 mM linoleate significantly increased the degree of depression. However, these results are difficult to interpret since it is not known how completely 10 mM 2-deoxyglucose blocked glycolysis, and indeed it was found that 20 mM 2-deoxyglucose resulted in still greater depression of mechanical performance during hypoxia.

Table 3 shows that the positive inotropic effects of calcium and of paired stimulation were both relatively and absolutely greater during anoxia than in the oxygenated state. Resting force was not further raised by either intervention.

Mean control values in all the muscles studied were: developed force, 5.2 ± 0.1 g (n = 120); dF/dt, 66 ± 14 g/sec; time to peak force, 124 ± 1.1 msec; muscle length, 3.9 ± 0.6 mm; muscle weight, 3.4 ± 0.8 mg, mean cross-sectional area, 0.84 ± 0.02 mm². When developed force was normalized for cross-sectional area to give developed stress (6.0 ± 0.2 g/mm²), this was found to be inversely related to cross-sectional area over

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
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<tbody>
<tr>
<td><strong>Inotropic Responses in Muscles Stimulated at a Rate of 6/min under Oxygenated and Anoxic Conditions (N = 6)</strong></td>
</tr>
<tr>
<td>State</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Oxygenated</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Anoxic</td>
</tr>
<tr>
<td></td>
</tr>
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<td></td>
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</tbody>
</table>

*% of oxygenated control.
Values are means ± SE.
Normalized isometric tension development related to muscle cross-sectional area in 109 rat papillary muscles at 30°C. The linear regression lines for this relationship are compared in rat muscles (r = 0.84, P < 0.001) and in cat muscles (replotted from published data, reference 28).

Discussion

In these studies of rat papillary muscle preparations, FFA did not influence the performance of well-oxygenated myocardium but depressed contractility when the muscle was partially or completely deprived of oxygen. Depression of contractility by FFA was manifest in the presence or absence of glucose in the medium. Higher concentrations of FFA were somewhat more depressant than low concentrations, although in the absence of glucose, hypoxic muscles were depressed almost as much with FFA-to-albumin molar ratios of 2:1 as with ratios of 6:1. FFA also increased the degree of contracture that developed with oxygen deprivation. In addition, FFA adversely affected subsequent recovery of the muscles, since recovery on reoxygenation was directly related to the severity of the preceding depression.

Glucose, on the other hand, exerted a beneficial effect during oxygen deprivation and subsequent recovery, in accord with previous reports (1-4). This effect was also seen in the presence of FFA, and it is clear that glucose partially counteracted the depressant effect of FFA. The influence of glucose appeared to be greater in preventing the rise in resting force than in reducing the depression of contractility. The response to anoxia, with glucose in the medium, is essentially similar to that seen in similarly studied cat muscles (Tyberg et al., personal communication), except that anoxia induced only negligible rise in resting force in the cat muscles.

The positive inotropic response to calcium or to paired stimulation, at a rate of 6/min, was greater in anoxic muscles than in oxygenated muscles. In oxygenated rat muscles, increasing the external ionized calcium concentration above 2.5 mM, or paired stimulation at this calcium concentration, causes only minimal increase in contractility at rates as low as 6/min, when contractility is almost at its ceiling because of the negative con-
tractility-frequency staircase peculiar to this species (12). On the other hand, at higher frequencies or with lower initial external concentration of calcium, these interventions augment contractility (unpublished observations), presumably by increasing the amount of activator calcium delivered to the myofilaments (17). The marked increase in positive inotropic response during anoxia therefore implies that contractility under these conditions was limited not by the supply of energy available to the contractile process itself or by depression of its potential for contraction but by an interference with excitation-contraction coupling and the amount of calcium made available with activation. However, the inability of the potentiated hypoxic muscle to reach control levels probably indicates that the potential for active contraction is also limited. Similarly, it would appear that the rise in resting force observed with oxygen deprivation was unlikely to be due to tonic activity of the myofilaments associated with residual activator calcium, since resting force was not raised further by these calcium-mediated interventions.

The mechanism by which contractility in the oxygen-limited muscles is reduced in the presence of FFA remains to be established. The intermediary metabolism of the perfused rat heart has been explored extensively (18), but the findings have generally not been correlated with mechanical performance under conditions of oxygen deprivation, nor were any metabolic studies carried out in the present study.

In oxygenated hearts, glucose increases the rate of glycolysis (19), while FFA inhibit glycolysis (20). This latter effect occurs mainly through inhibition of phosphofructokinase by the accumulation of citrate arising from the oxidative breakdown of FFA (21, 22). Glycolysis provides the only known anaerobic source of energy in the heart to replenish the high energy phosphates (23), and thus it is possible that the differences in contractility observed with different exogenous substrates under hypoxic conditions might reflect associated changes in the rate of glycolysis. In anoxia the provision of exogenous glucose further increases glycolysis (4). FFA are taken up normally by the cells (24), but they have been reported to cause less accumulation of citrate (25). However, the influence of FFA on glycolysis under oxygen-limited conditions appears to have been little studied, although their inhibitory effect is said to be abolished by anoxia (26).

Pentenoic acid depressed contractility in hypoxic muscles to the same extent as linoleate or octanoate. Pentenoic acid is a nonmetabolized FFA and is thought to block the carnitine-dependent entry of long-chain FFA into the mitochondria, while not affecting the metabolism of short-chain FFA (13). Rat livers perfused aerobically with pentenoic acid in the absence of short-chain FFA accumulated glycolytic intermediates and contained low levels of CoA and Krebs cycle intermediates, while the redox pairs showed the cells to be in a highly oxidized state (Ruderman et al., personal communication). None of these findings would readily suggest that pentenoic acid might reduce the rate of glycolysis under anaerobic conditions, though these suppositions clearly need experimental confirmation. The present findings therefore suggest that FFA exert their depressant effect at a premitochondrial level. Thus the accumulation of FFA or their acyl CoA derivatives in the cytoplasm may itself have been responsible for the reduction in contractility seen in oxygen-limited muscles supplied with FFA.

Unbound FFA within the cell may be capable of lowering activator calcium ion concentration. An alternative possibility is suggested by reports that unbound FFA depress enzyme function in vitro (8) and impair the respiration of isolated mitochondrial preparations by what is thought to be a detergent effect (9). While the external FFA-to-albumin molar ratios in the present study were lower than those found to be deleterious in vitro, FFA probably accumulate in the cell in hypoxia and might then exceed the binding capacity (10, 11). Linoleate has a stronger detergent action than octanoate (8, 9) but is more tightly bound to albumin than octanoate.
(9), so that these two factors operating in opposite directions could account for the essentially similar findings with these two FFA. Their acyl CoA derivatives will also have detergent properties (8). Moreover, it has been shown that FFA are less tightly bound at low pH (27), and the intracellular pH will be low in hypoxia. The findings might then be attributable to a detergent effect of unbound FFA or their acyl CoA derivatives on membranes. This could interfere with the provision or translocation of energy, or with the control of activator calcium movement to and from the myofilaments. The critical limit to contractility in hypoxia would appear to be imposed by the supply of activator calcium, which implies that FFA interfere further with this process. FFA depress both contractility and oxygen consumption simultaneously in rat hearts perfused under probably slightly hypoxic conditions, thus pointing to a primary depressant action of FFA on contractility or oxidative respiration or both (unpublished observations). The possibility that FFA directly impair the normal function of the sarcolemma or sarcoplasmic reticulum under hypoxic conditions cannot therefore be excluded.

It may be noted that force development in oxygenated papillary muscles from the rat, when normalized to give developed stress in grams per square millimeter increased with decreasing muscle cross-sectional area down to areas of 0.35 mm². This is in marked contrast to similar preparations from the cat, in which normalized stress development remains constant with muscle areas up to 1.4 mm² (28) under comparable conditions of maximal contractility and similarly at the peak of the length-active tension curve (12). This implies that optimal oxygen requirements are higher in rat myocardium, and that the myocardial oxygen consumption for a given stress development is higher in the rat than in the cat. The theoretical maximum velocity of shortening of the contractile element (Vₘₐₓ) in rat papillary muscles is three times higher than that of cat muscles developing the same stress (12). In the dog Vₘₐₓ is a major independent determinant of oxygen consumption (29). Thus the equation which evaluates the factors controlling total oxygen consumption in the dog could be of general application between different species. It is likely then that most of the oxygenated muscles in the present study were in fact slightly oxygen-limited. The contractility of rat hearts perfused with oxygenated buffer but probably also somewhat oxygen limited was depressed by 1.75 mM linoleate but not by lower concentrations (unpublished observations), whereas the hypoxic papillary muscles in the present study were depressed by 0.5 mM octanoate, and the oxygenated muscles were depressed only at total FFA concentrations of 5 to 6 mM (pentenoic acid experiments).

The effective concentrations of FFA found to be depressant in the hypoxic muscles were thus within the clinical range (7, 30). Clearly, the influence of high arterial concentrations of FFA on myocardial function in patients with ischemic heart disease warrants further study. Clinical trials of therapy with glucose and insulin have yielded conflicting results (31-33), but as pointed out by Owen and his colleagues (34), these trials have been directed toward replacement of intracellular potassium (31-33, 35, 36) rather than the maintenance of high arterial concentrations of glucose. The arterial concentrations of FFA were not considered in these studies, but they are known to be raised by catecholamines and by heparin (30, 37). If the conclusions drawn from the present study apply to the intact animal, they could have important clinical implications.

**Acknowledgment**

The measurements of calcium binding were performed by Dr. A. Stam, to whom we are most grateful.

Part of this study has been briefly reported as a preliminary communication (Henderson, Most, and Sonnenblick, Lancet 2: 825, 1969).

**References**


