

# The Rate of Oxygen Uptake of Quiescent Cardiac Muscle

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**ABSTRACT** The rate of oxygen uptake of quiescent papillary muscle of the cat heart has been determined in a flow respirometer with the use of the oxygen electrode. The apparent rate of oxygen uptake as a function of the diameter of the muscle was also determined. It was found that papillary muscles from cat hearts use oxygen at a rate of 2.84 (microliters/mg. wet weight)/hour at a temperature of 35°C. Such muscles can be adequately supplied by diffusion when their surface is uniformly exposed to an atmosphere containing 95 per cent oxygen only if their diameter is 0.64 mm. or less. Papillary muscles from kitten hearts use oxygen at a rate of 4.05 (microliters/mg. wet weight)/hour at a temperature of 35°C. Such muscles can be adequately supplied by diffusion when their surface is uniformly exposed to an atmosphere containing 95 per cent oxygen only if their diameter is 0.53 mm. or less. If the muscles are small enough to be adequately supplied with oxygen by diffusion, the rate of oxygen uptake does not increase when the muscle is stretched.

## INTRODUCTION

Papillary muscles of cat, rat, and dog hearts have been widely used in the study of the pharmacology of cardiac muscle and in the study of the mechanical properties of cardiac muscle. The papillary muscle has many advantages in such studies because it approximates a strip which can be obtained without cutting any part of the muscle except the end at which it attaches to the ventricular wall. Because so much work has been done using this preparation it seems essential to determine its oxygen uptake. In the absence of a knowledge of the oxygen uptake it is not possible to be sure that the preparation is small enough to be adequately supplied with oxygen by diffusion. The problem is

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further made important by the fact that the few *in vitro* determinations of the oxygen uptake of resting cat papillary muscle reported previously suggest that its oxygen uptake is much lower than observations on the intact *in situ* heart would predict. The study reported here was intended to determine the oxygen uptake of cat papillary muscle at rest and to determine the maximum diameter which such a muscle may have and still be adequately supplied with oxygen by diffusion.

#### METHODS

*Preparation of Tissue* Adult cats or 6 to 8 week old kittens were anesthetized by intravenous injection of nembutal. The chest was opened immediately and the heart removed and placed under a stream of gas composed of 5 per cent CO<sub>2</sub> and 95 per cent O<sub>2</sub>. All subsequent dissection was carried out under the stream of gas. This procedure assists in maintaining oxygenation of the tissues and also prevents tissue CO<sub>2</sub> from falling rapidly (1, 2). The left or right ventricular chamber was opened and a small papillary muscle or trabecula was selected. A fine thread was tied around each end of the muscle and it was then cut free from its attachment or attachments to the heart and placed in a bath containing Tyrode's solution. The Tyrode solution used had the following composition in millimoles/liter:

NaCl	137.0
KCl	2.7
MgCl <sub>2</sub>	0.5
NaH <sub>2</sub> PO <sub>4</sub>	0.18
NaHCO <sub>3</sub>	12.0
Glucose	5.5
CaCl <sub>2</sub>	2.7

This solution has a pH of 7.2 at 35°C. when it is in equilibrium with a gas mixture containing 5 per cent CO<sub>2</sub>. In many experiments antibiotic was added to prevent bacterial growth. In those experiments each liter of Tyrode's solution contained 80 mg. of K penicillin G and 160 mg. of a mixture of dihydrostreptomycin and streptomycin. The presence of these antibiotics was found not to alter the rate of oxygen uptake. The papillary muscle was usually washed in a bath containing Tyrode's solution in equilibrium with 5 per cent of CO<sub>2</sub> and 95 per cent O<sub>2</sub> for about 30 minutes before it was inserted in the respirometer. This procedure served to wash the tissue free of blood and small bits of damaged tissue.

*The Respirometer* A plastic chamber similar to that described by Carlson *et al.* (3) and by Cranefield *et al.* (2) was used. The chamber includes a reservoir filled with Tyrode's solution which was equilibrated with a gas mixture containing 95 per cent O<sub>2</sub> and 5 per cent CO<sub>2</sub>. The reservoir ended in a small vertical capillary. This capillary in turn ended in a glass tube closed by a rubber tube and a small clamp. The muscle was placed in the reservoir and the thread attached to one end was drawn through the rubber tube which was then clamped. At that point the muscle was still

in the reservoir. The oxygen tension of the fluid flowing from the reservoir was determined and the muscle was then drawn into the capillary by releasing the clamp slightly and pulling on the thread. This maneuver could be accomplished without marked disturbance of the flow through the capillary. With the muscle in the capillary the tube was again firmly clamped. One end of the muscle was thus held in place because the thread attached to it was clamped at the bottom of the capillary. The thread tied to the other end of the muscle was brought through the top end of the capillary, into the reservoir, and straight up to the pin of an RCA-5734 mechanoelectronic transducer tube. Recording from the transducer was accomplished with a circuit described elsewhere (4). The tension was recorded on a Texas Instrument Recti-riter. The entire transducer assembly was mounted on a pair of micromanipulators. One of these micromanipulators made it possible to position the thread and the muscle in the center of the capillary. The other moved the transducer vertically so that stretch could be applied to the muscle. Tension was recorded in order to detect any spontaneous contractions. Such spontaneous contraction was easily seen and occurred infrequently. When it occurred the results were discarded from the series since the resting uptake was sought. Another capillary, at right angles to the vertical capillary, was used to draw solution past the muscle. This capillary also held the oxygen electrode. The solution was drawn through the system by a syringe identical with that described elsewhere (5).

A collodion-covered oxygen electrode was used in the usual fashion. Polarizing voltages of 0.6 to 0.9 volt were used, the electrodes were calibrated daily, and the operating voltage was selected from a point on the plateau of the voltage-current curve of the electrode. Calculations of oxygen uptake were made from the measured oxygen tension before and after insertion of the muscle and from the known flow rate. The flow rate was 1.23 cc./hour. The experimental methods generally closely resembled those employed in a previous determination of the oxygen uptake of mammalian peripheral nerve (2).

*Control of Temperature* The entire respirometer was contained in a large insulated box which was maintained at a temperature of 35°C. with the aid of electric lights and a fan. The lights were turned off and on by a thermostat which maintained the temperature constant to within 0.3°C. The temperature in the insulated box was checked by a thermometer from time to time and was also measured continuously from a thermistor embedded in the reservoir. The thermistor was monitored through a Tele-Thermometer manufactured by the Yellow Springs Instrument Company. The thermostat was a Thermistemp temperature controller also manufactured by the Yellow Springs Instrument Company, Yellow Springs, Ohio.

*The Diameter of the Muscle* Many experiments employing papillary muscles have been carried out in our laboratory. In all those experiments the length, diameter, and weight of the muscles were recorded. All those earlier observations were calculated out to see whether the diameter which would be predicted by the assumption that the muscle was cylindrical was the same as the measured diameter. Actual determinations of specific gravity yielded a value of 1.1 which was used in these calculations. It was found that the measured and calculated diameters agreed perfectly. In most of the experiments reported in the present paper the diameter was not measured but was

calculated from the length and weight on the assumption that the muscle was cylindrical. Our experience suggests that such calculated diameters never vary from the measured diameter by more than the experimental error measurement which is involved in measuring diameter directly. Muscles of diameter 0.3 to 0.4 mm. weighed as little as 0.2 mg. About 80 per cent of the muscles had lengths between 0.4 and 0.6 mm. The heaviest muscle used weighed 26.2 mg.

*Stretch* The muscles which were stretched were brought to a length which was 50 per cent greater than that which they had "at rest," *i.e.*, when under no tension. The length at rest and after stretch were measured directly. The diameter after stretch was calculated from the length and weight. This procedure is perhaps subject to a slightly greater error than is the calculation of diameter from the length and weight at rest. That is so because any irregularity in diameter might be exaggerated by stretch. Muscles which were stretched and examined under a microscope did sometimes show some slight departure from perfect cylindricality but there is no simple way to allow for that mathematically.

*Determination of Wet and Dry Weight* The muscle was removed from the respirometer and dried lightly with filter paper. The wet weight was then determined. Dry weights were determined by drying the muscle to constant weight in an oven. This usually required about 12 hours. The over-all average wet weight to dry weight ratio was found to be 4.0.

*Calculation of the Critical Diameter* The familiar equation (6) for diffusion in a cylinder,  $U = C - (a/4D)(R^2 - r^2)$ , where

- $U$  = oxygen concentration at  $r$
- $C$  = oxygen concentration at the surface of the muscle
- $a$  = rate of oxygen uptake of the muscle
- $D$  = the diffusion coefficient
- $R$  = radius of the muscle
- $r$  = distance from the center of the muscle

may be used to determine the critical radius for adequate oxygenation by diffusion provided that the value of  $a$ , the rate of oxygen uptake, is known. In determining the critical radius  $U$  and  $r$  are set equal to zero and the equation reduces to  $R = (4DC/a)^{1/2}$ . The diffusion coefficient of Krogh (7) was used. In accordance with Krogh's procedure an increase of 1 per cent for each degree was allowed in correcting the value for 35°C. The value actually used was  $5.175 \times 10^{-4}$  cm.<sup>2</sup>/min.; in calculations of critical diameter it was therefore necessary to express  $a$  in (microliters O<sub>2</sub>/ml. tissue)/min. and  $C$  in microliters of O<sub>2</sub>/ml. of solution.

## RESULTS

### *Apparent Oxygen Uptake As a Function of Diameter*

There is no way in which the critical diameter for adequate oxygenation of the muscle by diffusion can be calculated unless the true rate of oxygen uptake

is known. It was therefore necessary to determine the oxygen uptake of a series of muscles of various diameters. The results of that study are shown in Fig. 1. The muscle diameters were collected in groups covering a range of 0.1 mm. and the corresponding apparent oxygen uptake is shown above the mean diameter of the group. Thus, *e.g.*, the muscles whose diameters ranged from 0.9 to 1.0 mm. were grouped and the apparent oxygen uptake was

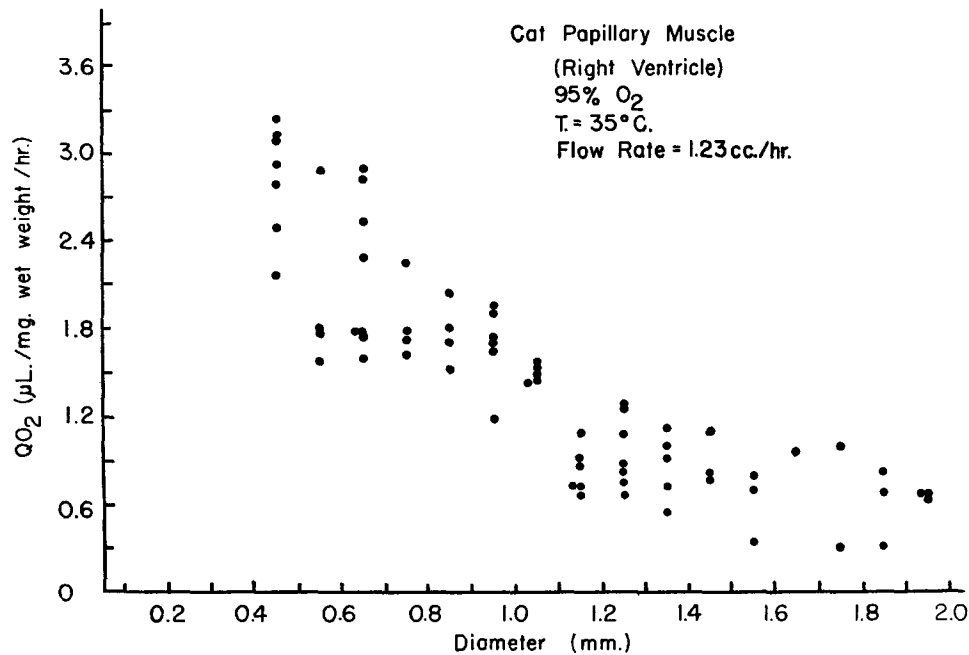


FIGURE 1. Apparent rate of oxygen uptake of quiescent, unstretched papillary muscles of different diameters. Oxygen uptake is shown in (microliters/mg. wet weight)/hour. Each point shows the uptake of a single papillary muscle. The diameters are combined into groups with a range of 0.1 mm. and the oxygen uptakes are plotted above diameters which represent the midpoint of each diameter group. The papillary muscles were taken from the hearts of adult cats. The uptakes were determined at 35°C.

plotted at a diameter of 0.95 mm. The diameters are in some cases measured diameters and in most cases calculated diameters (see Methods). Each point in Fig. 1 represents the rate of oxygen uptake of an individual muscle. In Fig. 3 the average values of oxygen uptake for each diameter group are shown. The oxygen uptake is referred to as apparent oxygen uptake to emphasize the fact that diffusion limitation, if present, will cause the oxygen uptake per gram of tissue to appear to be lower than the true oxygen uptake (5). In general the apparent oxygen uptake is greater when the muscle is smaller. There is a deceptive plateau in the curve in the range of diameters from 1.15 to 1.45 mm.; had no smaller muscles been studied the conclusion might have been

drawn that the rate of uptake is 0.9 (microliter/mg. wet weight)/hour or 3.6 (microliters/mg. dry weight)/hour and that a muscle of diameter 1.15 to 1.35 mm. is small enough to be supplied with oxygen by diffusion. At least two published studies have drawn that conclusion (8, 9). When smaller muscles are studied it becomes obvious that such a conclusion is unwarranted.

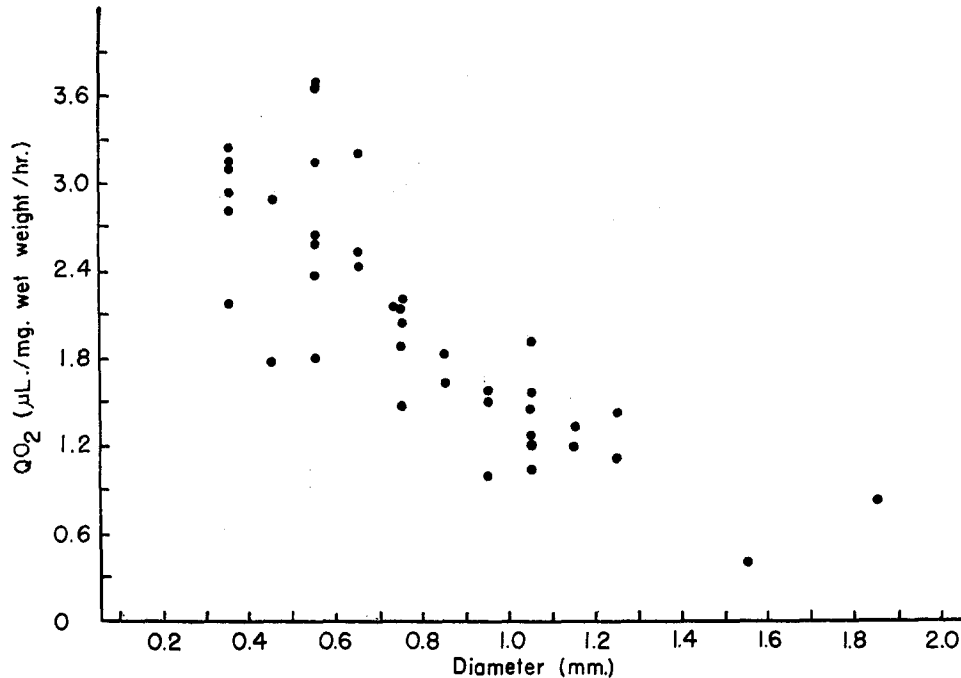


FIGURE 2. Apparent rate of oxygen uptake of quiescent papillary muscles stretched to a length 50 per cent greater than their rest length. Oxygen uptake is shown in (microliters/mg. wet weight)/hour. Each point shows the uptake of a single papillary muscle. The diameters are the diameters after stretch; they are combined into groups with a range of 0.1 mm. and the oxygen uptakes are plotted above diameters which represent the midpoint of each diameter group. The papillary muscles were taken from the hearts of adult cats. The uptakes were determined at 35°C.

The smaller muscles have a much higher apparent uptake than do the larger ones. Whether even the smallest muscles measured are small enough to be adequately supplied by diffusion is considered in the Discussion. The values of the rate of oxygen uptake observed by us were stable for several hours and were reproducible when the muscle was removed from the respirometer and later replaced in it. Even the very large muscles which are clearly inadequately supplied with oxygen by diffusion show a steady and reproducible apparent rate of oxygen uptake. Our conclusion, which is discussed further below, is that the true rate of uptake of oxygen by resting cat papillary muscle is 2.84 (microliters/mg. wet weight)/hour at a temperature of 35°C.

*The Effect of Stretch on Apparent Oxygen Uptake*

If muscles of large diameter show a low apparent oxygen uptake it might be supposed that the increase in length or the reduction of diameter which results from stretching the muscle would increase the apparent resting uptake simply by permitting a larger amount of respiring tissue to be supplied by

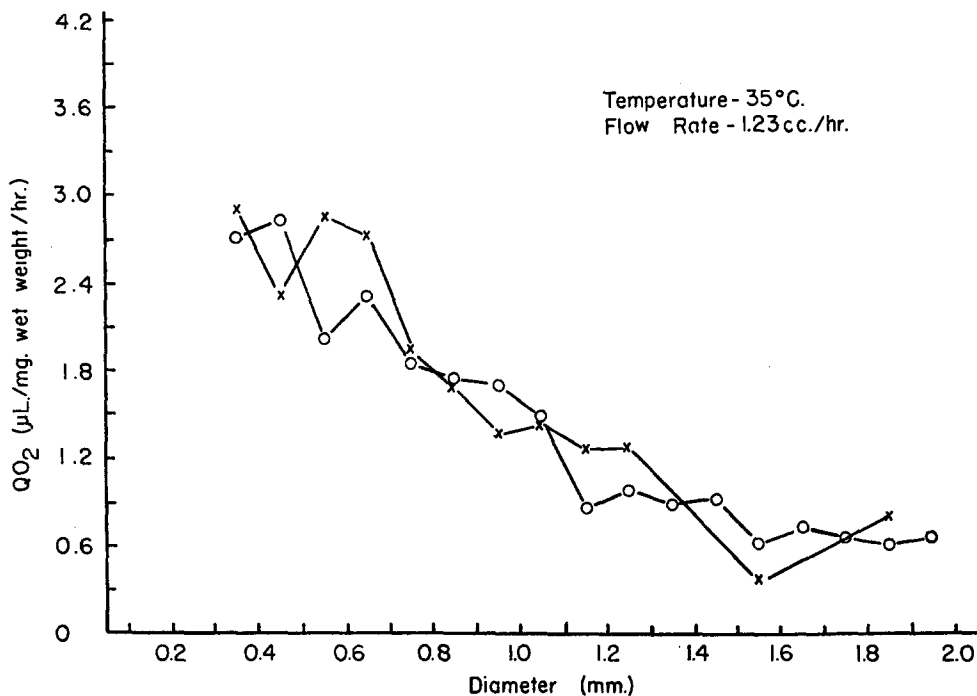


FIGURE 3. Apparent oxygen uptake, in (microliters/mg. wet weight)/hour, of quiescent unstretched papillary muscles (O—O) and of quiescent papillary muscles stretched to a length 50 per cent greater than their rest length (X—X). Diameters are combined into groups with a range of 0.1 mm. and the average oxygen uptake for each diameter range is plotted above the diameter which represents the midpoint of the diameter range of the group. The oxygen uptake of the unstretched muscles is plotted above the diameter of the unstretched muscle; the oxygen uptake after stretch is plotted above the diameter after stretch. Not all muscles were stretched and there are thus more muscles represented in the averages of the unstretched groups than in the averages of the stretched groups. The papillary muscles were taken from the hearts of adult cats. The uptakes were determined at 35°C.

diffusion. It also might be that stretch *per se* increases resting oxygen uptake. The results of applying enough stretch to increase the muscle length by 50 per cent (and therefore to decrease the muscle diameter by about 20 per cent) are shown in Figs. 2 and 3 and in Table I. Fig. 2 simply presents the actual observed values of the rate of oxygen uptake of stretched muscles. In Fig. 2 the

muscles are combined into groups, each group corresponding to a range of diameters of 0.1 mm. The values of the rate of oxygen uptake are plotted above the midpoint of the diameter range of each group. It can be seen that in range, scatter, and values of oxygen uptake Fig. 2 is very similar to Fig. 1

TABLE I  
ACTUAL AND CRITICAL DIAMETER AND OXYGEN UPTAKE OF  
PAPILLARY MUSCLES BEFORE AND AFTER STRETCH\*

No. of muscles	Diameter, unstretched	Critical diameter, unstretched	Diameter after stretch	Critical diameter after stretch	Rate of oxygen uptake	
					Before stretch	After stretch
6	0.45 (0.42-0.48)	0.615 (0.60-0.63)	0.37 (0.34-0.39)	0.62 (0.60-0.63)	2.90 (2.18-3.25)	2.90 (2.18-3.25)
2	0.545 (0.50-0.59)	0.615 (0.60-0.61)	0.44 (0.41-0.48)	0.605 (0.60-0.61)	2.32 (1.76-2.88)	2.32 (1.76-2.88)
6	0.65 (0.61-0.68)	0.60 (0.58-0.61)	0.53 (0.50-0.55)	0.59 (0.58-0.60)	2.23 (1.58-2.82)	2.84 (1.80-3.70)
4	0.74 (0.71-0.76)	0.58 (0.56-0.60)	0.61 (0.58-0.62)	0.56 (0.55-0.58)	1.84 (1.61-2.26)	2.68 (2.42-3.22)
1	0.86	0.56	0.71	0.55	1.71	1.88
5	0.93 (0.91-0.96)	0.56 (0.52-0.59)	0.75 (0.72-0.78)	0.54 (0.48-0.58)	1.64 (1.18-1.94)	1.98 (1.46-2.20)
2	1.025 (1.02-1.03)	0.565 (0.55-0.58)	0.85 (0.83-0.87)	0.555 (0.55-0.56)	1.45 (1.42-1.49)	1.72 (1.61-1.82)
3	1.17 (1.15-1.19)	0.57 (0.54-0.60)	0.95 (0.94-0.96)	0.54 (0.49-0.58)	0.89 (0.66-1.10)	1.36 (0.99-1.57)
5	1.25 (1.23-1.29)	0.57 (0.52-0.59)	1.02 (1.00-1.05)	0.53 (0.46-0.56)	1.03 (0.86-1.28)	1.44 (1.05-1.91)
2	1.34 (1.33-1.36)	0.48 (0.41-0.55)	1.10 (1.08-1.11)	0.44 (0.35-0.53)	1.02 (0.92-1.12)	1.23 (1.20-1.26)
2	1.45 (1.42-1.48)	0.58 (0.57-0.59)	1.15 (1.10-1.21)	0.54 (0.53-0.55)	0.79 (0.77-0.81)	1.23 (1.13-1.32)
1	1.52	0.52	1.24	0.40	0.77	1.43
1	1.83	0.57	1.50	0.56	0.30	0.40
1	2.25	0.57	1.84	0.32	0.63	0.81

\* All values for diameter and critical diameter are given in millimeters; rate of oxygen uptake is given in (micro-liters/mg. wet weight)/hour; when there are two or more muscles in a group the average and the range of the values in the group are given. The critical diameters given are the critical diameters for adequate supply from a solution containing the amount of oxygen actually present in the effluent; this is discussed in the text.

which shows the same information for unstretched muscles. In Fig. 3 the average rate of oxygen uptake is shown for stretched and unstretched muscles. The averages for the unstretched muscles are the averages of all measurements made on unstretched muscles and therefore include the uptakes of some muscles which were not subsequently stretched. It can be seen that the two curves are similar in shape and cross one another more or less at random. The only place where the rate of oxygen uptake of the stretched muscles seems to be markedly and significantly higher than that of the unstretched muscles is



in the diameter range 0.5 to 0.7 mm. The probable significance of this finding is taken up in the section on critical diameter. The values shown in Figs. 2 and 3 suggest that stretch does not uniformly or markedly increase the rate of oxygen uptake of papillary muscles.

The effect of stretch on the rate of oxygen uptake can be evaluated more critically from the information in Table I. Since the discussion of Table I involves a consideration of the critical diameter it is discussed in the section on critical diameter. It may be pointed out here that no group of muscles, when

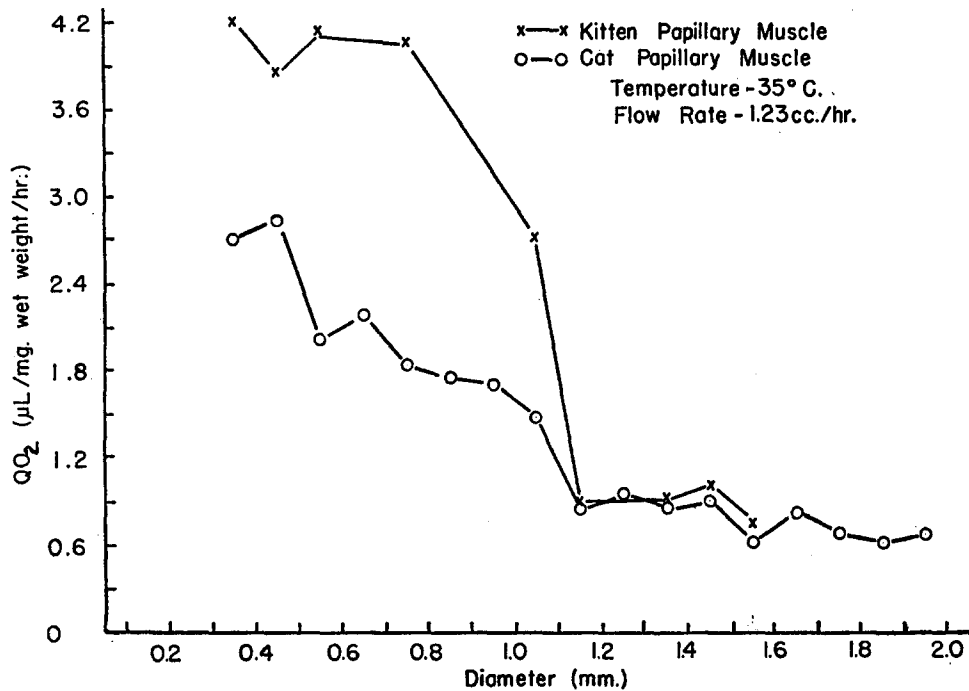


FIGURE 4. The apparent oxygen uptake, in (microliters/mg. wet weight)/hour, of quiescent papillary muscles from kitten hearts (X—X) and from cat hearts (O—O). Diameters are combined into groups with a range of 0.1 mm. and the average oxygen uptake for each diameter range is plotted above the diameter which represents the midpoint of the diameter range of the group. The uptakes were measured at 35°C.

stretched, attained a rate of oxygen uptake significantly higher than that of unstretched muscles of the smallest diameter studied, namely 2.84 (microliters/mg. wet weight)/hour.

#### *The Oxygen Uptake in Young Animals*

In an attempt to obtain extremely fine papillary muscles a series of experiments were carried out on kittens which were 6 to 8 weeks old. It became apparent that the results could not be averaged in with the studies on cats be-

cause the uptake is much higher. Fig. 4 shows the apparent oxygen uptake of papillary muscles from kitten hearts as a function of diameter; for comparison, it also shows the uptake of papillary muscles from cat hearts. It can be seen that the papillary muscles from kitten hearts show a much higher rate of uptake in the range of diameters where the muscles are presumably small enough to be supplied by diffusion. The muscles from kitten hearts had the same distribution of weights and were about the same length as those from cat hearts. The muscles from kitten hearts also show the deceptive plateau at large diameters which was mentioned above. The rate of oxygen uptake by sufficiently thin papillary muscles from kitten hearts is 4.05 (microliters/mg. wet weight)/hour at a temperature of 35°C.

#### *The Critical Diameter for Adequate Diffusion of Oxygen*

The calculation of the maximum diameter which a cylindrical piece of metabolizing tissue may have and still be adequately supplied with oxygen by diffusion cannot be made unless the rate of oxygen uptake is known. If an unduly low rate of uptake is used in the calculation, an unduly large critical diameter will be obtained. For example, if the apparent rate of uptake shown by muscles in the diameter range 1.15 to 1.45 mm. were used, namely 0.9 (microliters/mg. wet weight)/hour, the result is obtained that a muscle exposed over its entire surface to an oxygen tension of 95 per cent of atmospheric pressure will be adequately supplied by diffusion if its diameter is less than 1.13 mm. Such a result is clearly incorrect since smaller muscles are seen to take up far more oxygen. In our experiments a clear plateau in the low diameter range was not obtained. Apparent oxygen uptake increased with decreasing diameter up to the smallest diameters used. In calculating a critical diameter we have used the highest apparent oxygen uptake observed by us, that of muscles of a diameter of 0.45 mm., 2.84 (microliters/mg. wet weight)/hour. Muscles of that diameter, when stretched did not show an increase in apparent oxygen uptake, whereas muscles of larger diameter did. No group of muscles, even when stretched, showed an uptake greater than 2.84 (microliters/mg. wet weight)/hour. Finally, papillary muscles from kitten hearts show much higher apparent uptake at diameters of 0.75 mm. than do those of cat hearts. This suggests that metabolic rate rather than diffusion limitation is responsible for the difference and in turn suggests that the smaller muscles were in fact small enough to be supplied by diffusion. For the reservations which must be attached to this argument, see the Discussion. If the value for uptake which is seen at a diameter of 0.45 mm. is used, it is found that a muscle exposed to 95 per cent oxygen over its entire surface will be adequately supplied by diffusion only if its diameter is 0.64 mm. or less. The critical di-

iameter corresponding to the oxygen uptake of the papillary muscle from kitten hearts is 0.53 mm.

It is further true that in our experimental situation the muscle is not uniformly exposed to a surface oxygen tension corresponding to 95 per cent of atmospheric pressure. As the bathing solution is drawn past the muscle it gives up oxygen to the muscle. The end of the muscle farthest from the reservoir will be supplied with oxygen at its core only if its diameter is small enough to permit diffusion from a medium containing the observed oxygen tension of the effluent; *i.e.*, the actual value measured by the oxygen electrode. It is possible to calculate the critical diameter for the actual experimental conditions and if this is done for unstretched and stretched muscles, further interesting results emerge. Table I shows average values by 0.1 mm. diameter groups for initial diameter, the critical diameter for adequate diffusion supply by the actual effluent oxygen tension, the diameter after stretch, the critical diameter for adequate diffusion supply after stretch, and the apparent rate of oxygen uptake before and after stretch. It should be noted that the critical diameter for adequate supply after stretch differs from that before stretch because stretch causes the apparent oxygen uptake to increase and the tension of oxygen in the effluent falls. The critical diameters are calculated using a rate of oxygen uptake of 2.84 (microliters/mg. wet weight)/hour.

It can be seen from Table I that two groups of muscles showed no increase in apparent uptake when stretched, the groups with initial diameters of 0.4 to 0.5 mm. and 0.5 to 0.6 mm. The average rate of uptake did not change and in fact each individual muscle showed exactly the same rate of uptake before and after stretch. Those groups are the only two groups the diameter of which before stretch was less than the critical diameter for adequate oxygen supply for diffusion from a solution containing the amount of oxygen actually observed in the effluent. All fibers of larger diameter were larger than the critical diameter for adequate diffusion and they all showed an increase in apparent oxygen uptake when stretched.

The table further reveals that the muscles which showed the largest increase in apparent uptake when stretched, namely those in the initial diameter ranges 0.6 to 0.7 mm., are the only muscles which upon stretch attained a diameter less than the critical diameter appropriate to the new effluent oxygen tension. Those muscles, after stretch, attained a rate of oxygen uptake of 2.84 (microliters/mg. wet weight)/hour; *i.e.*, a rate equal to that of the smallest unstretched muscles. The muscles in the initial diameter range 0.7 to 0.8 mm. increased their rate of oxygen uptake markedly to 2.68 (microliters/mg. wet weight)/hour when stretched. Those muscles, after stretch, were almost small enough to be adequately supplied with oxygen by diffusion. All larger muscles, even when stretched, were much too thick to be adequately

supplied with oxygen by diffusion and they did not, when stretched, increase their rate of oxygen uptake enough to even approach the rate shown by those muscles which were small enough to be supplied by diffusion. These results, taken with those mentioned in the section on stretch, have led us to conclude that stretch has little or no effect on the rate of oxygen uptake except in so far as it causes a muscle to become longer and thinner and thus to approach the diameter at which it may be adequately supplied by diffusion. The effect of the change in length as such is considered in the Discussion. We must qualify this statement by saying that it would be desirable to conduct more experiments on stretch using very thin papillary muscles. It is unfortunately very difficult to find papillary muscles less than 0.64 mm. in diameter.

#### DISCUSSION

The rate of oxygen uptake found in this study of cat papillary muscle is significantly higher than values reported previously (8, 9). As a result the critical diameter proves to be much smaller than the value of 1.2 mm. reported previously (8). A critical diameter of 1.2 mm. for a muscle exposed uniformly to 95 per cent O<sub>2</sub> corresponds to an apparent oxygen uptake of 0.9 (microliters/mg. wet weight)/hour, the value seen in the deceptive plateau mentioned above. The only question which seems to arise concerning the value of the oxygen uptake is whether it may be even higher than we have found. No clear plateau was found in the small diameter end of the diameter-uptake curve and it is possible that somewhat smaller fiber bundles might have shown even larger rates of uptake. However, the fact that the smallest muscles studied showed an uptake which did not increase when the muscle was stretched suggests that the true uptake was determined. The same conclusion is reached by observing that those muscles which showed a marked increase in uptake upon stretch nevertheless did not show an uptake greater than that shown by the smallest unstretched muscles.

Our value of 2.84 (microliters/mg. wet weight)/hour represents an uptake of 11.32 (microliters/mg dry weight)/hour since our wet weight to dry weight ratio was 4.0. This value is very close to the value of 12.8 (microliters/mg. dry weight)/hour obtained on slices of rat ventricle by Pearson *et al.* (10). The same authors found that the tissue slice thickness which was optimal for maximum oxygen uptake was 0.5 mm. Whalen (9) reported an uptake of 7.7 (microliters/mg. wet weight)/hour in studies on rat atria; he reported low values for cat papillary muscle but he accepted a value of 1.2 mm. as the critical diameter. Whalen in fact states "Two muscles which approached the limiting thickness were eliminated from consideration because they showed a decrease in contraction strength greater than 50%." Our values may also be

compared with values obtained on intact *in situ* hearts. Such a comparison cannot be direct since most studies of the rate of oxygen uptake of the intact *in situ* heart were conducted on beating hearts. McKeever *et al.* (11) report an uptake in the intact normally beating heart of the dog of 8 to 10 (cc./100 gm.)/minute which is about the same as the value given by Bing *et al.* (12, 13). An uptake of 9 (cc./100 gm.)/minute is 5.4 (microliters/mg. wet weight)/hour or about 22 (microliters/mg. dry weight)/hour. Lorber's data (14) have been shown by Whalen (9) to indicate that the weakly contracting cat heart has an uptake of about 15 (microliters/mg. dry weight)/hour. Katz *et al.* (15) found that the apparent rate of oxygen uptake of contracting dog heart is sensitive to the level of arterial oxygen tension. They found values as high as 20 (microliters/mg. wet weight)/hour, by far the highest values reported in the literature. We have not studied the effect of contraction on the rate of uptake. In a single experiment in which spontaneous contraction occurred at a rate of 55 per minute for only 2 minutes the rate of uptake rose to 3.58 (microliters/mg. wet weight)/hour. In general therefore we feel that the high level of oxygen uptake which we note in the resting cat papillary muscle accords well with various *in vitro* studies on rat ventricle and rat atrium and with various studies on the beating mammalian heart *in situ*.

The finding of a higher value of oxygen uptake in the papillary muscle of kitten hearts is not wholly unexpected. Various authors have found that the tissues of young animals have a higher rate of uptake than do the tissues of older animals. One of us (P.F.C.) has made a similar observation on rat peripheral nerve.

The results of stretching the muscles are interesting in that they support our general belief that most previous studies have been carried out on muscles the diameter of which was too great to permit adequate supply of oxygen by diffusion. It is further interesting to note that we did not obtain any evidence to support the belief that stretch *per se* acts to increase the rate of oxygen uptake in cat papillary muscle. We do not regard the present study as definitely showing that stretch does not increase uptake but we feel fairly sure that it does not have a marked effect and that most of the reports that it has are based upon experiments on muscles which were brought closer to the critical diameter by being stretched and which increased their uptake for that reason.

A consideration of the diffusion equation for the cylinder is in order at this point. If  $U = C - (a/4D) (R^2 - r^2)$ , where the symbols have the meaning given in the section on method, and if the whole muscle is uniformly exposed at its surface to an oxygen tension which is too low to permit adequate supply of the muscle by diffusion, oxygen tension will fall to zero at some distance from the center of the muscle which we may designate  $r_i$ . Then  $U = 0$  at  $r = r_i$  and we may solve the equation for  $r_i$  obtaining the result  $r_i = (R^2 - 4DC/a)^{1/2}$ . The total uptake of the whole muscle will be

$$(a)(\pi)(l)(R^2 - r_i^2) = 4lDC.$$

In other words when there is an anoxic core throughout the length of the muscle the total uptake depends only on the external oxygen concentration and on the length of the muscle. If the weight of the muscle is constant, as it is when diameter is changed by means of stretch it will be seen that the apparent rate of oxygen uptake can increase in two ways. If the muscle has an anoxic core throughout its length and if by stretch the diameter is reduced enough so that the anoxic core entirely disappears, then the increase in uptake may be attributed to the reduction in diameter. If, however, the muscle has an anoxic core throughout its length both before and after stretch, the formula shows that the increase in uptake should be proportional to the increase in length. It can be seen from Table I that we have results of both kinds.

The finding that the critical diameter for a cylinder of cardiac muscle uniformly exposed to 95 per cent O<sub>2</sub> to be adequately supplied with oxygen by diffusion is only 0.64 mm. is extremely important. It is not easy to find papillary muscles which are that small and it seems likely that many of the studies which have been made on the pharmacology and on the mechanical properties of papillary muscle have been conducted on muscles with an anoxic core. It should be emphasized that our studies were made on quiescent muscle and that the rate of oxygen uptake of contracting cardiac must be higher than the value we found for resting muscle. If it is only twice as high, then the maximum diameter permissible for studies on contracting muscle would be about 0.45 mm. The critical diameter for an ambient oxygen tension of 20 per cent of atmospheric at a rate of uptake of twice that which we found at rest is less than 0.2 mm. It is thus evident that the normally beating and working heart is in need of an extremely rich capillary supply and a very high blood flow, both of which it in fact has. It is moreover true that the oxygen concentration of venous blood returning from ventricular myocardium is much lower than the oxygen concentration of mixed venous blood. All these observations support the finding that the uptake of oxygen by the quiescent papillary muscle is high. The finding that quiescent papillary muscle uniformly exposed to 95 per cent O<sub>2</sub> cannot be adequately supplied with oxygen by diffusion unless its diameter is less than 0.64 mm. and that contracting papillary muscle probably cannot be adequately supplied unless its diameter is less than 0.45 mm. goes far to explain many other observations. It is not surprising that the papillary muscle preparation *in vitro* is extremely variable in its response and in its viability. Nor is it surprising that many of the highest oxygen uptake rates which have been obtained during *in vitro* studies have been found in studies on atrial tissue which is thin and highly trabeculated. It seems likely that the whole heart perfused *in vitro* may frequently be anoxic even when perfused with oxygenated blood unless every possible precaution is used to assure full

patency of the capillary bed and a coronary flow rate at least as great as that of the *in situ* heart.

It is evident that studies in which uptake was determined to be low and in which that low uptake was then used to calculate a critical diameter are fallacious. It is difficult to attach any meaning to the determination of changes of the rate of uptake as the result of activity or as the result of the action of drugs if the muscle is too large to be supplied by diffusion. If the muscle diameter exceeds the critical diameter some unknown portion of the center of the muscle will be anoxic. Any change in the rate of oxygen uptake in the part of the muscle which is supplied by diffusion will result in an unknown change in the size of the anoxic core. It might be claimed that the finding of an increase or decrease in apparent uptake is meaningful even if the muscle has an anoxic core. But if the presence of an anoxic core affects the metabolic state of the non-anoxic part, as it well might through the release of various metabolites, then a change in the size of the anoxic core would alter the response of the non-anoxic part in an unpredictable manner.

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