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Factors Affecting the Metabolism of Resting Rabbit Papillary Muscle*

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Abstract. The rate of resting heat production of 12 right ventricular rabbit papillary muscles was measured myothermically. Resting heat rate was measured at 4 temperatures (15, 20, 25 and 30° C) in either 45 % or 95 % O_2 while the muscle was passively stretched with various pre-loads. The metabolic substrate was pyruvate (10 mmol · l^{-1}). The mean resting heat rate, averaged across all treatment conditions, was 2.88 mW/g with no significant difference between the two oxygen concentrations. The calculated Q_{10} of the resting heat rate was surprisingly low – only about 1.4 – but is shown to be in general agreement with literature values from whole heart oxygen consumption studies when the time-dependent decline is taken into account. Stretching the muscle beyond its rest length increased the rate of resting heat production. This response appeared unrelated to muscle diameter. The results are discussed in terms of the possible diffusion limitation of isolated papillary muscle preparations.

Key words: Cardiac resting heat rate – Myothermic technique – Stretch effect – Q_{10} – Diffusion limitation – Anoxic core hypothesis – Temperature – P_{O_2}

Introduction

Cardiac resting metabolism is thought to reflect those regenerative processes which must occur continuously in order to maintain the structural and functional integrity of the heart. This basal component accounts for a sizeable fraction of the total cardiac metabolism – about 25 % in man and over 40 % in the rat (Loiselle and Gibbs 1979). The resting metabolism of cardiac muscle is four to five times higher than that of mammalian skeletal muscle (Gibbs 1978) and as yet no explanation for this difference exists.

Previous work from this laboratory has shown that cardiac glycosides, which inhibit the Na^+-K^+-pump, have little or no effect upon basal metabolism (Gibbs and Gibson 1969) while calculations based upon flux data in the literature (Gibbs and Chapman 1979) suggest that the sodium pump accounts for less than 10 % of the observed resting heat rate [although more recent flux data (McCall 1979) may allow this figure to be doubled]. Nor does the high rate appear to be due to a high resting level of sarcoplasmic reticular Ca^{2+}-pumping (Chapman et al. 1977; Gibbs and Loiselle 1978; Wendt and Loiselle 1981). The effect of stretch on increasing the resting metabolism of papillary muscles has been reported as both large (Whalen 1960; Lee 1960) and radius-dependent (Cranefield and Greenspan 1960; McDonald 1966) in oxygen consumption studies but variable in myothermic studies (Gibbs et al. 1967). Studies on the effect of temperature upon the resting metabolism of isolated cardiac muscle are rare and report commonly accepted Q_{10} values (Fuhrman et al. 1950; Greenspan and Cranefield 1963), although Lochner et al. (1968) have reported a low value for the Q_{10} of the resting oxygen consumption of whole heart. The following study examines the effects of varying temperature, oxygen partial pressure, and pre-load upon the metabolic rate of resting cardiac muscle.

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Methods

Hearts from adult rabbits, killed by a blow to the neck, were removed, back-perfused through the aorta and placed in a dish of warmed (35° C) oxygenated (95 % O_2, 5 % CO_2) Krebs solution. The wall of the right ventricle was reflected exposing the septum. A papillary muscle was selected, tied at either end with silk thread (Ethicon 5/0) removed and mounted on a thermopile under a 1 g pre-load as previously described (Gibbs and Gibson 1969). Average muscle characteristics are presented in Table 1. The thermopile chamber contained 55 ml of a modified Krebs solution of the following concentrations (mmol · l^{-1}): NaCl 118, KCl 4.75, $NaHCO_3$ 24.8, $MgSO_4$ 1.18, KH_2PO_4 1.18 and $CaCl_2$ 2.54. The metabolic substrate was pyruvate (10 mmol · l^{-1}) and the pH was 7.4.

The muscles were equilibrated for $2\frac{1}{2}$ h in 95 % O_2 at either 20° C or 25° C. During this period they contracted isotonically against the 1 g pre-load at a rate of 0.25 or 0.3 Hz respectively. Excitation was achieved by point stimulation from a silver electrode cantilevered from the frame of the thermopile over the base of the muscle to a second silver electrode located in the plane of the thermopile under the apex of the muscle. At the end of the equilibration period the rate of exponential heat loss from the muscle/thermopile system was determined by heating the muscle with a 10 ms burst of pulses from a high frequency (100 kHz) generator, and the electronic heat loss corrector (Gibbs and Gibson 1969) was set appropriately. At the conclusion of the experiment calibration of the heat record was achieved by liberating a known amount of heat into the muscle by discharging a capacitor through the electrodes. The muscle was then removed from the thermopile and its length under a 1 g load measured. The ties were cut off and the weight determined to the nearest 0.1 mg. All values of heat rate are reported as mW per gram of muscle.

Temperature stability was achieved by immersing the muscle/thermopile chamber in a water bath the temperature of which was maintained (± 0.01° C) by a Haake Ultrathermostat Model N.B.S. coupled to a Haake Model

Table 1. Average muscle data
A Physical characteristics. W = Muscle weight; l = length; A/d = cross-sectional area/diameter calculated assuming muscle a cylinder of unit specific gravity; HL = rate of heat loss from muscle/thermopile

	W (mg)	l (mm)	A (mm^2)	d (mm)	HL (s^{-1})
95% O_2	4.2 ± 0.7	4.8 ± 0.4	0.91 ± 0.18	0.97 ± 0.11	0.14 ± 0.0094
45% O_2	3.8 ± 0.8	5.8 ± 0.6	0.63 ± 0.10	0.87 ± 0.09	0.14 ± 0.0074

B Mechanical performance. Mean relative extent of active shortening (percent) under a 1 g pre-load during the 1¼ h equilibration period preceding each temperature treatment. Statistically significant decline in extent of shortening at 25 and 30° C at each level of FO_2; effect of order of presentation of temperature treatments (no. 1, no. 2, etc) not statistically significant at either level of FO_2. Note: no. 1 was performed before subjecting the muscles to large passive loads

		15° C/no. 1	20° C/no. 2	25° C/no. 3	30° C/no. 4
95% O_2	Temperature	16.6 ± 2.4	16.5 ± 1.9	15.0 ± 1.3	11.2 ± 2.3
	Order	15.3 ± 1.4	14.9 ± 2.8	14.8 ± 3.0	14.4 ± 1.2
45% O_2	Temperature	15.6 ± 1.6	14.3 ± 2.2	11.8 ± 1.2	6.9 ± 1.0
	Order	12.4 ± 1.6	12.8 ± 2.2	11.4 ± 2.0	11.9 ± 2.7

K11 Refrigeration Unit. Output of the thermopile was amplified by an Astrodata 120 Nanovolt amplifier, passed through a filter network (20 Hz cut-off) and the heat loss corrector, and displayed on a Chart recorder (Gould Brush Model 260).

Changes in passive muscle length, produced by varying the pre-load, were measured by an angular displacement transducer (Brush Metrisite) mounted on the fulcrum of the isotonic lever. Length changes could be determined to an accuracy of ± 0.25 µm.

Statistical Design

A Two-way (Split Plot) Randomized Blocks Analysis of Variance Design (Edwards 1963) was employed. Each of 6 muscles (Blocks) was examined (repeated measures) in 95% O_2/5% CO_2 under 24 treatment conditions: 4 temperatures (15, 20, 25 and 30° C) and 6 levels of resting or pre-load (¼, ½, 1, 2, 4 and 6 g). Six different muscles received the same 24 treatments but in 45% O_2/5% CO_2/50% N_2. The dependent variables were muscle length and rate of resting heat production. Muscles experienced a given load for 5 min; muscle length was determined at the end of this period whereas measurements of resting heat rate were made at the 3rd, 4th and 5th min. The latter time-related information is henceforth referred to as the sequence effect; it tests the stability of the resting heat rate under the different loads. Thus a $4 \times 6 \times 3$ (temperature × load × sequence) Factorial Design arrangement was presented to each of 6 muscles in each of 2 plots (oxygen fraction: FO_2). The resulting 863 ($4 \times 6 \times 3 \times 6 \times 2 - 1$) degrees of freedom for resting heat rate and 287 ($4 \times 6 \times 6 \times 2 - 1$) degrees of freedom for muscle length were partitioned into main and interaction effects by Analysis of Variance. Statistical significance was adjudged at the 99% level ($\alpha = 0.01$). It should be noted that this design affords very high power for testing the effects of temperature, load and sequence but poor power for examining the effects of FO_2 (oxygen fraction) – equivalent only to an unpaired *t*-test with $n = 12$.

Experimental Protocol

Preliminary studies made it clear that equilibration was extremely slow and the chances of survival reduced if a muscle was initially equilibrated at 15° C. Hence the initial plan to present the temperature treatments according to a 4 × 4 Latin Square arrangement was abandoned. Instead muscles were initially equilibrated at either 20° C or 25° C (3 muscles at each initial temperature in each FO_2 group) with the remaining 3 temperatures presented as shown in Table 2A. Since the effect of temperature would now be confounded with the known time of day effect (Chapman and Gibbs 1974) it was necessary to extend the initial equilibration period to 2½ h by which time the decline in resting heat rate has reached a plateau (Loiselle and Gibbs 1979). Muscles in the 45% FO_2 group were equilibrated for 1¼ h in 95% O_2/5% CO_2 followed by 1¼ h in 45% O_2/5% CO_2/50% N_2.

Thus a muscle was equilibrated for 2½ h at either 20 or 25° C before any measurements were made. There then followed a 15 min experimental period in which the resting heat rate was measured at 3, 4 and 5 min and the muscle length at 5 min under each of 3 loads. The muscle was returned to

Table 2. Statistical design. Muscles were randomly assigned to one of the 12 rows shown in **A** and one of the 6 rows shown in **B** with the restriction that **B** was duplicated for the two levels of FO_2

A Order of presentation of temperature (° C)

95% O_2	25	15	30	20
	20	15	25	30
	25	30	20	15
	20	25	30	15
	25	20	15	30
	20	30	15	25
45% O_2	20	25	15	30
	25	20	30	15
	20	15	30	25
	25	30	15	20
	20	30	25	15
	25	15	20	30

B Order of presentation of load (g)

2	4	¼	½	6	1
4	½	2	1	¼	6
½	1	4	6	2	¼
1	6	½	¼	4	2
6	¼	1	2	½	4
¼	2	6	4	1	½

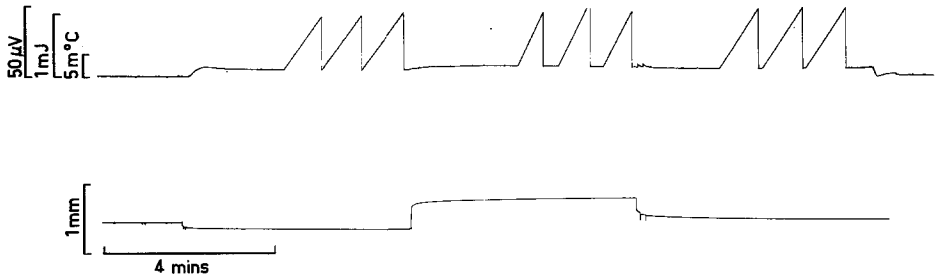

Fig. 1. Original records at 30° C in 95% O_2 of heat production (*upper trace*) and changes in muscle length (*lower trace*) associated with 3 pre-loads: $^1/_2$, 6 and 1 g from left to right. Extreme left hand side: muscle under solution under a 1 g load at completion of a 15 min recovery period. Extreme right hand side: muscle returned to solution. Notice transients on heat trace when solution is lowered and raised respectively. At the 3rd, 4th and 5th min (sequence effect) under each load the output of the thermopile was integrated; resting heat rate is given by the slope of the resulting sawtooth. After release from 6 to 1 g the muscle received 2 stimuli and responded with two isotonic contractions. The muscle weighed 5.8 mg and was 4.8 mm long

solution to re-equilibrate for 15 min and a second experimental period followed during which the remaining three loads were presented. The temperature was then changed and the muscle permitted to equilibrate for $1^1/_4$ h before the next experimental period. The total duration of an experiment was thus $9^1/_4$ h.

During all equilibration periods muscles were stimulated isotonically under a 1 g pre-load. Stimulation rates at 15, 20, 25 and 30° C were 0.125, 0.2, 0.25 and 0.3 Hz respectively. The order of presentation of experimental loads (shown in Table 2B) was arranged as a fully balanced 6 × 6 Latin Square (Edwards 1963) such that each load followed and preceded each other load exactly once. The same 6 × 6 Latin Square was used for both levels of FO_2.

Resting heat rates were measured as follows. With the muscle/thermopile under solution the output of the amplifier was offset to zero. When the baseline was steady the solution was drained from the muscle/thermopile chamber. At the 3rd, 4th and 5th min after draining the integrator was activated. The slope of the resulting line (Fig. 1), normalized for muscle mass, yields the resting heat rate in mW/g.

Results

Resting Heat Rate

For brevity the Analysis of Variance summary (Table 3) gives only the results for the main effects and *significant* interactions, together with their error terms. These results are presented graphically in Figs. 2 and 3. Note that neither the FO_2 effect (95% vs 45% O_2) nor the sequence effect (heat rate at the 3rd, 4th or 5th min) were significant. The former result probably reflects the low statistical power associated with this comparison (see Methods). Likewise the effect of order of presentation of neither temperature (Fig. 2B) nor load (Fig. 2D) was significant. Only two interaction effects (Fig. 3) were statistically significant: load × FO_2 and load × sequence. As can be seen in Fig. 3A there was a tendency for the larger loads to cause greater resting heat rates in 95% O_2 than in 45% O_2 – i.e., the stretch effect is oxygen concentration-dependent. Figure 3B shows the tendency for the resting heat rate, during the 5 min measurement period, to fall, remain steady or rise for low, intermediate and high loads respectively. Since the load effect (Fig. 2D, E) is averaged over the three sequence periods, it is thus possible that it has been slightly underestimated in this study because the resting heat rate is still falling (light loads) or rising (heavy loads) slightly at 5 min. However, it was deemed imprudent to keep the muscle out of solution for more than 15 min (during which time 3 loads were presented) – particularly at 30° C.

Table 3. Summary of variance analysis: Resting heat rate

Source of variation	*df*	SS	MS	*F*
Total	863	835.03		
Between muscles	11			
FO_2	1	39.08	39.08	1.21
Error	10	323.23	32.23	
Within muscles	852			
Temperature	3	172.87	57.62	16.28*
Error	30	106.16	3.54	
Temperature order	3	6.19	2.06	0.23
Error	30	272.07	9.07	
Load	5	55.49	11.10	17.65*
Load × FO_2	5	11.33	2.27	3.60*
Error	50	31.43	0.63	
Load order	5	2.52	0.50	0.45
Error	50	33.27	1.11	
Sequence	2	0.02	0.01	0.83
Error	20	0.24	0.01	
Load × sequence	10	0.56	0.06	4.20*
Error	100	1.31	0.01	

Results for interactions shown only if significant (*); *df* = degrees of freedom; SS = sum of squares; MS = mean square; *F* = treatment MS/error MS

Muscle Length

Table 4 summarizes the Analysis of Variance for changes in muscle length. Only the results for main effects are shown as there were no significant interactions. Note that the Sum of Squares for the load effect accounts for 85% of the total variance. This result is plotted in Fig. 4 where the extreme linearity between the pre-load (expressed logarithmically) and the resulting change in muscle length is apparent. Note also that the order of presentation of the loads had no significant effect on the average changes in muscle length. This, together with the same result for the resting heat rate, justifies the use of the fully balanced 6 × 6 Latin Square arrangement for presentation of the load sequence (Table 2B). Similarly the order of presentation of temperatures (Table 2A), although not a Latin Square arrangement for reasons given in the Methods, is vindicated. Thus the non-significant *F*-value for the order of presentation of temperatures (Table 3) implies a

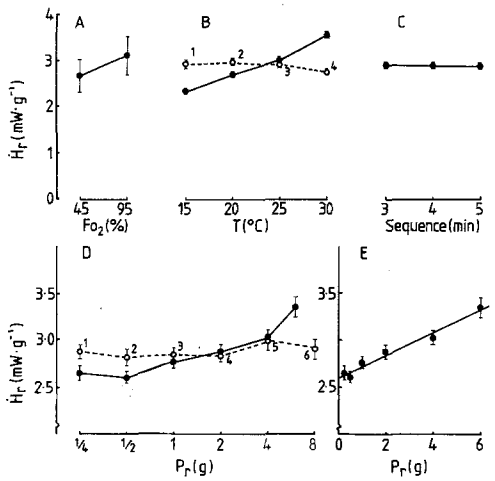

Fig. 2A—E. Analysis of variance: Treatment main effects upon resting heat rate, $\dot{H}_r$. **A** Oxygen fraction, FO_2 (not significant). **B** Temperature, T (*filled circles, solid line*) and order of presentation of temperature (*open circles, broken line*; not significant). **C** Sequence (see Methods) (not significant). **D** Passive load, P_r (*filled circles, solid line*) and order of presentation of load (*open circles, broken line*; not significant); note logarithmic scale. **E** Load data re-plotted on a linear scale: $\dot{H}_r = 2.6 + 0.12\,P_r$; correlation coefficient, $r = 0.985$; standard error of regression, $s_{y \cdot x} = 0.44$

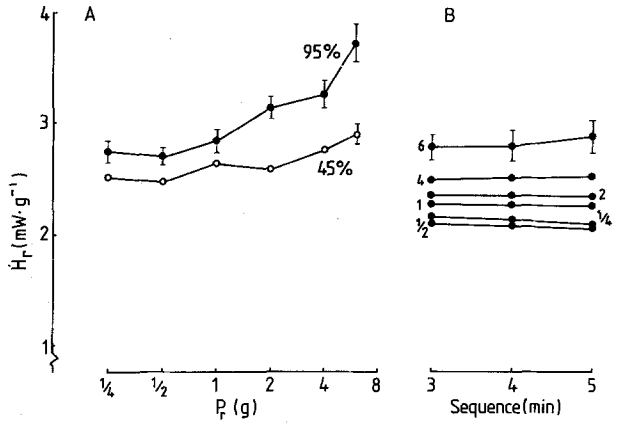

Fig. 3A and B. Significant first-order interactions in the analysis of variance. **A** Resting heat rate (averaged across all temperatures and sequences) versus load (expressed logarithmically) at $FO_2 = 45\,\%$ (*open circles*) and 95 % (*closed circles*). **B** Resting heat rate (averaged across all temperatures and both FO_2 values) versus sequence (3rd, 4th or 5th min under a given load — see Methods) under the different loads ($^1/_4$—6 g)

negligible time-of-day effect, the result of waiting $2\,^1/_2$ h before making the first measurement.

Muscle Size

The variation of resting heat rate with muscle diameter is shown in Fig. 5A where the data for all 12 muscles (6 in 45 % O_2 and 6 in 95 % O_2) under a passive load of 1 g at 30° C is shown. (A choice of any other combination of treatments would yield a similar result.) The data are well-fitted by a straight line; the rate of resting heat production declines with increasing muscle diameter. The increment in resting heat production with stretch, on the other hand, is not significantly related to muscle diameter (Fig. 5B). As a result (Fig. 6) the stretch-effect is unrelated to the basal metabolic rate.

Table 4. Summary of variance analysis: Muscle length. Meaning of symbols same as in Table 3

Source of variation	*df*	SS	MS	*F*
Total	287	36.440		
Between muscles	11			
FO_2	1	0.148	0.148	1.50
Error	10	0.989	0.099	
Within muscles	276			
Temperature	3	0.052	0.017	1.93
Error	30	0.269	0.009	
Temperature order	3	0.030	0.010	0.91
Error	30	0.335	0.011	
Load	5	31.052	6.210	136.66*
Error	50	2.306	0.046	

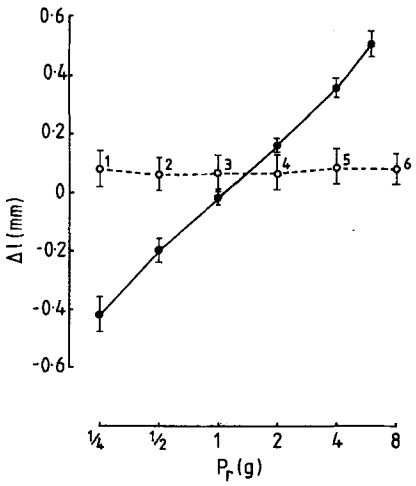

Fig. 4. Change in muscle length, Δl [arbitrarily referred to the length under a load of 1 g at the end of $1\,^1/_4$ h equilibration in 95 % O_2 at either 20° C or 25° C (see Methods)] versus passive load, P_r, expressed logarithmically (*closed circles, solid line*): $P_r = 2^{5\Delta l}$. Effect of order of presentation of load (numbers, *open circles, broken line*) not significant; i.e., the effect of any given load was independent of whether it preceded or followed any other (and, in particular, the largest) load

Discussion

The rate of resting heat production, averaged across all treatment conditions, was 2.88 mW/g (Fig. 2). This value compares favourably with a figure of 2.65 mW/g for rabbit papillary muscles under small pre-loads in 10 mM pyruvate at 21° C (Chapman and Gibbs 1974) and suggests that resting metabolism accounts for at least 30 % of the total myocardial metabolism in the rabbit (Loiselle and Gibbs 1979).

As indexed by the extent of active shortening under a 1 g load during each equilibration period (Table 1 B), the muscles remained healthy throughout the $9\,^1/_4$ h experimental period. Whereas the extent of shortening showed the characteristic diminution with temperature, the effect of order of presentation of treatments was not significant. Hence repeated passive stretching of the muscles with a 6 g preload had no

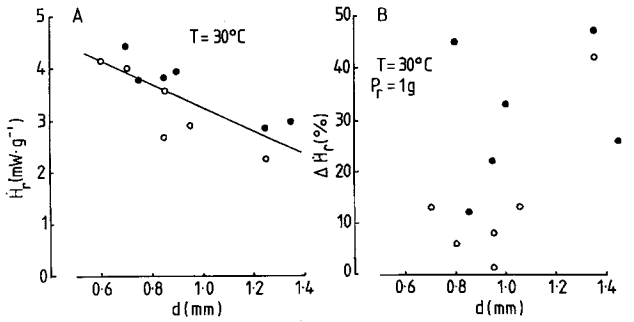

Fig. 5. A Resting heat rate, $\dot{H}_r$, at 30° C under a passive load of 1 g, versus muscle diameter, *d*: $\dot{H}_r = 5.47 - 2.19\,d$; $r = -0.778$ (significantly different from zero); $s_{y.x} = 0.410$. **B** Stretch-induced increment in resting heat rate, $\Delta\dot{H}_r$, at 30° C (from the value under a load of $^1/_2$ g to the value under a load of 6 g) versus muscle diameter (*d*). *Open circles:* 45% O_2; *closed circles:* 95% O_2; $r = 0.50$; $s_{y.x} = 14.5$. If data replotted vs muscle cross-sectional area: $r = 0.47$; $s_{y.x} = 14.8$; still no significant correlation

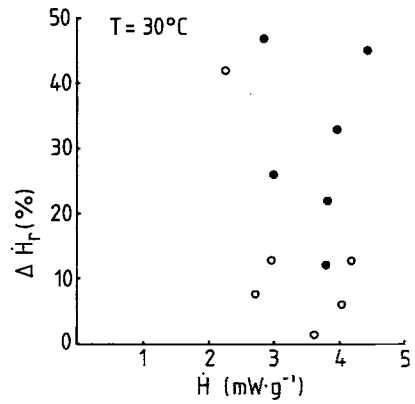

Fig. 6. $\dot{H}_r$ = Resting heat rate under a 1 g load at 30° C; $\Delta\dot{H}_r$ same meaning as in Fig. 5; $r = -0.417$; $s_{y.x} = 15.20$; *r* not significantly different from zero

detrimental effect on their subsequent mechanical performance.

Muscle Size

The resting heat rate varies inversely with muscle size (Fig. 5A). This observation could be taken as evidence of oxygen diffusion limitation in large muscles. However, there is no evidence of a plateau in the graph to the left of some "critical diameter" as demanded by the anoxic core hypothesis (Delbridge and Loiselle 1981). Furthermore, under the conditions of this experiment the critical radius according to the Hill model (see below) would not have been exceeded by any of the muscles studied. If muscles were indeed anoxic then large muscles (or, equivalently, those with lowest metabolic rates) should have shown a greater stretch effect. This was not the case; the stretch effect was unrelated to either muscle size (Fig. 5B) or the resting rate of heat production (Fig. 6). The cause of the inverse relation between muscle size and resting metabolism is unknown but bears a striking similarity to the equally ill-understood heat-stress relation in cardiac muscle (Delbridge and Loiselle 1981).

Using the model of A. V. Hill (1928) for diffusion into a cylinder (for a review see Gibbs 1978), the largest muscle diameter which just avoids an anoxic core (d_c) is given by $d_c = 4\,(Ky_0/a_0)^{1/2}$ where *K* is the Krogh diffusion coefficient (1.4×10^{-5} cm^2 · atm^{-1} · min^{-1}), y_0 is the oxygen partial pressure (atm), and a_0 is the resting metabolism expressed in ml $O_2 \cdot g^{-1} \cdot min^{-1}$. Assuming that the energetic equivalent of O_2 is 20 kJ · l^{-1}, the mean resting heat rate at 30° C observed in this study, 3.55 mW · g^{-1} (Fig. 2), would imply a critical diameter of 1.4 mm. Thus the mean muscle diameter of the 95% oxygen group, 0.97 mm (Table 1), is well below the critical value; in fact, no individual muscle exceeded it. Contrariwise, a muscle of diameter 0.97 mm could sustain a resting metabolic rate corresponding to 7.55 mW · g^{-1} without developing an anoxic core. Since this is over twice the observed mean value, and well exceeds any individual value, it seems improbable that the oxygen supply to the central regions of these muscles was compromised. Analysis of the effects of stretch supports this contention.

Stretch Effect

The effect of stretch upon the resting metabolism of skeletal muscle is well known. In a series of experiments with amphibian skeletal muscle Feng, working in A. V. Hill's laboratory, both quantified the effect and proved it to be of metabolic origin (Feng 1932). Although its underlying basis also remains obscure, a comparable effect exists in cardiac muscle. Gibbs et al. (1967) found the stretch response to be quite variable in a small sample of rabbit papillary muscles. Whalen (1960), studying both cat papillary muscle and rat trabeculae carneae, found stretch-induced increments in resting oxygen consumption, measured volumetrically, of 20 to 55% and Lee (1960), in an early usage of the oxygen electrode, obtained increments of 30 to 90% in cat papillary muscle. Cranefield and Greenspan (1960), also measuring oxygen consumption, attributed the stretch response to a purely physical basis, namely elongation causing a reduction in muscle diameter thereby providing oxygen to a previously diffusion-limited anoxic muscle core. McDonald (1966) used the existence of a stretch-induced increase in oxygen consumption as an index of oxygen insufficiency in resting papillary muscle, although the above discussion of muscle size and a recent theoretical treatment (Loiselle 1982) renders this hypothesis unlikely.

In the present study resting muscles were stretched by the application of standard loads ($^1/_4$, $^1/_2$, 1, 2, 4 and 6 g). Additionally, the change in resting muscle length caused by these loads was also measured. From Fig. 4 it is clear that the given increments in load (expressed logarithmically) caused quite regular increments in muscle length. Figure 2D shows, however, that they did *not* cause regular increments in the rate of resting heat production. For example, under the $^1/_4$ g load, with the muscle length minimal, the putative anoxic core region should have been maximal. Elongating the muscle by increasing the load to $^1/_2$ g should have reduced the region of anoxia and increased the resting heat rate. In fact, (see Fig. 2D) the rate was unchanged. Similarly, increasing the load from 4 to 6 g caused approximately the same increment in muscle length as for previous load steps (Fig. 4) whereas the increment in resting heat rate was roughly treble that of the previous three increments (Fig. 2D).

In Fig. 2E the load-resting heat rate relation is re-plotted and it is apparent that the resting heat rate ($\dot{H}_r$) is linearly dependent upon the resting or passive load (P_r): $\dot{H}_r = 2.6 + 0.12\,P_r$. Since the increment in muscle length (Δl) varies logarithmically with the load ($\Delta l = 0.2 \log_2 P_r$; Fig. 4) the rate

of resting heat production depends exponentially on the extent of stretch: $\dot{H}_r = 2.6 + 0.12\,e^{3.47\Delta l}$. (In the above regression equations P_r is measured in g and $\dot{H}_r$ in $mW \cdot g^{-1}$ while Δl is measured in mm with respect to resting muscle length under a passive load of 1 g.) If the stretch effect had a purely physical (i.e., diffusional) basis, the resting heat rate response to stretch would be expected to vary directly with the decrement in muscle radius and hence to show an inverse square root dependence upon Δl. If, on the other hand, the stretch effect occurs because elongation leads to the diminution of an anoxic central cylinder of tissue, then it should depend inversely upon the square of the radius or, equivalently, directly with the extent of stretch. However, the dependence of $\dot{H}_r$ upon Δl is neither linear nor square root but exponential in form. Thus these data do not support the hypothesis of an anoxic core underlying the stretch effect.

What then is the cause of the stretch effect? Distension of the heart reduces the resting membrane potential of rat ventricular fibres (Lab 1969) while stretch of papillary muscles and atrial trabeculae has been shown to cause membrane depolarizations of sufficient magnitude to induce spontaneous contractions (Kaufman and Theophile 1967). In the current study, an increase in passive load occasionally led to a single muscle twitch. These observations suggest that stretch may increase the transsarcolemmal sodium flux and hence increase the resting heat rate by stimulating the sodium pump. But, as recently discussed (Gibbs and Chapman 1979), the resting sodium flux can, at most, account for 7–20% of the resting metabolism. This calculation is supported by experimental evidence; administration of cardiac glycosides, which inhibit the Na^+ pump, has no effect on the resting metabolism. Thus Gibbs and Gibson (1969) showed that ouabain (10^{-6} mol·l^{-1}) did not alter the rate of resting heat production of rabbit papillary muscles while Coleman (1967) showed that acetylstrophanthidin (in a dose sufficient to augment the contractile state) did not alter the resting oxygen consumption of cat papillary muscles. It thus appears unlikely that the stretch-induced increment in metabolic rate can be accounted for by an increased rate of sodium pumping.

It is possible that passive stretch increases the flux of calcium ions into the cytoplasm (from either sarcolemmal or sarcoreticular sites) in a mechanism analogous to that suggested to explain length-dependent activation (Noble 1979). An increased cytoplasmic calcium concentration would stimulate the calcium pump of the sarcoplasmic reticulum and thus increase ATP consumption. Again, experimental evidence fails to support this contention. Thus, the combination of caffeine and high Ca^{2+} concentrations, which maximally stimulates the resting activity of the sarcoreticular Ca^{2+} pump (Endo 1975), causes only a slight increase in the rate of resting heat production of cat, guinea-pig or rat papillary muscles (Gibbs and Loiselle 1978; Loiselle et al. 1982) or in the rate of resting oxygen consumption of cat papillary muscles (Cooper 1976). Similarly catecholamines, which are known to augment sarcoreticular Ca^{2+} pumping (Mayer 1974) fail to alter the resting oxygen consumption of cat papillary muscles (Coleman et al. 1971) or the resting heat rate of rabbit papillary muscles even in the presence of caffeine (Chapman et al. 1977).

A third possible explanation for the stretch effect involves protein metabolism which (see below) is known to be high in cardiac muscle. Passive stretch enhances the uptake of amino acids into cardiac tissue (Lesch et al. 1970). As one of us (Gibbs 1978) has suggested, this may reflect a hypertrophic response to an increased load and may be the correlate of passive stretch-induced hypertrophy of skeletal muscle (Holly et al. 1980).

FO_2 Effect

The non-significant effect of roughly halving the FO_2 (from 95 to 45%) is somewhat surprising although it may reflect the fact that the experimental design optimized statistical power for the effects of temperature, load and sequence at the expense of oxygen fraction (see Methods and Results). As it stands this result suggests that isolated papillary muscles of the diameter used in this study are not diffusion limited when aerated with 95% O_2. The lack of significance of the temperature X FO_2 interaction (Table 3) lends further credence to this suggestion since any limitation of diffusion would be exacerbated in low O_2 fraction at higher temperatures where the resting heat rate is elevated. In fact, the Hill model of diffusion, above, would permit a muscle of diameter 0.87 mm (Table 1) a resting metabolic equivalent of 4.45 mW $\cdot g^{-1}$ in 45% O_2 without developing an anoxic core. This value is well in excess of the mean observed in 45% O_2 (2.6 mW $\cdot g^{-1}$) and even comfortably exceeds the mean value at 30° C in 95% O_2 (3.75 mW $\cdot g^{-1}$).

Temperature Effect

The effect of temperature upon the resting heat rate is the most baffling result of this study. The Q_{10} ranges from 1.28 (between 15 and 25° C) to 1.42 (between 25 and 30° C). These values are little more than one-half the commonly accepted value of the Q_{10} for most metabolic processes. In a study of the effect of temperature upon the rate of oxygen utilization by rat ventricular slices (Fuhrman et al. 1950), Q_{10} values ranged from 1.32 to 4.55 between 10 and 37.7° C. Although mention is made of a progressive decline in oxygen consumption after about 1 h, all measurements were made during an initial equilibration period of 30–60 min when rates were reported as steady. Only 3 slices were studied at each of 10, 15, 20 and 25° C. However, between 30° ($n = 7$) and 38° ($n = 27$) the calculated Q_{10} (see their Table 1) is 1.5 and between 30° ($n = 7$) and 35° ($n = 5$) the Q_{10} is 1.3. In a study of the rate of oxygen uptake by quiescent canine Purkinje fibres, Greenspan and Cranefield (1963) reported (see their Table 1) a Q_{10} of 2.3 between 25 and 35° C (with the fibres bathed in 60% O_2) but noted that above 30° C the uptake tended to increase steadily with time whereas below this temperature it remained constant for as long as 12 h. Neither of these effects occurred in the present study.

In an extensive investigation of arrested, perfused, whole rat and guinea-pig hearts, Lochner et al. (1968) measured the resting oxygen consumption over a wide range of temperatures. They report a mean Q_{10} (for 9 hearts) of 1.44 between 24 and 34° C which is in remarkable agreement with the value of 1.42 found in this study (see Fig. 2) between 25 and 30° C. Their mean Q_{10} value between 14 and 34° C can be calculated to be 1.46 (from mean values of oxygen consumption reported as 1.6 and 3.4 ml O_2/100 g/min respectively) whereas the mean Q_{10} value between 15 and 30° C in this study was 1.33. A recent report (Loiselle 1981) confirms these low Q_{10} values in arrested Ringer-perfused whole rabbit hearts in the range 17–37° C.

A Q_{10} of around 1.5 for resting cardiac heat production might be explained by a combination of metabolic and

physical (diffusion) processes underlying basal metabolism. Diffusion processes have a very low Q_{10} – only about 1.2 at body temperature. So if diffusion processes (for example, ion fluxes) accounted for a sizeable fraction of the resting heat, then the problem of a low Q_{10} could be resolved. The difficulty with this argument, as detailed above, is the lack of evidence implicating the ion pumps (the sarcolemmal Na^+-K^+-ATPase and the sarcoplasmic reticular Ca^{2+}-ATPase) in contributing significantly to the resting metabolism. Indeed, aside from the influence of stretch (i.e. either muscle length or resting load) as shown in this study, the manipulation of metabolic substrate remains the only agent known to markedly affect resting heat rate. Thus Chapman and Gibbs (1974) showed that, with respect to glucose, pyruvate roughly doubles the rate of resting heat production of rabbit papillary muscle with lactate and acetate causing intermediate increases.

As suggested by one of us (Gibbs 1978) and mentioned above, protein metabolism may be responsible for a sizeable fraction of the resting heat. Amino acids are known to increase both the oxygen consumption and glucose oxidation of isolated cardiac myocytes (Burns and Reddy 1978). The rate of protein synthesis is high in small animal species (Earl et al. 1978) and is not diminished by prolonged cardiac arrest provided oxygenation is adequate (Schreiber et al. 1977). The aforementioned stretch-induced increase in the rate of uptake of amino acids into cardiac muscle tissue (Lesch et al. 1970) could then be viewed as merely another facet of this process.

Further studies are required to determine whether the Q_{10} and stretch effects might be enhanced by the provision of essential amino acids in the bathing medium. Rat papillary muscle might be a more appropriate model to investigate because of its high rate of protein turnover (Earl et al. 1978) and its high rate of resting metabolism (Loiselle and Gibbs 1979).

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