

THE RESPIRATION OF
THE ANTERIOR BYSSUS RETRACTOR MUSCLE OF *MYTILUS*
EDULIS (ABRM) AFTER A PHASIC CONTRACTION

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SUMMARY

1. The oxygen consumption of isolated anterior byssus retractor muscle of *Mytilus edulis* (ABRM) has been measured at rest and after phasic contractions induced by a.c. stimulation.

2. The respiration was measured with a Clark oxygen electrode in successive periods of 5 or 15 min, at 20° C.

3. The resting respiration is 71.8 ± 2.4 n-moles O_2/g wet weight.min (mean \pm s.e., $n = 70$). It is increased by a release and decreased by a passive stretch.

4. After phasic stimulation of up to 30 sec the respiration is increased and returns to a slightly higher level than the resting level in an exponential fashion with a time constant of about 10 min.

5. The duration of stimulation does not change the time course of the excess respiration but it affects its magnitude. The amount of extra oxygen consumed, in n-moles O_2/g , is made up of a constant amount, 449 ± 102 , and an amount that depends on the duration of stimulation (t , sec), which is given by $t \times 13.2 \pm 4.3$. When due account is taken for the tension developed, these parameters become 83.1 ± 20.7 and $t \times 1.24 \pm 0.66$ n-moles O_2/g muscle and kg/cm^2 of tension. This regression analysis is based on forty-eight data, with a residual error based on 5 degrees of freedom.

6. Release of the tension after the last stimulus of a 30 sec tetanus reduces by half the extra oxygen consumed during the recovery whereas the same release applied 5 min later has a much smaller effect. This suggests that relaxation is an active process.

7. From these measurements of the recovery metabolism the energy cost of the contraction was estimated and compared with this cost in vertebrate striated muscle. The constant item has about the same magnitude, but the item related to the duration of stimulation is about 250 times smaller.

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INTRODUCTION

Depending on the type of stimulation, the contraction of the ABRM of the common mussel *Mytilus* can have a phasic or a tonic character (Winton, 1937). Our understanding of this duality of response has been increased by studies of the mechanical properties during phasic and tonic contractions, by characterization of the contractile proteins and by investigations of the ultrastructure of the muscle; but very little is known of the metabolic cost of the two types of contraction. The purpose of this paper is to provide some information on the energy cost of the phasic contraction, and another paper will be devoted to the question of energy expenditure during the tonic contraction.

The early measurements of Abbott & Lowy (1955) and the recent detailed study of Baguet & Aubert (1966) have shown that the heat production during the phasic contraction is fairly low, much lower than that of fast striated muscle. However, owing to technical limitations it has not been possible to give a complete picture of the energy cost of the contraction.

In the experiments reported here, the oxygen consumption has been measured before and after phasic contractions of varied duration. The analysis of the data led us to divide the energy used during the contraction into two fractions: one related to the development of the tension and its disappearance; the other related to the duration of stimulation. As the same kind of division has been described in the case of the frog sartorius, a comparison of the energy output of these two muscles is possible in quantitative terms.

METHODS

Material

The mussels (*Mytilus edulis*) were kept in a large tank of circulated sea water in which they were allowed to recover for at least 5 days. Crabs were kept in the tank, as they eat mussels in poor conditions but leave the healthy animals untouched. The scatter of the data was thus greatly reduced by the natural elimination of ill or dying animals. The anterior byssus retractor muscle was dissected according to the procedure described by Jewell (1959), with all the ganglia carefully removed. In all experiments natural sea water was used; the pH was 7.3 and the temperature was 20° C.

Mounting of muscle

The muscle holder (Fig. 1*a*), made of stainless steel, was a vertical L-shaped rod (2) attached at its top to a disk (4) held on a rigid stand (7). The shell fragment (1) of the preparation was tied to the lower part of the rod, and the byssal extremity of the muscle was connected to the tension recorder by means of a stainless steel wire (8) passing through a tiny hole drilled in the top disk. The muscle was then stretched to the reference length, i.e. the length *in situ* when the valves were half open.

The muscle was surrounded by a glass tube (3, internal diameter 0.8 cm; length 5.3 cm), filled with sea water and tightly adjusted at its upper extremity on the central part of the

top disk acting as a stopper. At its lower end, the glass tube was supported by a propylene screw (5) in the central hole of another disk. Two arms (6) linked together the top and the lower disks. The whole assembly was plunged into a thermostatic bath (20° C), the top edge of the tube remaining 5 mm above the level of the bath.

Care was taken to avoid trapping air bubbles in the sea water. The volume enclosed in the muscle chamber was about 1.9 ml., depending on the size of the muscle.

Stimulation and tension recording

The muscle assembly was lifted out from the bath, and the tube withdrawn. The exposed muscle was then stimulated tetanically with alternating current from the mains (50 c/s) through an isolating transformer and two platinum wires held on its surface. The strength of the stimulus for maximal response was between 10 and 15 V. Immediately after the end of the tetanus, the tube filled with sea water was replaced around the muscle, and the whole assembly was plunged back into the bath.

The isometric tension was measured by four strain gauges glued on both sides of a steel beam and connected in a bridge circuit. The output was fed into a multiple reflexions galvanometer and recorded with a spot-follower (Nachlaufschreiber of Lange, Berlin).

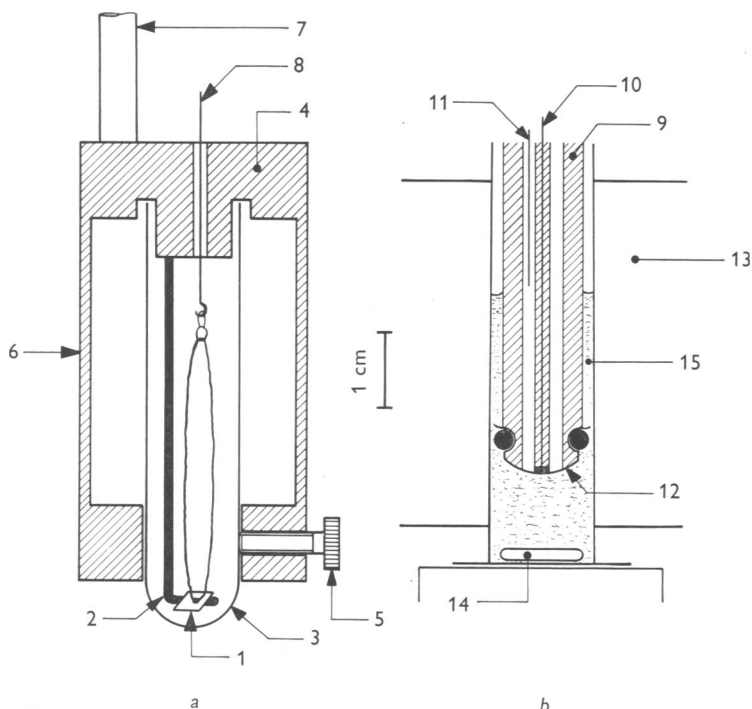


Fig. 1. (a) Muscle holder assembly: 1, muscle (ABRM) and the shell fragment; 2, L-shaped stainless steel rod; 3, glass tube; 4, top disk acting as a stopper for the tube; 5, propylene screw supporting the glass tube; 6, arms linking top and bottom disk; 7, attachment to a rigid stand; 8, stainless steel wire connected to the tension recorder. (b) Apparatus for the measurement of dissolved oxygen: 9, Clark electrode; 10, platinum electrode (negative); 11, Ag-AgCl reference electrode (positive); 12, polythene membrane held by an 'O' ring; 13, glass cylinder and water jacket; 14, protected iron rod and magnetic stirrer; 15, sea water sample to be analysed.

Measurement of the oxygen consumption

(a) *The Clark electrode.* The respiration of the muscle was estimated from the oxygen content of the surrounding sea water during a given period of time. The concentration of dissolved oxygen was determined by means of a Clark oxygen electrode (Yellow Spring Instrument Co., Ohio, U.S.A.), which is illustrated by Fig. 1*b*. The electrode was separated from the solution to be analysed by a thin ($50\ \mu$) polythene membrane.

In order to get high precision with this electrode, the following precautions were found to be necessary: 1, The platinum end must be kept perfectly polished; we used polishing cloth coated with diamond dust (particle size: $1\ \mu$) as used in preparing metal surface for microscopic observation. 2, The solution surrounding the electrode should be gently and regularly stirred. Too violent stirring creates pressure variations on the membrane with unreliable results. 3, All measurements of known and unknown concentrations of oxygen were made at the same temperature, and the calibration measurements repeated several times during the course of an experiment. Between the measurements, the electrode was kept at the same temperature. 4, There often occurred small tears in the membrane, which impaired the function of the electrode. The membrane was then replaced with a spare that had been kept in the same fluid as the internal solution of the electrode. It was a sterile and buffered saline mixture: 0.1 M-KCl; 0.25 M-KH₂PO₄; 0.25 M-Na₂HPO₄. With these precautions, repeated measurements of the same oxygen concentration agree to within 1%.

(b) *Calibration.* By equilibrating sea water with oxygen at different partial pressures, it was found that the current drawn by the Clark electrode increases linearly with the oxygen concentration up to the partial pressure of atmospheric air at least. The electrode could therefore be equilibrated by taking readings in (a) oxygen-free sea water (prepared by adding dithionite (Na₂S₂O₄) which reduces the dissolved oxygen) and (b) fully aerated sea water. Using the formula given by Sendroy, Dillon & Van Slijke (1934), which takes into account the temperature and salinity of the solution, it was calculated that fully aerated sea water at 20° C contains 5.45 ml. O₂/l. or 244 n-moles O₂/ml.

(c) *Experimental procedure.* The respiration of the muscle was measured over periods of either 5 or 15 min. At the beginning of each new period, the glass tube that serves as the muscle chamber (3, Fig. 1*a*) was filled with fully aerated sea water at 20° C. At the end of the period, the muscle chamber was removed and its contents poured into a narrow electrode chamber surrounded by a constant-temperature water jacket (Fig. 1*b*). Tests with samples of oxygen-free sea water showed that no contamination with oxygen from the atmosphere occurred during this transfer. The Clark electrode was then placed in the chamber and the solution was stirred by a small piece of protected iron, moved by a magnetic stirrer. After a reading had been obtained, the volume of sea water coming from the muscle chamber was determined by weighing it. Each determination was preceded and followed by a calibration reading on fully aerated sea water. The zero reading on oxygen-free sea water was only checked at the beginning and end of a series of determinations, as it showed very little variation.

(d) *Calculation of results.* From the calibration and the difference in the readings obtained with the Clark electrode in aerated sea water and in the water from the muscle chamber, the change in oxygen concentration could be determined. Knowing the volume of the sea water in the muscle chamber and the period of incubation, the respiration rate of the muscle could then be calculated in n-moles/min (n-mole O₂/min). For purposes of comparison, this value was transformed into one of the following:

(1) Respiration related to muscle weight, obtained by dividing the respiration rate, R , by the wet weight of the muscle in grams, M (units of R/M , n-mole O₂/g.min).

(2) Respiration related to muscle weight and to tension developed per unit cross-sectional area. If the maximum tension developed is P_0 (kg-force) and the muscle length l_0 (cm), the

tension developed per unit cross-sectional area can be obtained as $P_0 l_0 / M$ (kg/cm^2). The respiration rate per unit weight, R/M , divided by this gives the required measurement. In practice, this can be obtained as $R/P_0 l_0$, which is the respiration rate of 1 g of muscle when developing a tension of 1 kg/cm^2 (units of $R/P_0 l_0$, n-mole $\text{O}_2/\text{kg} \cdot \text{cm} \cdot \text{min}$).

RESULTS

The respiration of the resting muscle

After dissection, the muscle was left in aerated sea water for 2 hr. After that time, the respiration remained at a very stable level for many hours, provided the muscle was not stimulated. The mean value, based on seventy determinations running from November 1963 to March 1965, was 71.8 ± 2.4 (s.e. of mean) n-moles $\text{O}_2/\text{g} \cdot \text{min}$ at 20°C . No seasonal variation was observed.

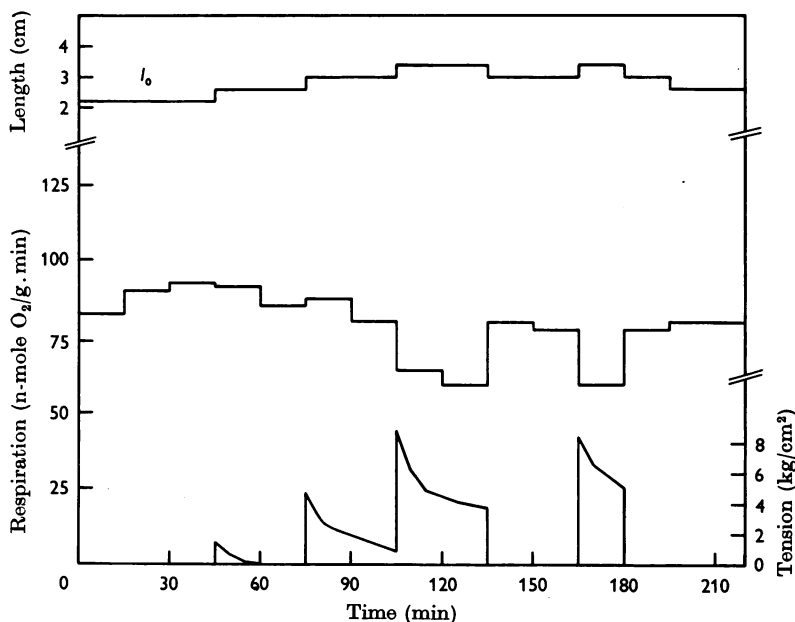


Fig. 2. Effect of altering the muscle length on the resting respiration of the ABRM. Upper trace, length; middle trace, respiration; lower trace, passive tension.

Influence of length. When the resting muscle was shortened below the reference length l_0 , the respiration immediately rose and remained high for at least 30 min. In six different muscles a decrease of 25% of l_0 raised the respiration by amounts ranging from 3% to 60% (mean 15%). The effect was reversible.

Figure 2 illustrates the results obtained in one of three similar experiments when the muscle was stretched in steps of 4 mm every 30 min up to well above its reference length. Though the largest stretch generated

a passive tension similar to that in an isometric contraction, the respiration was significantly depressed; and when the passive tension was released by a shortening of the muscle, the respiration returned in a reversible manner to its higher original level.

The respiration after a phasic contraction

After the resting respiration had been determined for two or three periods of 15 min, the muscle was stimulated tetanically. The respiration was then measured every 5 min during the first 20 min and every 15 min afterwards. The results presented here are based on several series of experiments: in some series every muscle was tetanized for either 10 sec or 30 sec. In another, each muscle was tetanized for both 10 and 30 sec in

TABLE 1. The time course of the supplementary respiration induced by phasic contraction of different durations

	Duration (sec)	Time after stimulation (min)						
		0	5	10	15	20	35	50
Excess respiration (n-moles O ₂ /g.min)	10	49.4	24.3	16.2	10.1	3.35	1.57	
	20	53.5	33.6	19.8	13.8	5.60	4.02	
	30	69.7	33.1	21.8	16.1	5.85	2.55	
Excess oxygen consumed (n-moles O ₂ /g)	10	0	247	368	449	499	549	573
	20	0	268	435	534	603	688	749
	30	0	348	513	622	702	790	829
% of the excess oxygen consumed	10	0	43	64	80	87	96	100
	20	0	36	58	71	81	92	100
	30	0	42	62	75	85	95	100

These mean values were obtained from six different muscles, each stimulated three times; during 10, 20 and 30 sec. These durations of stimulation were given to each muscle in an order imposed by a double latin-square design. The analysis could then remove the 'individual' effect as well as the 'position' effect. The standard error was calculated only for the first four periods of 5 min; in each case, it is of the order of 7% of the mean. Average size of muscles: $M = 40.8$ mg; $l_0 = 2.95$ cm; $P_0 = 7.0$ kg/cm² after 30 sec.

a cross-over design. Finally, in one series of six muscles, each one was stimulated for 10, 20 and 30 sec at 90 min intervals in a different order according to a double 'latin square' design (Cochran & Cox, 1950). This design offered a better way of assessing the effect of duration, but the results of the different series were consistent. They are presented in Table 1.

Figure 3 shows the time course of the respiration in a typical experiment. Tetanization induced a high rate of oxygen consumption, which then appeared to decrease more or less exponentially towards a new steady level reached after about 50 min.

This new level was always higher than the previous resting level, roughly 10% higher. The increase was not significantly related to the duration of the previous tetanus; it also appeared after stimulation with acetylcholine,

as will be shown in a later paper, and seemed to be more important in the spring. It was found that this new respiration rate remained constant for at least 2 or 3 hr. It is thus very improbable that it represented a delayed portion of the recovery metabolism; for the energy expenditure during the tetanus would then be enormous, many times larger than direct estimation of the initial heat would suggest (Baguet & Aubert, 1966). It is more likely to be the result of small alteration of the muscle fibres, or of their membranes, induced by the stimulation or by its mechanical effect.

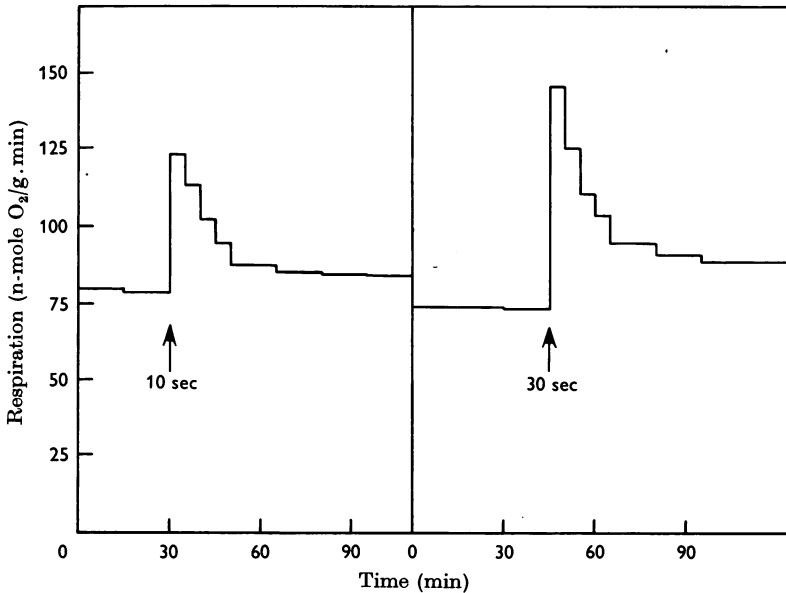


Fig. 3. Oxygen consumption of the ABRM after phasic stimulations (arrows) of 10 and 30 sec. The maximal isometric tensions reached were 6.4 and 7.1 kg/cm² respectively. On this figure the permanent shift of the base line is apparent.

To estimate the additional use of oxygen induced by the contraction, we have assumed that the change of the resting respiration occurred very soon after the stimulus, say within 5 min. We thus subtracted the final value of the resting respiration from all the measurements, except the first block of 5 min which was corrected for the initial value. This procedure did not affect the comparisons between factors like duration of stimulation or change of length of the muscle, especially if those 'treatments' were compared within the same group of muscles.

Influence of the duration of stimulation

The time course of the supplementary respiration induced by stimulation was not affected by the duration of the tetanus, at least up to 30 sec

stimulus. This is shown for instance by the comparison of the results in the latin-square series (Table 1). There, as in all the other series of experiments, 40 % of the extra oxygen was used up during the first 5 min, and about 70–80 % in the first 15 min. This is consistent with the assumption of an exponential time course, with a time constant of the order of 10 min.

Table 2 shows the total amount of oxygen consumed in excess of the resting level during 50 min after phasic contractions of different durations. The figures are the means of the different series of experiments. As Fig. 3 already shows, it is obvious that a longer tetanus is associated with a larger oxygen requirement, but no direct proportionality is apparent.

TABLE 2. The quantity of oxygen consumed above the resting level by the ABRM during 50 min after phasic contractions of different durations. The figures given are the mean values of each series of experiments

Duration of stimulation (sec)	Total O ₂ consumed (n-moles/g)	Max. isom. tension (P ₀) (kg/cm ²)	n	Date
10	580	7.1	6	16–17 Dec. 1964
	573	5.7	6	9–13 Feb. 1965*
	572	5.6	6	15–20 Mar. 1965
Mean	575	6.1	18	
20	749	6.6	6	9–13 Feb. 1965*
30	900	7.5	6	10–12 Nov. 1964
	985	6.8	6	18 Nov.–11 Dec. 1964
	829	7.0	6	9–13 Mar. 1965*
	645	6.8	6	15–20 Mar. 1965
Mean	840	7.0	24	

n, Number of measurements in each experimental series. * Experiments made according to a double latin-square design.

In view of the fact that data on the energetics of contraction in vertebrate striated muscle can be fitted by an equation of the type $C = A + bt$, where C is the energy cost of the contraction, t is the duration of stimulation, and A and b are constants at a given temperature (Hartree & Hill, 1921; Maréchal & Mommaerts, 1963), we have set out to test the hypothesis that our data might be similarly represented. The 'latin square' series (9–13 Feb. 1965 of Table 2) was specially designed to get quantitative information on these two parameters. It yielded the following regression equation: $C = 461(\pm 117) + 12.7(\pm 5.0)t$, where C is in n-moles O₂/g, t is in seconds of stimulation, and the numbers in parentheses are the s.e. of the mean, based on 8 degrees of freedom.

This analysis did not take into account the fact that tension still increased when stimulation was prolonged from 10 to 30 sec (Table 2). In order to compare oxygen consumption of muscles giving the same mechanical response, individual data were corrected for tension developed as

explained in the Methods section. The regression analysis performed on these corrected data gave the following: $C' = 96.3 (\pm 20.7) + 1.17 (\pm 0.88) t$, where C' is in n-moles $O_2/kg. cm.$

The other series gave quite similar results; from the eight groups collected in Table 2, we got the following equations: $C = 449 (\pm 102) + 13.2 (\pm 4.3) t$, where C is in n-moles $O_2/g.$; and $C' = 83.1 (\pm 20.7) + 1.24 (\pm 0.66) t$, where C' is in n-moles $O_2/kg. cm.$ The s.e. of the mean of these last equations were based on 5 degrees of freedom.

For the four equations given above, the variance analysis showed no significant deviation from linearity ($P > 0.2$).

In the limits of our experimental conditions and of precision of our results, the recovery metabolism of the ABRM, measured by the oxygen consumption, was related by a simple function to the duration of mechanical activity.

The effect of a release after stimulation

According to Hill (1964), the suppression of the tension by a quick release applied at the top of an isometric twitch reduces the heat production of a frog sartorius by about 15 %. In the case of the ABRM, a large amount of heat is liberated during the relaxation of an isometric tetanus and, at each moment, the rate of heat production is proportional to the remaining tension (Baguet & Aubert, 1966). For technical reasons, in those thermal experiments it was found impossible to test directly the effect of a release. But if some energy expenditure occurs effectively during relaxation, then a fraction of the oxygen consumed after a tetanus is probably related to this process, and it should be possible to get quantitative estimation of that fraction by comparing the respiration of muscles relaxing normally under tension and of similar muscles submitted to the release method of Hill (1964).

In our experiments the muscles were shortened by 20 % of their initial length (l_0) at a speed of 0.25 cm/sec, immediately after the last stimulus of a 30 sec tetanus. The tension dropped immediately to zero during the release and did not recover. As can be seen in Table 3A, the excess respiration decreases quite significantly after such a release, the average saving of oxygen being as high as 47 % of the total.

Two different factors were mixed up in the preceding experiment: the change of length and the early disappearance of the tension. Their effects can be dissociated by shortening the muscle later during the recovery, at a time when all active tension has spontaneously disappeared. Table 3B shows the results obtained in another set of experiments, made later during the year, when the same release was applied 5 min after the end of stimulation. Some members of the control series gave unusually large results, which made the mean and the standard error of the mean rather high.

Nevertheless, it can be seen that the reduction of respiration induced by the change of length was not significant, and in any case remained modest (21 % when extra oxygen consumed is related to the muscle weight and 12 % when it is related to both muscle weight and tension developed per unit cross-sectional area). Moreover, the averages of the 'released' series compare very well with the averages of the first control group of Table 3A, and indeed with those of the 30 sec group in Table 2.

Thus most of the effect seen in Table 3A must be related to the early disappearance of tension.

TABLE 3. Effect of a release of the ABRM (20 % l_0) on the amount of oxygen consumed above the resting level, after a 30 sec tetanus. The amount of oxygen is referred either to the weight of the muscle (unit, n-moles O_2/g) or to the weight and tension developed per unit cross-sectional area (unit, n-moles $O_2/kg.cm$), as described in the Methods section. (The figures are the mean values \pm the s.e. of the mean. n , Number of experiments in each experimental series.)

	O_2/M (n-moles O_2/g)	$O_2/P_0 l_0$ (n-moles/kg.cm)	n
A. Release at the end of stimulation			
Controls	829 \pm 61	129 \pm 17	6
Released	439 \pm 59	68 \pm 10	6
Difference	390 \pm 85	61 \pm 19	
P	0.001	0.01	
B. Release 5 min after stimulation			
Controls	1,095 \pm 131	149 \pm 13	6
Released	862 \pm 85	131 \pm 8	6
Difference	233 \pm 156	18 \pm 15	
P	0.15	0.25	

DISCUSSION

The resting respiration of the ABRM is higher than that of vertebrate striated muscles. For instance, with the same technique we found about 30 n-moles $O_2/g.min$ for the frog sartorius at 20° C. As reported by Brecht, Utz & Lutz (1955), this high level of resting metabolism is usually observed in smooth muscles of both vertebrates and invertebrates; it could be related to the high surface-to-volume ratio of these fibres with a small diameter.

The influence of a change of length on the resting respiration is very striking, for it is exactly the opposite of that observed by Feng (1932) on the sartorius of *Rana temporaria*. In the ABRM an increase of length causes a reduction of respiration. It is obvious that the effect cannot be explained by some stimulation of the muscle, even though it is accompanied by the appearance of tension. There is no obvious correlation between the rise of passive tension and the fall of respiration (see Fig. 2); and in most cases a shortening of the muscle from a length at which hardly

any tension is measurable will produce a significant increase of respiration. This effect is not understood, but it is important in the discussion of the release experiments after a phasic or a tonic contraction.

Stimulation entails an increased requirement of oxygen. The excess consumption above the resting level is spread over a period of 35–50 min with a maximum immediately after stimulation. The amount of this excess oxygen, but not the time course of its use, changes with the duration of the stimulus. Thus metabolites accumulate during phasic activity and are oxidized later according to a first-order reaction with a time constant of about 10 min. No saturation of the oxidative processes is apparent up to 30 sec stimulation, despite a contrary assertion (Baguet, 1965) put forward at a time when the permanent shift of the base line was misinterpreted.

It has been shown that the total oxygen consumed (C) above the resting level, after phasic contraction, can be fitted by an equation of the type $C = A + bt$, where A and b are constants and t is the duration of stimulation (the range of durations investigated was from 10 to 30 sec). This analysis of the recovery metabolism gives some information about the energy expenditure during the tetanus. The energy cost of the contraction can be divided into one part independent of the duration of stimulation, and into another part which increases linearly as stimulation is prolonged. The contractile metabolism of the frog sartorius was similarly divided by Hartree & Hill (1921) who measured the heat liberated and by Maréchal & Mommaerts (1963) who estimated the break-down of phosphocreatine.

The term bt is probably associated with a steady rate of energy expenditure occurring during the tetanus, once the muscle is fully activated. In the case of the ABRM, the complete activation may appear very delayed because the tension is still rising even after 20 sec of stimulation (Table 2). But, on the other hand, the heat production during the tetanus, which is a good criterion of the activation, reaches its maximal rate 3.6 sec after the onset of stimulation (Baguet, Maréchal & Aubert, 1962). The proposed interpretation of the term bt is probably correct, except for the first seconds of stimulation.

The term A of our equation indicates that, in addition to this stable metabolism described above, stimulation causes a fixed energy expenditure which is probably related to phenomena occurring once for every contraction during the development of tension and the relaxation. But, because the stable metabolism does not reach immediately its maximal level, our method of analysis introduces a deficit in the estimation of this fixed energy cost. Various items sum up in the constant A .

An estimation of one of them can be based on the data of Table 3A. The

release of tension at the beginning of relaxation reduces the oxygen requirements by about 390 ± 85 n-moles/g. This suggests that the largest fraction of term A (461 ± 117 n-moles/g; from experiments made at the same period of time) is associated with the spontaneous disappearance of tension, unless other factors can account for the reduction, such as an effect of stress on the resting metabolism or an effect of length on the recovery metabolism.

Now, the release of passive tension in a resting muscle increases the respiration and so does the mere shortening of the resting muscle (Fig. 2). This effect is thus in the opposite direction to the one we are discussing, and, moreover, most of it is taken into account by our procedure for the estimation of the excess respiration: except for the first period of 5 min, the final value of the resting respiration is taken as base line. On the other hand, Table 3B shows that a shortening of the muscle during the recovery period, but after complete relaxation of the active tension, has only a small and insignificant effect on the oxygen consumption. It can be concluded that the reduction of the oxygen consumption, when the physiological relaxation is cut short by the release method, is probably not an effect of length on the recovery metabolism, and cannot be explained by the removal of a stress applied passively on the muscle structures.

Though the existence of this 'energy of relaxation' seems demonstrated for the ABRM, its physiological meaning is not clear. One possible explanation is that the slow disappearance of the tension during the relaxation prolongs the active state by some positive feed-back mechanism of the kind already proposed by Pringle (1960) and by Hill (1964). Some unpublished results of F. Baguet on the heat production during phasic contractions in presence of 5-hydroxytryptamine (5-HT) suggest that another possible mechanism has to be considered: in presence of this drug, the tension of the ABRM drops rapidly after the tetanus, but, during this fast relaxation, the total heat liberated as well as its rate of liberation is greater than in absence of the drug. This last observation is difficult to explain in the present form of the feed-back hypothesis, and suggests that some energy is required to suppress the active tension.

Recent results of Minihan & Davies (1965) on energy requirement for the relaxation of ABRM in *tonic* contraction lend support to this hypothesis. They observed a break-down of $0.2 \mu\text{moles/g}$ of phosphoarginine during the relaxation induced by 5-HT. Such an energy expenditure should entail a recovery consumption of 33 n-moles O_2/g (assuming $\text{P/O} = 3$). This value is about 10 times lower than that found here in the case of the relaxation from phasic contraction (390 n-moles O_2/g), but the discrepancy is hardly surprising considering the differences in experimental conditions: mainly the tension sustained by the muscle and the

type of contraction. Nevertheless, a common conclusion arises: in the ABRM, relaxation is not only the disappearance of metabolic activity induced by contraction, but seems also an active process requiring energy.

In order to get a better appreciation of the cost of a contractile activity, it is interesting to compare different types of muscles in respect to their mechanical response and to the energy needed to produce it. Using the results presented here, together with some other published data, it is now possible to make this comparison in quantitative terms for a slow smooth muscle (ABRM) and a fast striated muscle (frog sartorius). Both muscles having the same geometry, the comparison of mechanical results is valid, if they are brought back to a common basis: we shall consider a muscle weighing 1 g and developing 1 kg/cm² of active tension at 20° C.

As previously mentioned, the total energy cost (C) of a phasic contraction of duration t can be summarized by the equation $C = A + bt$. This formula can be applied to measurements of heat liberated, phosphagen split, or oxygen consumed, but, in order to compare the values of the coefficients obtained from different types of study, all the measurements must be converted to a common unit. Heat has been chosen for the present purposes, and the figures given refer to millicalories per gram of muscle and per kg/cm² of tension developed which can be calculated as heat/P_0l_0 (unit, mcal/kg.cm), as explained in the Methods section. One problem is that most of the measurements on the frog sartorius have been made at low temperature, and these must be converted to their expected values at 20° C for comparison with the results obtained from the ABRM.

Heat measurements on the frog sartorius by Hill & Woledge (1962) at 0° C give 1.95 mcal/kg.cm.sec for b , and an extrapolation of their data (their Fig. 3) to zero duration of stimulation gives 4.73 mcal/kg.cm for A . Assuming a temperature coefficient of $Q_{10} = 3.1$ for the rate of maintenance metabolism (Aubert, 1956; Hill & Woledge, 1962), the value of b at 20° C can be estimated as 18.8. For reasons given by Maréchal (1964), it is unlikely that the value of A would be similarly increased: indeed, an extrapolation of the data of Hill & Woledge (1962) obtained at 17° C indicates a value of 3.05, which is actually lower than the value for A at 0° C. The equation for heat measurements at 20° C might therefore be tentatively estimated as $C = 3.05 + 18.8t$ mcal/kg.cm., t in sec.

Measurements of phosphocreatine splitting in the frog sartorius at 2° C by Maréchal (1964) have been converted to heat unit by assuming that for phosphocreatine $\Delta H = -9.65$ kcal/mole (Gellert & Sturtevant, 1960). At 2° C, b is 2.41 mcal/kg.cm.sec, and A is 3.76 mcal/kg.cm. Again, assuming that the value of b increases with a temperature coefficient of 3.1, and that the value of A is not changed by an increase in temperature, the equation for phosphocreatine splitting at 20° C can be estimated

as $C = 3.76 + 18.5t$ mcal/kg.cm. The above equations relate to heat liberated during the contraction period (initial heat). In the case of the ABRM, the measurements of oxygen consumption were made during the recovery period, and these must be used to estimate the equivalent initial heat. Now the caloric equivalent of the oxygen consumed during the recovery is comparable with the total heat liberated during both the contraction phase and the subsequent recovery (because the energy liberated during the contraction phase derives from anaerobic processes, i.e. hydrolysis of phosphate bonds). In the frog sartorius (Hill, 1939) and in the pharynx retractor of the snail (Bozler, 1930), the initial heat appears to be about half the total heat liberated. We have assumed that this is also the case in the ABRM. Our procedure has therefore been to convert the oxygen consumed (regression equation from data of Table 2, corrected for tension) during the recovery period into heat (assuming 5 kcal/l. O_2 or 0.112 mcal/n-mole O_2), and then to take half this figure as the estimate for the initial heat. This procedure yields the following equation for the initial processes of the ABRM at 20° C: $C = 4.65 + 0.07t$ mcal/kg.cm.

Comparing this equation with those obtained for the frog sartorius, it can be seen that the time-dependent component of the cost of the contraction (b) is about 250 times less in the ABRM, but the constant term is about the same. Thus the ABRM can sustain contraction very economically. This 'advantage' is nevertheless balanced by a 'disadvantage': a slow rate of the basic contractile processes which does not allow a rapid mechanical response. The tension raises to a plateau in about 50 msec in the frog sartorius and in 10 sec or more in the ABRM. Moreover, from the values quoted by Abbott & Lowy (1958) and assuming a Q_{10} of 2.05 (Hill, 1938), it is estimated that the velocity constant of the force-velocity relation of Hill (1938) is between 100 and 150 times smaller in the ABRM than in the frog sartorius. From the point of view of energetics, the main difference between these two muscles lies thus in the power output of their fundamental contractile processes.

A low stable metabolism may be an intrinsic property of the muscular proteins. In the case of rabbit muscle, Bárány, Bárány, Reckard & Volpe (1965) showed that rate of ATP splitting by myosin solutions from fast and slow muscles is directly related to the speed of contraction. Alternatively this low metabolic rate may be the result of the control imposed on the enzymic activity by some regulatory mechanism, the sarcoplasmic reticulum, for example, as suggested by the results of Sreter & Gergely (1964).

Morphological evidence suggests that the fundamental contractile process might be similar in various kinds of muscles. It was shown by Hanson & Lowy (1959, 1961) that the contractile apparatus of tonic

molluscan smooth muscles, including the ABRM, contains two kinds of filaments, one containing actin, the other containing myosin plus tropomyosin A. Linkages between these filaments were also observed. The similarity of this ultrastructural organization with that of the striated muscle led these authors to propose that in these smooth muscles contraction is performed by a sliding filament mechanism similar to that proposed for striated muscle (for review, see Lowy, Millman & Hanson, 1963). The comparison made above suggests that in steady-state conditions the interaction between the filaments is about 250 times slower in the ABRM than in the frog sartorius. The general similarity of the contractile mechanism might explain the rather surprising fact that the constant term of our equation, which is an integral value, not a rate, has about the same magnitude in the two types of muscle.

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REFERENCES

- ABBOTT, B. C. & LOWY, J. (1955). Heat production in a smooth muscle. *J. Physiol.* **130**, 25–26P.
- ABBOTT, B. C. & LOWY, J. (1958). Contraction in molluscan smooth muscle. *J. Physiol.* **141**, 385–397.
- AUBERT, X. (1956). *Le couplage énergétique de la contraction musculaire*. Brussels: Arscia.
- BAGUET, F. (1965). Consommation d'oxygène et contraction phasique chez un muscle de Lamellibranche. *Archs int. Physiol.* **73**, 389–391.
- BAGUET, F. & AUBERT, X. (1966). La thermogénèse d'un muscle lisse de Lamellibranche en contraction phasique. *Archs int. Physiol.* (In the Press.)
- BAGUET, F., MARÉCHAL, G. & AUBERT, X. (1962). La thermogénèse d'un muscle lisse de Lamellibranche en contraction phasique. *Archs int. Physiol.* **70**, 416–417.
- BÁRÁNY, M., BÁRÁNY, K., RECKARD, T. & VOLPE, A. (1965). Myosin of fast and slow muscles of the rabbit. *Archs Biochem. Biophys.* **109**, 185–191.
- BOZLER, E. (1930). The heat production of smooth muscle. *J. Physiol.* **69**, 442–462.
- BRECHT, K., UTZ, G. & LUTZ, E. (1955). Über die Atmung quergestreifter und glatter Muskeln von Kaltblütern in Ruhe, Dehnung, Kontraktion und Kontraktur. *Pflügers Arch. ges. Physiol.* **260**, 524–537.
- COCHRAN, W. G. & COX, G. M. (1950). *Experimental Designs*. New York: J. Wiley.
- FENG, T. P. (1932). The effect of length on the resting metabolism of muscle. *J. Physiol.* **74**, 441–454.
- GELLERT, M. & STURTEVANT, J. M. (1960). The enthalpy change in the hydrolysis of creatine phosphate. *J. Am. chem. Soc.* **82**, 1497–1499.
- HANSON, J. & LOWY, J. (1959). Structural features relating to the mechanism of tonic contraction in certain molluscan smooth muscles. *J. Physiol.* **149**, 31–32P.
- HANSON, J. & LOWY, J. (1961). The structure of the muscle fibres in the translucent part of the adductor of the oyster *Crassostrea angulata*. *Proc. R. Soc. B* **154**, 173–196.
- HARTREE, W. & HILL, A. V. (1921). The regulation of the supply of energy in muscular contraction. *J. Physiol.* **55**, 133–158.
- HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B* **126**, 136–195.
- HILL, A. V. (1939). The heat of recovery. *Proc. R. Soc. B* **127**, 297–350.
- HILL, A. V. (1964). The effect of tension in prolonging the active state in a twitch. *Proc. R. Soc. B* **159** 589–595.

- HILL, A. V. & WOLEDGE, R. C. (1962). An examination of absolute values in myothermic measurements. *J. Physiol.* **162**, 311-333.
- JEWELL, B. R. (1959). The nature of the phasic and the tonic responses of the anterior byssal retractor muscle of *Mytilus*. *J. Physiol.* **149**, 154-177.
- LOWY, J., MILLMAN, B. M. & HANSON, J. (1963). Structure and function in smooth tonic muscles of Lamellibranch molluscs. *Proc. R. Soc. B* **160**, 525-536.
- MARÉCHAL, G. (1964). *Le métabolisme de la phosphorylcréatine et de l'adénosine triphosphate durant la contraction musculaire*. Brussels: Arscia.
- MARÉCHAL, G. & MOMMAERTS, W. F. H. M. (1963). The metabolism of phosphocreatine during an isometric tetanus in the frog sartorius muscle. *Biochim. biophys. Acta* **70**, 53-67.
- MINIHAN, K. & DAVIES, R. E. (1965). Energy requirements for relaxation from tonic contractions ('catch') in an invertebrate muscle. *Nature, Lond.* **208**, 1327-1329.
- PRINGLE, J. W. S. (1960). Models of muscle. *Symp. Soc. exp. Biol.* **14**, 41-68.
- SENDROY, J., DILLON, R. T. & VAN SLIJKE, D. D. (1934). Studies of gas and electrolyte equilibrium in blood. XIX. The solubility and physical state of uncombined oxygen in blood. *J. biol. Chem.* **105**, 597-632.
- SRETER, F. A. & GERGELY, J. (1964). Comparative studies of the Mg activated ATPase activity and Ca uptake of fractions of white and red muscle homogenates. *Biochem. biophys. Res. Commun.* **16**, 438-443.
- WINTON, F. R. (1937). The changes in viscosity of an unstriated muscle (*Mytilus edulis*) during and after stimulation with alternating, interrupted and uninterrupted direct currents. *J. Physiol.* **88**, 492-511.