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ENERGY COST OF TONIC CONTRACTION IN A LAMELLIBRANCH CATCH MUSCLE

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SUMMARY

1. The oxygen consumption of isolated anterior byssus retractor muscle (ABRM) of *Mytilus edulis* was measured during tonic contraction induced by acetylcholine (ACh).

2. The respiration was measured with an oxygen electrode during 95 min, divided into one period of 5 min and six successive periods of 15 min.

3. Tonic contraction induced a prolonged increase of the basal respiration that slowly diminished with a time course roughly similar to that of the tonic tension.

4. For each period of measurement, the excess respiration over the resting level could be analysed into a constant amount and an amount that depended on the maintained tonic tension. The analysis was performed by fitting regression equations of the type $Y = Q + bP$, where Y is the excess respiration in n-moles O_2/g.min, and P, the isometric tension (kg/cm^2); term b of the equation expresses the amount of oxygen consumption directly proportional to the tonic tension.

5. During the first 20 min of contraction, terms b of the equations are not significant, and most of the excess respiration (terms Q) is independent of the tension. The oxygen consumed during this time is supposed to reflect the recovery metabolism for the energy cost of the development of the tension.

6. From the 20th to the 80th min of contraction, terms Q are reduced and terms b are significant and constant. The excess respiration during this period is equal to 16·9 ($\pm$ 0·5) n-moles O_2/g.min + $P \times$ 6·8 ($\pm$ 0·5) n-moles O_2/kg.cm.min ($\pm$ S.E. of the means, n = 24).

7. During a tonic contraction suppression of tension by a release reduced the oxygen consumption which increased again when tension was restored by stretching the muscle back to its original length. This con-

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firmed the role of tension in determining the intensity of respiration during the catch.

8. The oxygen consumption related to this tension restored by stretching the muscle, varied from 8·0 to 12·3 n-moles O_2/kg.cm.min. These figures are of the same order of magnitude as the coefficient b obtained in the case of tonic contraction without modification of tension by length changes.

9. These results are taken as a demonstration that the maintenance of tonic tension is an '*active*' phenomenon with a metabolic counterpart.

INTRODUCTION

Certain muscles of bivalve Molluscs possess tonic properties, such that they can sustain large tension over prolonged periods of time. This property is of use to the animal when the valves have to be kept closed in order to prevent dessication in low tides or to resist predators.

When these smooth muscles are isolated from the animal and detached from all nervous connexions, they still have tonic properties: such is the case of the anterior byssus retractor muscle (ABRM) of the mussel *Mytilus*. When stimulated by direct current (Winton, 1937) or by acetylcholine (Twarog, 1954) this muscle develops tension that disappears only hours after the initial stimulation. On the other hand, when the same muscle is stimulated by alternating current, it gives a *phasic* contraction, so called because relaxation is achieved in half a minute after the end of the stimulation. The duality of the contractile response of the ABRM has been investigated by Jewell (1959) and Lowy & Millmann (1963). These authors showed that *tonic* and *phasic* contractions are identical during the development of the tension. In both cases, the muscle is able to restore its tension after a quick release, but a few minutes afterwards the situation is quite different; after a.c. stimulation the muscle is completely relaxed, while after ACh, it still maintains high static tension and offers high resistance to stretch, the '*quick-release-recovery*' has disappeared, and the muscle exhibits the inert behaviour of a damped elastic body. This tonic response which takes hours to disappear has often been called '*catch*'.

This kind of behaviour raises a crucial problem: does maintenance of tension during the catch require a continuous supply of energy to the contractile machinery? At the beginning of this century Parnas (1910) claimed that the maintenance of catch did not require any expenditure of energy. However, this fundamental question needs to be re-examined by modern techniques.

We re-investigated this question with isolated ABRM by measuring the mechanical response together with the metabolism. The latter was estimated by the oxygen consumption. For measuring very small expendi-

ture of energy, as is likely to be the case here, this method is the most favourable approach because the reduction of molecular oxygen to water is the ultimate reaction yielding energy for the cellular processes (in aerobic conditions). On the contrary, the use of high energy phosphate compounds is likely to be undetectable by the available methods if breakdown is rapidly compensated by rephosphorylation; moreover, this method requires the destruction of the muscle.

To complete this investigation of the energetics of the tonic contraction, one of us (F.B.) has measured the heat production of the ABRM during the catch, by using a microcalorimetric technique. Some of his results are mentioned here, but will be published in detail later.

Preliminary reports of the results presented in this paper have already been communicated (Baguet & Gillis, 1964; Baguet, 1965).

METHODS

Material and methods have been described in detail in a previous paper devoted to the oxygen consumption following phasic contraction of the ABRM (Baguet & Gillis, 1967). Only the main features are reported here.

ABRMs were dissected out according to the procedure described by Jewell (1959), and all ganglia were removed. Histological examinations made for us by Dr M. A. Gerebtzoff of the University of Liège (Belgium) showed that no nervous cells remained within the muscle after dissection.

Isolated ABRMs were kept in a small chamber filled with sea water saturated with atmospheric air, at pH 7·3 and 20° C. Tonic contraction was obtained by adding acetylcholine to the sea water at a final concentration of 10^{-5} M. In different series of experiments, the duration of treatment with ACh was 0·5, 5 or 95 min. Tension was recorded isometrically with strain gauges.

The sea water in the chamber was withdrawn at given intervals (see below) and its oxygen content measured with an oxygen electrode. Results were calculated as follows: from the calibration and the difference in the readings obtained with the oxygen electrode in aerated sea water and in the water coming 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 average respiration rate of the muscle could then be calculated in n-moles O_2/min. For the purpose of comparison between muscles, this value was converted 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-moles O_2/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), and assuming that the density of the muscle is 1 g/cm^3 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 maintaining tension of 1 kg/cm^2 (units of $B/P_0 l_0$, n-moles O_2/kg.cm.min).

The resting respiration was routinely measured during three periods of 15 min before treating the muscle with ACh. On a few occasions, the muscles were not stimulated and the resting respiration was measured every 15 min during 12 hr. In these experiments, respiration remained stable with small variations not exceeding $\pm 5\%$.

During tonic contraction, the oxygen consumption was measured during 95 min, in the following way: first after one period of 5 min, and, afterwards, after each of six periods of 15 min. The measurements were then stopped and the muscle left in aerated sea water during 125 min. After that time, the muscle was completely relaxed and a new contraction could be produced by ACh.

RESULTS

Oxygen consumption during tonic contraction

Figure 1 gives the time course of the tonic tension and of the respiration in an experiment in which the ABRM remained in the presence of ACh during 95 min. Tonic contraction was associated with an increase of the oxygen consumption. This respiration in excess of the resting level was maximal during the first 5 min but was not maintained afterwards and slowly diminished during the rest of the experiment, but it was still present after 95 min. At the end of the experiment, the tonic tension was reduced to 10 % of its maximal value.

What are the energy consuming processes responsible for this increase of the oxygen consumption? As it is known that the development of the tension uses the same amount of phosphorylarginine in phasic and tonic contraction (Nauss & Davies, 1966), a part of this excess respiration was probably the recovery metabolism paying off an oxygen debt built up during the development of the tension at the start of the tonic contraction. Nevertheless, the excess respiration during tonic contraction lasted much longer than after a phasic contraction (compare Fig. 1 with Fig. 3 of Baguet & Gillis, 1967), so that it cannot be explained by the initial energy expenditure of the development of tension. We suggest that this prolonged increase of the respiration is due to the maintenance of the tonic tension. The experiments described in the next section were designed to check this hypothesis.

The oxygen consumption related to the maintenance of the tonic tension. In order to study how the excess respiration was related to the level of the maintained tension in the course of a tonic contraction, the experimental conditions were modified; the duration of exposure to ACh was reduced from 95 to 0·5 min or 5 min, so that the '*phasic-like*' part of the contraction was unchanged but the tonic part was different as far as the magnitude of the tension and its time course were concerned. The same effect was obtained in another series of experiments in which the muscles, while exposed to ACh during 95 min, were released by 20 % l_0, 0·5 min after the onset of stimulation, so that tension dropped and recovered to a lower level.

For all the experiments, the seven measures of respiration (see Methods) and the average tonic tension maintained during each period of measurement are given in Table 1. Maximal isometric tensions and resting respira-

tions are also included in Table 1. The results can be summarized as follows. (1) Whatever the duration of exposure to ACh, tonic contraction induced an increase of the respiration above the resting level. This increase slowly diminished but was still present after 95 min. (2) Tonic tension remained at a high level for longer times when the duration of exposure to ACh increased, and so did the respiration. (3) The time courses of tension and excess respiration were roughly similar.

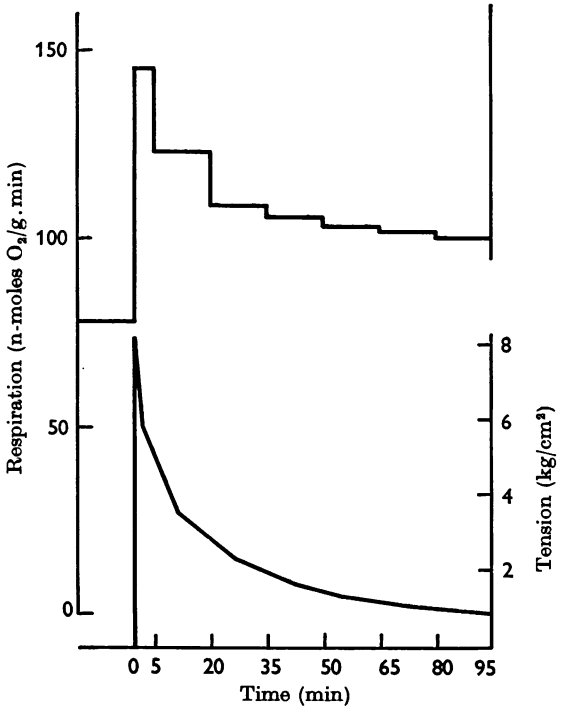

Fig. 1. Time course of the oxygen consumption (upper trace) and of the isometric tension (lower trace) during a tonic contraction of the ABRM in the presence of ACh (95 min).

This last observation did not prove that tonic tension and excess respiration were directly related, but simply that they had the same time dependence. Nevertheless, it was possible to test the existence of a relation by analysing the results in the following way. Each column of Table 1 which corresponds to one period of measurements has a set of six paired data of tension and respiration. It follows that, for a given set of data, the relation between tension and excess respiration can be tested by a regression analysis. It was assumed that the oxygen consumption above the resting level could be divided into two parts, one independent of the maintained tension, another directly related to it. This model was de-

scribed by a linear equation of the type $Y = Q + bP$, where Y is the excess respiration (calculated from data of Table 1), and P, the isometric tension given in Table 1. The analysis tested whether parameters Q and b of the equation were significantly different from zero. By applying the same

TABLE 1. *A*: The time course of the tension (kg/cm²) during tonic contraction of ABRM obtained by stimulation with acetylcholine (10^{-5} M) during various durations (0·5, 5 and 95 min).

B. The time course of the oxygen consumption (n-mole O_2/g.min) of ABRM during these tonic contractions. The results are the means of n experiments

Duration of contact with ACh (min)	Max. isometric tension (kg/cm²)	Time since contact with ACh (min) 0	5	20	35	50	65	80	95	S.E.*	n.	Date
		A. Mean isometric tension during tonic contraction (kg/cm²)										
0·5	9·5	6·0	2·0	0·9	0·4	0·2	0·0	0·0		0·35	3	Nov. 1964
5·0	9·4	8·1	5·0	2·8	1·6	1·0	0·5	0·3		0·44	5	Nov. 1963
95·0	7·5	6·2	4·1	3·0	2·3	1·8	1·6	1·3		0·31	4	Mar. 1964
95·0	7·0	5·7	3·7	2·6	2·1	1·7	1·4	1·2		0·35	6	Sep. 1964
95·0	8·6	6·8	4·6	3·3	2·8	2·4	2·1	1·8		0·14	6	Feb. 1965
95·0	8·0	2·2	1·3	0·8	0·5	0·3	0·3	0·2		0·18	6	Feb. 1965†
	Resting respiration (n-moles O_2/g.min)	*B*. Mean respiration during tonic contraction (n-moles O_2/g.min)										
0·5	60	140	112	85	80	76	73	69		2·6	3	Nov. 1964
5·0	76	140	118	113	107	99	98	92		4·1	5	Nov. 1963
95·0	80	141	120	115	113	113	114	116		5·1	4	Mar. 1964
95·0	89	163	127	121	118	116	115	114		2·7	6	Sep. 1964
95·0	77	150	135	114	112	112	110	111		1·5	6	Feb. 1965
95·0	82	137	126	101	101	101	101	98		3·8	6	Feb. 1965†

* Standard error of the mean calculated by variance analysis.

† In this series of experiments, muscles were released by 20 % l_0 after 0·5 min of stimulation with ACh, tension dropped and recovered to a lower level.

TABLE 2. Evolution of the parameters of the regression of excess respiration on tension ($Y = Q + bP$, see text) during tonic contraction of ABRM. The figures are the means of six data, and are given with their standard error

	Time (min) 0	5	20	35	50	65	80	95
Q (n-moles O_2/g.min)	55·9 ±5·2	34·8 ±3·7	17·1 ±2·9	17·8 ±2·2	15·3 ±2·3	15·4 ±2·3	11·8 ±2·7	
b (n-moles O_2/ kg.cm.min)	2·0 ±2·1	3·8 ±2·0	6·1 ±0·9	6·2 ±0·9	8·2 ±1·0	9·1 ±1·6	13·5 ±2·3	

procedure to the results of each column of Table 1, seven values were obtained for parameters Q and b which are given in Table 2. The analysis of the results thus proceeded in two steps; first, by testing the tension-excess respiration relation for each period of measurement; secondly, by studying how the relation was modified by time, by comparing the results obtained in the first step with one another.

It is apparent from Table 2 that as far as the first 20 min of contraction

are concerned, most of the oxygen consumed in excess is independent of the tension because terms Q, though decreasing, are a very large fraction of this excess respiration, and coefficients b are small and not significant. On the other hand, during the following hour, the oxygen consumption related to the tension is no longer a negligible item: the coefficients b are much larger and significant ($P < 0{\cdot}001$), while terms Q are reduced to one third of the initial value.

As also shown in Table 2, the values of both parameters change with time, in opposite directions, and eventually reach a rather constant value. From the 20th to the 80th min of the contraction, the slight variations of the parameters are not significant when tested by variance analysis. A single regression equation was therefore calculated for this part of the tonic contraction: $Y = 16{\cdot}9\ (\pm 0{\cdot}5) + 6{\cdot}8\ (\pm 0{\cdot}5)P$; where Y is excess respiration in n-moles O_2/g. min, and P, the isometric tension in kg/cm^2 ($\pm$ S.E. of the means, $n = 24$). The data used to calculate this general equation are plotted in Fig. 2. The regression coefficient b thus gives the amount of oxygen consumed by 1 g of muscle for maintaining 1 kg/cm^2 of tension for 1 min; its units are n-moles O_2/kg.cm.min. During the last period of 15 min, when tension was very low, the regression of respiration on tension was still significant, but coefficient b was larger than previous ones, 13·5 ($\pm$ 2·3) n-moles O_2/kg.cm.min, though its standard error was much larger also. The results of this last period were not included in the general regression equation.

The physiological meaning of terms Q and b will be discussed in detail later. It will be shown that term Q includes, especially during the first 20 min, the recovery metabolism related to the energy cost of the development of the tension. The meaning of term b is more straightforward; it gives a quantitative measure of the relation between tonic tension and excess respiration and indicates that this kind of contractile response is not passive but involves some metabolic activity.

Oxygen consumption and modifications of tension by length changes

If maintenance of tonic tension induces an increase of respiration, it is expected that the suppression of this tonic tension would reduce or abolish this increase. In a preliminary series of experiments, we found that suppression of tension by a release reduced by half the excess respiration produced by tonic contraction (Baguet & Gillis, 1964). A new series of experiments was designed to study, on the same muscle, the effect on the respiration of the modification of the tension by length changes.

In these experiments, the muscle remained 95 min in ACh-containing sea water, and, with the same time schedule as above, oxygen consumption was measured under three conditions: (A) contraction with continuous

tonic tension without length changes, as control experiment; (B) after 5 min of contraction, suppression of tension by a sudden release of about 20 % l_0; (C) same as in (*B*), but restoration of tension at regular time intervals by bringing the muscle back to its original length; the muscle was left at l_0 for 2 min only and then was released again. The last 90 min of the experiments were divided as usual into six periods of 15 min for

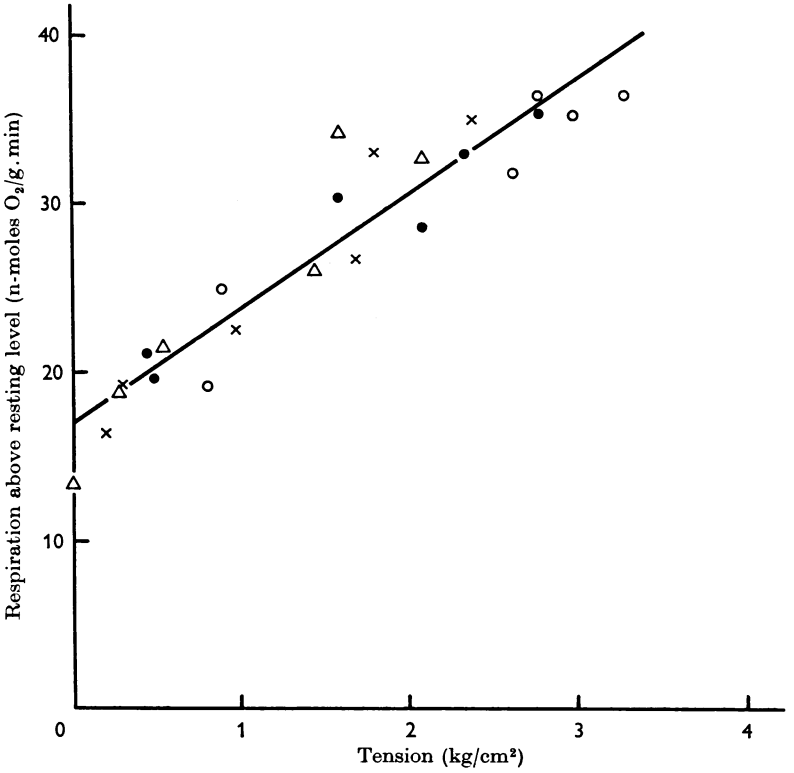

Fig. 2. Relation between excess respiration and the average isometric tension at different periods during tonic contractions of ABRM. ○: from the 20th to the 35th min of contraction; ●: from the 35th to the 50th min; × : from the 50th to the 65th min; △: from the 65th to the 80th min. All those data were used in calculating the regression of excess respiration on tension, represented by the straight line.

oxygen consumption measurements; tension was restored 3 times, at the beginning of each alternate period (Fig. 3). The speed of stretching was 0·25 cm/sec.

A complete experimental series included three muscles, each muscle receiving the three treatments at intervals of 125 min, according to latin square design. The results presented here came from two such complete series.

The average tensions recorded in each experimental condition are given in Fig. 3. As might be expected the development of the tonic tension during the first 5 min was very similar in each case. The tension restored by the stretch in condition (C) reached a peak which usually exceeded the maximal isometric tension for the first and the second stretch. This high tension was nevertheless not sustained and decreased rapidly to about

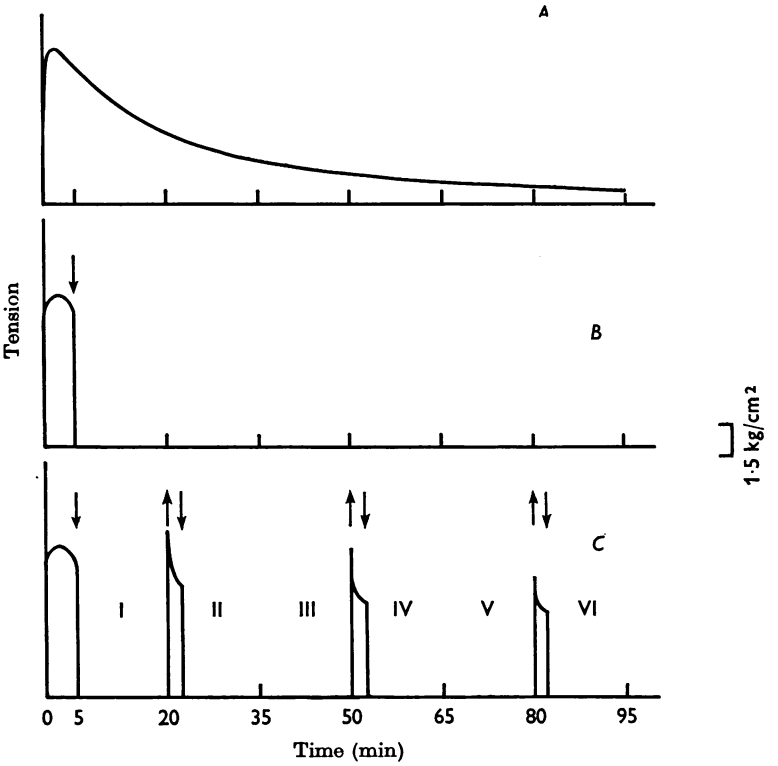

Fig. 3. Time course of the isometric tension, in kg/cm², during tonic contractions of ABRM, in the presence of ACh. (A), Normal response, without modification of tension by length changes. (B), After 5 min of contraction, the muscles were released (↓) by 20 % l_0, and left at that short length throughout the course of the experiment. (C), The muscles were released as in (B), but at the beginning of period II, IV and VI, they were stretched (↑) to their original length, and released (↓) again 2 min later. Traces are drawn according to the mean values of mechanical responses of six muscles. Average size of the muscles: M = 41 mg; l_0 = 2·7 cm.

5 kg/cm² at the end of the second minute when the muscle was released again. Table 3 gives the oxygen consumption above the resting level for the three types of experiments.

The suppression of tension in (B) caused a significant reduction (about 50 %) of the excess respiration, but did not abolish it. The remaining excess

was nearly stable throughout the experiment. From the 20th to the 80th min of contraction, it was, on the average, 14 n-moles O_2/g.min. This value is close to the term Q in the regression equations of the last section, for the same period of contraction.

When the tension was restored as in (C), at the beginning of periods II, IV and VI the excess respiration nearly doubled when compared with periods I, III and IV. This difference between alternate periods was tested by variance analysis and was significant ($P < 0{\cdot}01$). When no tension was produced, as in periods I, III and V, the excess respiration was about the same as in (B).

TABLE 3. Respiration above the resting level of the ABRM during ACh stimulation in three different conditions of tonic tension. Mean of six experiments made according to a latin square design

	Time (min)								
	0	5	20	35	50	65	80	95	
Period			I	II	III	IV	V	VI	
'Maintained tension' (A)	74		38	32	28	26	26	25	n-moles O_2/g.min
'Abolished tension' (B)	75		18	15	15	13	13	11	n-moles O_2/g.min
'Tension restored by stretch' (C)	100		22	24+	15	27+	16	23+	n-moles O_2/g.min

+ Underlined figures: respiration during the periods when tension was restored by stretch.

The results of these series of experiments demonstrated again the link of the tonic tension and the oxygen consumption. It was interesting to check whether the relationship between excess respiration and tension observed for continuous tonic tension (coefficient b, first part of the results) also held for this interrupted tension. The excess respiration during experiment (B) was taken as a *'base line'* for zero tension respiration. The quantity of oxygen consumed above this *'base line'* during the last 90 min of experiments (A) or (C) was divided by the integral tension × time recorded in each experimental condition. This ratio has, of course, the same units as the regression coefficient b calculated in the first part of the results: n-moles O_2/kg.cm.min. This procedure gave the genuine effect of tension on respiration: it eliminated the recovery metabolism for the development of tension which was equally present in either term of the difference (A) − (B) or (C) − (B), in as much as it was not affected by the release (Baguet, Gillis & Dainoff, 1967). Side effects due to the difference between muscles were also discarded because each muscle contributed equally to the results of the three experiments.

Table 4 gives the figures needed to calculate, by the procedure just described, the relation between tension and oxygen consumption. Quantities of oxygen were calculated from the data of Table 3; the values of the

integrals tension × time were measured directly on the original records (not given here).

In the case of experiment (A) in which the tension course was not modified by length changes, this method of calculation yielded a value of 7·2 n-moles O_2/kg.cm.min, which falls within the limits of error of coefficient *b* obtained by the regression method. Both estimates of the relation between tension and oxygen consumption thus agree quite well.

In the case of tension restored by stretch (experiment C), the same procedure gave a value of 12·2 n-moles O_2/kg.cm.min, i.e. about twice as large as that obtained from tonic contractions without length changes.

TABLE 4. Estimation of the relation between oxygen consumption above the resting level, and tonic tension of the ABRM, produced by different experimental procedures. Values in column 2 and 3 were calculated from data of Table 2, taking only the last 90 min of the experiments. Values of tension × time for the last 90 min were obtained directly from the original records of tension (not given here)

	1 Total O_2 consumed (90 min) (n-moles/g)	2 Total O_2 consumed above zero tension level (n-moles/g)	3 Tension × time (90 min) (kg.min/cm²)	4 Ratio column 2 / column 3 (n-moles/ kg.cm.min)
'Maintained tension' (A)	2625	1350	187	7·2
		(A)–(B)		
'Abolished tension' (B)	1275	—	—	—
'Tension restored by stretch' (C)	1905	630	51·0	12·3
		(C)–(B)		
		412+	51·0	8·0+

+ Estimation of the effect of treatment (C) by the difference in oxygen consumption between the periods with and without tension in experiment (C); see explanation in the text.

Another method for estimating the relation between tension and respiration was obtained by the difference between the amounts of oxygen consumed during the even (with tension) and the odd (without tension) periods in experiments (C) alone. This procedure did not require another experimental series, such as (B), as a base line. On the other hand, the first odd period was not included in the calculations because it may have included a large part of the recovery metabolism for the development of tension; it therefore could not be taken as a base line, otherwise the difference between odd and even periods would have been underestimated. This method yielded a value of 8·0 n-moles O_2/kg.cm.min, in better agreement with the result of the regression method. As we have no objective reasons to prefer one or the other method of calculating the tension–respiration relation in the '*stretch*' experiments, the results of these experiments must be taken only as a semi-quantitative confirmation of the metabolic cost of the tonic tension measured during unmodified tonic contractions.

DISCUSSION

There are few published data concerning the energy cost of the tonic contraction. In vertebrate smooth muscles, tonus is definitely associated with an increase of oxygen consumption as was shown by Bülbring (1953). In invertebrates, the tonic ability of Molluscan muscle is particularly remarkable. The energy expenditure of this contraction was first investigated by Parnas (1910) who found no significant increase of the oxygen consumption, and concluded that tonic contraction was a '*passive*' phenomenon. Unfortunately in the experimental conditions of Parnas it is very likely that the animals were anaerobic during the largest part of the experiment.

Brecht, Utz & Lutz (1955) also measured the oxygen consumption of the adductor muscle of *Anodonta*, stimulated electrically. No significant increase of the respiration was observed but tension was very weak—1, 4 g. Unfortunately, these authors did not mention either the length or the weight of the muscle so that a comparison with our results is not possible.

The use of phosphorylarginine during the maintenance of tonic tension of ABRM was investigated by Nauss & Davies (1966) who found no significant net break-down over a period of 15 min. According to the results presented in this paper, the expected break-down of phosphorylarginine is too small to be detected by the methods used by these authors.

The energy cost of the tonic contraction

Tonic contraction of the ABRM obtained by ACh stimulation induced a prolonged increase of the basal respiration. The results suggest that, at any time during the contraction, the excess oxygen consumption may be divided into two fractions, one independent of the remaining tonic tension, and another directly related to it. According to this model, for each period of measurement of the respiration, regression equations of the type $Y = Q + bt$ were calculated, where Y is the oxygen consumption above the resting level and P, the isometric tension during that period. Evolution of parameters Q and b during the course of tonic contraction were reported in Table 2. The physiological meaning of these parameters will be discussed now.

Term Q. During the first 5 min of contraction, the term Q included most of the excess respiration (from 70 to 100%, as can be calculated from Tables 1 and 2). During the following 15 min, the contribution of this term decreased, and thereafter remained rather stable at one third of its initial value during the rest of the experiment. One can thus divide term Q into a decreasing part and into a constant one, and the discussion below shows that the decreasing part of term Q can be taken as a measure of the

recovery metabolism for the energy spent during development of the tonic tension.

Some evidences suggest that phasic and tonic contraction are not different during the development of the tension: the mechanical properties are very similar (Jewell, 1959; Lowy & Millman, 1963) and the amounts of phosphorylarginine (PA) broken down are identical (provided that the work performed is the same; Nauss & Davies, 1966). It can then be expected that this energy expenditure will induce a similar oxygen consumption for both types of contraction.

It is known that after phasic contractions of 10–30 sec duration, the recovery metabolism is 80 % complete in 20 min and 100 % complete in 50 min (Baguet & Gillis, 1967). The decrease of term Q during the first 50 min of the tonic contraction has roughly the same time course (Table 2), and may represent the evolution of the recovery metabolism for the '*phasic-like*' activity at the beginning of the tonic contraction. This hypothesis is supported by the fact that the amount of oxygen consumption corresponding to the decreasing part of term Q during the first 50 min has the right magnitude: when it is calculated from the figures of Table 2, after subtraction of the constant part of term Q (see reasons below), it amounts to 477 n-moles O_2/g. This figure should correspond to a break-down of 2·86 μmoles PA/g (assuming P/O = 3) which is in close agreement with the break-down of 2·60 μmoles PA/g directly measured during the development of the tension in ACh stimulation (the last figure was obtained from the results of Nauss & Davies (1966) without taking into account the extra break-down due to the work performed).

One could get an idea of the duration of this '*phasic like*' activity at the beginning of the tonic contraction by using the empirical equation of Baguet & Gillis (1967) which relates the magnitude of the recovery metabolism to the duration of the phasic stimulation. This equation expresses the data of oxygen consumption in relation to the weight of the muscle and to the tension developed per unit cross-sectional area (n-moles O_2/kg.cm; see Methods). In experiments reported in Table 1, the weighted mean of the maximal isometric tension was 8·2 kg/cm^2. The recovery metabolism for the development of the tonic tension then becomes 477/8·2 = 58·1 n-moles O_2/kg.cm. Since tonic contraction is not followed by a fast relaxation of the phasic type, we shall not take into account the amount of oxygen consumed in relation to the energy cost of the phasic relaxation (53·0 n-moles O_2/kg.cm; Baguet, Gillis & Dainoff, 1967). The duration of the '*phasic like*' activity at the beginning of the tonic contraction should be 22·6 sec, obtained from the following equation

$$58{\cdot}1 = (83{\cdot}1^{+} - 53) + 1{\cdot}24^{+} \times t$$

(+ figures coming from Baguet & Gillis, 1967; other figures are explained in the text). This estimate of the duration is quite reasonable, though it is perhaps too small since even after 1 min of treatment with ACh, tension still recovers to some extent after a quick-release (unpublished observation).

After the 20th min of contraction term Q remained more or less constant and, at the end of the experiments, it represented most of the increase of

the respiration still present. In some instances, the measurements of the respiration were continued in ACh free sea water for 2 hr after the 95th min. Though the muscles were by then completely relaxed and soft, this increase of respiration remained at a stable value of 10–15 n-moles O_2/g.min, which is similar to the remaining excess respiration in experiments where tension was abolished by a release (Table 3). It is thus very unlikely that this permanent increase represents a delayed portion of the recovery metabolism for the energy spent during the contraction. We were unable to found a significant influence either of the duration of exposure to ACh or of the presence of the tonic tension on this phenomenon. A similar, though sometimes smaller, permanent increase of the respiration was also observed after phasic contraction (Baguet & Gillis, 1967); there again no causal factor was discovered. For these reasons, it was justifiable to distinguish two parts in term Q: one related to the development of the tonic tension, another to this permanent increase the significance of which remains obscure.

Term b. As shown in Table 2, after the 20th min of contraction, the regression coefficient b became significant and during the following hour it remained practically constant. The relation between tonic tension and excess respiration was thus clearly demonstrated as soon as the recovery metabolism for the development of the tension was over and the contraction was definitively of the tonic type. The simplest interpretation of these findings is that tonic tension is an '*active*' phenomenon in the sense that its maintenance requires energy and therefore increases the metabolism in direct proportion. This increase is very small indeed: the maintenance of 1 kg/cm^2 of tension by a tonically contracted ABRM induces a consumption of 6·8 n-moles O_2/g.min, which is only a small fraction of the resting respiration. It is very likely that such a small increase of metabolism does not create an oxygen debt so that the hydrolysis of ATP and of phosphorylarginine linked to the maintenance of tonic tension is immediately compensated by oxidative phosphorylations.

Using a microcalorimetric technique, one of us measured the heat produced during tonic contraction (F. Baguet, unpublished experiments). It was calculated that the maintenance heat of the same unit of tonic tension corresponded to a consumption of 9·0 n-moles O_2/g.min. This figure, obtained with a quite different technique, agrees rather well with the estimate reported here.

It is striking that we did not find a significant relation between tension and excess respiration during the first 20 min, when tonic tension was very large. It is possible that during this period the energy cost for maintaining high tonic tension was too high to be payed off immediately by respiration so that the oxygen consumption linked to this tension would

then be delayed and, by our method of analysis, be included in term Q instead of term b. Unfortunately, the time resolution of the technique used here was not good enough to obtain detailed information about the kinetics of the metabolism during the first 20 min which include the development of the tension and the transformation of the contraction from a '*phasic like*' type to a tonic one.

The role of tension in determining the intensity of respiration during the catch is also emphasized by the results of the '*release*' and '*stretch*' experiments. Abolition of tension by a sudden release reduced the oxygen consumption, and this latter increased again when tension was restored by stretching the muscle to its original length. Nevertheless, in these experiments changes of length were concomitant with modifications of tension, so that the interpretation of the results requires control experiment to evaluate the role of length changes in the observed effects. On resting ABRM, we found that respiration is increased by a shortening and decreased by a stretch (Baguet & Gillis, 1967). These effects are thus in opposite direction to the one we are discussing here, and one can accept that the modifications of tonic tension are mainly responsible for the variations of respiration in these '*release*' and '*stretch*' experiments.

It could be objected that the stretch not only restored tonic tension but activated the muscle in the sense that it elicited a phasic-like activity. Such a stretch-activation is observed in vertebrate smooth muscles (Bozler, 1948), in insect fibrillar muscle (but with a quite different time scale; Pringle, 1965) and in the ABRM in the presence of 5-hydroxytryptamine (5-HT) (Rüegg, 1965). In our experiments, activation via the excitation of the nerve cells can be ruled out because ganglia were removed during dissection. Moreover, on the mechanograms, we never observed the spontaneous and delayed redevelopment of the tension which is typical of stretch-activation.

The nature of the tonic tension

To explain the tonic phenomenon, various hypotheses resting on mechanical, structural and biochemical works have been proposed. According to some authors (Ritchie, 1928; Bayliss, 1928; Hoyle & Lowy, 1956) the maintenance of tonic tension is just a prolonged phasic contraction, repetitively triggered by transient depolarization of the membrane. A quantitative comparison between the energy expenditures associated with the maintenance of tension during phasic and tonic contraction does not support this hypothesis. In the first case, once the ABRM is fully activated, the maintenance of tension induces a consumption of 1·24 n-moles O_2/kg.cm.sec (Baguet & Gillis, 1967). Expressed in the same units, the oxygen consumption caused by tonic tension is only 0·11 n-moles O_2/kg.cm.sec, i.e. 11 times less than for phasic contraction.

The '*catch*' hypothesis is based upon the properties of tropomyosin A (TMA), a protein abundant in tonic muscles of molluscs (Bailey, 1957), and upon experiments with living and glycerinated ABRM (Rüegg, 1958, 1961, 1963; Johnson, Kahn & Szent-Gyorgyi, 1959; Johnson, 1962). According to this hypothesis, tonic tension is developed by the activation of the actomyosin system of the muscle, while it is maintained passively by TMA which becomes rigid by a change of state during stimulation. This form of the '*catch*' hypothesis which does not imply any energy cost linked with the maintenance of the tonic tension is not supported by our results. Nevertheless, as has already been suggested by Jewell (1959), the maintenance of this peculiar state of rigidity may require only a small supply of energy.

The '*linkage*' hypothesis is based upon morphological (Hanson & Lowy, 1959; Lowy & Hanson, 1962) and mechanical studies (Lowy & Millman, 1963; Millman, 1964). According to this hypothesis, tonic tension is developed by interaction between actin- and myosin-containing filaments, and is maintained by these linkages formed during the initial phase of ACh stimulation; tension decay is supposed to be a single process of breaking of linkages. Use of ATP in this breaking process has been postulated by Nauss & Davies (1966) who observed that the fast relaxation of a tonically contracted ABRM produced by 5-HT involved a break-down of 0·2 μmoles ATP or PA/g that would induce a consumption of 33 n-moles O_2/g (assuming P/O = 3). In absence of 5-HT tension disappears slowly and during this time 1350 n-moles O_2/g are consumed (first row of Table 4). This result shows clearly that breaking of linkages is far from being an important factor in the energy expenditure during tonic contraction.

In the terms of the '*linkage*' hypothesis, our results suggest that tonic tension is the result of a continuous and slow interaction of actin and myosin in the form of a turn-over of linkages between the myofilaments. This turn-over would be 11 times slower than during phasic contraction and about 2750 times slower than during tetanic contraction of a vertebrate fast muscle like the frog sartorius (for the rate of the energy expenditure during phasic contraction of the ABRM is about 250 times slower than during tetanus of the frog sartorius (Baguet & Gillis, 1967). The absence of the '*quick release recovery*' in the tonic contraction of the ABRM (Jewell, 1959) seems compatible with such a slow turn-over.

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REFERENCES

BAGUET, F. (1965). The respiration of a catch-muscle (ABRM) during a tonic contraction. *J. Physiol.* **179**, 35–36*P*.

BAGUET, F. & GILLIS, J. M. (1964). Consommation d'oxygène d'un muscle de Lamellibranche en contraction tonique. *J. Physiol., Paris* **56**, 528–529.

BAGUET, F. & GILLIS, J. M. (1967). The respiration of the anterior byssus retractor muscle of *Mytilus edulis* (ABRM) after a phasic contraction. *J. Physiol.* **188**, 67–82.

BAGUET, F., GILLIS, J. M. & DAINOFF, G. (1967). Energétique du relâchement phasique d'un muscle lisse de Lammellibranche. *Archs int. Physiol.* **75**, 523–528.

BAILEY, K. (1957). Invertebrate tropomyosin. *Biochim. biophys. Acta* **24**, 612–619.

BAYLISS, L. E. (1928). The energetics of plain muscle. *J. Physiol.* **65**, 1*P*.

BOZLER, E. (1948). Conduction, automaticity and tonus of visceral muscle. *Experientia* **4**, 213–218.

BRECHT, K., UTZ, G. & LUTZ, E. (1955). Über die Atmung quergestreifter und glatter Muskeln von Kalblütern in Ruhe, Dehnung, Kontraktion und Kontraktur. *Pflügers Arch. ges. Physiol.* **260**, 524–537.

BÜLBRING, E. (1953). Measurements of oxygen consumption in smooth muscle. *J. Physiol.* **122**, 111–134.

HANSON, J. & LOWY, J. (1959). Evidence for a sliding filament contractile mechanism in tonic smooth muscles of Lamellibranch Molluscs. *Nature, Lond.* **184**, 286–287.

HOYLE, G. & LOWY, J. (1956). The paradox of *Mytilus* muscle. A new interpretation. *J. exp. Biol.* **33**, 295–311.

JEWELL, B. R. (1959). Nature of the phasic and tonic responses of the anterior byssus retractor muscle of *Mytilus*. *J. Physiol.* **149**, 154–177.

JOHNSON, W. H. (1962). Tonic mechanisms in smooth muscles. *Physiol. Rev.* **42**, suppl. 5, 113–143.

JOHNSON, W. H., KAHN, S. J. & SZENT-GYORGYI, A. G. (1959). Paramyosin and contraction of 'catch muscles'. *Science, N.Y.* **130**, 160–161.

LOWY, J. & HANSON, J. (1962). Ultrastructure of invertebrate smooth muscles. *Physiol. Rev.* **42**, suppl. 5, 34–47.

LOWY, J. & MILLMAN, B. M. (1963). The contractile mechanism of the anterior byssus retractor muscle of *Mytilus edulis*. *Phil. Trans. R. Soc.* **246**, 105–148.

MILLMAN, B. M. (1964). Contraction in the opaque part of the adductor muscle of the oyster (*Crassostrea angulata*). *J. Physiol.* **173**, 238–262.

NAUSS, K. M. & DAVIES, R. E. (1966). Changes in inorganic phosphate and arginine during the development, maintenance and loss of tension in the anterior byssus retractor muscle of *Mytilus edulis*. *Biochem. Z.* **345**, 173–187.

PARNAS, J. (1910). Energetik glatter Muskeln. *Pflügers Arch. ges. Physiol.* **134**, 441–495.

PRINGLE, J. W. S. (1965). Locomotion: flight. In *Physiology of Insecta*, ed. ROCKSTEIN, M., vol. 2, pp. 283–329. New York: Academic Press.

RITCHIE, A. D. (1928). *The Comparative Physiology of Muscular Tissue*. Cambridge University Press.

RÜEGG, J. C. (1958). The possible function of invertebrate tropomyosin. *Biochem. J.* **69**, 46*P*.

RÜEGG, J. C. (1961). On the tropomyosin-paramyosin system in relation to the viscous tone of lamellibranch 'catch' muscle. *Proc. R. Soc.* B **154**, 224–249.

RÜEGG, J. C. (1963). Actomyosin inactivation by thiourea and the nature of viscous tone in a molluscan smooth muscle. *Proc. R. Soc.* B **158**, 177–195.

RÜEGG, J. C. (1965). Physiologie und Biochemie des Spertonus. *Helv. physiol. pharmac. Acta*, suppl. XVI, 1–76.

TWAROG, B. M. (1954). Response of a molluscan smooth muscle to acetylcholine and 5-hydroxytryptamine. *J. cell. comp. Physiol.* **44**, 141–163.

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.