

## THE HEAT PRODUCTION OF SMOOTH MUSCLE.

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OUR knowledge of the physiology of muscular contraction is based mainly upon studies of vertebrate skeletal muscle. The study of a very different type of muscle might be expected to be of value (*a*) for the understanding of its special properties, and (*b*) to show which of the properties of skeletal muscle are common also to other types and may, therefore, be regarded as properties of muscular tissue in general. In the course of comparative studies on unstriated muscle<sup>(2)</sup> an easily obtainable and very favourable preparation was found in the retractor of the pharynx of the large edible snail (*Helix pomatia*): this muscle was used for all the experiments on heat production reported here.

*Preparation.* The muscle is a part of the columellar muscle. It is attached at one end to the axis of the shell and at the other to the ventral side of the pharynx. The shell is first removed, care being taken not to break the middle part of its axis, which is later used as an attachment for the muscle. After removing the front part of the mantle, which is very slimy, the body wall is cut dorsally from near the top up to the head. The genital organs are removed. If the stomach be then lifted with forceps, the pharynx and the retractor muscle attached to it beneath the oesophagus become visible. The pharynx is then cut off the body near the mouth. This should be done as quickly as possible since it involves cutting the connection of the muscle with the central nervous system, which forms a ring round the pharynx. Strong stimulation of the ganglia produces a vigorous contraction of the retractor muscle which persists for a long time, even after complete removal from the body. The axis of the shell with the muscle attached to it is cut off. Only as much of the axis is left as is necessary for the attachment. The other parts of the columellar muscle attached at the same place are removed as far as possible.

The axis is a narrow tube-like structure, and by pushing a thread or a wire through the opening an excellent attachment can be obtained. The other end of the muscle is fixed by means of the pharynx.

In preparing the muscle great care must be taken not to stimulate it, and to keep it entirely uninjured. This can easily be done, as it lies free in the body cavity and is barely connected to other tissues except at the two ends. Nevertheless, even after very careful preparation and much experience, the muscle is often obtained in a contracted state. Generally, however, it will relax after half an hour if immersed in snail's blood. This, therefore, is collected from the same animal before the dissection. One can easily obtain several c.c. of the blood by making a hole in the shell at the second turn on the side towards the top, where an artery is to be found. The blood flows out naturally as soon as this artery is cut: squeezing the animal will accelerate the flow.

It is important to keep the animals in perfect condition, otherwise the muscle preparation is very liable to go into long-continued contractions after a short stimulus. The animals seem to be in the best condition for the experiment in the winter. They should be kept in a refrigerator, and a few days before they are to be used should be brought into a warm room, kept moist, and fed with cabbage as soon as they creep out.

No salt solution was found as a satisfactory substitute for blood. The best results were obtained with a frogs' Ringer's solution containing three times the usual amount of calcium chloride.

*Method.* The method of heat measurement was that of A. V. Hill(8). Only a few modifications, which were required to adapt it to the new type of muscle, need be mentioned.

The muscle is fairly sensitive to stimulation and responds to single weak induction shocks. It is, however, not always certain that the heat produced by the current is quite negligible. Stimulation, therefore, was carried out by two electrodes lying close together above the thermopile. The stimulating current did not pass through the part of the muscle lying on the thermopile. A third electrode, below the thermopile, served for calibration. It was found that, using sufficiently strong stimuli, the mechanical response was the same whether the muscle was stimulated only at one end or by a current passing from one end to the other. No measurable effect of the heat produced by the stimulating current between the upper pair of electrodes could be detected at the thermopile, as was shown by tests with the dead muscle. The end attached to the columella was connected to the tension lever by a thread. The other

end was fixed below the thermopile by a curved pin pushed through the pharynx.

The calibration was carried out as described by Hill. Considerable difficulties arose, however, from the fact that it is very difficult to kill the muscle without causing it to alter its shape. By "electrocution" only the parts near the electrodes are killed: the middle parts still contract afterwards. Pure carbon dioxide, even when saturated with alcohol vapour, does not make the muscle sufficiently non-irritable. The method finally adopted was the following. The muscle was stimulated with a faradic current or a series of condenser discharges, beginning with weak stimuli and very gradually increasing them. Short periods of rest were interposed. If this process was continued for about an hour the response to a strong current was only about one-third of that of the fresh muscle. If, then, the chamber was filled with pure carbon dioxide, the muscle no longer responded to the alternating current used for calibration. It was not killed by this procedure: it recovered if left in air. That no appreciable heat, however, was produced actively by the muscle, under these conditions, was concluded from the fact that the heat liberated by the alternating current was exactly proportional to the square of that current as measured by the hot wire in the same circuit. With completely dead muscles it was ascertained that the result of the calibration is the same in carbon dioxide as in air, as was found by Hill.

Some difficulty was experienced at first, with the sensitive arrangement desirable for these experiments, in obtaining a constant zero. The influence of slow changes in the bath temperature could be largely eliminated in the following way. This influence appears to be chiefly due to heat radiation from the walls of the chamber. The thermopile, therefore, was adapted so that this radiation affects the hot and the cold junctions equally. This was done by varying the amount of wax on the cold junctions, removing some of it if warming of the bath caused a galvanometer deflection to the warm side, adding to it if the opposite effect was observed. With the thermopile first employed there is the disadvantage that it is less sensitive when there is little wax on the cold junctions, since their heat capacity is small and they are rather quickly warmed by the heat conducted from the hot junctions.

This disadvantage was avoided by winding the wire of the thermopile on a copper frame, shown in Fig. 1. The cold junctions lie close to a large heat capacity with a good heat conductivity, and the wires need only be covered with a thin sheath of wax. The cold junctions are not exactly

opposite to the warm junctions, more than two-thirds of each turn is silver-plated. This (a) diminishes the electrical resistance, and (b) delays the conduction of heat to the cold junctions, because the heat conductivity of constantan is so much less than that of silver. Both ends of the frame are covered by a copper plate, so that the interior of the thermopile is completely closed. The muscle side is covered by a very thin sheet of mica fixed by a thin layer of paraffin, in order to obtain a perfect and permanent insulation against water and electrical leaks.



Fig. 1. Cross-section of thermopile.

This very sensitive thermopile "settles down" in air and with no muscle on it in a quarter of an hour. With a muscle on the hot junctions, however, almost invariably a negative or positive deflection, sometimes a large one, persists for hours, and diminishes but slowly even when every precaution is taken to avoid leaks. It was finally found that the cause of this phenomenon was a difference between the vapour pressures (a) of the Ringer's solution at the bottom of the chamber, and (b) of the muscle. This deflection can be made positive or negative at will by using either diluted or concentrated Ringer's solution. Water will condense on the muscle in the first case, evaporate from the muscle in the second case, and thus give apparent heat production or absorption. The importance of this factor has already been realized by Hill(9). The zero becomes much more steady by introducing into the chamber a Ringer's solution of appropriate concentration, which must be found by trial. It is not possible, however, under these circumstances to measure the rate of resting heat production. To do so requires that the muscle should first be soaked in the Ringer's solution and brought into equilibrium with it, which is possible with frog's muscle, but, in the absence of a suitable solution, not with the muscle employed in the present investigation.

Some possible errors may be mentioned. (a) There is no distinct maximal response as in the skeletal muscle of a frog. If the stimulus be increased gradually the response increases more rapidly at first, and then more slowly over a considerable range. Strong stimulation must be avoided. It produces long-continued activity accompanied by heat production. This is the case, even if only a small part of the muscle be stimulated directly with excessive current. There is the possibility that not all the fibres respond to stimulation of moderate intensity. This, however, would hardly give an appreciable error in the heat measurement, because the muscle is so very thin, thinner indeed than the insulation,

that it allows a very rapid conduction of heat from any part of it into the thermopile. (b) Parts of the muscle near the stimulating electrodes might contract more strongly than those on the thermopile. One of my records of action current shows that the excitation produced by a weak but just adequate stimulus (slight mechanical response) applied at one end is not conducted to the other end, whereas that due to a stronger stimulus is. This condition, however, is rare. With stronger stimuli there is never a difference in the response, whether the whole muscle be stimulated or only one end. Repetition of such stimuli produces the same high maximum tension, which may be more than 5 kg. per sq. cm. of cross-section. Unequal tension in different parts would produce a deformation of the muscle which does not normally occur. The weak make shocks of a faradic current should be eliminated. (c) The most serious source of error is probably the occurrence of responses which outlast the stimulation. This can be avoided to a large extent by careful dissection, and by the use of moderate stimuli. Preparations which do not give a perfectly smooth relaxation curve after each response should be discarded.

The muscle has the form of a flat band, 4 to 6 cm. long, about 2 to 3 mm. broad, and about 0.3 mm. thick. It is uniform in cross-section and parallel-fibred. A detailed description of its mechanical response and its mechanical properties is to be given elsewhere (2). It is characteristic that the rise of tension is very rapid, the maximum being reached after 0.3 sec. in a twitch at room temperature. Relaxation, however, is very slow and lasts several seconds at least. Measurement of action current records shows that the wave of excitation spreads at a rate of 40 to 60 cm. per second. The contraction, therefore, of the whole muscle is practically instantaneous.

Snyder (13) has recently published a paper on the heat production of smooth muscle from the tortoise. His method seems to be open to many objections, and his actual observations (though to a less extent his conclusions) differ so much from those recorded here that a comparison of the results seems impossible.

#### RESULTS.

*Delayed heat.* The galvanometer returns to zero much more slowly after the contraction of a live muscle than after the corresponding heating of a dead one, which shows that a considerable part of the heat is produced after the end of the mechanical response. The area of the galvanometer deflection curve for a contraction produced by 1 second stimulus was measured. It was assumed that the initial heat, the part which is

associated with the mechanical response, is measured by the maximum deflection of the galvanometer. A "control" heating of  $2\frac{1}{2}$  seconds was employed. For the same maximum deflection, then, the total area of the deflection-time curve, in the case of the contraction of the live muscle, divided by the area for the control heating of the dead muscle, is equal to the ratio of the total heat to the initial heat.

The method is correct only if the initial heat be produced within a short interval, otherwise the maximum deflection is not a true measure of it. It is necessary, therefore, to choose conditions in which relaxation is rapid. This is the case only in fresh muscle in perfect condition. The experiments were carried out at a fairly high temperature, 20–22° C. This assists rapid relaxation. The recording system, moreover, was made slow by employing thicker insulation, so that the maximum deflection was reached only in about 7 seconds. These precautions are sufficient to ensure that no appreciable amount of initial heat is produced after the maximum deflection is attained. A slight error may arise owing to the fact that the later part of the relaxation heat may not be measured quite accurately in the maximum deflection. There is, however, certainly very little heat produced in this last phase of relaxation (see below). Any error introduced in this way would tend to make the ratio of total to initial heat, as observed, too great, by allowing the initial heat to appear to be slightly smaller than it actually is.

The results of eleven experiments are given in Table I.

TABLE I. Ratio of total heat (in air) to initial heat, in snail's muscle.

Date, 1929	Ratio	Date, 1929	Ratio
16. ii.	2.2, 2.2, 2.2	11. iii.	1.8, 1.9, 1.9, 1.9
28. ii.	1.8, 1.8	14. iii.	1.9, 1.9
1. iii.	2.0, 2.1	18. iii.	1.8, 1.8
2. iii.	2.3, 2.3, 2.3	20. iii.	2.4, 2.4, 2.4, 2.4
3. iii.	2.25, 2.3	21. iii.	2.1
10. iii.	2.3, 2.4		

Mean value of ratio = 2.1.

The deflection curves are perfectly smooth (Fig. 2) and agree almost exactly in different experiments. The delayed heat starts at a high rate and is finished in 4 or 5 minutes, which is quicker than in frogs' muscle. It must be remembered that the actual amount of energy liberated in these contractions is very small, about 1500 g. cm. per g. The delayed heat lasts longer after long-continued contractions, and after the liberation of greater amounts of initial heat. It was also found that washing the muscle in Ringer's solution reduces the rate of recovery

heat production; the same is true for  $\text{CO}_2$ , even in fairly low concentrations (see below).

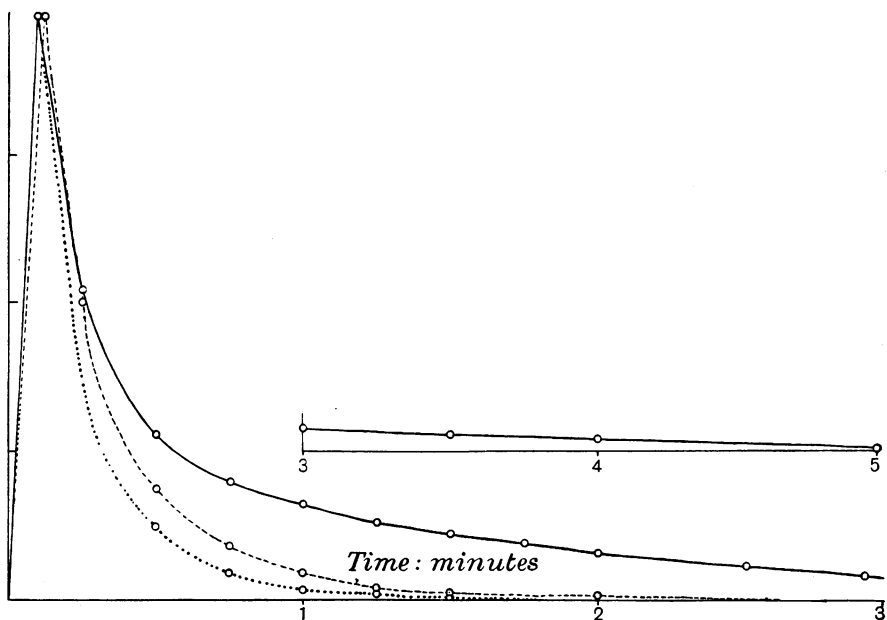


Fig. 2. Experiments of 2. iii. 1929. Galvanometer deflection curves: full line for contraction in air; broken line for contraction in nitrogen— $\text{CO}_2$  mixture; dotted line for control heating for 2.5 seconds. (Total heat)/(initial heat)=2.3. Inset: continuation of curve in air.

The delayed heat, or at any rate the greater part of it, disappears in the absence of oxygen. The muscle was deprived of oxygen by placing it in nitrogen, purified by the method of Kautsky and Thiele(10). This has the peculiar effect of making it extremely irritable. After a weak short stimulus it usually gives a strong contraction lasting for several minutes. After being in pure nitrogen for some time it often starts contracting spontaneously. To avoid this the nitrogen was mixed with carbon dioxide (about 25 p.c.) which acts as a weak narcotic. This, however, has the disadvantage of making the muscle extremely slow, relaxing completely only after several minutes. The maximum galvanometer deflection, therefore, cannot be expected to represent the initial heat with any accuracy. Even so, however, the deflection of the galvanometer invariably comes back more quickly than in air (Fig. 2). In nitrogen the area of the deflection curve is generally 30 to 40 p.c. greater than that of a control heating curve of the same maximum deflection.

In one case in which the relaxation was relatively quick, it was only 11 p.c. greater. It is unnecessary to give these results in detail, as they cannot be regarded as quantitative.

We may conclude that, in the snail muscle, as in the skeletal muscle of frog or tortoise, the heat consists of two parts, the initial heat, which is anaerobic, and the delayed heat, which is produced only in the presence of oxygen. The ratio of these to one another is the same as in the frog's sartorius.

*The isometric heat coefficient.* The energy used for producing tension has been expressed by Hill in the quotient  $H/Tl$ , where  $H$  is the initial heat,  $T$  the maximum tension, and  $l$  the length. This has been determined for snail's muscle for different durations of the stimulus. The initial heat was measured by the maximum deflection of the galvanometer: the same precautions, therefore, were necessary as before, in order to be sure that the initial heat was produced within a sufficiently short time. If we plot  $H/Tl$  against duration of stimulus we obtain a straight line (Fig. 3):

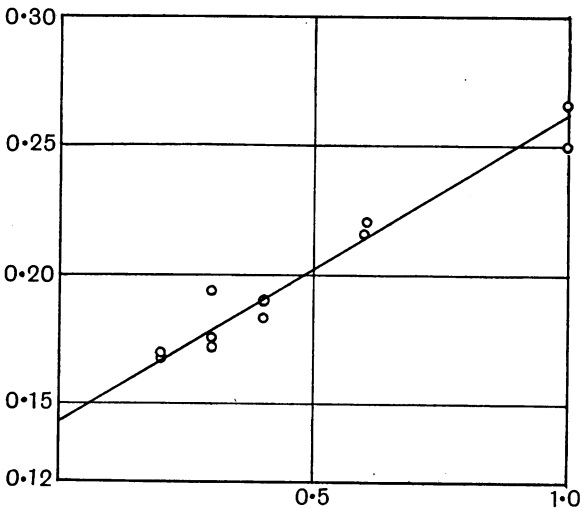


Fig. 3. Experiment of 17. ii. 1929.  $H/Tl$  for different durations of stimulus. The points shown are single observations. Time in seconds.

this relation, which is the same as for the frog's sartorius, has been discussed by Hartree and Hill(5). The heat can be regarded as consisting of two parts. One part is related to the production of the tension: this is constant and is given by the value of  $H/Tl$  for short durations of the stimulus. The other part is related to the maintenance of tension



already produced: this is proportional to the duration of the stimulus. We obtain therefore the equation

$$H/Tl = a + bt,$$

where  $a$  and  $b$  are constants, and  $t$  the duration of the stimulus. For the frog's sartorius  $a$  is 0.16, and has been found to be of the same order of magnitude for other kinds of vertebrate skeletal muscle<sup>(7)</sup>. It is interesting that it has almost the same value for snail's muscle. The values obtained in nine experiments were

0.15, 0.15, 0.11, 0.14, 0.15, 0.17, 0.14, 0.15, 0.12; mean = 0.142.

The values must be extrapolated to zero time as shown in Fig. 3, since it was not possible to apply stimuli shorter than 0.15 sec. at the low frequency of stimulation used in the present experiments.

The constant  $b$  determines the slope of the line. It is very different for different muscles. Hill has shown that it depends on the time relations of contraction. Less energy is spent in maintaining a tension in slow than in quick muscles:  $b$  therefore is much smaller for the tortoise's than for the frog's muscle. It is also smaller for the same muscle at low temperatures than at high. In snail's muscle  $H/Tl$  has been found to rise on the average to 0.29 at a duration of stimulus of 1 second, whereas in frog's muscle at the same temperature it rises to about 1.0 (Hartree and Hill<sup>(5)</sup>). The rate of increase, therefore, of  $H/Tl$  with time is five to six times greater in frog's than in snail's muscle.

If the measurements be repeated on a given muscle, the values of  $H/Tl$  obtained usually agree very well with one another. It happens, however, in some muscles that the same stimulus when repeated suddenly produces a much larger response, with a value of  $H/Tl$  as much as 40 p.c. higher than before. A probable explanation of these occasional differences, which are much larger than the possible error of the measurement, was found when the contractions were recorded at a higher speed of the revolving drum. One may then observe that sometimes a second response follows the first, just before the latter should have reached its maximum. This is shown by a distinct discontinuity of the tension record, and by a late maximum of the curve. The great sensitivity of the muscle to mechanical stimuli may be the cause, slight movements of the muscles over the electrodes producing mechanical irritation during the contraction, and a resulting second response. This would have the same effect as a greater duration of stimulus, and would explain the higher tension and the greater value of  $H/Tl$ .

*The economy of maintaining tension.* It is characteristic of the type

of muscle studied that it can maintain a tension very economically. This is probably its most important difference from skeletal muscle. The energy required for maintaining tension, therefore, is in this case of special significance.

The tension maintained in a contraction is measured by the "tension-time" (Hartree and Hill(5)), that is, by the area of the tension-time curve  $\int Tdt$ , where  $T$  is the tension,  $t$  the time. The efficiency in maintaining tension then is expressed by the quotient  $\frac{\int Tdt}{H}$ . It is independent of the mass of the muscle, since both the tension and the heat are proportional to this. This quotient may be called the "coefficient of economy of maintaining tension," or, more shortly, the "economy." It has the dimension of time, which indicates that it depends on the time relations of the processes concerned. By  $H$  is meant only the initial heat in this connection.

Accurate determinations of the "economy" are difficult, because it changes fairly rapidly during a contraction. To avoid this only short contractions with small expenditures of energy must be used. The "economy" of fresh muscles was measured in a number of experiments in two different ways: firstly, a short tetanus was produced by stimulating for 0.1 to 1.0 second; the initial heat was measured by the maximum deflection; secondly, after complete recovery it was stimulated for 1 minute by one shock every 2 to 3 seconds and the total heat determined by measuring the area of the deflection-time curve. The initial heat of this contraction was taken as half the total heat. The results of a few experiments are given in Table II.

TABLE II. Coefficient of economy of maintaining tension: snail's muscle.

Date	Stimulus (sec.)	Tension- time (g. sec.)	Heat (g. cm.)	Half re- laxation (sec.)	"Economy" (sec.)
27. iii.	0.8	43.2	8.35*	1.5	12.0
	1.0	37.2	7.82*	1.5	11.4
	60	228	72†	1.8	14.4
4. vi.	0.1	24.6	2.15*	2.5	26.0
	60	229	33.0†	2.9	32.0
6. vi.	0.5	40.7	11.7*	1.2	8.2
	0.6	58.8	10.25*	1.6	13.0
	60	342	82†	2.3	19.2
17. vi.	1	37.8	5.83*	3.0	16.8
	60	170	42.6†	4.9	21.0
18. vi.	0.6	38.4	5.5*	3.0	18.6
	60	580	121†	6.5	25.0

\* From maximum deflection.

† From area.

Hartree and Hill<sup>(5)</sup> have pointed out, and shown experimentally, that in skeletal muscle the "economy" depends on the time relations of the mechanical response to a single stimulus; the slower the muscle the more tension-time is produced with the same expenditure of energy. To compare the time relations in the several muscles shown in Table II, the time required for the tension to fall to half its maximum value is given.

In agreement with its slower time relations the "economy" of the snail's muscle is found to be considerably higher than that of the frog's muscle. Values for the "economy" of the frog's sartorius are given by Hartree and Hill<sup>(5)</sup> in a graph showing the relation of heat and tension-time for different durations of stimulus and different temperatures. At 15° the "economy" for short stimuli (up to 0.2 sec.) is about 0.4. The higher values for longer stimuli may be partly due to fatigue. At 14° the tension falls during relaxation to half its maximum value in about 0.065 sec., as can be read off from curves given by the same authors. We will assume the value 0.060 for 15° C. In the experiment of 27. iii. 29 in Table II, the time to half relaxation was 1.5 sec. for the fresh muscle, which is 25 times as great as the value just given for the frog. The "economy" is  $12/0.4 = 30$  times greater. Other experiments give similar results. The comparison is of course only rough, but it shows that the values are of the order which might have been expected from the difference of time relations. There is, therefore, in this connection no difference of principle, but only of time relations, between muscles of the snail and of the frog.

In Table II the "economy" is found to be the same, as closely as could be expected, whichever method of measurement was adopted and with different durations and frequencies of stimulation. Such differences as occur may be partly due to a recovery heat coefficient other than 2, but chiefly to a change in the condition of the muscle due to previous stimulation. This is indicated by the change of the speed of relaxation: the muscle is slower at the end of a long contraction and the "economy" correspondingly greater.

This progressive change in "economy" with stimulation is a phenomenon of special interest. If the muscle be stimulated repeatedly it produces the tension with about the same amount of energy, but relaxation becomes slower and "economy" greater. In the experiments shown in Fig. 4 the muscle first gave the contraction *A* with a stimulus lasting 0.35 sec. This was repeated several times and then contraction *B* was obtained with a stimulus lasting 0.4 sec. The response of the galvano-

meter recording the heat production was recorded photographically as shown in the upper curves *A* and *B*, and a control curve was obtained

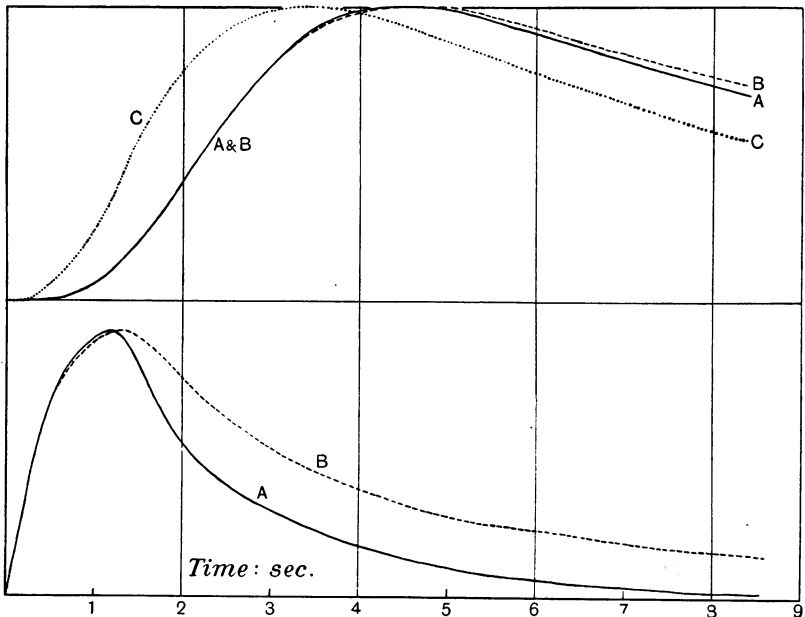


Fig. 4. Experiment of 15. vi. 1929, at  $14.4^{\circ}\text{C}$ . Lower curves, tension records of two contractions, *A* (full line) that of fresh muscle, *B* (broken line) after a period of exercise. Upper curves, galvanometer deflection records for the two contractions *A* and *B* shown below, and for 0.3 sec. heating (*C*).

by heating the muscle for 0.3 second (curve *C*). The galvanometer deflection curves for the contractions have their maxima 1.6 sec. after the control, and after their maxima the curves fall off almost as rapidly as the control. This shows that by far the largest part of the initial heat is produced within less than 2 seconds, and that the heat produced during the later stages of relaxation is small. This was the case, although relaxation was rather slow in both cases and the temperature low ( $14.4^{\circ}\text{C}$ ). The curves for both contractions fall off in very nearly the same way. This shows that insufficient heat was produced in the later stages to account for the much slower relaxation in contraction *B*, thus excluding the possibility that the change in time relations is simply the effect of further weak activity of the muscle during relaxation. An accurate analysis of the curves by the method of Hartree and Hill<sup>(4)</sup> would show this more clearly, but a quicker recording system than that used here would be required.

There are, however, cases of slow contractions in which the galvanometer deflection curves fall off appreciably more slowly than the control curves. A considerable amount of heat is produced during relaxation, probably due to continued weak activity. Only, therefore, if the relaxation be fairly quick is it safe to measure the initial heat by the maximum deflection. The slow, incomplete and irregular relaxation of contractions produced by too strong stimuli is always accompanied by a large production of heat. The galvanometer deflection reaches a maximum later than in a normal contraction, falls very slowly and shows humps which correspond to the irregularities of the relaxation curve.

The extent of the change in "economy" due to exercise can be studied by stimulating the muscle with a series of shocks at a low frequency, one shock every 2 to 3 seconds. This produces at first a very discontinuous tetanic contraction, the mechanical response becoming more and more a smooth tetanus as time goes on, owing to a progressive slowing of relaxation. The galvanometer deflection soon reaches a maximum and then begins to return while the tension is still rising, which shows that the rate of heat production is diminishing even while the mechanical response is increasing.

A constant deflection of the galvanometer measures the rate of heat production if it remains constant for as long as a minute. This is the time necessary for the thermopile to get into a steady state, heat production being balanced by heat loss. During the first minute of the contraction, however, it is no measure of the rate of energy expenditure for maintaining tension, for (a) the muscle has not only to maintain but also to produce tension; this latter part is not quite negligible even for a fairly slow rise of tension; and (b) the recovery heat production will occur only a certain time after the beginning of the contraction. I have therefore measured the "economy" of the fresh muscle as described above, using contractions of short duration and measuring the total tension-time produced. The total energy liberated in such a contraction is small and the condition of the muscle may be regarded as constant during the short period involved.

The tension and the rate of heat production become approximately constant after the muscle has been stimulated at a low frequency for several minutes, and the muscle can be kept in practically a constant condition for at least 1 hour. It is justifiable, therefore, to assume that the muscle does not run into oxygen debt. This is shown also by the fact that after the end of the stimulus the galvanometer deflection comes back to about half during the first minute of relaxation and returns to

zero after about 15 minutes. The constant deflection of the galvanometer, therefore, measures the rate of total energy expenditure in maintaining tension, *i.e.* including recovery heat. The results of two experiments are given in detail in Table III.

TABLE III. "Economy" of maintaining tension in long contractions:  
snail's muscle.

Exp. 27. iii. 29. 20.2° C.

1 sec. tetanus, tension-time	37.2 g. sec.,	heat	7.82 g. cm.,	"economy"	11.4
60 sec. " "	228 g. sec.,	"	72 g. cm.,	"	14.4

Long tetanus, maximum deflection 425 mm., reached in 3 min. 15 sec., tension 12.5 g.

After 7 min., tension	13 g.,	deflection	140,	"economy"	87
" 13 min., "	15.5 g.,	"	201,	"	70
" 19 min., "	13.8 g.,	"	181,	"	72

Rest for 20 min.

Long tetanus, maximum deflection 515 mm., reached in 2 min. 15 sec., tension 15.5 g.

After 7 min., tension	17 g.,	deflection	180,	"economy"	89
" 10 min., "	14.1 g.,	"	208,	"	66
" 16 min., "	10.5 g.,	"	112,	"	88

Rest for 20 min. The same procedure repeated a third time gave the same result.

Exp. 6. vi. 29. 22° C.

0.5 sec. tetanus, tension-time	41 g. sec.,	heat	11.7 g. cm.,	"economy"	8.2*
60 sec. " "	342 g. sec.,	"	82 g. cm.,	"	19.2

Long tetanus, maximum deflection 475 mm., reached in 3 min. 45 sec., tension 20 g.

After 6 min., tension	23 g.,	deflection	231 mm.,	"economy"	72
" 18 min., "	18.5 g.,	"	160 mm.,	"	87

Relaxation, rest for 1 hour.

Long tetanus, maximum deflection 660 mm., reached after 1 min. 30 sec., tension 29 g.

After 4 min., tension	29 g.,	deflection	302 mm.,	"economy"	68
" 9 min., "	15.7 g.,	"	108 mm.,	"	102
" 11 min., "	13.5 g.,	"	81 mm.,	"	127

\* Similar short contractions are interposed before the next longer contraction.

In all the experiments moderate exercise for a few minutes produced an increase in "economy" of six to ten times, and on occasions even as high as fifteen times. This change is not connected in any way with signs of deterioration of the muscle. Contraction may continue for some hours, and relaxation may in the end be many times slower. The difference in mechanical response between fresh muscle and muscle after exercise is illustrated in Fig. 5. The muscle stimulated by one shock every 3 seconds gave at first a very incomplete tetanus, but at the end a perfectly smooth contraction. The change is reversible. After 15 to 30 minutes' rest relaxation is very nearly, if not quite, as quick as at the beginning of the experiment, and the "economy" is low. The observation, therefore, can be repeated several times on the same muscle, as was done in the experiments given in Table III.

Another factor which has nearly as great an influence on "economy," under physiological conditions, as exercise is carbon dioxide tension.

The muscle chamber was filled with different mixtures of  $\text{CO}_2$  and air, starting with low concentrations of  $\text{CO}_2$ . Short tetani only (1.0 sec.)

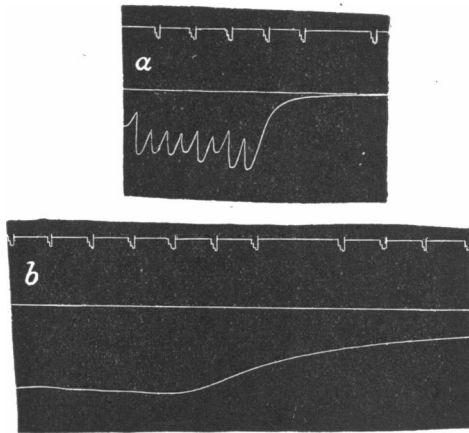


Fig. 5. Experiment of 6. vi. 1929, at  $22^\circ \text{C}$ . Tension records. The muscle was stimulated by one shock every 3 seconds; *a*, response of fresh muscle; *b*, after 10 minutes of exercise. Stimulus discontinued in middle of *a* and *b*. Time in 6 sec. units. 1 mm. = 1.1 g. tension.

were used to exclude the possibility of changes in the condition of the muscle due to exercise. The total heat was measured from the area of the deflection-time curve. Between every pair of contractions there was a pause of at least 10 minutes, which allowed complete recovery. At the end the chamber was again filled with air, and new measurements made to see whether the condition of the muscle had altered irreversibly. The slightly diminished oxygen concentration in the chamber in the  $\text{CO}_2$  experiments had hardly any appreciable effect on the muscle. Some tests were made with a mixture of 5 p.c.  $\text{CO}_2$  and oxygen, which gave exactly the same effect as the same concentration of  $\text{CO}_2$  in air.

The results were very consistent. If "economy" be plotted against  $\text{CO}_2$  tension a relation is found which is approximately linear, but increasing rather more steeply at the beginning. The results of two experiments are shown in Fig. 6. Table IV gives the results of all the experiments made (except preliminary observations).

The magnitude of the change of "economy" due to  $\text{CO}_2$  varies in different experiments. At a  $\text{CO}_2$  concentration of 3 p.c. it rises 2.8 to 6.6 times, with 6 p.c.  $\text{CO}_2$  5.2 to 9.9 times. The effect is completely reversible. The last value obtained in air is generally slightly lower than

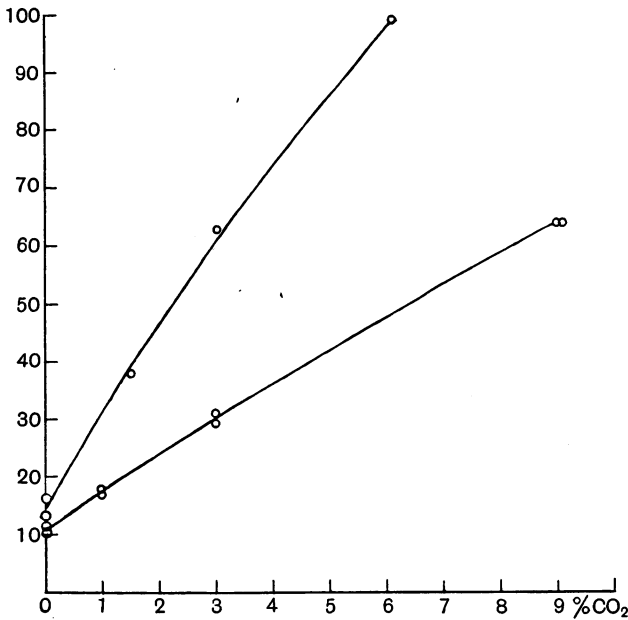


Fig. 6. "Economy" of maintaining contraction at different partial pressures of CO<sub>2</sub> in air. Upper line, experiment of 20. viii. 1929; lower line, experiment of 12. viii. 1929.

TABLE IV. Effect of CO<sub>2</sub> on the "economy" of maintaining tension: snail's muscle.

Exp. 12. viii. 29. 18.6° C.

CO <sub>2</sub> (p.c.)	0	1	3	9	0
Heat (g. cm.)	37	40	45	40	68
Tension-time (g. sec.)	64	115	216	400	113
"Economy," absolute	11	18.4	31	64	10.7
"Economy," relative	1	1.7	2.8	5.8	0.98

Exp. 17. viii. 29. 19° C.

CO <sub>2</sub> (p.c.)	0	1.5	3	6	0
Heat (g. cm.)	43.5	42.5	69	56	61
Tension-time (g. sec.)	67.5	161	350	465	75
"Economy," absolute	13.5	33	59	70	10.6
"Economy," relative	1	2.4	3.3	5.2	0.8

Exp. 19. viii. 29. 16° C.

CO <sub>2</sub> (p.c.)	0	1.5	3	6	0
Heat (g. cm.)	51.8	36.3	35.9	53.6	51.3
Tension-time (g. sec.)	114	183	310	724	92
"Economy," absolute	16.3	38	63	100	13.3
"Economy," relative	1	2.3	4.0	6.1	0.8

Exp. 20. viii. 29. 16° C.

CO <sub>2</sub> (p.c.)	0	1.5	3	6	0
Heat (g. cm.)	44.5	43.5	55	55.6	28
Tension-time (g. sec.)	55	143	415	680	39.2
"Economy," absolute	8.2	25	54	81	10
"Economy," relative	1	3.1	6.6	9.9	1.2



TABLE IV (contd.).

Exp. 26. viii. 29. 19.5° C.	0	1.5	3	6	0
CO <sub>2</sub> (p.c.)					
Heat (g. cm.)	30.6	43.2	33.5	40.7	48.5
Tension-time (g. cm.)	69.5	317	458	842	107.5
"Economy," absolute	11.7	38	67	107	11.4
"Economy," relative	1	3.2	5.7	9.2	0.98

the first, but they agree sufficiently well considering that 3 to 4 hours elapsed between the first and the last determination.

In these experiments also the change in "economy" is associated with a change in the time relations of relaxation. The muscle relaxes much more slowly in the presence of CO<sub>2</sub>; even concentrations of 0.5 to 1 p.c. CO<sub>2</sub> have a noticeable effect. The rate of rise of tension, however, does not seem to be influenced. Unfortunately, at the time of these experiments (September), the muscles were not in good condition, and usually did not give a smooth relaxation curve. A new weak contraction often set in some seconds after the end of the stimulus. It is not possible, therefore, to give exact data to the time relations.

The accuracy of the measurements at higher CO<sub>2</sub> tensions is diminished by two factors: (a) The rate of production of recovery heat is greatly diminished. During the first minutes after the contraction the deflection of the galvanometer comes back more quickly with the muscle in CO<sub>2</sub> mixtures than with the muscle in air, although relaxation may be much slower: in the end, however, the galvanometer returns more slowly. This means that the recovery heat is spread over a longer period and is, therefore, more difficult to measure. The same effect has been noted for the frog's sartorius by Hartree and Hill(6). They found the rate of recovery heat production to be diminished to one-half in the presence of 25 p.c. CO<sub>2</sub>. The effect is apparently much greater with the snail's muscle. It is noticeable at 6 p.c. CO<sub>2</sub> and considerable at 9 p.c. It cannot be ascribed simply to a slightly smaller oxygen tension in the gas mixture, but must be regarded, as in frog's muscle, as due to a diminution in the rate of oxidation by the increase of acidity. (b) A small residual tension may often be noticed for many minutes at higher CO<sub>2</sub> concentrations, due apparently to continued weak activity of the muscle. Under these circumstances the determination of the area of the tension-time curve is less exact. The heat associated with this continued activity of the muscle is of course included in the total heat. The possible errors which arise from (a) and (b) are certainly small at the low CO<sub>2</sub> concentrations used here, as compared with the magnitude of the changes produced by the CO<sub>2</sub>. At higher concentrations, however, quantitative experiments have not been made for the reasons given.

An attempt has been made to determine the carbon dioxide dissociation curve for the snail's muscle, which seemed to be of interest in connection with the great effect of  $\text{CO}_2$  on this muscle. The method used was that employed by Fenn(3) for the tissues of the frog. A muscle is brought into equilibrium with various concentrations of  $\text{CO}_2$  in one of the chambers of a differential micro-respirometer. The combined  $\text{CO}_2$  is then liberated by dropping sulphuric acid on to the muscle. Unfortunately the values obtained on different days do not agree sufficiently to give a smooth dissociation curve. This is probably due to the very variable water content of the animals employed. More constant conditions would probably give more consistent results. The values obtained, however, are of interest because they show the very large carbon dioxide combining power of the snail's muscle. If left in air the muscle contained 60 to 133 volumes of  $\text{CO}_2$  p.c., and in 6 p.c.  $\text{CO}_2$  it contained 140 to 180 volumes p.c. This is many times more than found by Fenn and by Stella for frog's muscle, and considerably more than has been found for vertebrate blood. The high  $\text{CO}_2$  combining capacity may be connected with the great effect of  $\text{CO}_2$  described above.

#### DISCUSSION.

Smooth muscles differ from skeletal muscles mainly in two respects. (a) They are able to maintain a tension for a very long time with a very small expenditure of energy (see *e.g.* Parnas(11), Bethe(1)). (b) Their properties vary very much from one set of conditions to another. The differences are so great that it has been found difficult to apply to smooth muscle the principles established for skeletal muscle, and it has been doubted whether the mechanism of contraction is the same in the two cases.

A closer study, however, of the mechanical response of two smooth muscles, which will be published separately in detail(2), has shown that the differences are not so great as is often assumed. After every isometric contraction there is a relaxation, which differs from that of skeletal muscle only in its time relations. Under certain conditions the response may be as rapid as that of slow skeletal muscles, and the similarity between both kinds of muscles is then most striking. One might be tempted to think that the snail's muscle is not typical of smooth muscle in its physiological properties. The same time relations, however, of the contractions have been found also for the smooth part of the adductor muscle of *Pecten*, which has always been regarded as a typical smooth muscle. It should be noted that the responses of these muscles can be successfully studied only if they are in perfect condition. It is not possible to draw definite conclusions concerning the time relations of the contraction from experiments in which it is uncertain whether the muscle is still active at the end of stimulation, or in which the fibres do not contract simultaneously. Failure to obtain muscles in good condition may be one reason for the inconsistent results of earlier experiments.

In addition to the similarity of mechanical response, the myothermic experiments described here give results which in important points agree surprisingly closely with those obtained from the frog's sartorius. The isometric heat coefficient for short contractions, and the ratio of total to initial heat in the presence of oxygen, are practically the same. The fact that these values agree, for two muscles so different in their structure and taken from animals so different in their organization as the frog and the snail, may be regarded as a new proof of the fundamental significance of these values for muscular tissue in general. The present experiments have thrown light also on the question of the great economy of smooth muscle. They provide no evidence that tension can be maintained without heat production. The muscle sometimes gives long-continued contractions after a short stimulus, or it may contract spontaneously. In either event there is a production of heat which seems to be as large as that occurring when the muscle is stimulated. A number of authors have adopted the view that the great economy is due to the slow time relations of the response to a single shock. Hill confirmed this view by showing that slow muscles, like those of the tortoise, are more economical than fast muscles like those of the frog, and that the same muscle becomes more economical if its time relations are made slower, *e.g.* by lowering the temperature. The same standpoint has been adopted and developed in detail by Ritchie (12).

The snail's muscle may be fairly quick and relatively uneconomical after a period of rest. Moderate exercise, however, may make it much slower and fifteen times more economical. As the result of exercise alone the economy may rise to values of more than 100 in absolute units. One may calculate that the isolated muscle could maintain a tension of 12 g. for one day, *i.e.* 1.2 kg. per sq. cm. of cross-section, by supposing that the glycogen content of the muscle is the same as that in the frog (1 p.c.) and that this is the source of energy. It is true that some muscles, like the adductor muscles of certain Lamellibranchs, are probably still more economical, as judged by the time during which they can keep their shells closed. Under more normal circumstances, however, the change of economy might be even greater than in the case considered. I refer especially to the effect of CO<sub>2</sub>, which may be one of the main factors determining the economy in the normal muscle. The necessity of maintaining tension for a considerable time arises particularly when the snail has retracted into its shell, or in the case of Lamellibranchs when the animal has closed its shell. The CO<sub>2</sub> tension may be particularly high under such conditions, and the concentrations used in the present experiments are probably still within the physiological range.

The change of time relations of the contraction due to exercise is not peculiar to the type of muscle studied here: it is probably of the same nature as that associated with fatigue in skeletal muscle. Hill has shown that  $H/Tl$  for a single shock is not changed in a moderate condition of fatigue. For the same maximum tension, however, the tension-time, and therefore the economy, are increased because the contraction is slower. This change has been studied in detail by Bronk and has been described in the last number of this *Journal*. Frogs' sartorii may become about three times more economical after severe exercise, and in fatigue. The change is probably small under physiological conditions. In the normal animal the importance of this change is certainly much greater in the case of the snail, not only because it is considerably greater but also because it occurs after very moderate exercise and relatively small expenditure of energy, long before any signs of exhaustion are apparent.

It seems likely that the change after exercise, as well as that under the influence of  $\text{CO}_2$ , is due to the same factor—an increase of acidity inside the muscle. Other factors, however, probably also are involved. The adductor muscle of *Pecten* (2) can relax in the intact animal very quickly, in less than 1 second, after being contracted for a considerable time. We can explain this fact only by supposing that a change of the time relations of relaxation, in a direction opposite to that resulting from exercise or  $\text{CO}_2$ , can occur under nervous influence. The question would seem to be of peculiar importance in the physiology of this muscle.

It seems, then, that the muscle can adapt the time relations of its mechanical response within rather wide limits to varying requirements. These changes in time relations will probably make more intelligible many of the properties of smooth muscle.

#### SUMMARY.

The energetics of the retractor of the pharynx of the edible snail (*Helix pomatia*) have been studied with A. V. Hill's myothermic methods. The main results are as follows:

1. For short durations of stimulus  $H/Tl$  has an average value of 0.14, practically the same as for the frog's sartorius. It increases in a linear manner with the duration of the stimulus.
2. The ratio of total to initial heat in the presence of oxygen is about 2.1. The delayed heat is largely, probably completely, absent in the absence of oxygen.
3. The "coefficient of economy in maintaining tension" is expressed by the ratio  $\int Tldt/H$ , where  $T$  is tension,  $t$  time,  $l$  length of muscle,

*H* initial heat. It is much greater than in the frog. It increases considerably after moderate exercise, in some experiments as much as fifteen times. This change is due to an alteration in the time relations of relaxation.

4. A considerable increase in "economy" (several fold) is effected by CO<sub>2</sub> in quite low concentrations.

5. No evidence has been found for the idea that tension may be maintained without heat production. The great "economy" of the muscle under certain circumstances can be explained by the slow time relations of the mechanical response.

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