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# THE PRESSURE DEVELOPED IN MUSCLE DURING CONTRACTION

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There is much speculation, but little is known for certain, about the connexion between chemical breakdown in muscle and the physical events of contraction and relaxation. It is not even certain that in the elementary unit of muscular activity, the single twitch, contraction and relaxation are associated with any of the chemical processes as yet recognized. It is true that during the course of a maintained contraction chemical changes can be shown to occur, particularly the breakdown of creatine phosphate and (if the stimulus is prolonged) the formation of lactic acid: but a maintained contraction is the resultant of many elementary contractions and relaxations, fused more or less together to outward appearance, and overlaps the early stages of recovery from all except the last of these, so that any chemical changes observed at the end may be connected rather with recovery. It is assumed by some that the breakdown of adenosine triphosphate (ATP) provides the energy for contraction, or, by others, for relaxation: but since no changes at all have been found in the ATP of living muscle, except in extreme fatigue verging on rigor, it is not possible to say when (or if) its breakdown actually occurs in a normal twitch.

The initial heat production in the twitch of a frog's muscle, being 2 to $3 \times 10^{-3}$ cal./g., corresponds, if the breakdown of ATP or phosphagen be supposed to account for it, to about $2 \times 10^{-7}$ mole of phosphate, or about 6 $\mu$g. of P, set free per g. of muscle. This assumes Meyerhof's (1947) figures of 11,000 cal./mole of phosphate from phosphagen, or of 12,000 cal. from ATP. Contraction and relaxation occupy about 1 sec. at 0° C., and about 0·2 sec. at 20° C. The supposed chemical changes, therefore, are so small and so rapid that direct chemical methods cannot possibly cope with them. Very special value, therefore, is attached to any other method, with sufficient sharpness of resolution in time and sufficient sensitivity and specificity, which might be used to establish a connexion between contraction and relaxation on the one hand and chemical changes on the other. One such method is that of measuring the heat production. This is sensitive and accurate, and can be made very rapid, but it does

not distinguish specifically between one chemical change and another. The heat production in a twitch occurs in the contractile phase only: in relaxation no heat at all appears unless mechanical energy is degraded into heat. The measurement of pH is more specific, but as hitherto employed it is affected by the lag of the electrode (Dubuisson, 1939, p. 465) and by the time taken in diffusion from the inside of the muscle: together these must require several seconds before a true reading is obtained. The measurement of volume changes is another method which is sensitive and rapid, though not specific: indeed it provides the only evidence yet available (apart from the heat production) that chemical changes have actually occurred in a single twitch by the time that relaxation is complete. Unfortunately, its application is affected by a serious complication which it is the purpose of this paper to describe.

The complication is this. A considerable mechanical pressure is developed inside a muscle when it contracts, which is bound to cause a compression, followed in relaxation by a decompression, of its contents, particularly its contained water. The early and reversible changes of volume accompanying contraction and relaxation must be largely, if not entirely, due to this. The later observed effects, the remainder at the end of relaxation and any subsequent changes, are unaffected by this compression and may safely be attributed to chemical processes. The early volume changes, however, must be so largely affected by it that they cannot be used directly as an index simply of chemical breakdown, and the hoped for resolution in the early stages is not realized.

The first measurement of volume change during contraction was made by Ernst (1925). Versfelt (1927) confirmed its existence and attributed it to mechanical compression of the contents of the muscle: indeed he estimated the pressure developed during contraction of a frog's gastrocnemius as about 100 mm. Hg. The most significant results, however, were obtained by Meyerhof and his colleagues and are referred to below.

Water is a rather compressible substance, its volume change at 15° C. being about $4{\cdot}9 \times 10^{-5}$ c.c./c.c. × atm., as compared with mercury $0{\cdot}37 \times 10^{-5}$, copper $0{\cdot}05 \times 10^{-5}$, and iron $0{\cdot}04 \times 10^{-5}$. In the papers of Meyerhof & Möhle (1933), Hartmann (1934) and Meyerhof & Möhle (1935) are a number of records of the volume changes of a frog's gastrocnemius undergoing an isometric tetanus. There is a rapid diminution of volume immediately after the stimulus begins, the volume continues to diminish as the stimulus goes on, and when the stimulus ends there is a sudden increase of volume coinciding in time with relaxation. The net result, when relaxation is complete, is a diminution of volume which can safely be attributed to chemical breakdown, probably chiefly of creatine phosphate. The reversible effect seen in contraction and relaxation can most readily be assessed by measuring the sudden increase in volume during relaxation. The rapid reversible decrease of volume at the beginning of contraction is complicated by the possibility of rapid onset of the decrease associated with chemical breakdown: they cannot easily be

separated. During relaxation, however, the volume returns rapidly to a nearly constant base-line.

The following results (Table 1) were measured off from all the figures in the three papers for which a calibration is given: in each case the contraction was the first of a series, providing usually the greatest reversible effect.

TABLE 1. Increase of volume in relaxation

| Author | Tetanus (sec.) | Volume increase (c.c./g. muscle) | Note |
|---|---|---|---|
| Meyerhof & Möhle (1933) | 4 | $0{\cdot}8 \times 10^{-5}$ | |
| | 2 | $1{\cdot}5 \times 10^{-5}$ | |
| | 4 | $2{\cdot}6 \times 10^{-5}$ | Muscle previously fatigued |
| | 0·2 | $7{\cdot}4 \times 10^{-5}$ | * |
| | 2·0 | $7{\cdot}5 \times 10^{-5}$ | * |
| Hartmann (1934) | 3 | $0{\cdot}7 \times 10^{-5}$ | |
| | 2 | $1{\cdot}3 \times 10^{-5}$ | I.A.A. |
| | 2 | $0{\cdot}9 \times 10^{-5}$ | I.A.A. |
| | 2 | $0{\cdot}6 \times 10^{-5}$ | |
| Meyerhof & Möhle (1935) | 0·8 | $1{\cdot}9 \times 10^{-5}$ | |

Of these results, two, marked by an asterisk, are much larger than the rest. They are given on the same page (478), and were obtained presumably under similar conditions; probably there is some abnormality about them and they have been omitted from the mean. The next largest reading was taken under similar conditions on a muscle which had been subjected to a previous series of stimuli. Two records (I.A.A.) were on muscles poisoned with iodoacetic acid. A mean value for all, except the two marked with asterisks, is $1{\cdot}3 \times 10^{-5}$ c.c./g. of muscle. If we assume that the compressibility of muscle is the same as that of water, this would require a mean pressure of about 200 mm. Hg to produce it.

In single isometric twitches a similar measurement of four records in papers by Meyerhof & Möhle (1935) and Meyerhof & Hartmann (1934) gave for the volume increase in relaxation $1{\cdot}35 \times 10^{-5}$, $3{\cdot}7 \times 10^{-5}$, $1{\cdot}8 \times 10^{-5}$ and $0{\cdot}9 \times 10^{-5}$ c.c./g. The scatter is too wide to allow a fair average to be taken. The mean given by Meyerhof & Hartmann is $2 \times 10^{-5}$, but from this must be subtracted the remainder after relaxation, leaving (say) $1{\cdot}5 \times 10^{-5}$. The reversible volume changes for an isometric twitch are obviously about the same as for a tetanus.

In the isometric twitch of a frog's gastrocnemius, the rise of temperature (Hill, 1931) is not more than 3 to $4 \times 10^{-3}$° C. If this could be attributed to the dephosphorylation of ATP or of creatine phosphate it would require (at 12,000 cal./mole of phosphate split off) about $3 \times 10^{-7}$ mole of one or the other to be broken down: this (at 10 c.c. volume constriction per mole—Meyerhof 1947, p. 824) would give a diminution of volume of about $3 \times 10^{-6}$ c.c./g. of muscle, which is only about one-seventh of the average ($2{\cdot}0 \times 10^{-5}$) observed by Meyerhof & Hartmann. Clearly, known chemical changes are inadequate to account for the volume constriction observed in a twitch.

Can an average pressure of 200 mm. Hg be developed inside a frog's gastrocnemius during an isometric contraction? Direct evidence that it can is given below, but strong indirect evidence exists already in the well-known cessation of blood flow in a strongly contracting muscle. Barcroft & Millen (1939), for example, found that the flow of blood in a human gastrocnemius was completely stopped by an isometric contraction which was not more than one-third maximal. If the arterial pressure (say 130 mm. Hg) was overcome by the pressure set up inside the muscle in such a sub-maximal contraction, a maximal one should have been capable of producing a pressure of about 400 mm. In a dog's gastrocnemius Anrep & Saalfeld (1935, fig. 2) showed an immediate cessation of arterial flow during a tetanus, though the contraction was a weak one (only 2·7 kg.). How are these pressures produced? The structure of the gastrocnemius is very complex, with fibres only half the length of the muscle as a whole (Hill, 1931) pulling in various directions. The external form of the gastrocnemius shows that the fibres and their tendons cannot be lying straight, and if they are curved they must necessarily exert a pressure inwards when they contract, depending on their tension and radius of curvature. The smaller size of the frog's gastrocnemius does not imply a smaller pressure: with a similar form and the same intrinsic strength of contraction the greater curvature would exactly compensate for the smaller number of fibres. We should expect, therefore, in a frog's gastrocnemius, a pressure to be developed, during an isometric contraction, of the order of several hundred mm. of Hg.

All the recorded experiments on volume change during contraction were made on frogs' gastrocnemius muscles, except those of Fischer (1941) on a frog's sartorius. Fischer's results are not described in detail, and the changes found varied in size, and even in direction, with initial length: it is difficult to draw any conclusion from them.

In order to settle the matter it was necessary to measure this pressure, if it existed, directly. The method employed depends on a novel principle, the rise of temperature associated with the adiabatic compression of oil. Like air, any fluid with a positive coefficient of thermal expansion will show a rise of temperature when compressed. One should choose a liquid with as large a thermal coefficient as possible. Various fluids exist (e.g. pentane) with a coefficient of about $1{\cdot}6 \times 10^{-3}$ c.c./c.c. $\times$ ° C., but these are all unsuitable to be injected into a muscle. Thin medicinal paraffin oil, however, is very suitable and it has a fairly high coefficient (about $7{\cdot}6 \times 10^{-4}$; compare water at 17° C., $1{\cdot}5 \times 10^{-4}$). The rise of temperature on compression can be calculated from the formula

$$\delta T = \frac{\alpha T \delta P \times 10^6}{(\text{density})\,(\text{specific heat})\,4{\cdot}18 \times 10^7},$$

where $\alpha$ is the coefficient of thermal expansion, $T$ is the absolute temperature (taken as 290° K.), $\delta P$ is the pressure in megabars (which can nearly enough

be taken as atmospheres) and $4{\cdot}18 \times 10^7$ is the mechanical equivalent of heat (see e.g. Poynting & Thomson, 1904). Inserting the constants for paraffin oil the rise of temperature per atmosphere works out at about 0·012° C.

## METHODS

A fine hypodermic needle was blocked at its tip and a small hole was ground near the tip on one side. It was fitted to a small syringe, the piston of which was modified to carry a 'thermistor' mounted on fine manganin wires above it. The piston was movable, but could be fixed by the turn of a screw. The syringe and needle were filled completely with the paraffin oil referred to above, no air bubbles being allowable since these would buffer the oil against change of pressure. The syringe was mounted on a screw stand by which its needle could be driven forward accurately and conveniently into a gastrocnemius muscle held isometrically on a board. When the needle had been inserted, usually at an angle of about 25° with the axis of the muscle, the hole near its tip being well inside, about 0·01 c.c. of oil was forced in by pressing on the piston, which was then fixed. This oil was intended to spread around the needle and to ensure that its small hole was not blocked by tissue lying across it: it was necessary that any pressure developed should be communicated at once to the oil in the needle and piston. When the muscle contracted, the pressure inside it rose; this raised the pressure in the syringe (the flow in the needle must have been very small), the temperature of the oil rose and lowered the resistance of the thermistor, and a galvanometer (see below) deflected. When the muscle relaxed, the pressure fell, the oil cooled and the galvanometer returned.

The thermistor (type V 597, kindly given me by Dr E. J. Baldes of the Mayo Foundation, Rochester, Minnesota) is a glass-coated spheroid of about 0·3 mm. diameter with two 25 $\mu$. platinum alloy leads. It contains a semi-conducting material with a very high-temperature coefficient of resistance, about 3·3 % per 1° C. The specimen used had a resistance of about 2000 Ω at 17° C. Being so small it takes up the temperature of the oil very quickly, in less time than is needed to read the galvanometer.

The arrangement was calibrated directly. By means of a pump, an air-pressure reservoir, a two-way tap and a manometer, the pressure inside a thick rubber tube containing oil could be suddenly raised or lowered by a known amount above or to atmospheric. The needle was forced through the rubber into the oil, so that known changes of pressure could be suddenly produced in the syringe. The pressure was turned on (or off) and the deflexion of the galvanometer was read; the result was expressed in terms of change of resistance in the thermistor. A rise of pressure of 1 atm. lowered the resistance by 0·74 Ω. A resistance was kept in series with the thermistor, which could be suddenly switched in or out, so allowing the galvanometer deflexion to be expressed in terms of pressure change. As finally used, the galvanometer gave 1 mm. deflexion for a pressure change of 10 mm. Hg.

For accurate work, the syringe would have to be maintained at a constant temperature and the thermistor connected to a resistance bridge. Since no great accuracy was desired but only to find out whether changes of pressure do occur in a stimulated muscle, and of what order of size, a simpler arrangement was used. The thermistor was placed in series with 33,000 Ω and a battery (24 V.), and the potential difference between the ends of the thermistor was led, through a good 8 $\mu$F. condenser, to an amplifier. The output of the amplifier was led through a similar condenser to the galvanometer (period 1 sec.). Slow changes of temperature, therefore, caused no deflexion of the galvanometer, which responded only to rapid changes. Calibration was carried out under the same conditions as the experiments on the muscle, so the deflexions were comparable.

The current in the thermistor (about 0·7 mA.) produced enough heat to warm the oil slightly in its neighbourhood. Movement of the oil, therefore, if allowed, would cool the thermistor, but pressure changes caused so little movement that no error of this kind occurred: a fall of pressure gave the same deflexion, but in the opposite sense, as an equal rise of pressure: movement of the oil would have caused a cooling of the thermistor in either case. The deflexions obtained with muscles were always sharply reversible, a rise of temperature (corresponding to a rise of pressure) on contraction, a fall of temperature (corresponding to a fall of pressure) on relaxation.

## RESULTS

The results were definite though somewhat variable. Gastrocnemius muscles of English *Rana temporaria* were used. Insertion of the needle into the muscle was bound to cause some injury and successive stimuli (tetani of 1 or 2 sec. duration) gave diminishing deflexions. One could not be sure (i) that the hole in the needle was in the most suitable place inside the muscle, (ii) that some leakage of oil did not occur along the line of the needle when the muscle contracted, or (iii) that the muscle did not draw away slightly from the needle (which was inserted from the lower end) even in a contraction intended to be isometric. All such causes would tend to reduce the rise of pressure recorded, and doubtless combined to give the variability observed.

The observed deflexions corresponded to pressures from 100 to 300 mm. Hg. For example, successive readings on one muscle were (*a*) 300, 200, 160, 200, 100: and with the needle inserted in a new place 170. Other series were (*b*) 250, 170, 170; 150, 150; (*c*) 130, 240, 200, 100, 100, 100; (*d*) 130, 90, 60, 140, 100; 130, 120, 120, 140, 110, 100; 170, 130, 150. No significance would attach to a mean value, particularly since every experimental influence tends to reduce the reading. It is clear, however, that a pressure of the order of 200 mm. Hg is, in fact, developed inside a frog's gastrocnemius during contraction—which is sufficient to account, at least in isometric contractions, for the reversible part of the volume changes observed.

## DISCUSSION

Meyerhof and his colleagues examined the volume changes in isotonic as well as in isometric contractions: in the isotonic ones the muscle was completely unloaded before contraction, and then allowed to shorten freely—either an unlimited amount, or until it came up against the tension recording device. The volume changes observed were much smaller in isotonic than in isometric contractions, as regards not only reversible effects but also the remainder after relaxation. The relative smallness of the reversible effects could be expected because the force exerted by the fibres of a muscle allowed to shorten freely would be much reduced and the pressure developed be correspondingly less. The smaller remainder after relaxation is an index of smaller chemical change. The heat production in a muscle initially unloaded and allowed to shorten freely may be very much less than when initially stretched. Even in single twitches, for example, Cattell (1932) found that a gastrocnemius initially unloaded and allowed to shorten freely gave out less than one-quarter of the heat observed in an isometric contraction under a standard initial load: this effect I have observed in a sartorius under similar conditions. In a tetanus the effect may be even greater, since the later stimuli are applied to a muscle already considerably shortened, and the heat may be far less than under

isometric conditions or with a considerable isotonic load. A smaller heat production implies a smaller chemical change. These two factors, therefore—smaller mechanical force and smaller chemical breakdown—can amply account for the fact that the volume changes found in unloaded isotonic contractions are much smaller than in isometric.

Whether the much smaller reversible volume changes under isotonic (unloaded) conditions also are due to compression cannot be decided by the present experiments, for the measurement of pressure would have been unreliable had the muscles been able to move considerably. Prof. Meyerhof writes me from Philadelphia: 'The best and most reliable measurements done formerly in Heidelberg are in the 1935 paper (pp. 538, 539). From these I calculate that the volume increase in relaxation (c.c. per g. of muscle) for the isometric twitch is $1{\cdot}3 \times 10^{-5}$, for the isotonic $0{\cdot}5 \times 10^{-5}$. For the tetanus the respective values are $1{\cdot}9 \times 10^{-5}$ and $0{\cdot}5 \times 10^{-5}$. . . . While I would agree that most of the reversible volume change in isometric contraction is water compression, I would think that most of the relatively small reversible change in isotonic contraction is not.' At any rate, the approximate identity in size of the reversible volume changes in twitch and tetanus shows that they cannot be associated with the chemical processes of which the heat is an index, for the latter is far greater in a tetanus. It is possible that the complex form of the gastrocnemius prevents its fibres from shortening freely as much as they could, in which case a pressure would be developed even when the muscle was unloaded. Without more evidence it is clearly unsafe to use the reversible volume changes as an index of chemical or physical processes, other than mechanical ones, accompanying contraction.

If, therefore, we admit that the rapid reversible changes of volume are due mainly to mechanical compression, we are left with the remainder after relaxation as a sign of chemical breakdown. There is a small remaining constriction after even a single twitch, which—from the form of the curves—appears to have accumulated during contraction, or relaxation, or both. In magnitude it would correspond approximately to the amount of ATP or phosphagen broken down, as calculated (see p. 518) from the heat production. After a tetanus the remaining constriction is larger; it has undoubtedly accumulated during contraction, as is seen from the continuing decrease of volume while the stimulus goes on. After relaxation the constriction usually diminishes—the volume increases again—corresponding probably to the resynthesis of phosphagen and the formation of lactic acid. In a muscle poisoned with iodoacetate, however, the constriction may go on increasing after relaxation (Hartmann, 1934, p. 175), corresponding to a continued breakdown of phosphagen (cf. Lundsgaard, 1934; D. K. Hill, 1940, p. 473; Dubuisson, 1939). If, therefore, accurate allowance could be made for the reversible volume changes due to mechanical compression, we could obtain by

subtraction, even during contraction and relaxation, a very significant curve of volume change almost certainly associated with chemical breakdown. The best way to apply this correction would probably be to assume (i) (as above) that, as regards magnitude, the sudden increase of volume during relaxation is due to decompression, and (ii) that, as regards time course, compression and decompression follow the recorded curve of isometric tension. Better still, however, would be to work with muscle containing only straight parallel fibres, in which changes of pressure would not, presumably, occur.

The result would be very interesting. Whether the early constriction (after allowance for the reversible changes) is due to phosphagen breakdown alone, or whether ATP breakdown provides any part of it, cannot be decided on the present evidence, but the continuing decrease of volume of an I.A.A. muscle after relaxation can scarcely be due to the resynthesis of ATP in the Lohmann reaction at the expense of phosphagen breakdown, since the volume changes, molecule for molecule, of these two processes should nearly balance each other (+10 c.c./mole for ATP resynthesis, −11·5 c.c./mole for phosphagen breakdown: Meyerhof, 1947).

If experiments on volume changes in muscle are to be repeated, it is suggested that they ought to be made at 4° C. At this temperature the density of water is a maximum, so that much less accuracy is required in the thermostat and the heat production of the muscle itself would cause no error. A further advantage of the low temperature would be that events in muscle go on much more slowly and do not require so sharp a resolution in the recording apparatus.

## SUMMARY

1. A method is described of measuring alterations of pressure by means of the reversible change of temperature caused by the compression of oil.

2. In the maximal isometric contraction of a frog's gastrocnemius an internal pressure is developed of the order of 100–300 mm. Hg.

3. The same pressure should be developed in a muscle of any size, provided it be of the same shape and the same intrinsic strength. It explains the cessation of blood flow during strong contraction.

4. This pressure is sufficient to account for the reversible changes of volume accompanying contraction and relaxation, which can be regarded as due to mechanical compression and decompression, chiefly of the water contained in the muscle.

5. This does not affect the accepted view that the decrease of volume remaining after relaxation, and its subsequent changes, are due to chemical breakdown.

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