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ON THE INCREASE IN RATE OF HEAT PRODUCTION CAUSED BY STRETCH IN FROG'S SKELETAL MUSCLE

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SUMMARY

1. The increase in rate of heat production caused by stretch in the unstimulated frog's sartorius (stretch response) has been measured using a conventional thermopile technique.

2. The rate of heat production was found constant between l_0 (the distance *in vivo* between the tendons when the legs were in a straight line) and $1{\cdot}2\ l_0$, and rose rapidly above this length to reach 3–5 times the basal rate at $1{\cdot}3\ l_0$. Stretching to greater lengths appeared to damage the muscles.

3. The stretch response is increased by several substances which increase the duration of the active state.

4. Unlike the rate of heat production at l_0, the stretch response is increased by procaine; while the presence of CO_2 greatly reduces it.

5. Evidence is presented supporting the hypothesis that the stretch response is associated with the appearance of tension in the sarcolemma.

INTRODUCTION

Since the end of the last century it has been believed that stretching frog's skeletal muscle causes a rise in its metabolic rate (see Ernst, 1963, for references). Largely as a result of the work of Feng (1932) and Euler (1935) in A. V. Hill's laboratory, much is known of the conditions which modify the effect. In fact it has become known as the 'Feng effect', though Feng's own term 'stretch response', will be used here. An important addition to these results is the finding of J. V. Howarth (unpublished) that the stretch response persists in frog sartorii after soaking in an isotonic K_2SO_4 solution. This finding throws doubt on the suggestion of Ling & Gerard (1949) that the stretch response reflects extra energy turnover needed to maintain the resting membrane potential at great lengths; and

also on the suggestion of Harris (1954) that it is caused by an increase in activity of the sodium pump. However, no satisfactory way of accounting for the effect has been found, and the following work was begun in order to collect more information about the effect, and in particular to investigate more carefully the relation between muscle length and rate of heat production.

A curious feature of the metabolic response to stretch is its variation in size from one amphibian species to another. The sartorii of several species of European frogs show the effect (though to different degrees (Feng, 1932)), while in the sartorius of the American frog, *Rana pipiens*, the stretch response appears to be absent (Baskin & Gaffin, 1965). However, the *gastrocnemii* of both European and American frogs probably show the effect (Ernst & Fricker, 1931; Eddy & Downs, 1921). No stretch response can be measured in sartorii of the common toad, *Bufo bufo* (A. V. Hill, L. Macpherson & J. V. Howarth; unpublished observations).

Little information is available about the distribution of the stretch response in mammalian muscles, but Pool & Sonnenblick (1966) have established its presence in cat papillary muscle; while according to Whalen, Dernberg & Jenden (1958), in the rat diaphragm the stretch response is very small.

METHODS

Pairs of sartorii from *Rana temporaria* were used with a conventional thermopile, which has been described by Woledge (1961). Normally only the pelvic element, comprising thirty-eight constantan–chromel couples, was used. At 20° C the temperature sensitivity was 2200 μV/deg.

The upper (tibial) end of the muscles was attached by a straight wire to a light lever. The lever could be loaded to provide a constant small extending force on the muscle (usually 1 g to 2·5 g). The length of the muscle under this small load was used as the reference length throughout the experiment and will be referred to as 'l_{1g}', '$l_{2.5g}$', etc. The '*in vivo*' length (measured as the distance between the tendons when the legs were gently pulled into a straight line) will be referred to as 'l_0'. The lever was mounted on the stage of a vertical screw stand. Also attached to this mounting was an RCA 5734 tension transducer whose anode pin was joined by a short length of light chain to the tip of the lever (Fig. 1). Before stretching the muscles the stage was raised until the chain just became taut. Further raising of the stage now stretched the muscles through a distance which could be read to within 0·1 mm from a calibrated scale. In experiments in which tension was not recorded the lever was fixed by means of the stop *s* (Fig. 1).

In series with the thermopile was a Zernicke Zb galvanometer whose deflexion was amplified photo-electrically as described by Hill (1965*a*). The output from the head amplifier as well as that from the transducer bridge was recorded by means of a hot wire pen writer. The maximum sensitivity of the temperature measuring system was greater than was needed; 0·05 μV, or $2{\cdot}3 \times 10^{-5}$ deg./cm deflexion on the chart. Under quiet conditions noise and disturbances were equivalent to less than 10^{-2} μV in the thermopile circuit. In the more recent experiments the output from the thermopile has been recorded directly by a Kipp Micrograph recorder. This system was convenient, but considerably less sensitive: the greatest sensitivity which could be used was 1 μV/cm deflexion.

The thermopile and its container were immersed in 14 l. of water at 17–20° C in a polystyrene insulated Dewar flask. The water was stirred by a current of moist air, and a thin layer of oil on the surface reduced temperature drifts due to changes in rate of evaporation.

To reduce errors due to thermoelectric or other fluctuations in the circuit external to the thermopile a reversing key could be put between the thermopile and the external circuit. Two gold plated, bifurcated contact Microswitches were found satisfactory. By arranging these switches to be closed by the armature of a large relay which itself was driven by a signal generator, the input signal could be reversed automatically at any desired frequency.

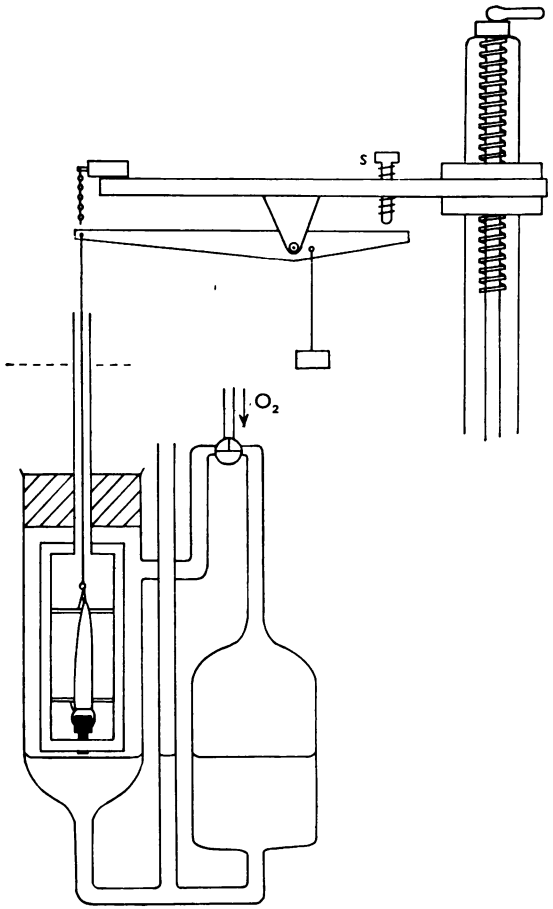

Fig. 1. Schematic diagram of arrangement for stretching and measuring changes in temperature and tension. Parts not to scale. For description see text.

The composition of the normal Ringer solution was (mM): NaCl 115; KCl 2·5; $CaCl_2$ 2·0; NaH_2PO_4 1; Na_2HPO_4 2. In some experiments isotonic K_2SO_4 solution (K_2SO_4 95 mM, saturated with solid $CaSO_4$), was used instead of Ringer solution.

Figure 2 shows a record of a stretch response (upper trace) and associated changes in resting tension (lower trace). The muscles were stretched 4 mm from l_{2g} at the point where the tension rises, and were released back to the initial length at the right of the record. The

rapid changes in temperature on stretch and release are due to thermoelastic effects (Hill, 1952).

Analysis of temperature records. When the temperature of the muscles is steady, the rate of heat production is equal to the rate of heat loss and is found in practice to be approximately

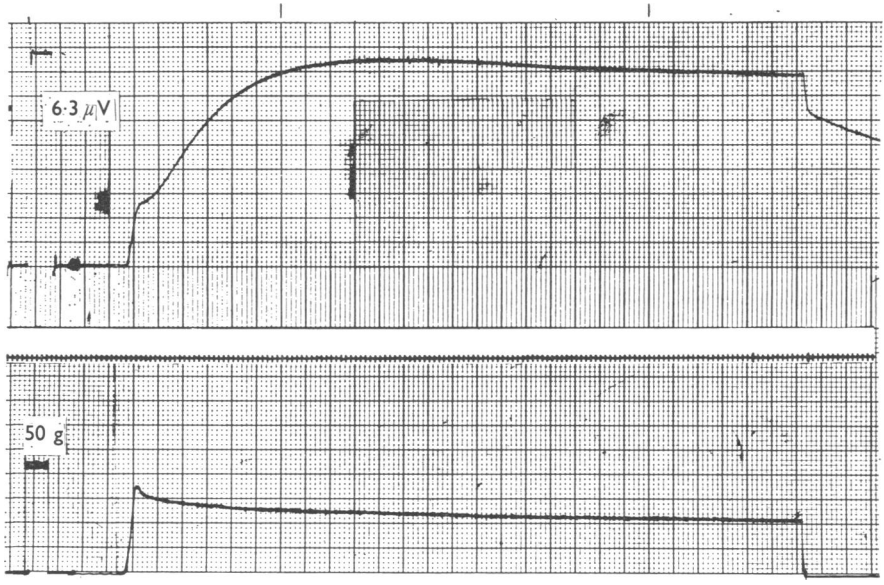

Fig. 2. A record showing changes in muscle temperature and tension following stretch. Upper trace shows change in muscle temperature; the calibration signal of 6·3 μV is equivalent to an increase in the steady rate of heat production by the muscle of 6·0 mcal.g^{-1}.min^{-1}. Centre trace; 1 sec time marks. Lower trace shows change in resting tension. Initial tension (base line) was 2·5 g.

proportional to the temperature difference between the hot and cold thermopile junctions. Under these conditions, the rate of heat production of the muscles, $\dot{h}$, is given by

$$\dot{h} = \frac{60\,yck}{\mu},$$

where $\dot{h}$ is rate of heat production (mcal.g^{-1}.min^{-1}), y is the output from the thermopile (μV), c is the specific heat of muscle (cal. g^{-1}.deg.$^{-1}$), k is the cooling constant (sec^{-1}), and μ is the temperature sensitivity of the thermopile (μV.mdeg.$^{-1}$).

During the first minute after stretch the *temperature* of the muscles is usually changing; to find the *rate of heat production* at any moment the rate of change of heat content must be added, thus

$$\dot{h} = \frac{60c}{\mu}[ky + \dot{y}].$$

where $\dot{y}$ is the rate of change of y.

In order to take account of the heat capacity of the thermopile, the calculated heat rate should be multiplied by the factor $(1+b/a)$ where $2a$ is the total thickness of the muscles ($> 1000\ \mu$) and $2b$ is the total thickness of the thermopile element, epimysia and trapped layer of Ringer solution (equivalent to about 80 μ of muscle). In view of uncertainty about the absolute values of a and b this factor has been ignored. Consequently all heat rates have been underestimated by between 5 % and 10 %.

The specific heat of muscle has been taken to be 0·88 cal $g^{-1}.deg.^{-1}$ at all temperatures (Hill, 1931).

k was determined in the usual way from the time course of fall of temperature following brief warning of the muscles by a 50 kHz sinusoidal current. A small correction was applied for the change of k with length (Clinch, 1965).

The smallest change in the rate of heat production which could be reliably detected depended on the stability of the base line on any given occasion; it varied between 0·02 and 0·2 mcal.$g^{-1}.min^{-1}$. The mean resting rate of heat production in oxygen at l_0 or l_{2g} was found to be 2·6 mcal.$g^{-1}.min^{-1}$ at 20° C (S.D. = 0·6, n = 30).

RESULTS

The relation between length and rate of heat production. The pair of muscles on the thermopile was allowed to rest under a constant initial tension of 1 g, and stretches were given from this reference length (l_{1g}). Progressive changes in the reference length throughout the experiment were found to be reduced by giving the muscles a short isometric tetanus at the start of the experiment, before beginning the series of stretches.

In order to find the relation between muscle length and rate of heat production the muscles were given a series of progressively increasing stretches above l_{1g}. After each stretch the new length was maintained for 60 sec before the muscle was released back to l_{1g}. If a stretch caused an appreciable increase in the rate of heat production, the muscle was briefly washed in Ringer solution before the next stretch. Figure 3 shows the results of eight experiments of this type. Each point shows the increase in rate of heat production (ordinate) measured 60 sec after stretching from l_{1g} to the length shown by the abscissa. In spite of the appreciable inaccuracies involved in measuring l_0 and l_{1g}, the results appear rather consistent. Stretching caused no change in the rate of heat production until the muscle length reached about $1{\cdot}2l_0$. Above this point the metabolic rate rose very rapidly, often reaching 3–4 times the basal rate by $1{\cdot}3l_0$.

In two experiments a Levin–Wyman ergometer was used to give stretches of constant amplitude from l_{1g}, but at different velocities: changes of velocity in the range used (0·5–20 mm/sec) were without effect on the final rate of heat production. Similarly, if the muscles were stretched from different initial lengths to a fixed final length, the rate of heat production at the final length was found to be independent of the actual length change involved.

The length of the muscles could not be increased much beyond $1{\cdot}3l_0$ without causing apparently irreversible change in the stretch response. Such an experiment is shown in Fig. 4. As before, the muscles were given progressively greater stretches from reference length (in this case $l_{2{\cdot}5g}$), separated periods of rest at $l_{2{\cdot}5g}$. This was continued until a point was reached where the stretch response failed to increase with an increase in

the applied stretch. Upon now giving a succession of diminishing stretches, the curve relating increase in metabolic rate to length was found to be shifted to the right. At $l_{2\cdot5g}+7$ mm each muscle bore about 30 g tension.

The length at which the increase in rate of heat production first occurs is close to the length at which an element showing normal thermoelasticity

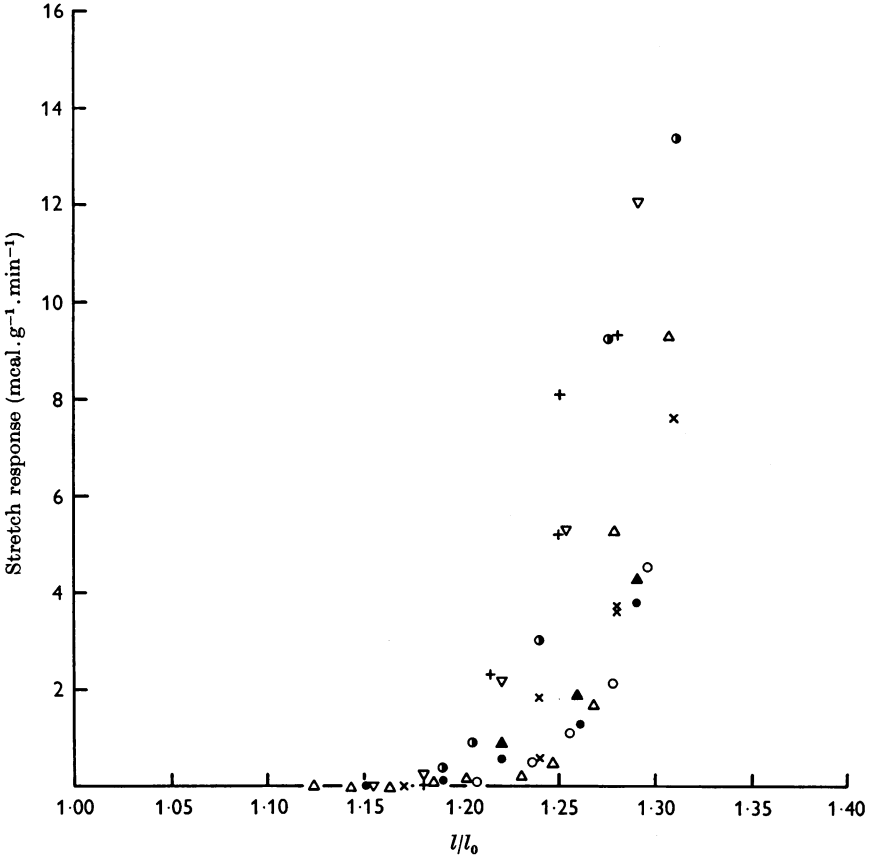

Fig. 3. The relation between size of stretch response and length for eight pairs of sartorii at 20° C. Each symbol refers to a separate experiment. Ordinate: increase in rate of heat production 60 sec after stretching from l_{1g}. Abscissa: muscle length expressed as l/l_0. All rates of heat production have been corrected for small temperature differences between experiments by using an assumed Q_{10} of 2·5.

○	$l_0 = 27{\cdot}2$	$l_{1g} = 30{\cdot}2$	$T = 18{\cdot}2°$ C	(25. v. 64)
●	$l_0 = 29{\cdot}0$	$l_{1g} = 32{\cdot}0$	$T = 19{\cdot}0°$ C	(9 v. 67)
△	$l_0 = 25{\cdot}4$	$l_{1g} = 28{\cdot}0$	$T = 15{\cdot}6°$ C	(19. v. 64)
+	$l_0 = 28{\cdot}0$	$l_{1g} = 31{\cdot}6$	$T = 21{\cdot}4°$ C	(5. v. 67)
▲	$l_0 = 29{\cdot}0$	$l_{1g} = 31{\cdot}5$	$T = 19{\cdot}7°$ C	(8. v. 67)
×	$l_0 = 29{\cdot}0$	$l_{1g} = 32{\cdot}0$	$T = 19{\cdot}9°$ C	(11. v. 67)
◑	$l_0 = 29{\cdot}0$	$l_{1g} = 32{\cdot}0$	$T = 19{\cdot}3°$ C	(15. v. 67)
▽	$l_0 = 28{\cdot}3$	$l_{1g} = 31{\cdot}5$	$T = 20{\cdot}3°$ C	(12. v. 67)

first manifests itself in the passively stretched sartorius (Hill, 1952). Hill also pointed out that below this length the resting tension–length curve is exponential, being described by an equation of the form $P = \alpha e^{\beta l}$, where P is the tension, l is length and α, β are constants. Above this point the tension rises more steeply with increasing length than predicted by the

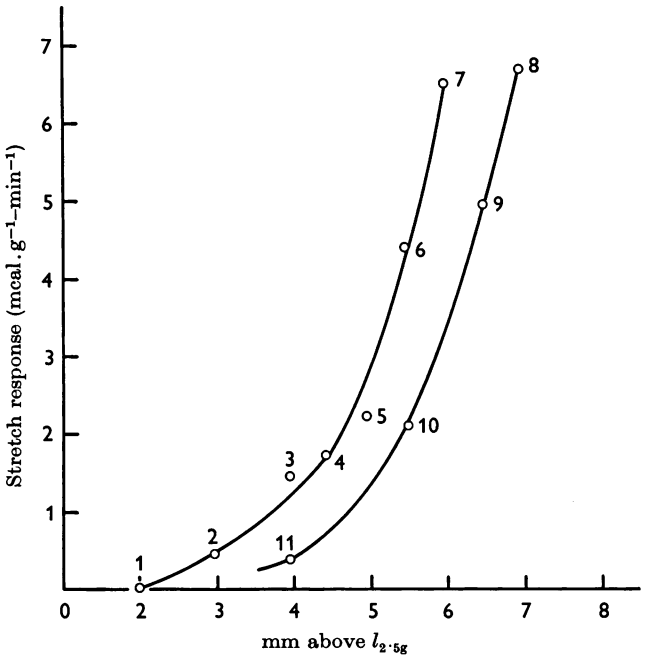

Fig. 4. The effect of overstretching the muscles. Numbers by points show the order in which stretches were given. Ordinates: size of stretch response estimated as the increase in rate of heat production 60 sec after stretching from $l_{2\cdot5g}$ (mcal.g^{-1}.min^{-1}). Abscissae; size of stretch above (mm). $l_{2\cdot5g}l_0$ = 34 mm.

equation. It was thought worth while to examine the resting tension–length curve for the divergence from the simple exponential relation, and to compare the length at which it occurred with that at which the stretch response was first noticeable.

Unfortunately, the resting tension in this region is rather small (a few grams), and measuring temperature as well as tension imposes inaccuracies on the tension measurements which become important when the tension to be measured is small. In measuring temperature the muscles must be in oxygen, not in Ringer solution, so the tension at the bottom of the muscle is less than at the top because of the muscle's weight (0·1–0·2 g), and more important, at low tensions a large amount of hysteresis exists, owing to adhesion of the muscle to the thermopile. Consequently, tensions

below 1 g could not be reliably measured. This limited the length of the straight part of the curve of log P against l and thus reduced the accuracy with which the divergence (if any) could be described. Nevertheless, in four experiments sufficient of the straight part of the curve was obtained to allow comparison of the point of divergence with the point of origin of the stretch response. In each case they were within 0·5 mm of each other. A single experiment is shown in Fig. 5; in A, log (tension/cm^2) is plotted against length above l_{1_g}. If the ordinates of the extrapolated linear part of the curve (dotted line) are subtracted from the corresponding measured tension, the 'extra tension' is obtained. In Fig. 5B this 'extra tension'

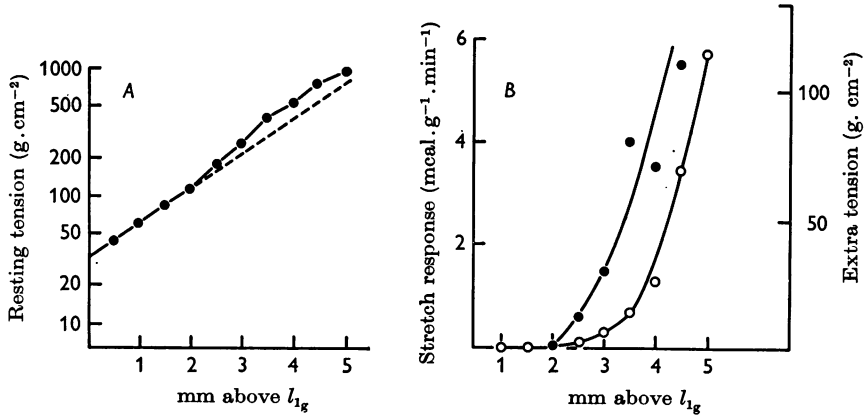

Fig. 5. A. Semilogarithmic plot of resting tension–length curve. Ordinate; log (tension/cm^2). Abscissa; length above l_{1_g}. Dashed line is extrapolated linear relation from short lengths. The difference between the observed tension and the value given by the extrapolated line gives the 'extra tension' at any length.

B. 'Extra tension' (filled circles and right ordinate), and stretch response (open circles; left ordinate), plotted against length above l_{1_g} (mm). Stretch response points refer to the increase in heat rate measured 60 sec after stretching from l_{1_g}.

and the stretch response are plotted against muscle length. Each point is the mean of three measurements, two from descending and one from an ascending series of stretches. When a divergence from linearity was seen in the log tension–length curve, the stretch response always started close to that point. However, since the curve does not diverge abruptly, the slope of the linear part of the curve should be accurately defined. This was often impossible, either because the tension points were too scattered, or because the curve diverged at low tensions (in small muscles) so that too little of the straight part of the curve could be obtained. It is easier to determine the point of divergence in the log tension–length curve from experiments where the muscle is freely suspended in Ringer solution. In four such experiments at 16° C, the divergence points were found to be:

$1{\cdot}16l_0$, $1{\cdot}19l_0$, $1{\cdot}17l_0$, and $1{\cdot}17l_0$. These lengths compare well with those at which the stretch response is first seen (Fig. 3).

The effect of substances which prolong the active state. A number of substances increase the force developed in an isometric twitch in skeletal muscle without changing the tension produced in a tetanus. Several of these 'active state potentiators' have been tested for effect on the stretch response. All but two increased it reversibly. Zn^{2+} (10^{-4} M) irreversibly diminished the response to stretch in each of three experiments, while UO_2^{2+} (10^{-5} M) was without effect. Both of these substances probably prolong the active state by slowing the falling phase of the action potential (Sandow, 1965).

Caffeine (Sandow, Taylor, Isaacson & Seguin, 1964); quinine (Benoit, Carpeni & Przybyslawski, 1964); NO_3^- and SCN^- (Hodgkin & Horowicz, 1960) are all known to lower the mechanical threshold, and all increase the stretch response to a greater or lesser extent. Imidazole (Sandow, Isaacson & Preiser, 1964), and raised extracellular K^+ (Kahn & Sandow, 1952), also increase the duration of the active state plateau though their effect on the mechanical threshold is unknown. Both increase the stretch response.

Figure 6 shows the effect of some of these substances on the metabolic response to stretch. Each graph refers to a separate experiment, and shows the changes in rate of heat production over a 1 min period following three identical stretches. Curve 1 in each case is a stretch response after soaking in normal Ringer solution. Curve 2 is the effect of the same stretch after soaking in Ringer solution containing the potentiator in the stated concentration, and curve 3 is the stretch response after soaking in normal Ringer solution again. All ordinates represent change in rate of heat production ($mcal.g^{-1}.min^{-1}$) plotted against time after stretch (sec).

Thiocyanate had the greatest effect on the stretch response, though in the concentration used it was poisonous to the muscle; in the experiment of Fig. 6, the testing stretch was very small, since greater stretches appeared to cause contracture; even at l_{1_g} the muscle began to shorten 15 min after stretch 2 was given. The fall of rate of heat production after release was very slow compared with that after a release in normal Ringer solution.

At 1 mM caffeine caused only a moderate increase in the metabolic response to stretch, though in each of nine experiments a definite increase was seen. Quinine sulphate (10^{-4} M) caused a great increase in the stretch response. The effect was very slow, the 'quinine stretch response' of Fig. 6 being obtained 20 min after adding the quinine, while after soaking for another 50 min the response to stretch was considerably greater (1 min after the stretch the rate of heat production was $5{\cdot}5\ mcal.g^{-1}.min^{-1}$). The reversal on returning to Ringer solution was much quicker: a steady size was reached in 30 min.

All these substances increased the metabolic rate at the initial length to a varying degree. The increase in rate of heat production at short lengths which is caused by raised $[K]_o$ has been studied by Solandt (1936) and by Hill & Howarth (1957). As well as increasing the metabolic rate at short lengths, increased $[K]_o$ is known to increase the stretch response (Feng, 1932). In view of a possible connexion between the two effects it seemed of interest to compare the time course of the rise of the stretch response and the metabolic rate at l_{2g} after increasing $[K]_o$. Figure 7 shows the result of an experiment in which a pair of muscles, initially in Ringer solution, was soaked in a modified Ringer solution containing 15 mM-K for 2 hr before

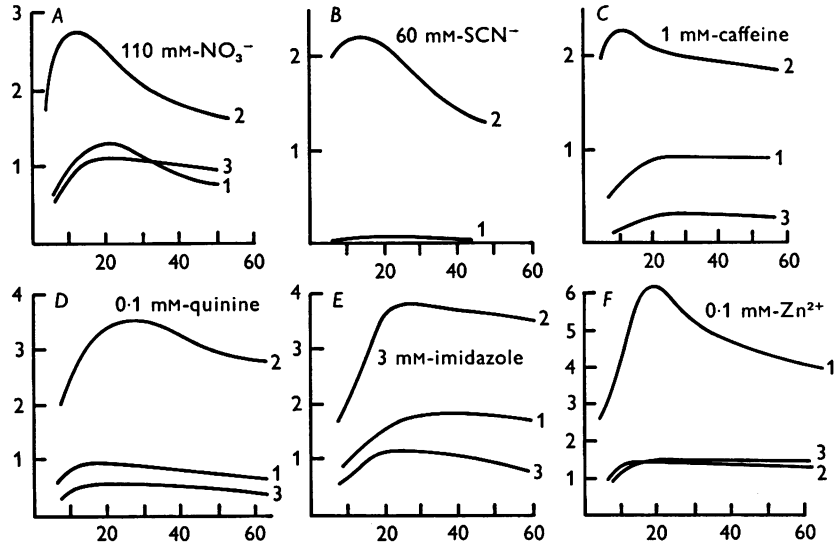

Fig. 6. The effect of some active state potentiators on the stretch response. Each graph represents a different experiment. In every case the curve labelled (1) is a stretch response after soaking in normal Ringer solution; (2) shows a stretch response following an exactly similar stretch after soaking in a Ringer solution containing the potentiator in the stated concentration, and curve 3 shows the stretch response after soaking in normal Ringer solution again. All ordinates represent *increase* in rate of heat production in units of mcal. $g.^{-1}$ min^{-1} (note different scales), and all abscissae represent time in sec after the stretch. Owing to the occurrence of transient thermoelastic effects, analysis of the temperature records was not begun until 5–8 sec after the stretch ended.

A. Nitrate Ringer solution; 3 mm stretches from $l_{2\cdot5g}$.
B. 60 mM thiocyanate; 2 mm stretches from l_{1g}.
C. 1 mM caffeine; 3 mm stretches from l_{2g}.
D. 0·1 mM quinine sulphate; 3 mm stretches from l_{2g}.
E. 3 mM-imidazole hydrochloride, pH 7·3; 4 mm stretches from l_{2g}.
F. 0·1 mM zinc sulphate; 3 mm stretches from l_{2g}.

In the experiment of Fig. 6*F* the muscle was smaller than usual so that the test stretch of 3 mm from l_{2g} gave initially a large response (curve 1).

returning to normal Ringer solution. At intervals the solution was replaced by moist oxygen, and the rate of heat production at l_{2g} was measured. The muscles were then stretched 3 mm and kept at this new length for 60 sec so that the usual measure of the stretch response could be obtained.

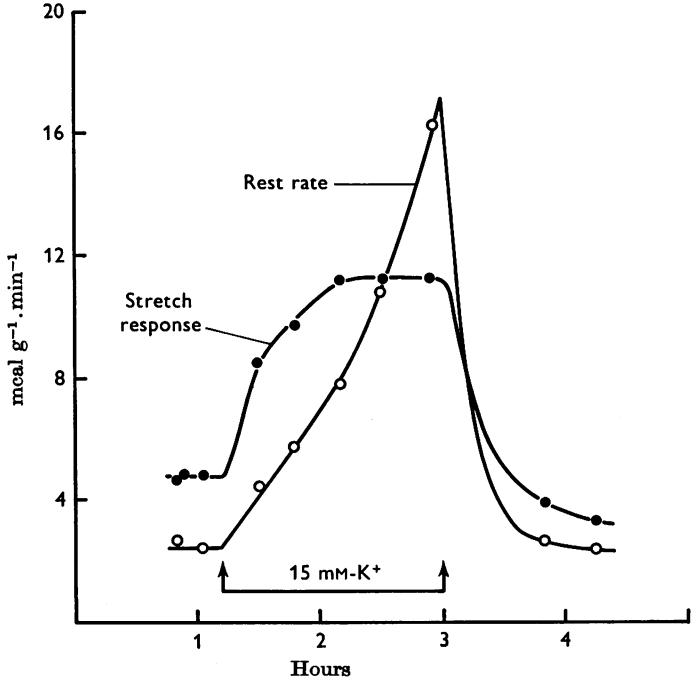

Fig. 7. The effect of high $[K]_0$ on the stretch response and on the rate of heat production at l_{2g}. Ordinates: stretch response (filled circles) and rate of heat production at l_{2g} (open circles), mcal.g^{-1}.min^{-1} Abscissa: time after start of experiment (hours). $[K]_0$ was raised to 15 mM between the times shown by arrows. The stretch response was measured as the increase in rate of heat production 60 sec after 3 mm stretches from l_{2g}.

The muscles were subsequently released back to l_{2g} and the solution was readmitted. In Fig. 7 the curve 'rest rate' refers to the total rate of heat production at l_{2g}: the curve 'stretch response' gives the increase in rate of heat production measured 60 sec after the 3 mm stretches from l_{2g}. It can be seen that the time course of onset of each of the two effects is quite different under the conditions of these experiments.

If the muscles can be treated as infinite plane slabs insulated on one side, then the diffusion equation given by Hill (1965*b*) can be used to test the hypothesis that the rate of rise of the stretch response in the K-rich solution is limited by diffusion of K ions into the muscle.

Neglecting all terms of the series except the first and rearranging this expression becomes

$$= \frac{4\,l^2}{k\pi^2} \ln\left[\frac{4/\pi \cos \pi x/2l}{1-y/y_0}\right],$$

where y is the concentration of K^+ a distance x from the insulated side of the slab whose thickness is l cm, when the other side has been exposed for t sec to a solution of constant K concentration y_0; k is the diffusion coefficient of K in muscle, taken as $4 \cdot 10^{-6}$ cm^2.sec^{-1} (Hill & Macpherson 1955).

If it is assumed that the stretch response in each fibre increases when the surrounding K concentration reaches 12–14·5 mM-K, then by putting this concentration (minus the original Ringer solution concentration of 2·5 mM) = y, and y_0 = (15–2·5) mM, and $x = \frac{1}{2}l = 0{\cdot}035$ cm, substitution in the equation gives a value for t which is an estimate of the half time of rise of the stretch response if this is limited by diffusion. The value of t obtained is 11–20 min for the stated conditions which compares well with the observed half time of about 18 min. The calculation suggests that the time course of the increase in the stretch response may have been limited by the rate of penetration of K into the muscle.

The reason for the slow rise of the rate of heat production at l_{2g} is not known, though it was always seen.

The effect of procaine. A further difference between the 'Solandt effect' and the stretch response lies in their response to local anaesthetics. Euler (1935) showed that cocaine increased the stretch response, while more recently Novotny, Vyskocil, Vyklicky & Beranek (1962) have found that procaine (1 mM) is effective in preventing the increased metabolism caused by 15 mM $[K]_o$. The effect of procaine on the stretch response and metabolism at l_{2g} is shown in Fig. 8. Like quinine sulphate, 3·5 mM procaine hydrochloride caused a slow but large increase in the stretch response. Unlike quinine, however, it was without effect on the metabolic rate at l_{2g}. To avoid the complication of a possible slow fall in membrane potential in the procaine solution (Draper, Friebel & Karzel, 1959), the experiment of Fig. 8 was carried out in isotonic K_2SO_4 solution, though exactly the same results were obtained in normal Ringer solution. The increase in the stretch response caused by procaine was partially maintained on returning to the control solution.

The influence of internal pH. Euler (1935) showed that the stretch response was sensitive to alteration of the extracellular pH, being increased by an increase in pH and vice versa. This has been confirmed, but in addition it has been found that the effect appears to be more sensitive to alteration of the cell's internal pH. If muscles are transferred from a phosphate buffered solution equilibrated with pure O_2, to a solution at the same pH which is equilibrated with a 5% CO_2, 95% O_2 mixture, the internal pH of the cells falls by about 0·3 units (Stella 1929; Caldwell, 1958). Under these conditions a large and reversible fall in the stretch response occurs. Table 1 shows the results of seven experiments of this type. For each solution used in a given experiment the table shows the external pH and the increase in rate of heat production 60 sec after a fixed stretch, Δl, from reference length. The first two experiments show the effect of changing from 5 to 0% CO_2; Expts. 3 and 4 have a further control period in Ringer solution equilibrated with 5% CO_2; and Expts. 5, 6 and 7

are of the form 0% CO_2; 5% CO_2; 0% CO_2. In all cases the stretch response was substantially smaller in the presence of CO_2, though in Expt. 5 the pH of the 5% CO_2 solution was made 0·7 units greater than that of the 0% CO_2 solution. To maintain the pH of the Ringer

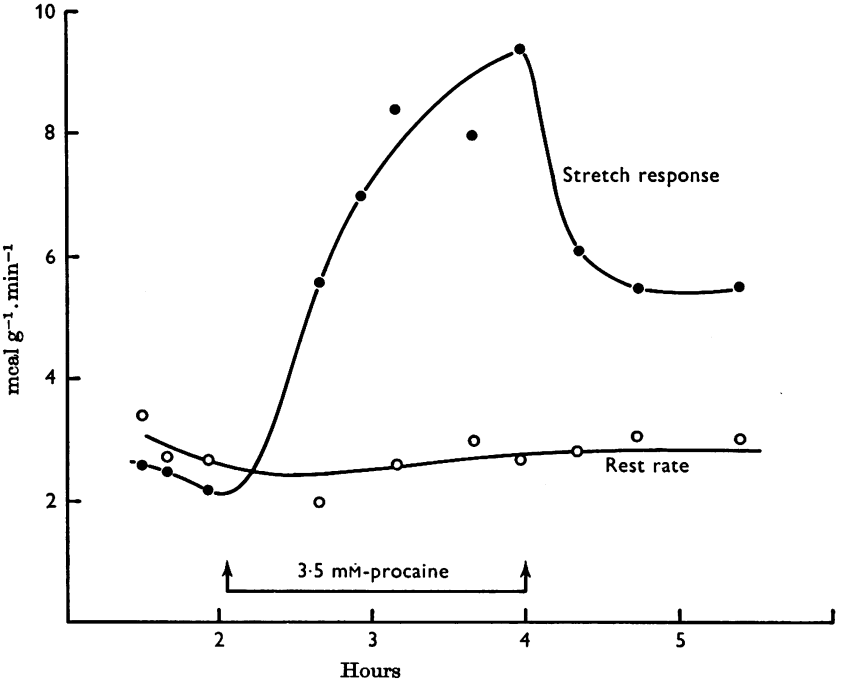

Fig. 8. The effect of 3·5 mM procaine hydrochloride on the stretch response (filled circles), and on the rate of heat production at l_{2g} (open circles). Ordinate: rate of heat production, mcal.g.$^{-1}$ min^{-1}. Abscissa: time after start of experiment. 3·5 mM procaine was present during the interval shown between the arrows. Throughout the experiment the muscles were in isotonic K_2SO_4 solution, except when this was removed to allow temperature readings to be made.

solution in the presence of CO_2, some of the Cl^- was replaced by HCO_3^- according to the relation given by Hill (1965*c*). That the reduction of the stretch response was not due to the presence of the bicarbonate was shown by substituting pure oxygen for the CO_2 mixture bubbling through the bicarbonate solution, whereupon the stretch response rapidly increased in size as the external pH rose.

Species variability of the effect. No stretch response could be detected in sartorii of the common toad, *Bufo bufo*. This is in agreement with the results of unpublished experiments of A. V. Hill, P. Macpherson & J. V. Howarth. Similarly, in agreement with Baskin & Gaffin (1965), though contrary to the findings of Whalen, Collins & Berry (1962), no increase in

rate of heat production was found on stretching sartorii of *Rana pipiens*. As Baskin & Gaffin point out, the system used by Whalen *et al.* to manipulate their muscles may have caused damage to the fibres during stretch with a consequent increase in oxygen consumption. Further evidence for the absence of a stretch response in *R. pipiens* sartorii has come from the

TABLE 1. Effect of 5 % CO_2 on the stretch response (SR) in seven pairs of sartorii. Figures in the 'SR' columns show the increase in rate of heat production after muscles had been held at the long length for 60 sec (units: mcal.g.$^{-1}$ min^{-1}). The muscles were allowed to rest in each solution for about 20 min before being stretched

		5 % CO_2		0 % CO_2		5 % CO_2		0 % CO_2	
Expt.	Δl (mm)	pH	SR	pH	SR	pH	SR	pH	SR
1	3·0	7·0	0·60	7·0	1·88	—	—	—	—
2	4·0	7·2	0·90	7·2	2·20	—	—	—	—
3	4·0	7·1	0·51	7·1	3·30	7·1	1·50	—	—
4	4·0	7·1	1·78	7·1	3·97	7·1	1·76	—	—
5	3·0	—	—	7·1	2·74	7·8	0·85	7·1	2·57
6	3·0	—	—	7·0	1·75	7·3	0·36	7·1	1·50
7	4·0	—	—	7·1	2·01	7·1	0·22	7·1	1·56

work of Sandberg & Carlson (1966), who found that stretches of up to 1·4 l_0 did not increase the rate of creatine phosphate splitting in iodoacetic acid-N_2 treated muscle.

DISCUSSION

In these experiments the metabolic rate of the resting sartorius was found to rise when the muscle's length exceeded about 1·2l_0. This length was found to be indistinguishable from the length at which 'extra tension' appeared. According to Hill (1952), the divergence from the simple exponential tension–length relation occurs at or close to the thermoelastic 'inversion point', where normal thermoelasticity is first seen in the passively stretched muscle. To account for this Hill supposed the muscle to contain an element with short range elasticity which at normal body lengths was slack, bearing tension only at and beyond the 'inversion point'. Hill suggested that this element could be the sarcolemma. Evidence that the sarcolemma does not bear tension at normal body lengths has come from the work of Casella (1950), and of Martin (1954) who showed that conduction velocity in the sartorius is independent of muscle length between 67 and 122 % l_0. He interpreted his finding as being compatible with the existence of a folded surface membrane of constant area, a conclusion supported by Hodgkin (1954). For single fibres from the semitendinosus muscle, Podolsky (1964) has shown that the sarcolemma begins to bear tension when the fibres' sarcomere length reaches 3·2 μ, and that above this length most of the resting tension is borne by the sarcolemma.

To determine the relation between mean sarcomere length and muscle length under the conditions of the present experiments, a conventional light diffraction method has been used, giving the relation between the mean sarcomere length, $\bar{s}$, and length, l/l_0, as

$$\bar{s} = 2{\cdot}40\, l/l_0 - 0{\cdot}02\, \mu \quad (r = 0{\cdot}97).$$

This expression gives a mean sarcomere length at $1{\cdot}2l_0$ of $2{\cdot}86\ \mu$, $0{\cdot}34\ \mu$ less than the length at which the sarcolemma of single *semitendinosus* fibres tightens. However, the whole muscle consists of a large population of fibres whose sarcomere lengths are distributed about the mean value. It is quite possible that some fibres could have sarcomeres of $3{\cdot}2\ \mu$ while the mean length is $2{\cdot}8\ \mu$. In order that 5% of the total fibre population should have sarcomeres at or above $3{\cdot}2\ \mu$ with a population mean of $2{\cdot}8\ \mu$, the S.D. of an individual fibre's sarcomere length would have to be only $0{\cdot}2\ \mu$ if the population were normally distributed. Unfortunately the sarcomere length at which the sarcolemma tightens in *sartorius* fibres is unknown.

It has been assumed that, at least in the part of the muscle which lies on the thermopile, for any single fibre the sarcomere length is constant along its length. This is in accordance with the findings of Carlsen, Knappeis & Buchthal (1961).

If the stretch response is associated with tightening of the sarcolemma in individual fibres, then it follows that the relation between rate of heat production and length may appear very different for a single fibre and for a whole muscle. If the metabolic rate of a single fibre were to rise more or less abruptly when the sarcolemma tightened, then the relation between increase in rate of heat production and length for whole muscle would be a sigmoid curve whose exact shape would depend on the S.D. of the sarcomere lengths in individual fibres. All that can be said is that the curves of Fig. 3 could be the initial parts of such ogives, but since it is impossible to stretch the muscles further without damage the rest of the curve cannot be experimentally observed. Lutz & Brecht (1958) claim that with further stretch the metabolic rate falls, only to rise again at extreme length. Unfortunately they did not repeat their measurements of oxygen consumption at shorter lengths after these large stretches so the possibility of damage cannot be excluded.

How could a rise in tension in the sarcolemma lead to an increase in metabolic rate? One possibility is that a change in surface energy, or a disorientation of fixed changes in the membrane might result in looser binding of some substance involved in excitation-contraction coupling processes. In this connexion it is interesting that all the Class A active state potentiators that have been tried (Sandow, 1965) have potentiated the metabolic response to stretch. These substances are thought to act by

increasing the effectiveness of a given membrane depolarization in releasing Ca ions into the cytoplasm (Sandow, 1965).

Further evidence that the stretch response is in some way concerned with the contractile process has come from experiments in which it has been found that the presence of the effect is associated with an increase in the time constant of relaxation in isometric twitches. At long lengths where the stretch response occurs, reduction of the effect by introducing 5 % CO_2 at constant external pH is associated with a decline in the time constant of the exponential part of relaxation by as much as 50 %. At normal body lengths ($< 1{\cdot}2l_0$), CO_2 is without effect on relaxation. It is interesting that this effect of CO_2 on the shape of the twitch at long lengths is almost absent in sartorii from *Bufo bufo*, which, as previously mentioned, do not show a stretch response. These experiments will be described more fully in another paper.

If stretch were to bring about activation of the actomyosin adenosine triphosphatase and active tension development similar to that seen in insect flight muscle (Jewell & Rüegg, 1966), then the stretch response would be explained as the metabolic turnover caused by the increased rate of ATP-splitting. On this view the 'extra tension' would be the active force developed, and since contracting muscle is known to exhibit normal thermoelasticity, the coincidence of the first sign of the stretch response (and 'extra tension') with the thermoelastic inversion point would be accounted for. However, it seems likely that a structural interpretation of the 'extra tension' and inversion point is correct. If sartorii of *R. temporaria* are cooled to 0° C, the divergence from the simple exponential tension–length relation occurs at a somewhat greater length than at room temperature (*ca.* $1{\cdot}25l_0$), though above this length the appearance of 'extra tension' is just as marked as at 20° C (Hill, 1952). The stretch response, on the other hand, becomes extremely small at 0° C. Similarly, while procaine and the active state potentiators markedly increase the stretch response, they are without effect on the resting tension–length curve. Toad sartorii do not show a stretch response at all, though they have tension–length curves very similar to those of the frog, with the usual appearance of 'extra tension' above $1{\cdot}2l_0$.

No explanation can be offered for the striking effect of procaine on the metabolic response to stretch; its action develops slowly, but this need not mean that it is acting intracellularly since, according to Falk (1961), its effect on the action potential is similarly slow.

It is difficult to see why the small fall in internal pH which is caused by 5 % CO_2 should be so effective in reducing the stretch response; even a CO_2 concentration as low as 1 % was found to cause a definite reduction of the effect. However, the stretch response appears to be accompanied by a rise

in extracellular pH (Margaria, 1934: Dubuisson, 1940) and it is possible that the CO_2/HCO_3^- buffer acts by preventing this alkalization, since the buffer capacity of this mixture is greater than that of the 3 mM phosphate buffer otherwise used.

According to Harris (1954), stretch causes an increased rate of Na extrusion from tracer-loaded sartorii of *R. temporaria*. Increased activity of the Na-pump is unlikely to be the cause of the stretch response since this persists unchanged after 24 hr soaking in isotonic K_2SO_4 solution. The most probable explanation is that stretch in some way causes an increase in free Na concentration in the cytoplasm. A similar situation exists in the Solandt effect, where the increase in metabolism is also accompanied by increased active extrusion of Na (Horowicz & Gerber, 1965). In this case the increase in rate of Na extrusion can be prevented by 10^{-5} M strophanthidin, without measurably affecting the increased metabolism (N.F. Clinch & J. M. Dutton, unpublished).

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