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THE BEHAVIOUR OF FROG MUSCLE IN HYPERTONIC SOLUTIONS

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It is well known that hypertonic solutions diminish, and in sufficient concentration abolish, the twitch of frog muscle, whereas *hypotonic* solutions within a certain range enhance the twitch (Overton, 1902; Fenn, 1936; Hodgkin & Horowicz, 1957). This paper describes some mechanical measurements made with muscles exposed to solutions up to the equivalent of three times the concentration of normal Ringer's solution, and to *hypotonic* solutions.

METHODS

All the experiments described were made with sartorii of English frogs, male and female: no seasonal variations were noticed. Similar results were obtained with the sartorii of toads.

Most of the experiments involved measurement of the speed of shortening or of the tension developed. Shortening was recorded by means of a light isotonic lever of low inertia bearing a vane which cast a shadow on a twin phototube (Hill, 1951). The potential change arising from movement of the shadow was amplified by a Kelvin and Hughes amplifier Type 6 and displayed on a smoked drum by a Kelvin and Hughes pen recorder Mk. V. Tension was recorded by means of an R.C.A. 5734 transducer triode and similarly displayed on a smoked drum or, when high amplification and speed were required, photographically from a cathode-ray tube.

In early experiments muscles were mounted on a multi-electrode assembly to eliminate the effect of any possible change in the rate of propagation of the contractile wave. This was later found unnecessary and a simple holder bearing two platinum electrodes was adopted, exposing both faces of the muscle to the solution, thus allowing quicker equilibration. Stimulation was direct.

The normal Ringer's solution was: (mM) NaCl, 115·5; KCl, 2·5; CaCl, 1·8. *Hypotonic* solutions were made by adding the calculated amount of distilled water. Hypertonic solutions were of two kinds in which the excess osmotic pressure was provided, (*a*) by sucrose and (*b*) by salts. 'Sucrose hypertonic Ringer' was made by dissolving solid sucrose in normal Ringer's solution, assuming 220 mM-sucrose to be isotonic. Salt-enriched solutions were made by dilution of stock solution of 10 × the concentration of standard Ringer's solution in all constituents. Buffering was provided by 2 mM sodium phosphate buffer at pH 7·2. Throughout this paper concentrations are expressed relative to Ringer's solution, e.g. 2·5 × R, or 0·7 × R. Where the word '*hypotonic*' appears it is italicized to make it more easily distinguished from 'hypertonic'.

Experiments were made at 0° C and in the region 18–20° C. There was no qualitative difference between results at the two temperatures.

RESULTS

The concentration of solutions used was often very far from that of Ringer's solution (from one half to three times) and the muscles underwent considerable shrinking or swelling. They suffered little or no permanent damage by such treatment, and recovery was rapid in normal Ringer's solution, even after 6 hr exposure.

Velocity of shortening

Isometric twitches recorded while a muscle is equilibrating with a hypertonic solution show a progressive decline of peak tension, and a later and slower take-off. This suggested that the velocity of shortening was reduced. The first experiments were made to examine that possibility.

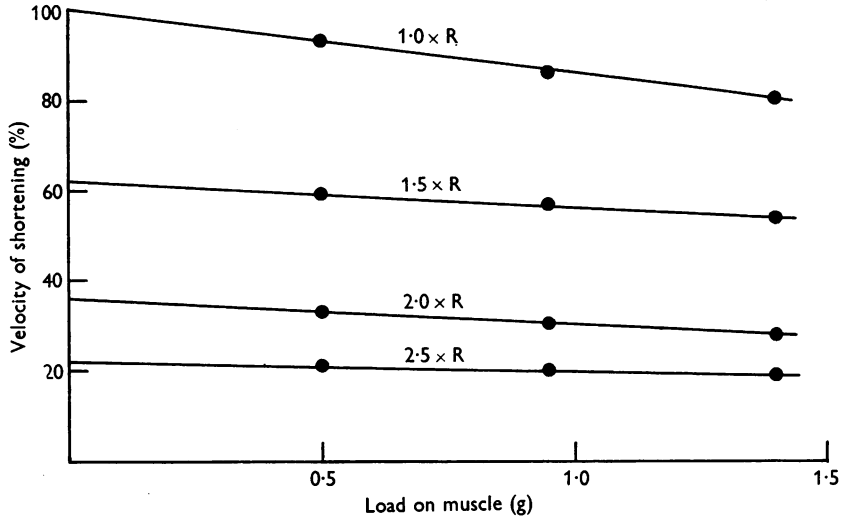

Fig. 1. Velocity of shortening as a function of load in tetanic contraction at 19° C. Muscles exposed to concentrations shown on curves. Velocity expressed as percentage of maximum velocity in Ringer's solution.

Speed of shortening depends on the load, and on the strength of a muscle, except for zero load when speed is independent of strength: so to avoid errors due to feebler contraction in hypertonic solutions, it was necessary to measure speed at zero load. The condition of zero load cannot be realized experimentally, so velocity has here been measured with the muscle shortening against very small loads (0·5–2 g). From these values a portion of the load–velocity curve was extrapolated to give velocity at zero load (intrinsic speed). The intrinsic speed so obtained was greatly diminished by hypertonic solutions. For the results shown in Fig. 1 different muscles were used for the different concentrations, so the velocity is expressed as percentage of the velocity at

zero load in normal Ringer's solution. In every case the muscles regained their original speeds within 15 min of being returned to normal Ringer's solution; recovery is, in fact, nearly complete in 5 min. Fig. 1 was obtained from experiments in which the concentration was raised by electrolytes, but similar results were obtained in sucrose hypertonic Ringer.

Experiments made with *hypotonic* solutions showed no effect on the velocity of shortening.

Isometric tension

The strength of a muscle is diminished in hypertonic solutions. At body length the isometric tension in a tetanus was reduced, in $2\frac{1}{2} \times$ R, to about 30% of that in Ringer's solution. Fig. 2 shows the relation between tetanic isometric tension of a single sartorius and the concentration of the bathing fluid. As was expected from observations of the speed of shortening, tension developed

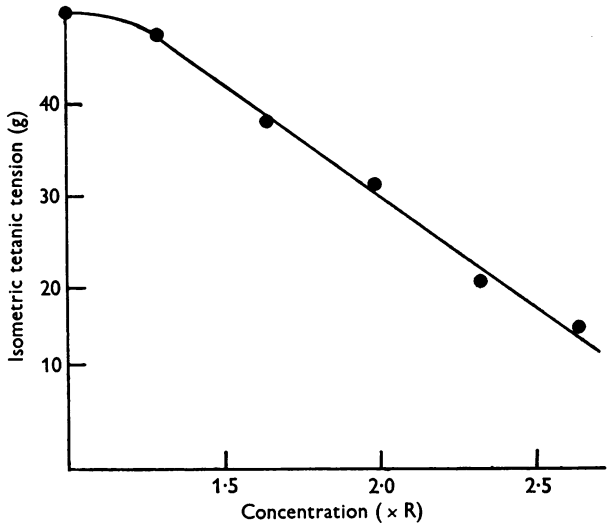

Fig. 2. Effect of concentration on the maximum isometric tension of frog sartorius. Tetanus; 18° C.

extremely slowly in concentrated solution. In $2\frac{1}{2} \times$ R a 6 sec tetanus was needed to achieve maximum tension at room temperature and a much longer one at 0° C.

Isometric tetanic tension, like velocity of shortening, is not changed by *hypotonic* solutions down to about $0{\cdot}6 \times$ R.

Latent period

If part of the latent period represents the time needed for a muscle to take up internal slack, it was to be expected that, in hypertonic solutions, when the velocity of shortening is reduced, the latent period would be extended: this

proved invariably to be the case. The latent period was measured by recording tension in the early part of a twitch with high amplification and measuring the time from stimulus to the first detectable rise of tension. Fig. 3 is a curve of latent period against concentration. No attempt was made to measure latency relaxation, but Professor A. Sandow has informed Professor A. V. Hill that latency relaxation could not be detected in a muscle after the twitch had been abolished by 3 × R.

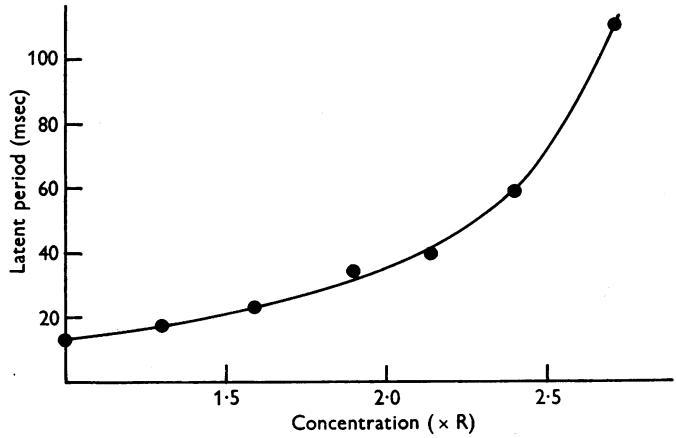

Fig. 3. The effect of concentration on the latent period. Frog sartorius; 0° C.

Abolition of the twitch

If the concentration of the surrounding fluid is raised to more than $2\frac{1}{2}\times$ to 3 × R, a muscle fails to twitch in response to a single stimulus. Nevertheless, a series of stimuli does evoke a response in the form of a slowly rising tension. Hill (1950) used small, rapid stretches to reveal the presence of a mechanical response before any actual tension had developed after a stimulus. The same technique was applied here to see if any mechanical response to a single stimulus could be detected after the usual twitch response had been abolished by hypertonic solution. Tension was recorded by means of a transducer mounted on the arm of a Levin–Wyman ergometer (Levin & Wyman, 1927) which applied small stretches to the muscle soon after the stimulus. A mechanical response showed as an increased resistance to stretch. The amount and speed of the stretch were varied and in each case the tension in a stimulated muscle was compared with that for a similar stretch without a stimulus. Each stretch began from the same initial length, the muscle having been previously tetanized at that length to take up any slack remaining from a previous stretch. Sometimes the stretch with stimulus preceded that without, sometimes the reverse. The result was always the same; the muscle exhibited greater resistance to stretch when stimulated than when not stimulated. Fig. 4 is a typical result

of such an experiment. This clearly demonstrates the presence of a mechanical response but provides no measure of it. It was possible that the slow rise of tension in a tetanus after abolition of the twitch was due to some sort of recruitment of activity by the successive stimuli. To test this the tension was

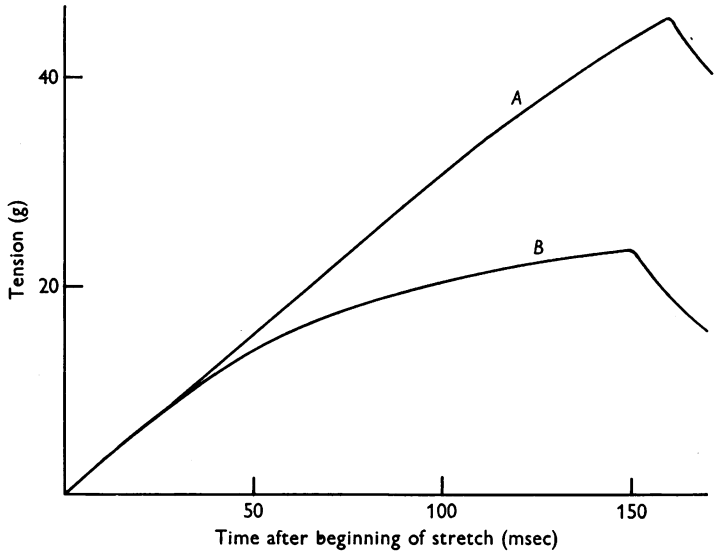

Fig. 4. Tension during 2·3 mm constant speed stretches; *A* stimulated by single shock, *B* not stimulated. The muscle was in 3 × R and produced no tension for a single stimulus without a stretch.

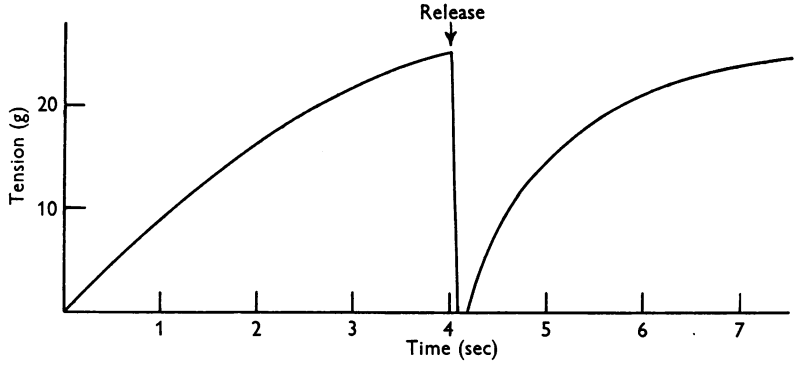

Fig. 5. Tension during a maintained tetanus showing redevelopment after a quick release of 2·3 mm. Frog sartorius in 3 × R.

recorded during a tetanus in which the muscle was suddenly released a few millimetres when the tension was high; the redevelopment of tension was then compared with the initial development. Fig. 5 is a typical record where it is seen that redevelopment, though not so slow as the original rise of tension, was still very slow compared with that of muscles in normal Ringer's solution.

Duration of the active state

Conventional methods for measuring duration of the active state (Hill, 1953; Macpherson & Wilkie, 1954; Ritchie, 1954) are not applicable to muscles in the more concentrated solutions because of their extreme slowness, but it is possible to use Ritchie's quick-release method with muscles which have undergone only moderate dehydration. In experiments with 1·5 × R at 0° C the active state was slightly and consistently prolonged. The twitch reached its peak 40–60 msec later than in normal Ringer's solution and the active state decayed with a time course similar to normal but delayed throughout by the same amount.

For muscles so dehydrated that the twitch was abolished a rough guide to the duration of the active state was sought by recording tension in quick stretches as described above. For this purpose the stretch was begun at various times after the stimulus. A stretch applied after the active state had completely decayed could not produce a tension different from that produced by a stretch without a stimulus. In these experiments, made at 0° C in 3 × R, a stretch beginning 400 msec after a stimulus always produced more tension than a stretch without a stimulus, but the result of a stretch after an interval of 600 msec did not differ from that of one without a stimulus. Ritchie (1954) found that the normal active state fell to zero at about 500 msec. The present experiments, then, do not show any marked effect of hypertonic solutions on the duration of the active state.

For muscles in *hypotonic* solutions the method of Macpherson & Wilkie (1954) was used to find the duration of the plateau of the active state. As in the case of hypertonic solutions the observed effects were small. At 18° C the plateau lasted for 16·5 msec in normal Ringer's solution and in 0·62 × R it was extended to 21·8 msec.

DISCUSSION

The most striking result of exposing the muscle to hypertonic solutions is the greatly reduced intrinsic speed. Not only was shortening slowed but the relaxation following isometric or isotonic contraction was prolonged. The muscles felt stiff when handled and displayed some degree of lateral rigidity. The considerable tension developed when the unstimulated muscle was stretched (cf. Fig. 4) is another indication of stiffness; a normal frog's sartorius would have developed little or no tension for a similar stretch. All these facts give the impression of a greatly increased viscosity.

One way in which such enhanced viscosity could arise is by close packing of the I and A filaments (Huxley, 1957) and other formed elements of the fibre. As water is withdrawn and the fibre diameter shrinks some packing must occur which may lead to friction between parallel sliding structures. Dr H. E. Huxley kindly prepared some sections for the electron microscope, but these

did not furnish any new information because of the expected difficulty, that shrinkage during fixing and staining was greater than that in the most concentrated solutions used during the present experiments.

Another factor to be considered is the new ionic concentration within the fibre which must initially increase in proportion to water loss. It would not be surprising if the contractile proteins were seriously affected by this change. Solutions of actomyosin and actomyosin threads are very sensitive to their ionic environment (Perry, 1956). For example, aqueous solutions of G-actin which are quite mobile polymerize and become viscous with the addition of univalent ions. Further, the ability of actomyosin threads to complete their cycle of contraction and relaxation depends on the relative concentration of calcium and magnesium ions. The decline of isometric tension may also be a result of the changed ionic concentrations within the fibre.

There is no doubt that at the higher concentrations the normal isometric twitch is entirely abolished (Fenn, 1936), although the action potential persists (Hodgkin & Horowicz, 1957; Ishiko & Sato, 1957). At first sight this suggested that the link between action potential and contractile process had been broken. The stretch experiments described above do not support that view. They reveal a mechanical response to a single stimulus which implies that the link is intact. The latter view is supported by the observation (Hill, 1958) that the 'activation heat', that part of the heat in a twitch which accompanies the setting up of the active process, is substantially unchanged by hypertonic solutions even when the twitch is absent and there is no shortening heat or work.

Hodgkin & Horowicz (1957) have drawn attention to the slowness of the rise of tetanic tension in dehydrated muscle. This would result if the active state was not fully established by a single stimulus but needed several stimuli to reach a maximum. This is not known to occur in normal frog sartorius, but there is evidence that it does in lamellibranch smooth muscle (Abbott & Lowy, 1958; B. R. Jewell, personal communication). The release experiments were made to see if such recruitment caused the slow tension development in hypertonic solutions. The release occurred when the tension, and therefore the activity, was maximal so subsequent redevelopment of tension would not then have to wait upon the further accumulation of activity but would rise rapidly to its former level. In fact this did not happen; redevelopment was very slow (Fig. 5). This indicates that recruitment of activity is not responsible for the slowness of the rise of tension in a tetanus. That is not to say that there was no recruitment at all; in fact the redevelopment was always, as in Fig. 5, slightly quicker than initial development of tension. However, that is also seen in normal frog muscle (Jewell & Wilkie, 1958), and Hill's observation of the full activation heat (Hill, 1958) for a single stimulus in $3 \times R$ tells against any idea of recruitment.

The duration of the active state, so far as it can be measured in hypertonic Ringer, is not much different from that of normal muscle. The present experiments also fail to show appreciable change in *hypotonic* solution. In some other experiments, when duration of the active state was not being measured, I have seen considerable increase of twitch tension and duration when the Ringer's solution was diluted. This was not due only to the paucity of ions, for the effect was reversed by making up the concentration again with sucrose. Other authors have recorded similar observations (Fenn, 1936; Lullmann & Muscholl, 1954) and it is tempting to explain them by supposing a prolonged active state. However, under the conditions of the present experiments the prolongation is too small to allow that explanation.

The frog's sartorius in hypertonic solution resembles certain smooth muscles in some of its properties, e.g. the slowness of contraction and relaxation and the response to stretch. Unlike the normal sartorius, the muscle in hypertonic solution shows considerable resistance to small stretches without a stimulus (Fig. 4). This is typical of many smooth muscles, notably those of the marine lamellibranchs. Also, as in these muscles, the decay of tension following such stretch, i.e. stress relaxation, is very long-lasting. Qualitatively at least the muscle in hypertonic solution obeys the relation described by Abbott & Lowy (1957) for several smooth muscles, i.e. the rate of stress relaxation increases with intrinsic speed.

SUMMARY

1. The mechanical properties of frog's striated muscle have been examined in solutions of different concentrations.

2. The velocity of shortening is greatly reduced by hypertonic solutions but is not affected by *hypotonic* solutions down to half the concentration of normal Ringer's solution.

3. The maximum tetanic tension is reduced by hypertonic solutions but not affected by *hypotonic* solutions.

4. In solutions of $2\frac{1}{2}$–3 times the concentration of normal Ringer's solution the twitch response to a single stimulus is abolished. Nevertheless, the action potential still provokes activity in the contractile proteins. It is suggested that failure to develop tension to a single shock is due to the great slowness. The muscle is not able to extend the series compliance sufficiently in the brief period of the active state.

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