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CHANGES IN TRANSPARENCY OF MUSCLE DURING A TWITCH

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The first sign of mechanical activity in a stimulated frog's muscle is a transient lengthening (the 'latency relaxation'; Sandow, 1944) which starts at about 2 msec. (18° C.) and reverses into a positive contraction a few msec. later. The heat production of the muscle starts roughly at the same time as the early relaxation (measured at 0° C.; Hill, 1948). The transparency change which accompanies the muscle twitch (Schaefer & Göpfert, 1937; Buchthal, Knappeis & Sjöstrand, 1939) has not hitherto been recorded with sufficient amplification for it to have been possible to discover when it starts, nor the manner in which it does so.

The work described below is concerned with the *earliest* transparency changes following the application of a single electric shock to a frog's skeletal muscle. In addition, brief notes are added in connexion with: (*a*) the long-drawn-out transparency change which *follows* the contraction (von Muralt, 1934*a*, *b*); (*b*) a transient effect on transparency caused by a rapid stretch of the muscle.

Urban & Peugnet (1938) have reported wave-length-specific absorption changes in contracting frog's muscle, and have attributed them to cytochrome *c* and the yellow enzyme. It is not thought that their observations bear much relation to the phenomena described here.

METHODS

When light passes through a muscle it is partly absorbed, but the loss of intensity in the incident direction is largely caused by scattering (Buchthal *et al.* 1939). The first series of experiments was made without attempting to distinguish between these two factors, and a simple optical arrangement was used (Fig. 1*a*); this was suitable for relating the time of onset of the earliest transparency change with the mechanical response. A further analysis demanded a differentiation between absorption and scattering. The measurement of absorption alone by means of a modified 'ball photometer' (Buchthal *et al.* 1939), although an ideal method in principle, was not practicable with a whole muscle. The arrangement adopted is shown in Fig. 1*b*. A small telescope, focused for parallel light, was mounted on an arm which could be rotated on a vertical axis. The muscle was held vertically on this axis. A photocell of the 'multiplier' type was fixed to the eyepiece of the telescope. By rotating the telescope it was possible to measure the intensity of the light transmitted

at a series of angles, 0–90°, from the incident direction. The effect of a change of scattering was found to reverse its sign as the telescope was swung round; e.g. an increase in scattering gives a lower transmission in the incident direction but a greater intensity laterally. An effect caused by a change in absorption should have the same sign at all angles.

The frog's sartorius muscle was used. The light source was a 6 V. 36 W. tungsten lamp, overrun at 8 V. to give a high intensity, and supplied by batteries. The light was condensed with a lens to give a parallel beam. The muscle was free in air and kept moist by flowing Ringer solution over its surface between observations; the illuminated patch was about 7 mm. long. In the later experiments with monochromatic light *Farrand* 'interference' filters were used, a set of thirteen of these filters covering the visible spectrum from 400 to 700 mμ.

The output of the photocell passed to a condenser-coupled amplifier, followed by a Mullard E 800 cathode-ray oscilloscope. The mechanical record of the latency relaxation for comparison with the optical events was made with a crystal gramophone pick-up. The muscle was connected with a glass lever, 1 cm. long, inserted in the needle holder of the pick-up, and was tightly stretched to minimize any optical effect due to localized thickening or movement as the contraction wave passed along the muscle.

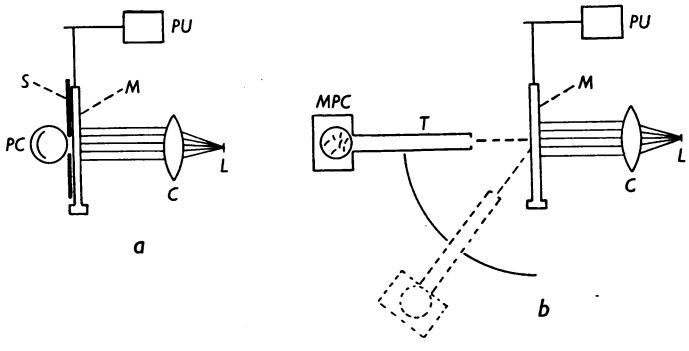

Fig. 1*a*. Simple optical arrangement. Tungsten lamp, *L*. Condenser lens, *C*. Muscle, *M*. Slit, *S*. The photocell, *PC*, is of the single-stage vacuum type. The crystal pickup, *PU*, measures the mechanical response.

Fig. 1*b*. The arrangement for 'directional' recording. *L*, *C*, *M* and *PU*, as in Fig. 1*a*. Telescope, *T*. Multiplier photocell, *MPC*. The long axis of the muscle is actually at right angles to the plane of the page, and not as shown.

Stimulation of the muscle. The muscle was stimulated by a single short discharge from a 0·03 μF. condenser, with the muscle shunted by a 1000 Ω. resistance. With a shock as brief as this the muscle may be assumed to be excited within 0·1 msec., and this delay time is considered as negligible. The condenser was charged and discharged by means of a Siemens high-speed relay. A second Siemens relay operated in series with the former by a hand key triggered the time base of the oscilloscope. The time interval between the moments of closure of the two relays was found to be less than 0·1 msec. The stimulating electrode consisted of a thin stainless steel wire stretched against the glass window in front of the photocell, the muscle being brought into contact with this window; or, in the 'directional' recording with the photomultiplier, the stimulating wire was held against the muscle with light pressure with a flexible support. The anodic connexion was made through the supporting stand. In both cases the point of stimulation lay in the centre of the illuminated patch of muscle. The muscle was not curarized.

Photocells. A single-stage vacuum cell (type VB39: Cinema Television Ltd.) was used for the experiments with the simple optical arrangement. In the 'directional' experiments the light entering the photocell was of much lower intensity, and a 9-stage photomultiplier (RCA931-A)

replaced the single-stage cell; it was run at 720 V., giving a 6000-fold amplification of the primary photocurrent.

The output from either type of photocell was passed through a series resistance, and the voltage across this resistance applied to the grid of the first amplifier valve. The values of the circuit components shown in Fig. 2. were determined by the requirements of a wide-frequency response combined with a low noise level.

Vibration of the building caused high-frequency mechanical interference which was sometimes greater than the electronic noise, but this was largely overcome by mounting the table on sponge rubber.

Electronic noise. The limiting factor in amplification was the electronic noise. There are two sources of noise: (*a*) the 'shot' noise caused by random fluctuations in the primary photocurrent; (*b*) the 'Johnson' noise from the resistance in series with the photocell. 'Shot' noise in the first valve is not important when using a high-gain amplifier. The use of a multiplier photocell effectively eliminates the 'Johnson' noise, because the 'shot' noise from the primary photocurrent greatly exceeds it after being amplified non-resistively in the photocell (Preisach, 1939). In a single-stage photocell the 'Johnson' noise preponderates over the 'shot' noise when the light intensity is below a certain level, and it is then that the multiplier photocell is superior. For this reason the photomultiplier was used in the 'directional' experiment when the light intensity was very low. Reduction of noise also requires that the width of the frequency band amplified should be as narrow as it is possible to make it without introducing distortion of the signal; and the light intensity should always be as high as possible. When using white light and 'non-directional' recording the noise was thus practically eliminated. On the other hand, when the transparency change was recorded by the 'directional' method, the earliest phase was not entirely free from noise. With monochromatic light the noise even with 'non-directional' recording was sometimes severe (see Fig. 7), but with 'directional' recording it overwhelmed the signal, and the latter combination could not be used.

Optimum thickness of muscle. The use of a thicker muscle would increase the modulation of the photocurrent, but this does not necessarily improve the signal-to-noise ratio. With an incident light of I_0 and an extinction coefficient of k, the intensity of the transmitted light is given by $I = I_0 e^{-kd}$, where d is the length of the light path in the muscle. The noise can be taken as having an amplitude $A\sqrt{I}$ (where A is a constant), and the signal expressed as BId (where B is a constant). The signal-to-noise ratio is therefore $B/A\sqrt{(I_0)}\,de^{-\frac{1}{2}kd}$. This quantity has a maximum for a certain optimum value of d. Calculation shows that for frog's muscle, and directional recording in the line of the incident beam, this optimum is about 0·7 mm. for blue light, and 1·9 mm. for red light; it is consequently only about twice the thickness of a sartorius muscle. The function has a rather broad maximum, and there was little to be gained by using a double thickness of muscle.

Frequency response. (*a*) *Transparency.* When the single-stage photocell was used the capacity from the grid of the first valve to earth was made up of 'stray' capacity. The time constant was measured by injecting an impulse into the system, and recording its delay on the oscilloscope. The R.C. of the circuit for $R = 1$ MΩ. (Fig. 2) proved to be 0·18 msec., which was small enough to give an undistorted record. In the case of the multiplier photocell with R at 100,000 Ω., shunted by a capacity of 0·001 μF., the calculated time constant was 0·1 msec. In both circuits the low-frequency response was determined by the coupling capacity (0·02 μF.) and the grid-leak resistance (100 MΩ.), the R.C. being 2 sec. The Mullard E800 oscilloscope itself has a low-frequency response corresponding to an R.C. value of 4 sec. (*b*) *Mechanical record.* The circuit used in conjunction with the crystal pick-up is shown in Fig. 2. The time constant of 100 msec. was sufficiently long to record the early relaxation with practically no distortion.

The delayed transparency change. A few experiments were made with d.c. amplification to record the transparency during the twitch and for some seconds afterwards. von Muralt (1934*a*, *b*) described a change produced by stimulation, and which decayed in a few minutes, but he did not use a high-frequency recording system and was unable to observe the onset of the change or the manner in which it followed on from the change which accompanies the twitch itself.

Rapid stretch. A curious transient change in transparency was seen in a muscle that was rapidly stretched. The stretch was applied by connecting the tibial end of the muscle with the moving arm of a Siemens high-speed relay, the movement being about 100 μ. and varied by adjusting the contact stops. The closing time of the unloaded relay is under 1 msec., but when loaded with a muscle under tension the delay is probably greater than this. A strip of rubber of approximately the same dimensions and opacity as the muscle was used as a control. The 'directional' recording system was employed. To damp out high-frequency oscillations which were caused by the sudden snap of the relay the muscle (or the rubber strip) was suspended with its surface in contact with a glass plate, the interface being kept moist with Ringer solution. Even when damped in this way the oscillations were fairly severe, as can be seen from the records (Fig. 9).

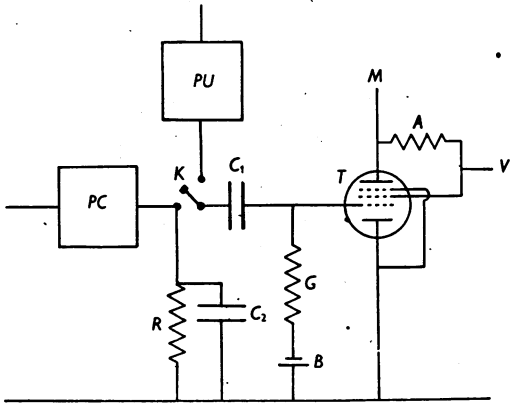

Fig. 2. Circuit for use with photocell, *PC*, or crystal pickup, *PU*. Coupling capacity, C_1, 0·02 μF. Grid leak resistance, *G*, 100 MΩ. Bias battery, *B*. Preamplifier valve, *T* (EF50, pentode): screen at voltage *V*, 250 V.; anode load, *A*, 20,000 Ω. Amplified voltage passes to Mullard oscilloscope, *M*. The key *K* selects either *PC* or *PU*. When the single stage photocell is used the resistance *R* is 1 MΩ., and the condenser C_2 is removed. When the multiplier photocell is used *R* is 100,000 Ω., shunted with capacity C_2, 0·001 μF.

RESULTS

Correlation of transparency with mechanical changes. To ensure the greatest possible freedom from noise the 'non-directional' optical system was used, with the single-stage photocell, and white light. Fig. 3 shows photographs of the transparency change and of the early relaxation. To give a closer comparison seven such records were meaned and plotted together (Fig. 4). The optical and mechanical changes appear to start at the same time, within a fraction of a msec. Fig. 3 also shows the transparency change with lower amplification and slow time base; the initial change in the vertical direction can just be seen. The residue at the end of the twitch, showing as a positive displacement from the base line, is not a real change in transparency, but is an artefact due to the amplifier being condenser-coupled; the real residue is actually in the opposite direction.

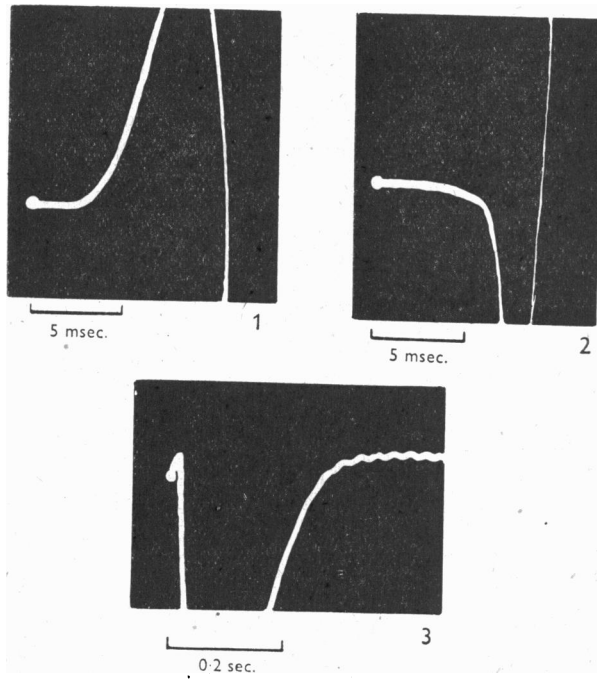

Fig. 3. 1, the opacity change with high amplification. 3, the same, but with low amplification. 'Non-directional' recording. The positive direction denotes an increase in photocurrent. 2, the mechanical record, showing the early relaxation.

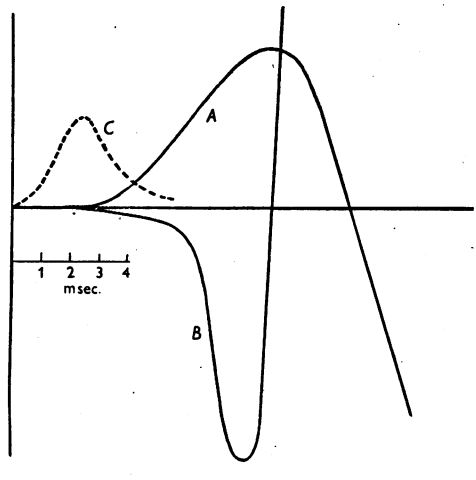

Fig. 4. Averaged records of opacity change, *A*; and of mechanical response, *B*. The time course of the action potential is also shown, *C* (not recorded, but as given by Katz, 1941).

The form and magnitude of the transparency change are unaltered when the stimulating electrode is removed out of the light beam. There can therefore be no possibility of the stimulating current itself being responsible for any part of the effect.

The action potential for the whole sartorius muscle is also shown in Fig. 4. (It was not recorded, but copied from Katz, 1941.) At present no particular significance can be attached to the fact that the peak of the action potential and the onset of the other events appear to be roughly coincident, for it has yet to be shown that this relation holds good under other conditions, e.g. at 0° C., and in any case the rising phase of the action potential of a single fibre (Kuffler, 1942) is probably more rapid than it is in the whole muscle.

Magnitude of the transparency change. If I denotes the resting photocurrent and ΔI the change on stimulation, the ratio $\Delta I/I$ is a measure of the effect. Two phases are considered, the early one during the early relaxation and the opposite phase which occurs later. With 'non-directional' recording the peak values are approximately:

	$\Delta I/I$ first peak	$\Delta I/I$ second peak
Violet light	1:1500	1:25
Red light	1:3000	1:50

The time course of the transparency change does not depend on the colour of the light.

Differentiation between absorption and scattering. The 'directional' method was used. The main transparency change, as seen with low amplification, is largely a scattering effect, and it reverses its sign as the telescope is swung from 0 to 90° (Fig. 5). The delayed transparency change, as described by von Muralt, also reverses; this is not clear from records taken with the relatively short time-constant amplifier, but the reversal is easily detected on the micro-ammeter which is in series with the output stage of the photomultiplier. Similarly, the effect of a small change of length is reversed as the telescope is moved from 0 to 90°.

On the other hand, the early optical change which starts with the early relaxation does *not* reverse in the same way (Fig. 5). At an angle of 0° to the incident beam it has the same sign as the much larger change which follows it; at 30° the secondary scattering effect is in the transition stage, and at 60° the two phases are in opposite directions (as they were with the 'non-directional' optical system). The effect at 90° is the same as at 60°.

The above observations lead to the conclusion that the early increase in transparency is essentially different from the later change. The latter, in view of its ready reversibility, must be caused by a change in the scattering power of the muscle, and its sign shows that it cannot be attributed to a localized thickening of the muscle at the electrode as the contraction commences, although it seems likely that changes in thickness of the muscle must to some extent affect

the transparency in the later stages. It is deduced also that the first effect cannot be due to the lengthening of the muscle during the early relaxation, as this should reverse with the angle; in any case, it can be calculated from Sandow's (1944) results that this lengthening should give less than one-thirtieth of the observed change at an angle of 0°. Consequently one might conclude that the

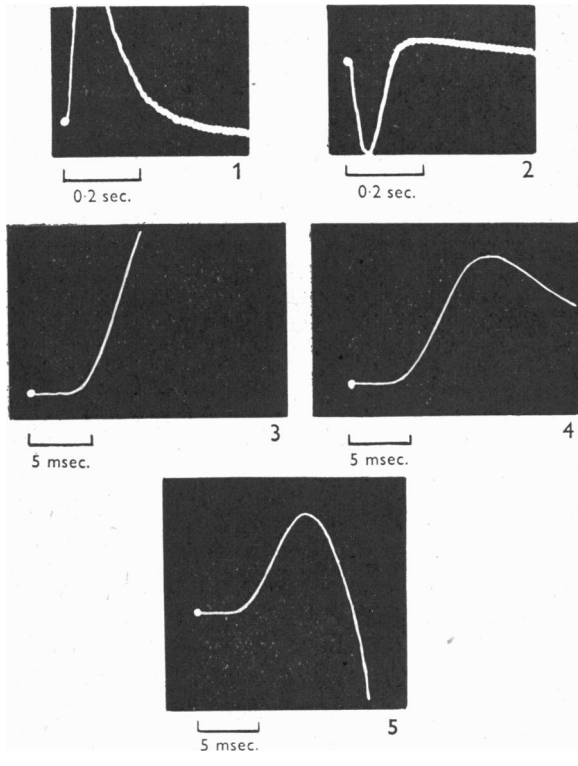

Fig. 5. The results with the 'directional' method. Records 1 and 2 show the response with low amplification, and with the telescope at 0° and 90° from the line of the incident beam. Records 3, 4 and 5 are with high amplification at 0, 30 and 60° from the line of the incident beam. The positive direction denotes an increase in photocurrent.

first phase of the optical change is caused by a decrease in *absorption*. Further evidence on this point was hoped for from an examination of the spectral distribution of the effect. The demonstration of absorption bands, or of a characteristic spectrum, would prove it to be an absorption phenomenon, as distinct from scattering, and might lead to the identification of the earliest reaction in stimulated muscle.

The spectral distribution of the first effect. At each wave-length the cathode-ray tube was photographed, the trace projected on squared paper and the

slope of the initial rise measured. The values so obtained were scaled to allow for variations in the light intensity at the different wave-lengths. The results of five experiments, with a total of 150 observations, are shown in Fig. 6. There does not appear to be a characteristic absorption spectrum, although it is possible there is a maximum around 450 mμ. The scatter in the results is largely caused by 'noise' which cannot be avoided when using monochromatic light.

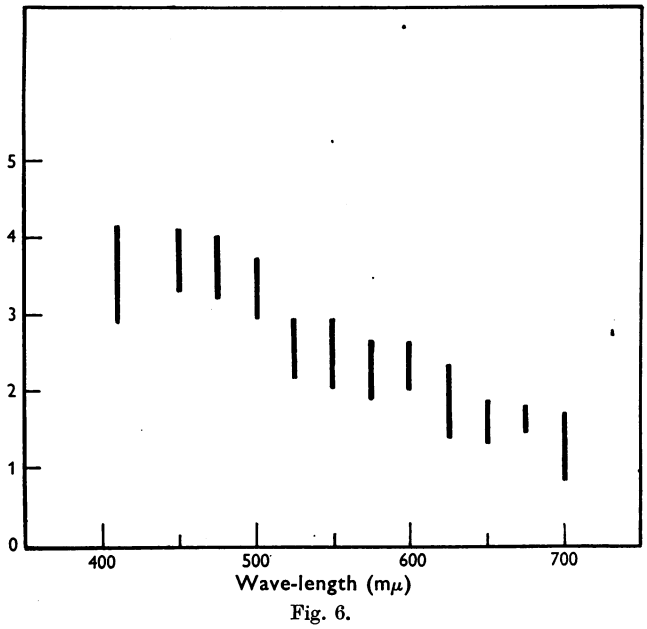

Fig. 6.

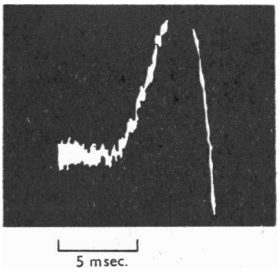

Fig. 7.

Fig. 6. The spectral distribution of the first transparency effect, showing the results of 150 observations distributed at wave-lengths from 400 to 700 mμ. At each wave-length the results are shown as a vertical line to represent the scatter, the length of each line being twice the average deviation from the mean. The vertical scale is in arbitrary units.

Fig. 7. A sample photographic record taken when using monochromatic light to show the 'noise'.

A sample record is shown in Fig. 7, and the importance of the 'noise' may be judged from this. It is proposed to make further observations using the intense mercury line at 365 mμ. as a source in the near ultraviolet, filtered from the visible radiation with a Wratten 18-A filter. This should help to decide whether there is truly a maximum at 450 mμ.

Polarized light. An attempt was made to locate the cause of the first transparency change in relation to the anisotropic and isotropic bands by working between crossed 'Polaroids'. To avoid overlap of the *A* and *I* bands of different fibres the sterno-cutaneous muscle was chosen for this purpose. This sheet of muscle is only one fibre thick. It was found that the early change in transparency was present, and of approximately the same magnitude as in

unpolarized light. The effect is therefore not confined to the isotropic bands. It is not yet possible to say whether the change is restricted to the anisotropic band, because the necessary *exact* comparison of the magnitudes of the signals with unpolarized light and with crossed polaroids has not yet been made; there is some difficulty in doing this owing to the great difference between the light intensities in the two cases.

The delayed transparency change. The transparency change recorded with a d.c. amplifier is shown in Fig. 8 for light received in the direction of the incident beam. It is seen that the change which remains after the twitch, and

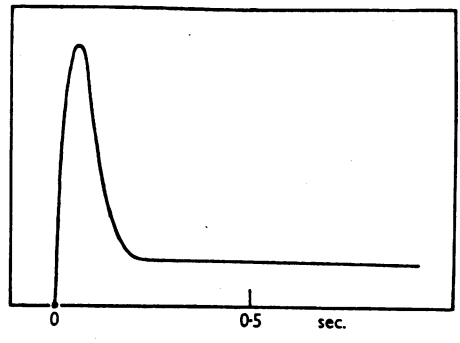

Fig. 8. The transparency change recorded with a d.c. amplifier, to show the delayed effect. 'Directional' recording in the line of the incident beam. The positive direction denotes an increase in photocurrent.

which decays in a matter of minutes as described by von Muralt, is in the same sense as the change during the twitch. Both phases are reversed in sign if the scattered light is measured.

Rapid stretch. The transparency changes caused by a rapid stretch of the muscle, and of the strip of rubber used as a control, are shown in Fig. 9. The effect with the rubber is attributable merely to a change in length. The muscle shows, in addition, a peculiar early transient effect. A muscle made inexcitable by soaking it in a potassium rich solution (20 times normal K) shows the same change. The effects following a rapid *release* of a previously stretched muscle are the reverse of these. By 'directional' recording it appears that the early transient effect is caused by an increase in the *scattering* of light, but this is not yet certain. An objection to the use of rubber for the control is that the viscosity or plasticity of the muscle has no counterpart in the rubber, for the latter behaves more like a simple spring. Perhaps the stretch effect in muscle can be reproduced in some other inert material, suitably chosen for its physical characteristics.

DISCUSSION

The early relaxation of a muscle is associated with a change in transparency of the muscle. The two events appear to start at precisely the same time. It seems fairly clear that the early transparency change has certain properties which are not shared by the change which occurs later, during the contraction proper. The latter phase is attributable largely to a change in the scattering power of the muscle, but there is evidence that the first phase, starting with the relaxa-

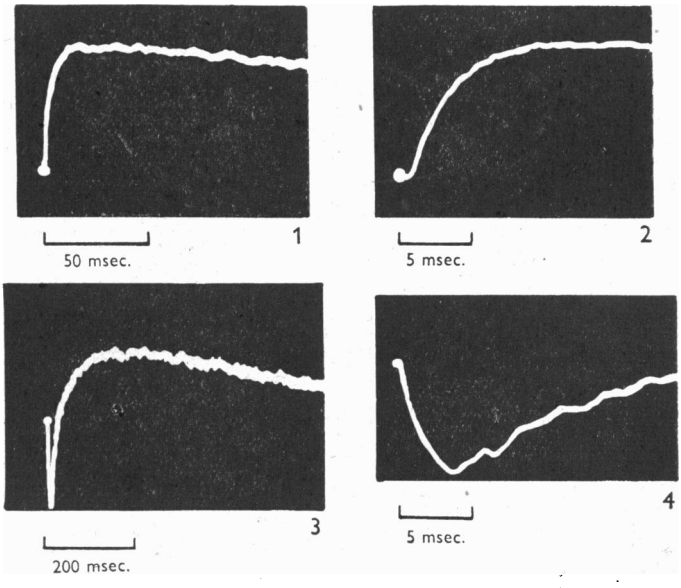

Fig. 9. Transparency changes as the result of a rapid stretch. 'Directional' recording at 50° from the line of the incident beam. The vertical direction denotes an increase in photocurrent. 1 and 2 show the effect with the 'control' rubber strip; 3 and 4 are for muscle, and show the early rapid transient change which is absent in the 'control'.

tion, may be caused by a decrease in the true absorbing power of the muscle substance. A determination of the spectral distribution has not confirmed this supposition, and the question is still undecided. Further experiments may show that in spite of the evidence to the contrary the first phase is really due to scattering. It may prove that the difference between the two phases is connected with some subtle dissimilarity in the mechanisms by which the light is scattered in the two cases. The many units which compose muscle—the molecules, the 'micelles', the fibrils, the fibres—may all play some part in scattering the light. Moreover, the scattering power of a particular type of unit may be determined not only by its chemical constitution or environment, but also by its degree of organization; the more complete the orientation of the particles among themselves the smaller will be their scattering power. Again, such

processes as the gelation of a colloidal solution, associated with changes in particle size, may give rise to changes in opacity (Krishnamurti, 1929).

The possibility has not been ignored of the change being due to an alteration in the reflecting power of the muscle membrane. To test this it will be necessary to record changes in intensity of light scattered and reflected backwards towards the source of light. The ideal way of distinguishing between absorption on the one hand, and scattering and reflectivity on the other, would be to record the complete three-dimensional 'polar diagram' of the effect.

SUMMARY

1. A study has been made of the changes in transparency of frog's skeletal muscle during a twitch. The source of light was a tungsten filament lamp combined, in some of the experiments, with monochromatic colour filters.

2. The mechanical response, starting as a transient relaxation, was recorded under the same conditions by means of a crystal pick-up. The transparency change and the early relaxation appear to commence at exactly the same instant, within a fraction of a msec., i.e. at about 2 msec. at 15° C.

3. An attempt was made to distinguish between *scattering* and *absorption* of the light. It is possible that the first phase, during the early relaxation, is an absorption phenomenon, in contrast with the later change which is largely a scattering effect. An examination of the spectral distribution of the first phase has not revealed any characteristic absorption bands.

4. Experiments were made to test whether a rapid stretch of the muscle gave rise to any transparency change other than that due to a change of thickness. The muscle was stretched about 100 μ. A strip of rubber was used as the control. An early rapid transient effect was observed, but it has not yet been investigated in detail.

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REFERENCES

Buchthal, F., Knappeis, G. G. & Sjöstrand, T. (1939). *Skand. Arch. Physiol.* **82**, 225.
Hill, A. V. (1948). *J. Physiol.* **107**, 29 *P.*
Katz, B. (1941). *J. Neurophysiol.* **4**, 207.
Krishnamurti, K. (1929). *Proc. Roy. Soc.* A, **122**, 76.
Kuffler, S. W. (1942). *J. Neurophysiol.* **5**, 309.
von Muralt, A. (1934*a*). *Pflüg. Arch. ges. Physiol.* **234**, 233.
von Muralt, A. (1934*b*). *Pflüg. Arch. ges. Physiol.* **234**, 653.
Preisach, F. (1939). *Wireless Engr.*, **16**, 169.
Sandow, A. (1944). *J. cell. comp. Physiol.* **24**, 221.
Schaefer, H. & Göpfert, H. (1937). *Pflüg. Arch. ges. Physiol.* **238**, 684.
Urban, F. & Peugnet, H. B. (1938). *Proc. Roy. Soc.* B, **125**, 93.