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THE EFFECT OF STIMULATION ON THE DIFFRACTION OF LIGHT BY STRIATED MUSCLE

By D. K. HILL

From the Postgraduate Medical School, University of London, Ducane Road, W. 12

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The previous paper (Hill, 1953) is concerned with changes in the scattering and diffraction of light by resting striated muscle when it is suddenly stretched. It was pointed out that in an earlier investigation (Hill, 1949) the question of diffraction had been overlooked, and the first phase of the optical change which was found to accompany the latency relaxation in stimulated muscle appeared then to be attributable only to absorption. A change of diffraction was not considered as an alternative possibility, although for reasons given (Hill, 1953) this could equally well have explained the results obtained. It is the purpose here to deal more fully with this early phase of the optical response. The previous paper gives a description of the technique which can be used for distinguishing experimentally between scattering, absorption and diffraction. As mentioned above, the present position is that the first optical change is equally attributable to absorption as to diffraction. Further tests can now be made to decide between these two alternatives. The early phase is followed after a few milliseconds by a second one, in which the optical change is predominantly due to scattering: it takes place during the contraction proper, ending with a residue which reverses slowly, in parallel with the chemical recovery. This second phase is not touched on in the present paper: considerations of diffraction do not allow anything to be added to what was reported previously (Hill, 1949). Doubtless the first phase continues through the second, but the situation is too complicated for analysis into the separate events. Movement of the muscle during contraction in some cases adds to the complexity of the second phase.

This is not the first time that attention has been directed to diffraction of light by stimulated muscle. Sandow (1936*b*) recorded the intensity of the spectra photographically. He used a sartorius muscle; changes were found to occur when the muscle was stimulated tetanically, but these could not be interpreted with any certainty. The changes with a sartorius are small, and the alteration in scattering which accompanies activity means that intensity

comparisons from photographic records have doubtful significance. For reasons which are discussed later diffraction changes are seen more easily in a thin muscle. The frog's sternocutaneus, about two to three fibres thick, shows a very marked reduction of the diffraction spectra, sometimes almost to extinction, when the muscle is tetanized. (It was actually this observation, made during the course of some other work, which gave the impetus to the present investigation.) The sternocutaneus was used for some of the experiments to be described here, and proved very satisfactory for the study of the early optical change which (anticipating the conclusion) appears to be due to a decrease of diffraction.

Buchthal & Knappeis (1940) studied the diffraction spectra of single fibres from the frog's semi-tendinosus. They reported that the intensities of the diffracted beams rise when the muscle is subjected to a short isometric tetanus. This conflicts with the observations on the sternocutaneus, but it is possible that the change seen in a tetanus depends on whether or not the muscle is held in a strictly isometric manner. A sternocutaneus cannot be stimulated isometrically because of the extensibility of the supporting skin and other tissue.

METHOD

Experiments were made with the sartorius, and also with the sternocutaneus muscle of the English frog. The sartorius was dissected in the usual way, leaving one end attached to a piece of the pelvic bone. It was soaked in Ringer's solution for several hours before mounting in the apparatus shown in Fig. 1. Experiments were done with the muscle stretched to about 120% of its normal extended length in the body.

The sternocutaneus was prepared in the following way. (It is a rather fine dissection for the naked eye, and magnifying spectacles make it much easier.) The pithed frog was laid on its back with the front limbs pinned out. The skin was pierced over the centre of the abdomen, and a cut was made towards the base of each front limb. When the V-shaped flap of skin so formed was raised, the sternocutaneus muscle could be seen, lying flat against the thoracic wall. A cross-cut was then made through the skin flap from one front limb to the other, above the level of the attachment of the muscle. The triangular piece of skin so formed, with the sternocutaneus attached to it, was held by its abdominal corner and raised away from the body. The muscles were then ready to be freed from their attachments to the body wall. First it was necessary to cut through a very thin membrane which runs downwards from the fascial layer covering the neck and mouth to join the skin near the origin of the sternocutaneus. Only one muscle of the pair was generally required, so the other was cut at this stage. Holding the remaining muscle taut by means of the flap of skin, the attachments to the thoracic wall and to the sternum were cut or broken. The muscle was freed down to the level of the xiphisternum medially, where one corner of it is inserted, and to a rather lower level laterally, where the muscle ends by joining the fascial sheaths of other abdominal muscles. The abdominal wall was then pierced near the lower lateral corner of the muscle. The muscle was held well away from the body by its skin attachment, and an area of abdominal wall together with part of the xiphisternum was excised by cutting around the insertion of the muscle. This piece of body wall was used for holding the muscle, and was cut to a suitable size. All unnecessary tissue above the level of insertion was cut away. The muscle was frequently moistened with Ringer's solution during the dissection, as it quickly dries up if exposed to air on both sides. The muscle was then laid on a flat surface, ventral side uppermost, but with the skin reversed, i.e. with the inside uppermost (the muscle lies more easily in this relation to the skin). All unnecessary parts of skin were cut away with the scissors, a portion of

the abdominal end (now turned away from the muscle) being retained for attachment. Two holes were pierced in the skin at the corners of the remaining flap; one hole was pierced in the xiphisternum, and two holes in the flap of abdominal wall. Lengths of cotton were attached to these points.

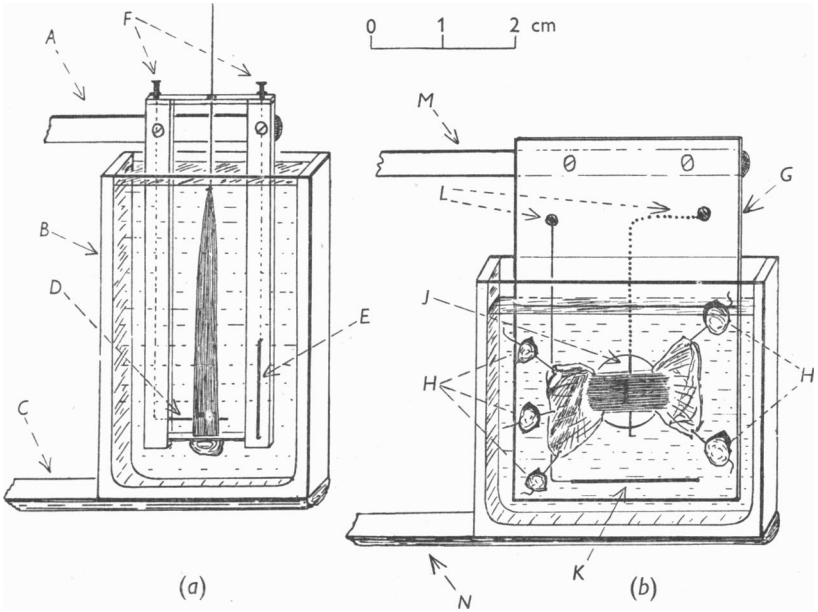

Fig. 1. Devices for holding the muscles. (*a*) sartorius muscle. The muscle is held in the Perspex frame by being passed through a slot which does not admit the pelvic bone. The tendon is tied with cotton which is secured to a clamp (not shown) held in a Palmer rack stand. The frame is fixed to a brass rod, *A*, held in another Palmer stand. It is lowered into a cell, *B*, with parallel glass windows about 1 cm apart, containing Ringer's solution. The cell is screwed to a brass rod, *C*, which is held in a third stand. The stimulating cathode, *D*, made of platinum, emerges from the Perspex frame and makes contact with one side of the muscle near the pelvic end. Immediately above the level of the electrode is shown a horizontal rectangular black area, which indicates the part of the muscle which is illuminated. The anode is silver wire, *E*, fixed to one limb of the Perspex frame. The leads to both electrodes (shown as dotted lines) are embedded in grooves in the Perspex, and run to brass terminals, *F*. (*b*) sternocutaneus. The muscle is held, with its fibres running horizontally, on a thin (1 mm) sheet of Perspex, *G*, by cotton tied at two points to the skin at one end, and to three points on the flap of body wall at the other end. The cotton is held down by plasticine, *H*. *J* indicates a circular hole in the Perspex across which is stretched the muscle. *J* also indicates the platinum stimulating cathode close to the surface of the muscle. The silver anode, *K*, is fixed to the Perspex sheet. The lead to the cathode (dotted line) is embedded in a groove in the Perspex. The leads run to terminals, *L*. Immediately to the left of the cathode is shown a rectangular black area which indicates the position of the incident beam of light. The Perspex holder is screwed to the brass rod, *M*, held in a Palmer stand, and is lowered into a cell, with parallel glass sides about 1 cm apart, containing Ringer's solution. The cell is fixed to the brass rod, *N*, which is held in another Palmer stand.

Mounting the muscle. The sternocutaneus was held as shown in Fig. 1. Before mounting, the Perspex sheet was detached from its supporting rod and put flat on the bench. The sternocutaneus was laid centrally over the circular hole, and five small pieces of plasticine were firmly applied at the appropriate points over the cotton attachments. The muscle was stretched out by

pulling on the cotton, until it was reasonably taut. When its position was finally adjusted so that it would lie horizontally, the plasticine was pressed down hard to prevent any further movement. The size of the muscle was then about 5 mm wide and 10 mm long. The platinum electrode which is fixed across the hole in the Perspex lay very close to the central region of the muscle but was not actually in contact with it. The Perspex sheet, with the muscle attached to it, was then screwed on to the supporting rod and lowered into the cell of Ringer's solution (Fig. 1) where it was left to soak for about 1 hr before use.

Optical system. This was similar to the one described in the previous paper. In order to obtain a clear-cut diffraction pattern it was designed to give a wedge-shaped beam of light, with the parallel sides of the wedge at right angles to the long axis of the muscle. Fig. 1 shows that the sartorius fibres were vertical, while the sternocutaneus was horizontal: a change from one muscle to the other therefore necessitated a rotation of the optical system through a right angle. The muscle was 5 cm from the lens, at the plane of focus of the filament. The position of the muscle holder was adjusted so that the light was incident on the muscle at a point within a fraction of 1 mm from the stimulating electrode. A mask of black paper was stuck to the window of the cell facing the lens, with a hole 1·7 mm square cut in it. This hole admitted only part of the beam of light, which was thus prevented from overlapping the edge of the muscle. The mask also reduced the amount of stray light entering the photocell. The exclusion of 'irrelevant' light in this way helped to reduce the level of noise. White light was used.

Recording system. This was similar to the one described in the previous paper, where it was shown how the aperture of the photocell can be set in any required position in relation to the spectra. The output current was amplified to drive a cathode-ray tube. The amplifier was condenser coupled with a time constant of 0·1 sec, which was too short for obtaining an undistorted record of changes during the twitch as a whole, but was adequate for the present experiments which cover less than the first 10 msec of the optical change. The response of the amplifier extended to 10,000 c/s without loss. The oscilloscope time-base unit (Attree, 1949) provided single sweeps from an initially suppressed spot. The sweep was triggered from one output channel of the stimulator unit (Attree, 1950), the other channel being connected via a short-circuiting key to the terminals on the muscle holder. The duration of the stimulating pulse was 0·3 msec. The time delay in triggering was less than 0·02 msec.

Procedure. The strength of the stimulating pulse was set to about twice the minimum value required for a maximal twitch as observed by eye. The stimulator was set to run repetitively at a frequency of about 1 shock every 2 sec; each impulse triggered the time-base, but stimulation of the muscle occurred only when the short-circuiting key was pressed. A 35 mm reflex camera was used to photograph the trace. With the room darkened, the response for the photocell in a particular position was recorded by opening the camera shutter in the interval between sweeps, pressing the stimulating key, allowing one 'live' sweep to occur, and then closing the shutter before the next sweep. Figs. 2–8 are photographic records taken in this way, with the addition of a scribed base-line and time marks recorded independently with an oscillator working at 1000 c/s.

RESULTS

The type of record is described by stating, first, which order of spectrum was admitted through the photocell aperture, followed by the word *direct* if the record was made with the aperture positioned at some point on the vertical (sartorius) or horizontal (sternocutaneus) plane containing the incident beam, or *lateral* if it was to one side or the other of this plane. When using a large aperture it was not always certain how many orders of spectra were being recorded. In such cases it is easier to state which orders were excluded; for instance, if the zero order was excluded, but the first and higher orders admitted, the words *less zero order* are used.

In describing the results, attention is directed only to the earliest part of the response. After about 3–4 msec (20–25° C) the situation becomes complicated, as already mentioned, by supervention of changes due to scattering and to localized shortening of the muscle. If the muscle is highly stretched (Hill, 1949) the effect of localized shortening can be greatly reduced, and this simplifies the picture. In the present experiments the sartorius was not highly stretched, and the results after the first 3–4 msec were often spontaneously variable, and also depended on factors such as stimulus strength and the exact relationship of the beam of light to the stimulating electrode. The results for the sternocutaneus after the first 3–4 msec were extremely variable, and 'polyphasic' forms of curve, due to localized contraction and lengthening, were often seen. In the case of the sternocutaneus these secondary effects are not avoidable by working with a tightly stretched muscle; the overall picture during the twitch appeared variable and incoherent under all conditions, and this muscle is clearly not suited to the study of optical changes except during the first 3–4 msec after the stimulus. By contrast, the change recorded during the first 3–4 msec has shown complete consistency, and for reasons discussed below it is much more clearly seen with the sternocutaneus than with the sartorius.

Sartorius. The earliest phase of the optical change is shown in Figs. 2–4. The gain of the amplifier had to be very high, and the noise ('shot' noise, from the photocell) is large. The noise is greater than in the earlier experiments (Hill, 1949) because the intensity of light on the muscle, and the aperture of the recording system with the present optical arrangements are relatively small (enforced by the need of obtaining and recording individual diffraction spectra). The dependence of noise on the intensity of light received is a point discussed in the earlier paper (Hill, 1949). The results suggest that the first detectable change is due to a decrease of diffraction. This conclusion is consistent with (*a*) an increase of light at *zero order, direct* (Fig. 2); (*b*) a non-reversal on moving to *zero order, lateral* (Fig. 3); (*c*) a reversal at *less zero, first and second orders, direct* (Fig. 4). By themselves, the results for the sartorius are not at all convincing. A reversal was obtained only by excluding the zero, first and second orders; it was not possible to detect any early change by excluding only the first or second orders, or by recording the first order alone. Even when the reversal is achieved, it appears to start rather late (2–2·5 msec) compared with the starting time of 1·7 msec for the record at *zero order, direct*, but this may be explained by a weak signal on a noisy base-line.

Sternocutaneus. The results are much clearer than for a sartorius. In view of what is said later regarding the behaviour of a multi-layer diffraction grating, this fact alone strengthens the argument in favour of the conclusion that diffraction is responsible for the change observed, for diffraction effects should theoretically be more easily detected in a thin muscle than in a thick

one, whereas the optimal thickness for detection of a change of scattering is (Hill, 1949) if anything rather greater than that of a sartorius.

Figs. 5 and 6, recorded for *zero order, direct* and *first order, direct* show a change starting at 1·2 msec which is attributable to a decrease of diffraction. The sartorius showed no change of the first-order spectrum alone; the sterno-

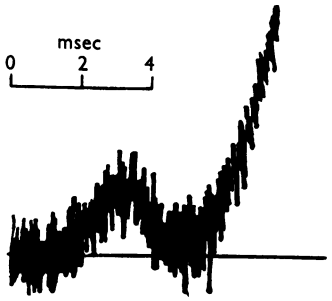

Fig. 2.

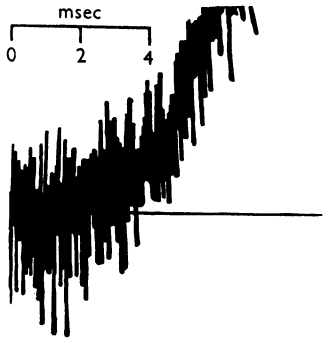

Fig. 3.

Fig. 2. Sartorius muscle. The response is shown for position *zero order, direct.* 21° C. The change starts at 1·7 msec. The stimulating shock is applied at time zero. The positive vertical direction corresponds to an increase of light at the photocell.

Fig. 3. Sartorius muscle. The response is shown for position *zero order, lateral.* 21° C. The change starts between 2·0 and 3·0 msec.

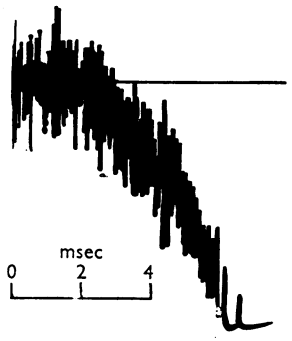

Fig. 4. Sartorius muscle. The response is shown for position *less zero, first and second orders, direct.* 21° C. The change starts between 2·0 and 2·5 msec.

cutaneus shows a large one. The angle between the zero and first order is about 12°. There is a reversal of the effect as between the two positions. No such reversal is found by recording laterally; even at about 60° off the direct plane the change starts in the same direction as it does with the photocell aperture on the direct plane. The absence of reversal laterally rules out a change of scattering as being the cause of the early phase. The reversal

obtained by recording the first-order spectrum rules out absorption. The same reversal is found by recording any other of the spectra singly, or any combination from which the zero order is excluded.

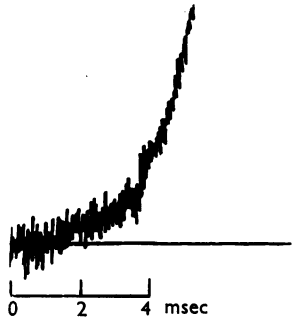

Fig. 5.

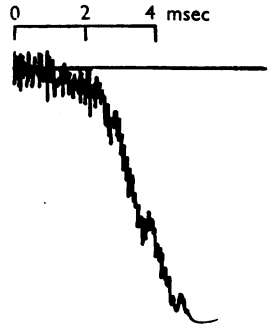

Fig. 6.

Fig. 5. Sternocutaneus muscle. The response is shown for position *zero order, direct.* 24° C. The change starts at 1·2 msec.

Fig. 6. Sternocutaneus muscle. The response is shown for position *first order, direct.* 24° C. The change starts at 1·2 msec.

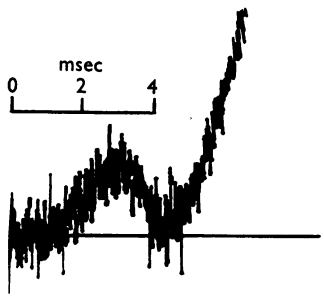

Fig. 7.

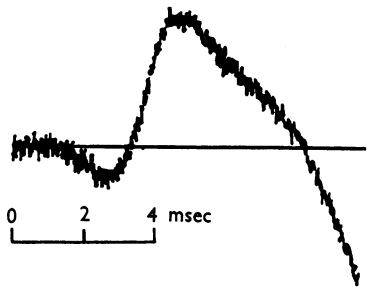

Fig. 8.

Fig. 7. Sternocutaneus muscle. The response is shown for position *zero order, direct.* 22° C. The change starts at 1·6 msec. This record gives an example of the variety of forms the response may take following the initial consistent response seen during the first 3–4 msec.

Fig. 8. Sternocutaneus muscle. The response is shown for position *first order, direct.* 22° C. The change starts at 1·3 msec. This record gives an example of the variety of forms the response may take following the initial consistent response seen during the first 3–4 msec.

Examples of the complex and variable results which may occur after 3–4 msec are given in Figs. 7 and 8.

The time after the stimulating shock at which the first effect starts can be estimated best from the results for the sternocutaneus. Noise leads to some uncertainty, but this can be reduced by averaging a number of results. A series of twenty-three records was made with the same muscle, and the times are given in Table 1. The average optical latent period was 1·37 msec

at 24° C. The delay in excitation of the illuminated zone of muscle was small, and probably negligible. The distance of the electrode from the edge of the illuminated area was less than 0·5 mm. For a transmission speed of 2 m/sec the maximum delay would be 0·25 msec, but the actual delay must have been less than this, or even zero, owing to spread of excitation around the electrode. Some records were made with the electrode in the beam of light, and the time of onset of the optical change appeared to be about the same.

TABLE 1. Times of the first detectable optical change in a series of twenty-three records taken with a sternocutaneus muscle. 24° C.

Type of record	Time of first detectable change (msec)	Type of record	Time of first detectable change (msec)
a	1·5	*c*	1·2
a	1·6	*c*	1·2
b	1·3	*a*	1·3
b	1·3	*b*	1·8
a	1·4	*a*	1·3
b	1·4	*a*	1·3
a	1·2	*b*	1·3
a	1·1	*a*	1·7
b	1·1	*b*	1·5
b	1·1	*a*	1·6
a	1·5	*b*	1·2
a	1·5	Average	1·37

Types of record: *a, zero order, direct*; *b, first order, direct*; *c, less zero order, direct.*

The intensity of spectra from a multi-layer grating

The mechanism by which a muscle fibre diffracts light is similar to that of the laminary grating, in which the opaque strips of the ordinary slit grating are replaced by transparent strips of such a thickness that some one wave-length in the spectrum suffers a retardation of $\frac{1}{2}\lambda$. The resulting interference between rays which pass through the retarding strips and those which pass between them, leads to a loss of intensity in the direct beam. The light which is lost in this way is diffracted, and is seen in the various spectral orders. A whole muscle, several fibres thick, may be considered as a series of gratings superimposed upon one another. Sandow (1936*a*) has pointed out that, although the spacing of the spectra will be the same as for a single fibre, the distribution of intensities in the pattern will be altered by repeated diffraction at successive layers, and relatively more and more light will be thrown into the lateral spectra of greater order. This question of the distribution of intensity in the various orders from a multiple grating, and of the effect of a change in the diffracting power of a single fibre on the diffracting power of the muscle as a whole, happens to be of some interest in connexion with the work described in the present paper, and will be considered in greater detail.

The formal mathematical treatment would be excessively complicated. The alternative is to make various approximations, and to calculate what is happening layer by layer. This serves the immediate purpose, which is to show in a general sort of way how the intensities of the various orders of spectra from a multi-layer grating depend upon the intensities from a single layer. Suppose that unit quantity of parallel light falls upon the first layer of a multi-layered grating. The quantities of light emerging from the first layer are arbitrarily taken as $3a$ in the first order, $2a$ in the second order, a in the third order, zero in the higher orders. This leaves $1–12a$ in the direct beam. Seven beams of light are now incident at different angles upon the second layer. Each of these forms its own seven beams after diffraction by the second layer, and by summation the totals in the various orders from the second layer may be found. It is assumed that

all orders higher than the fifth will be lost; this means that after diffraction by the second layer the sixth order is ignored. The process is continued for a third layer and again for a fourth. Some results have been worked out for selected values of a. These are given in Table 2. They illustrate the fact that the output of a multi-layer grating may bear very little relation to the output of a single layer. Taking, for instance, the first-order beam of a four-layer grating, the intensity is seen to rise initially in the range $a=0{\cdot}1$–$0{\cdot}2$, but in the range $a=0{\cdot}2$–$0{\cdot}5$ it falls again. It follows that under some conditions an examination of changes (or the absence of changes) in the intensities of spectra from a multi-layer grating will reveal very little of what is happening in the individual layer. According to Buchthal & Knappeis (1940), the intensities of the spectra from a single frog's muscle fibre are in the following proportions: zero order, 4; first order, 1·0; second order, 0·31; third order, 0·21. A value of $a=0{\cdot}05$ is therefore probably about in the middle of the range which ought to be considered.

TABLE 2. Intensities in the individual spectral orders for gratings of different numbers of layers

Spectral order	One layer, a				Two layers, a			
	0·01	0·02	0·05	0·08	0·01	0·02	0·05	0·08
0	0·88	0·76	0·40	0·04	0·772	0·589	0·230	0·180
1	0·03	0·06	0·15	0·24	0·054	0·098	0·160	0·121
2	0·02	0·04	0·10	0·16	0·037	0·067	0·118	0·109
3	0·01	0·02	0·05	0·08	0·019	0·035	0·070	0·083

Spectral order	Three layers, a				Four layers, a			
	0·01	0·02	0·05	0·08	0·01	0·02	0·05	0·08
0	0·689	0·466	0·171	0·110	0·613	0·377	0·142	0·109
1	0·074	0·120	0·146	0·120	0·090	0·134	0·132	0·103
2	0·051	0·084	0·116	0·105	0·062	0·096	0·109	0·094
3	0·019	0·035	0·070	0·083	0·027	0·046	0·078	0·077

The incident light has magnitude unity. The quantities of light emerging from the first layer are taken as $3a$ in the first order, $2a$ in the second order, a in the third order. Spectral order 0 represents the direct beam; 1, 2, 3 are the diffracted orders on one side only of the direct beam. The figures for the intensities in the fourth and fifth orders are not included in the table.

On these lines it is possible to account for the difference in behaviour on the one hand of the sternocutaneus, with its two to three layers of fibres, or of a single fibre, and on the other hand of a muscle such as the sartorius, with about eight layers. In the former, the intensities of all the orders of spectra show a marked decrease in a tetanus, while in the sartorius the change is almost undetectable. In the experiments described above, both the sartorius and the sternocutaneus were used. The conclusion has been advanced that the optical effect being studied is due to a change of diffraction: it might therefore be expected that the 'signal' would be considerably greater, in relation to the electrical noise, for the sternocutaneus than for the sartorius. This is evidently so, as may be judged from the records shown. The first phase of the optical change is practically absent for the sartorius when the photocell is placed to receive only the first-order beam; it has not been possible to record it. Compared with this, the sternocutaneus gives a large response (Fig. 6) when the first-order spectrum is recorded alone.

While on the subject, it is worth mentioning that in the pattern produced by a simple optical grating the intensities of the successive orders of spectra follow an irregular sequence. Some orders may even be absent. If x is the width of the slit, and y the width of the opaque strip in the ordinary grating, then if $x=y$ the spectra of even order, 2, 4, 6, etc., will be absent. If $x=\frac{1}{2}y$ the spectra of order 3, 6, 9, etc., will be absent; if $x=\frac{1}{3}y$, spectra 4, 8, 12, etc., will be absent. Similar reasoning may be applied to a laminary grating or to a single layer of muscle fibres. If the A and I bands have the same width the even-order spectra should be absent. Under certain conditions a single fibre does, in fact, show a markedly diminished second-order spectrum (A. F. Huxley, personal communication), suggesting that the A and I bands have become nearly equal

in width. The same thing may be seen with a frog's sternocutaneus muscle. In a muscle as thick as the sartorius, no loss of intensity in the second-order spectrum is ever observed. The explanation of this follows from the above considerations of the behaviour of a multi-layered grating, for it has been shown that the intensities of the spectra from such a grating are within wide limits almost independent of the intensities which would be produced by any one layer by itself.

DISCUSSION

Stimulation of a striated muscle results in a reduction of the amount of light diffracted by the striations. The striations are caused by a localization of material of high refractive index in the anisotropic segments of the fibre, and the loss of diffracting power means that the high-μ material undergoes some dispersal when the muscle is stimulated (cf. Hill, 1953). This is perhaps an undue simplification of the situation regarded from the morphological point of view. Barer (1948) has reviewed the literature on the structure of the striated muscle fibre, and refers to earlier work concerning the effect of stimulation on the band structure of striated muscle. More recently, Hanson (1952), and Ashley, Porter, Philpott & Hass (1951) have described changes in cross-striation of isolated myofibrils during contraction induced by adenosine-triphosphate. (Horvath (1952) has failed to show any alteration in the striations of glycerinated psoas muscle treated with ATP.) The band structure as revealed by the electron microscope is complex, and the changes which have been claimed as taking place are equally so. In speaking here of 'dispersal' of high-μ material, the morphological implications are ignored, for the present experiments give no information on the subject.

The diffraction starts to decrease at about 1·4 msec at 24° C (the average from a series of twenty-three records made with the sternocutaneus muscle). The latency relaxation of a sartorius starts at about the same time, at 1·6 msec at this temperature (Sandow, 1950). An attempt was made, using a piezoelectric crystal, to record the latency relaxation of a sternocutaneus, but it was not possible with this very small muscle and heavy attachments to detect any latency relaxation. The action potential of a single muscle fibre has a rising time of about 1·0 msec at 13° C (Hodgkin, 1951). Both the diffraction change and the latency relaxation therefore appear to start within a fraction of 1 msec after the peak of the action potential, during the falling phase. The contraction proper is delayed for another 1·5 msec.

What connexion might there be between the latency relaxation and the loss of diffracting power? There may be nothing more than a remote common cause, though there is a temptation to speculate on the possibility of there being a closer link. It is conceivable, for instance, that the rearrangement of high-μ material responsible for the change in the striations is the direct cause of the breakdown of molecular bonding which allows the partial release of tension in stressed protein chains, resulting in the latency relaxation. But at present there is no evidence to establish any connexion of this sort.

Although the function of the striations is obscure, their presence is undoubtedly associated with the ability of a muscle to hold a high resting tension. It is therefore not altogether surprising to find a loss of diffraction accompanying the loss of resting tension (latency relaxation), which can be thought of as being due to some kind of plasticization, or breakdown of molecular bonding.

One of the questions which has to be answered is this: why does 'vulcanization' involve the aggregation of high-μ material in the form of striations? Or, admitting a direct causal connexion: why does 'vulcanization' result from aggregation of high-μ material, and what mechanism produces the aggregation, or the dispersal following stimulation? There is a fair amount of evidence (reviewed by Barer, 1948) that potassium, present in relatively high concentration within the fibre, is concentrated in the *A*-bands. If it is indeed largely potassium which constitutes the high-μ material referred to above, it would be interesting to consider whether longitudinal dispersal of this polar material could conceivably be brought about by the electrical changes, or ionic movements, which occur at the excitable membrane during the passage of the action potential. It appears that the dispersal starts during the falling phase of the action potential, and there is evidence (Hodgkin, 1951) that during this phase the membrane possesses an abnormally high permeability to potassium, and that there is consequently a loss of potassium from the fibre at this time. It is possible that this outward flow of potassium involves longitudinal dispersal of the aggregates in the *A*-bands. It is probably too much to hope that this might provide some clue to the missing link between excitation and contraction.

SUMMARY

1. There is a decrease in the intensity of white light diffracted by a frog's striated muscle when it is stimulated. A photoelectric cell is used for recording. The change starts at 1–2 msec after the stimulus is applied (20–25° C); this is about the time at which the latency relaxation begins. The contraction proper, and the change of scattering which accompanies it, start later at 3–4 msec.

2. The sternocutaneus muscle of the frog shows this change more clearly than the sartorius. The reason why the thinner muscle shows the better response is discussed.

3. It is suggested that the change of diffraction is caused by longitudinal dispersal of material with high refractive index which is normally aggregated in the anisotropic bands. It appears, from the timing, that the change starts during the falling phase of the action potential; the significance of this is discussed. Consideration is also given to the relationship between the change of diffraction and the latency relaxation.

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