PROGRESS IN BIOPHYSICS AND BIOPHYSICAL CHEMISTRY

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A Pergamon Press Book

THE MACMILLAN COMPANY
NEW YORK

1957

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MUSCLE STRUCTURE AND THEORIES OF CONTRACTION

A. F. Huxley

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Introduction

It has seemed natural to all who have studied voluntary muscle with the microscope to suppose that the striations are an important clue to the mechanism of contraction. Until the early years of this century, they were almost the only clue, apart from the process of shortening or tension development itself, and interest was accordingly centred on the changes that the striations undergo during contraction. But no coherent theory of contraction emerged from these studies; perhaps it is for this reason that the hard-won knowledge of muscle structure fell into neglect as rapid progress began to be made in muscle chemistry and in the energetics of contraction.

Up to the outbreak of the second war, these branches of muscle physiology were able to proceed successfully without reference to the details of structure. It appears now that the discoveries that were made in muscle biochemistry up to 1939 were relevant to recovery processes rather than to the contraction mechanism itself. This is not to belittle their importance: from the point of view of muscle physiology they led to the discovery of ATP and to the idea that this substance is the immediate source of energy for contraction, while in a broader context they laid the foundations of our understanding of the glycolytic cycle and of the ways in which energy is made available for the activities of all kinds of cells. It does however mean that these discoveries had little immediate relevance to the problem of how the contraction itself is brought about; conversely the characteristic features of muscle structure were irrelevant to the processes that were being elucidated. This position was brought to an end by the discovery of the interactions of "myosin" and ATP, and of the composite nature of "myosin." It could be said that muscle chemistry had then become ripe for integration with information about structure, but in the meantime the knowledge that had been gained by the nineteenth-century microscopists had been largely forgotten. This is illustrated for example by comparing the brief accounts of the striations, and of their changes during contraction, that are to be found in modern text-books, with the full and accurate description in say the 1888 edition of Michael Foster's text-book.

The chemistry of the muscle proteins did indeed retain some contact

with microscopical information; for example NOLL and WEBER (1934) showed that the birefringence of the *A* bands could be accounted for by the birefringence of "myosin" threads. This contact seems to have been lost at the time when it might have been most fruitful; there was no attempt to locate myosin proper and actin with respect to the striations until more than a decade after the separation of these proteins.

Knowledge of the mechanics and heat production of muscle reached a stage at which they were ready to link up with other lines of information on the contractile process in 1938, when A. V. HILL published his well-known analysis of the relations between tension, speed of shortening, and heat production. However, in spite of HILL's repeated calls for chemical studies to parallel his analysis of the time course of energy liberation, this work remained isolated until the recent demonstration by FLECKENSTEIN *et al.* (1954) and MOMMAERTS (1954) that the amounts of ATP and creatine phosphate in a muscle do not fall during the contraction phase of a twitch.

As regards the processes by which the contractile mechanism is "turned on," it was clear by the end of the war (KUFFLER, 1946; KATZ, 1950) that the reduction of the resting potential which occurs on excitation is the normal stimulus for contraction, and a number of physical changes were known to begin during the "latent period" between excitation and the development of tension or shortening (see pp. 300–304).

Although the basic facts concerning the striations, which had been known for 80–100 years, were unduly neglected at this time, it must be admitted that our knowledge of muscle structure had not made much progress since the turn of the century, and in relation to the available techniques it was less advanced than other branches of muscle physiology. Even the application of the electron microscope did little at first beyond confirming (and restoring to respectability) the picture that had been handed down by the nineteenth-century microscopists; indeed, several of the new conclusions that were drawn from the early work with the electron microscope have since turned out to be unfounded. But in the last few years a number of important points about the striations themselves, and about the spatial distribution of the structural proteins, have come out, and as a result it is now possible to make statements about the intimate structure of muscle which are sufficiently detailed to have a bearing on the interpretation of the mechanical, thermal and chemical events. It remains for the future to decide how far the views that I am adopting here about the structure of muscle are correct, but I do not think that they are any more likely to be in error than are the current statements of its mechanical and chemical behaviour.

Up to now, the theories of muscle contraction that have been put forward have often been based on only a single aspect of muscular activity. The lactic-acid theory, which might be classified as primarily chemical, did indeed take account of what was then known of heat production and work done by the muscle. But ASTBURY's (1947) theory was purely structural and RAMSEY's (1944) takes account of little except the energetics of contraction, while most of the other recent theories are chemical and make no attempt to accommodate either the relationships which HILL found to govern the release of energy as heat and work, or the known features of structure. POLISSAR's theory (1952a,b,c,d) is very comprehensive as regards mechanical and thermal data, and could probably accommodate the known chemical events, but it takes no account of muscle structure and it does not fit with some of the important facts about heat production (WILKIE, 1954).

These theories have done good service by suggesting lines for further work and by bringing together observations from more or less diverse fields. Their onesidedness has been a natural consequence of the fact that the different branches of muscle physiology had hardly made contact with one another; this did mean however that there was little chance that a generally satisfying theory would be produced. But this is no longer the position; it should by this stage be possible to produce hypotheses which try to account simultaneously for the mechanical, thermal, chemical and structural changes which are known to occur during contraction. Later in this article I shall try to make good this statement by putting forward a hypothesis which does this to some extent. It achieves a fair agreement with observation in several respects, but I do not suppose it is by any means the only scheme that could be devised at the present day with an equal degree of success.

Much of the new evidence on muscle structure has been recently reviewed (PERRY, 1955; HANSON and H. E. HUXLEY, 1955) and I shall therefore give only a short account of it here. This forms the first section of the article, while the second reviews older observations and opinions in the light of this work. The third section deals with activation and the function of the *Z* membrane; in the fourth, there is an account of the hypothesis to which I have just referred, and in the fifth are discussed various phenomena for which fresh interpretations are suggested by the structural data.

The article makes no attempt at completeness as a review, and it contains a higher proportion of historical matter and of speculation than is customary in review articles. It deals mostly with structure and with the physical changes that accompany contraction, while the chemical and electrical events are referred to only in so far as they have a direct bearing on these central topics.

I. Structure of the Myofibril

I. 1. *Interpretation of the striations*

The essential facts about the structure of a striated muscle fibre at rest that were established by the nineteenth-century microscopists are the following.

(1) The transversely striated appearance of a fibre examined in ordinary light is due to an alternation of zones of higher and lower refractive index.

(2) The interior of the fibre consists of fibrils embedded in a varying amount of probably liquid sarcoplasm, which also contains nuclei and granules. When the fibrils are separated from one another, each shows the banding pattern characteristic of the whole fibre.

(3) The zones of higher refractive index (*Q* or *A* bands) are birefringent (uniaxial, with the optic axis parallel to the length of the fibre, and with positive sign), while the zones of lower refractive index (*J* or *I* bands) are optically isotropic or nearly so.

(4) Each *I* band is bisected by a narrow line of high refractive index, which appears to represent a more or less continuous membrane stretching across the fibre and attached to the sarcolemma.

(5) The centre of the *A* band sometimes has a lower refractive index than the edges, and is then called the *H* band.

These points were first clearly stated by Bowman (1840) for (1) and (2), Brücke (1858) for (3), Dobie (1849) and Krause (1869) for (4), and Hensen (1869) and Engelmann (1873a) for (5). They were clearly understood by Engelmann, Rollett, and others who investigated fresh fibres by visual microscopy towards the end of the last century, but the first has been a source of much confusion in more recent times. The striations do not differ appreciably in the absorption of light; consequently they are invisible if the fibre is examined in ordinary light with a wide condenser aperture. The refractive index difference between *A* and *I* can be made to show up by stopping down the condenser: the striations then become visible but the appearance depends on the focusing of the microscope. If it is focused exactly on a thin part of a fibre, almost nothing is seen; if the microscope tube is raised slightly, high refractive index regions appear bright, but if the tube is lowered the image is reversed and high refractive index regions become dark. To speak of the "dark" and "light" bands is therefore meaningless unless the position of focus of the microscope is specified; in the last century it became customary to use the low position ("tiefe Einstellung"), so that *A* and *Z* could be called dark; it is convenient but also potentially misleading that this agrees with the appearance of a fibre stained with the commonly used basic dyes. The interpretation of the image of a "phase object" such as this, in which light is retarded but not absorbed,

became less important with the improvement of section-cutting and staining methods, and it seems nowadays not to be universally understood even by those who work with the microscope. The interpretation is still more difficult in a thick specimen such as a frog muscle fibre, and it is very difficult to tell, for example, whether the dark bands seen by Buchthal *et al.* (1936) were in fact the *A* bands as these authors assumed.

I. 2. *Structure of the A, I and H bands*

It was widely supposed before the second war that the high refractive index and birefringence of the *A* bands were due to the presence there in a more or less solid state of the "myosin" which can be extracted by strong salt solutions or dilute acids (Kühne, 1864; Danilewsky, 1881). Although this view was strongly supported by the quantitative work of Noll and Weber (1934), it seems to have been discarded after the war, partly because the "myosin" had by then been shown not to be a single substance, but chiefly, I think, because it was difficult to reconcile this localization of what was thought to be the contractile

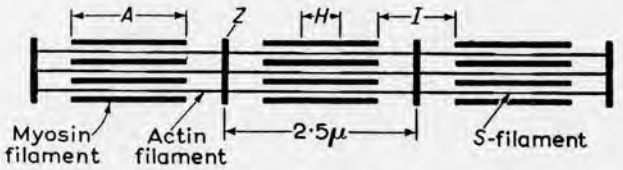

Fig. 1. Diagram showing the arrangement of the filaments, within a myofibril, which has been suggested by recent observations described in the text. The degree of stretch corresponds to the extended length in the body. Transverse dimensions are enormously exaggerated in comparison with longitudinal ones. When the length of the muscle is changed, the actin and myosin filaments slide past one another in each of the zones where they overlap, and only the *S*-filaments are actually stretched or shortened.

substance with the current idea that contraction was produced by the folding of protein chains that extended throughout the length of the muscle. Recently, good evidence has appeared that these characteristics of the *A* band are indeed due largely to the localization there of the myosin (as opposed to actin) component of the old "myosin." At the same time, observations on the changes of the striations during stretch and contraction, and electron microscope studies of thin sections of muscle, led to ideas on the structure of the myofibril that fit in well with a localization of the chief materials of the fibril in different parts of the system of striations.

The resulting picture of the structure of a fibril is shown schematically in Fig. 1. The main features of this were arrived at independently by

H. E. HUXLEY and HANSON (1954) and by A. F. HUXLEY and NIEDERGERKE (1954), although more of its details are due to the former than to the latter authors. Fig. 1 is in fact almost the same as the corresponding diagram of HANSON and H. E. HUXLEY (1955). Most of the evidence on which this scheme is based has been thoroughly reviewed by HANSON and H. E. HUXLEY (1955): I shall therefore do no more than summarize it here.

The essentials of the scheme shown in Fig. 1 are:

(1) The *A* band owes its high refractive index and its birefringence to a set of rods or filaments arranged alongside one another. The length of each of the rods is constant at about 1·5 μ so long as the muscle does not shorten enough to bring the ends of the rods into contact with the *Z* lines.

(2) A second set of filaments extend from the *Z* line through the *I* and into the end of *A*, as far as the beginning of the less dense *H* region. These keep a constant length of about 1·0 μ so long as the muscle does not shorten to a sarcomere length below 2 μ.

(3) When the muscle lengthens or shortens, the filaments of these two types slide past one another.

(4) When the fibre shortens so much that the *I* filaments of the two ends of one sarcomere come into contact, they shorten further by folding or crumpling near the point where they have come into contact, and possibly at other places. Similarly, the *A* rods may fold at their ends if the fibre shortens so much that they come into contact with the *Z* line.

(5) Myosin is a principal constituent of the *A* rodlets, and actin of the *I* filaments.

(6) Corresponding *I* filaments of the two ends of a sarcomere are joined by a very extensible connection, the *S* filament.

(7) In transverse section, the filaments are arranged as shown in Fig. 2.

The chief points of the evidence which led to this picture being put forward are as follows:

(1) *Existence of two sets of filaments.* The two sets of filaments are beautifully shown in the region of the *A* band where they overlap, in electron micrographs of both longitudinal and transverse sections of frog and rabbit muscle (H. E. HUXLEY, 1953a).

(2) *Constancy of length of A-band rodlets.* (*a*) A. F. HUXLEY and NIEDERGERKE (1954) showed that, in a living isolated muscle fibre from the frog, photographed with an interference microscope, the *A* band stays of constant width, within the errors of measurement, during stretch, passive shortening and quick and slow contractions, so long as the sarcomere length does not fall below 1·8–2 μ. They also found the same in passive stretch and relaxation using polarized light (unpublished: see p. 266).

(*b*) H. E. HUXLEY and HANSON (1954) similarly found by phase contrast microscopy that the width of the *A* bands of fibrils from glycerinated muscle is independent of sarcomere length, in stretch and in contraction induced by ATP treatment, so long as the sarcomere length did not fall below 1·7 μ.

(*c*) HARMAN (1954) recorded spontaneous contraction and relaxation of isolated fibrils by cine photography with the phase microscope, and

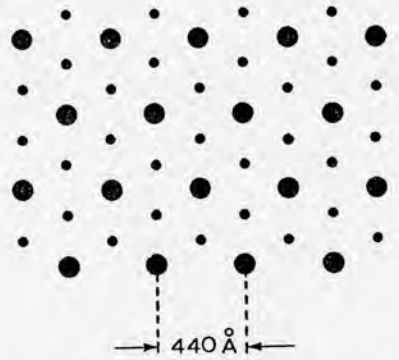

Fig. 2. Diagram showing the arrangement of the filaments in a cross-section through the outer part of an *A* band, where both myosin (larger) and actin (smaller) filaments are present. The dimensions given are appropriate for a muscle at its extended length in the body.

found that shortening took place by approximation of the *A* bands, with obliteration of the *I* bands.

(3) *Constancy of length of I filaments.* The gap between the *I* filaments belonging to the two ends of any one sarcomere shows up as the *H* band in the intact fibril, and as an almost completely empty region when the *A* filaments have been dissolved away. In both cases the gap increases in width as the fibre is stretched (H. E. HUXLEY and HANSON, 1954), and the length of the filaments that remain after extraction of the *A* filaments is independent of the degree of stretch (HANSON and H. E. HUXLEY, 1955, p. 247). In living muscles under the interference microscope, the *H* zone is conspicuous only when the fibre is moderately stretched (neither shortened not greatly stretched), as would be expected on this picture, but the optical conditions are not good enough to measure its width.

(4) *Constancy of X-ray spacings.* The 415 Å period of the low-angle X-ray diagram was found by H. E. HUXLEY (1952, 1953b) to be unchanged when the muscle was stretched. A spacing of about this value is seen in both *A* and *I* bands under the electron microscope (HALL, JAKUS and SCHMITT, 1946; DRAPER and HODGE, 1949), so it is

not clear whether the X-ray observation is really evidence for constancy of length in the *A* or the *I* filaments, or both. The high-angle X-ray pattern also appears to be little affected by stretch or contraction (ASTBURY, 1947).

(5) *Location of myosin in A, and actin in I, filaments.* HASSELBACH (1953) and HANSON and H. E. HUXLEY (1953) found that solutions known to dissolve myosin would readily remove most of the *A* band from fibrils of rabbit muscle; the central part of the *A* band disappeared only on prolonged extraction. HASSELBACH showed by viscosimetry that the material that went into solution was about 95 per cent myosin and 5 per cent actin. The *I* regions of the fibrils are not much affected by this extraction, and the *A* region is reduced to the same density as the *I* (except that, if the fibril is sufficiently extended to possess *H* bands, these become almost empty after the extraction). The fact that the actin is known to be present in untreated fibrils makes it probable that the remaining filaments consisted largely of actin: this was confirmed by HANSON and H. E. HUXLEY (1955), who showed that they disappeared on treatment with a solution known to dissolve actin.

(6) *Behaviour of filaments at short sarcomere lengths.* At sarcomere lengths between about 1·6 and 2·0 μ, the *A* band retains its ordinary length of 1·5 μ, but the *I* filaments are too long to do this. They appear to shorten by localized folding and not by uniform contraction, since narrow dense bands appear, particularly at the centre of the *A* band where the ends of adjacent *I* filaments meet (H. E. HUXLEY and HANSON, 1954; A. F. HUXLEY and NIEDERGERKE, 1954). On still further shortening, the dense bands at the *Z* lines become exaggerated as "contraction bands," which can naturally be interpreted as due to folding of the ends of the *A* filaments where they meet the *Z* line proper.

(7) *Existence of S filaments.* H. E. HUXLEY and HANSON (1954; HANSON and H. E. HUXLEY, 1955) found that a fibril from which the *A*-band material had all been dissolved away could still be stretched, leaving an apparently empty gap at the middle of each sarcomere, and would shorten again passively when released. There must therefore be some highly extensible connections between the *I* filaments of the two ends of any one sarcomere.

(8) *Arrangement of the filaments.* The arrangement shown in Fig. 2 is taken from H. E. HUXLEY's (1953a) very beautiful electron micrographs of transverse sections of rabbit and frog muscle. To a large extent, this arrangement was suggested by the same author's low-angle X-ray observations (1952, 1953b), which are also the source for the dimensions.

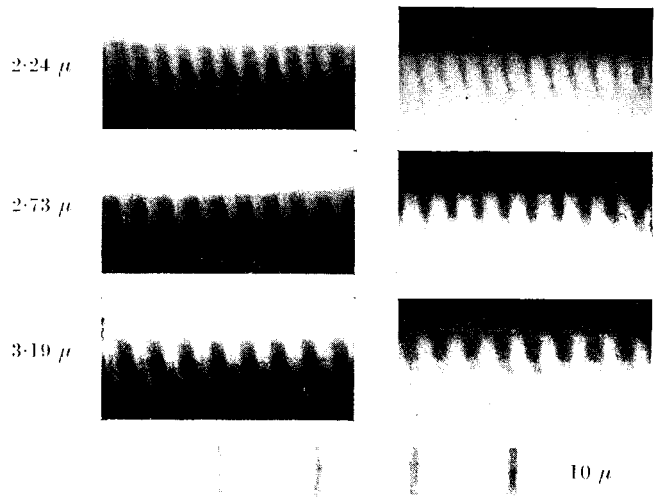

Fig. 3. Polarized light photomicrographs of the edge of an isolated living muscle fibre from the frog, unstimulated. *Right*: no compensation: background and *I* bands dark, *A* bands bright. *Left*: compensated so as to make *A* bands appear dark: background and *I* bands bright. Three degrees of stretch are shown, the sarcomere length in each case being indicated to the left of the photographs. The width of the *A* bands is seen to be unchanged while the *I* bands increase in width as the fibre is stretched. Water immersion objective, n.a. 0·75; condenser aperture limited by a square whose side (parallel to the axis of the fibre) gave an n.a. of 0·41. From a cine film taken in collaboration with Dr. R. E. TAYLOR.

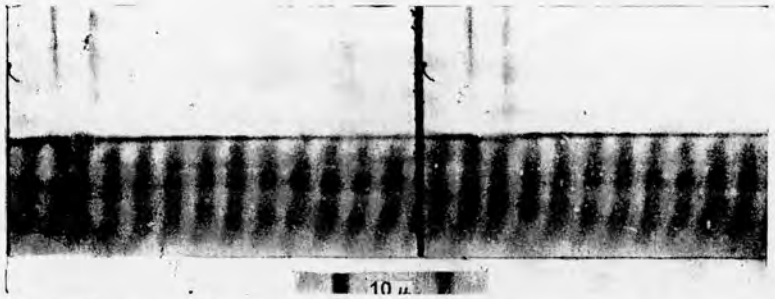

Fig. 4. Shortening of a single *I* band induced by depolarizing a small area of the membrane with a micropipette (tip diameter 4 μ) in contact with the fibre surface (the tip of the pipette is not well shown in this reproduction). Two successive frames from a cine film, 16 frames/sec; *right*, just before, and *left*, just after, the start of a negative pulse applied to the pipette. Isolated fibre from the frog; polarized light, compensated so that *A* bands appear dark; other optical conditions as in Fig. 3. Reproduced by permission of the *Journal of Physiology* from A. F. Huxley and Taylor, 1955b.

1. 3. *Discussion of the evidence*

This evidence appears sufficient to show that the scheme under discussion is correct at least in its main outlines. There are obvious deficiencies, however: for instance, isolated fibrils contain tropomyosin and at least one unknown protein as well as actin and myosin (Perry, 1953), and the material at the centre of the *A* filaments evidently differs in some way from that composing the ends which are easily removed by fluids which dissolve myosin. Points of this kind do not however alter the main thesis that changes of length involve two sets of filaments, one composed largely of myosin and the other largely of actin, sliding past one another.

Some of the important points in this evidence have however been recently questioned. Hodge (1955) has published some extremely clear electron micrographs of dipteran flight muscle in cross-section which show only a single set of filaments in the *A* bands. He regards this as contradicting H. E. Huxley's (1953a) evidence for a double set, but it seems unsafe to deny the existence of a structure on the grounds that it has not been seen in a particular preparation treated in a particular way. The material and the treatment are both very different from H. E. Huxley's, and it would be most valuable to discover what is the reason for the difference between their electron microscope pictures. A clue may perhaps be given by the fact that in his X-ray studies H. E. Huxley found evidence of the secondary filaments only in muscle that was in rigor, not in fresh muscle. Hodge also claims that his longitudinal sections show that the filaments of the *A* and *I* bands are continuous with one another. Apart from the fact that his *I* bands are not well preserved and the continuity is not easy to trace, at any rate in the published reproductions of his micrographs, this is again negative evidence in that filaments originally separate might become adherent during the preparation. It is clear however that these points need further investigation.

Another criticism of the evidence presented here concerns the interference microscope observations of A. F. Huxley and Niedergerke (1954). Buchthal *et al.* (1936) found, on isolated frog fibres under the ordinary light microscope, that the *A* band appeared to be more extensible than the *I*, which is the reverse of the findings reviewed above. As mentioned on p. 261, measurements of this kind on a thick specimen like a frog fibre (approx. 100 μ diameter) in ordinary light are not reliable; this was the reason for developing the interference microscope used by A. F. Huxley and Niedergerke. But Carlsen and Knappeis (1955) now claim to have substantiated this result using polarized light, with which it is certainly possible to obtain a much more trustworthy image than with ordinary light. They agree that in fixed

or glycerol-extracted material the *A* band appears to stay of constant width, but state that in fresh isolated fibres from the frog the birefringent regions increase in width during stretch more than the isotropic bands. If this was true, it would invalidate much of the evidence I have quoted. But NIEDERGERKE and I also used polarized light on living fibres on several occasions, and found the *A* band width to be independent of the degree of stretch, just as we did with the interference microscope; Dr. R. E. TAYLOR and I have confirmed this again since the appearance of CARLSEN and KNAPPEIS's paper. A series of photomicrographs showing the constancy of width of the birefringent band is shown in Fig. 3. There is therefore a direct disagreement between CARLSEN and KNAPPEIS's results and our own; it is impossible to say what is the cause of the discrepancy, since CARLSEN and KNAPPEIS do not state the conditions under which their observations were made (numerical aperture of objective and of illuminating cone; whether compensation was used or not; thickness of specimen) and do not publish any of their photographs. It is possible that they used too low a condenser aperture; I have found with frog fibres of ordinary thickness that polarized light may then give an image almost indistinguishable from the ordinary light image. NIEDERGERKE and I did not publish any of our polarized light results in 1954 because this method, though better than methods using ordinary light, is less reliable than the interference method for thick specimens.*

II. EARLIER EVIDENCE ON THE WIDTH OF THE *A* BAND AND ITS COMPOSITION

Some of the evidence for the idea that filaments of actin and myosin slide past one another when a muscle changes length was given in the last section. This included only the recent evidence which actually led to the idea being put forward. Several of the separate points that are established by this evidence (e.g. constancy of *A* band width, presence of myosin in *A* bands) were repeatedly suggested, and often clearly stated, both in the voluminous literature of last century and in more recent work. Probably none of this old evidence is important at the present day for helping to decide how far this idea is correct, but it is perhaps instructive to examine some of this work in the hope of seeing how it came about, in the first place, that the idea we are

* The reason is that the oblique rays in the plane of the long axis of the fibre traverse a greater thickness of muscle substance than the oblique rays in the plane perpendicular to the fibre; the total retardation of the extraordinary component by the parts of the fibre which are above and below the plane of focus is therefore greater for the former than for the latter groups of rays. The corresponding error with the interference microscope is eliminated by adding protein to the immersion fluid so as to bring its refractive index close to the average value for the fibre contents; there is no equivalent procedure for the polarizing microscope.

discussing was not put forward much sooner, and in the second place, that even the separate points which had formerly been accepted, and which are now confirmed, were generally rejected or disregarded from the end of the second war, or in some cases earlier, until a year or two ago. Many of the old observations are also of considerable interest in themselves, and have not been repeated in recent years.

II. 1. *Constancy of A band width*

It seems to have been generally agreed throughout the second half of last century that the width of the *I* band varied more than that of the *A* when fibres or fibrils in different degrees of stretch or contraction were compared. Some of the sources of this opinion are the following:

(1) DOBIE (1849) reported that the band with lower refractive index (*I*) was extremely narrow in fibrils which were slightly shortened, and could sometimes be lengthened by stretching the fibril if the preparation was very fresh.

(2) KRAUSE (1869, p. 172) observed that the width of the *A* band of vertebrate muscle fibres, which he gave correctly as 1·5 μ (p. 12), did not decrease during contraction.

(3) ENGELMANN (1880) measured the width of the birefringent bands (*A*) of fibres of fixed insect muscle. In "contraction waves" he found that *A* changes less than *I* on shortening; there was very little change in *A* during shortening to 60 per cent of the initial length; beyond that, *A* and *I* both shortened but *I* continued to form a reducing proportion of the muscle length.

(4) KÖLLIKER (1888, p. 706) states that *A* enlarges at the expense of *I* during contraction, until neighbouring *A* bands come into contact with the intervening *Z*.

(5) RETZIUS (1890, p. 72) observed, in fixed and stained preparations of muscle from beetles, that contraction (shortening) is associated with first a narrowing, and then complete disappearance, of the *I* band (defined as the weakly stained region) before contraction bands appear.

(6) ROLLETT (1891) states that the low refractive index band becomes relatively longer in passive stretch (p. 61) and shorter on contraction (p. 69).

(7) HEIDENHAIN (1911, p. 623) states that the *I* band is more extensible than *A*.

The testimony on this point is thus remarkably uniform. With the exception of KRAUSE, however, none of these authors laid particular stress on this phenomenon; more emphasis was generally placed on the changes which occur in more extreme degrees of contraction (formation of contraction bands, etc.). KRAUSE, however, on the basis of the apparent constancy of the width of the *A* band, put forward the theory

that the high refractive index and the birefringence of the *A* band were due to the presence there of submicroscopic rods in a parallel arrangement. The length of these rods was supposed to stay constant (1·5 μ) as the muscle shortened, their lateral separation increasing so as to keep the volume of the whole fibril constant. KRAUSE believed that the *I* bands were liquid during life, so that this fluid was supposed to enter the spaces between the *A* rodlets as they moved away from one another during contraction. He did not specify the forces that produced these movements, but suggested that the rods of each *A* band attracted those of the neighbouring *A*'s.

In recent years, however, it has been generally believed that contraction takes place in the *A* band (e.g. FULTON, 1955, p. 129). How then has a universal and, it now appears, well-founded opinion been reversed, in spite of having been embodied in a theory of contraction by one of the most distinguished of the nineteenth-century microscopists?

The most important factors in this change seem to have been two theoretical points raised by ENGELMANN. In the first place, he dismissed KRAUSE's theory on the ground that muscles can shorten far beyond the point at which the *A* bands of adjacent sarcomeres would come into contact if they stayed of constant width (ENGELMANN, 1873b, p. 161). This is admittedly a difficulty, though it should have been clear, independently of any theory, that the shortening process changes in some way at the stage where contraction bands begin to form. The second theoretical point is that ENGELMANN enunciated the principle that all formed contractile elements are birefringent (see for example ENGELMANN (1906), which is largely a review of his own work on muscle). From this generalization, which is probably valid as it stands, and is clearly of great significance, he concluded that the birefringent parts of a striated fibre must be the contractile parts, and hence that they must be the parts which shorten during contraction. He thus seems to have persuaded himself of the reverse of what he had found by direct measurement; in his article of 1906 he refers only in the most cursory way to his paper of 1880. It is worth noting two features of this argument: first, that an experimental observation has been overthrown on theoretical grounds, and second, that the argument involves the tacit assumption that muscle contains a single "contractile substance" which shortens during contraction. Both of these points will turn up again in a different connection (p. 271).

It is these opinions of ENGELMANN's that seem to have been generally accepted, but it is difficult to say how far this was due to his authority and how far to the well-known paper that was published by HÜRTHLE in 1909. He obtained cine-photographs of spontaneous contractions in insect muscle fibres, mostly with polarized light, and claimed that his results both confirmed ENGELMANN's claim that the shortening

occurs in the *A* band, and contradicted the generally accepted view that a "reversal of striations" (development of dense bands in the regions where *I*'s had been in the resting fibre) takes place when muscles shorten greatly. But the difference between his and his predecessors' results seems in fact to lie in the condition of his fibres in the resting state. His *I* bands appear to have been about $\frac{1}{8}$ the width of his *A* bands, while the usual figures given for this ratio are $\frac{1}{2}$ or more. The only explanation that suggests itself for this difference is that his fibres, even in the resting state, were already shortened almost to the point where contraction bands are formed, and further shortening must necessarily involve the *A* band. Similarly, he did not find a reversal because in these fibres at rest the *Z* line (with the accompanying *N* bands, which are composed of interstitial granules) appeared as the densest part of the sarcomere, this being no doubt connected with the narrowness of *I*. He observed the formation of dense contraction bands at the level of *Z* (in agreement with all other observers) but did not regard this as constituting a reversal because they were at the same position as the densest part of the resting pattern. His differences from former observations are therefore more apparent than real, and depend on some imperfectly explained difference in the initial state of the fibres; nevertheless, his conclusions have been widely quoted not only as supporting ENGELMANN's contention that shortening takes place only in the *A* bands (e.g. MEYERHOF, 1930, p. 295) but as disproving the reality of the reversal phenomenon and thence of the formation of contraction bands.

The other well-known work which appears to support ENGELMANN's view is that of BUCHTHAL, KNAPPEIS and LINDHARD (1936). They photographed isolated fibres from the frog in ordinary light, and found that their dark bands changed in width more than their light bands, during both passive stretch and isotonic contraction. As was mentioned on pp. 261 and 265, this is an extremely difficult specimen from the optical point of view, and I can only conclude, from the disagreement with our interference microscope results (A. F. HUXLEY and NIEDERGERKE, 1954) that they were not justified in assuming that their dark band corresponded accurately in width to the actual high refractive index bands of the fibres. This work was, however, generally accepted as further evidence in support of the view that it is the *A* band which is contractile. The observations of HORVATH (1952), which appear to show that the *A* and *I* bands of a glycerol-extracted fibre maintain a constant ratio during contraction, are subject to the same criticisms.

With the advent of the electron microscope, these confusions ought to have been cleared up at once, but they were not. HALL, JAKUS and SCHMITT (1946, p. 38), in their well-known paper, do indeed state that the *A* band hardly changed in width on passive stretch, but they also

state that this confirms Buchthal *et al.*'s results, while in fact it is in direct contradiction with them. Unlike the other features of their photographs, this particular result did not attract much attention: even the authors themselves do not mention it in a subsequent review article (Schmitt *et al.*, 1947). Draper and Hodge (1949) published very good micrographs of separated fibrils from fixed toad muscle: measurement of their plates shows that the *A* band was very constant at about 1·5 μ width in all fibrils that were not so much shortened as to produce contraction bands, but the authors do not comment on this. Hoffmann-Berling and Kausche (1950), using similar preparations from frog muscle, did clearly state that only the *I* band extended noticeably on stretching, though all their absolute dimensions are much too small ($1\frac{1}{2}$—2 times), suggesting that either their preparations had shrunk or the magnification was not correctly determined. Bluhm and Sitaramayya (1951) also found the width of the *A* band in fibrils from rat diaphragm to be unchanged on stretch and during isometric contraction, but to shorten somewhat during isotonic contraction. These two papers do not appear to have received the notice they deserved. The figures given by Philpott and Szent-Györgyi (1953) for the ratios of widths of the various bands in thin sections do show greater changes in *I* than in *A*, but *A* does not seem to stay constant. These results are again unsatisfactory, because the authors assumed without a check that the absolute sarcomere length in each photograph was given by the degree of stretch applied to the muscle before fixation.

It is thus unfortunately the case that there is at present no really satisfactory electron-microscope evidence on the question whether the *A* band width remains constant; on the whole, this idea is supported by the electron-microscope work, and is certainly not excluded by it. Dempsey *et al.* (1946), in their histochemical study of muscle, also found that the *I* band became narrower in moderate contraction, until it almost disappeared.

II. 2. *Localization of myosin*

"Myosin" was first extracted from muscle by Kühne (1864), who named it and characterized it by its solubility properties and by the great ease with which it is denatured. Engelmann (1873b, p. 174) mentions in passing that he believes myosin to be the anisotropic substance, "on account of its histochemical reactions." T. H. Huxley (1880), in a general description of muscular tissue, was able to write: "In fibres which have been acted upon by solution of salt, or dilute acids, the inter-septal zones [*A* bands] have lost their polarizing property. As we know that the reagents in question dissolve the peculiar constituent of muscle, *myosin*, it is to be concluded that the inter-septal substance is chiefly composed of myosin." (The "septa" on which his

nomenclature of the striations is based are the *Z* membranes; the "inter-septal zones" correspond to the *A* bands, and are contrasted with the non-birefringent "septal zones" which correspond to the *I* bands.) Similar observations were made by Schipiloff and Danilewsky (1881), who showed also that the high refractive index of the *A* bands disappeared when the myosin was extracted. They also found that a drop of myosin solution became birefringent on drying.

This view seems to have been generally adopted (e.g. the review by Biedermann, 1927, p. 427), but there seems to have been no fresh evidence until 1930 when von Muralt and Edsall showed that myosin solutions gave flow birefringence, and that the particles were of a length roughly equal to the width of an *A* band (Edsall, 1942). Finally, in 1934 Weber made oriented threads of myosin, and Noll and Weber showed that their birefringence would account for both the intrinsic and form components of that of muscle.

The interpretation of these results was of course complicated by the discovery in Szent-Györgyi's laboratory (Straub, 1943) of the composite nature of the material that had formerly been known as myosin, but there does not seem to be any justification for the way in which the evidence for localization of at least one component came to be disregarded. The chief reason seems to have been that the interactions between actomyosin and ATP provided plausible theories of contraction which required both the actin and the myosin to be uniformly distributed along the whole of the fibrils, so that, on activation, they could form actomyosin filaments which were supposed to shorten by folding under the influence of ATP. The only evidence adduced in favour of this uniform distribution was that the early electron micrographs, especially those of Hall *et al.* (1946), appeared to show continuous filaments running through both *A* and *I* bands. Rozsa, Szent-Györgyi and Wyckoff (1950) did suggest that the filaments might be actin and that the extra material which causes the electron-scattering power of the *A* band might be myosin, but Szent-Györgyi (1953, p. x) later gave up this view.

The position appears to be closely parallel to that which led, after 1910 or so, to the assumption that shortening takes place in the *A* band (see p. 268). The experimental evidence, which on its own account requires at least one component of actomyosin to be localized in the *A* bands, was discarded on theoretical grounds, and the argument again involved the tacit assumption that there is a single contractile substance (actomyosin) which itself shortens during contraction. A further parallel is that in both cases the theoretical view received support from some technically very impressive observations (in the first case, Hürthle's cine-photomicrographs; in the second, the early electron-microscope observations) which, however, would probably not have

been adequate to overthrow the older evidence without the theoretical backing.

II. 3. *The idea of sliding molecules*

The idea that passive stretch of a striated muscle takes place by long molecules sliding past one another, rather than by unfolding or stretching of chains, was put forward at least twice before the evidence discussed on pp. 262–264 had been obtained. FISCHER (1947, p. 787) made this suggestion on the ground that the birefringence of striated muscle changes very little on stretch, and MORALES (1948) did so in order to explain the great extensibility of resting muscle and the absence of change in the wide-angle X-ray pattern; all these appear to be important pieces of evidence. Neither of these authors assumed that active shortening took place by sliding. MORALES did point out that the decrease of active tension with stretch might be accounted for by the decrease of the zone of overlap, but his argument differs from that of A. F. HUXLEY and NIEDERGERKE (1954) in that he postulated only that the strength of connection would decrease, not the number of sites where tension is generated. BOZLER (1936) suggested that the behaviour of smooth muscle could best be explained by assuming that contraction involved changes in the relative position of the molecules, and not in their shape.

III. "ACTIVATION" AND THE *Z* MEMBRANE

III. 1. *The link between excitation and contraction*

A great deal is known about the electrical events which accompany excitation of a muscle fibre, and about the contraction process, but very little about the steps that link them together. The interior of a resting muscle fibre is electrically negative to the surrounding fluid by 60–100 mV, the potential difference existing across a very thin surface membrane. When the fibre is excited through its nerve supply, this potential difference undergoes a transient reduction or reversal. In the most familiar kinds of muscle fibre (those which are capable of an all-or-none twitch in response to direct electrical stimulation), this decrease of membrane potential ("depolarization") propagates itself along the whole length of the fibre by a process which is essentially the same as the conduction of an impulse along a non-myelinated nerve fibre. In other cases (the slow fibres of frogs, and many arthropod muscles) the depolarization spreads passively for only a short distance from each neuromuscular junction, but the whole of the length of each muscle fibre is nevertheless brought into action because it receives numerous nerve terminations distributed along its length. In all cases, however, the electrical change is a reduction (or reversal) of the

potential difference across the surface membrane. It is also found that, if this potential difference is reduced by any other treatment of the fibre (passage of current; KCl solutions; application of acetylcholine to an endplate), the part of the fibre which is depolarized contracts (KUFFLER, 1946). This suggests strongly that the membrane potential change is the essential thing for setting off the contractile process, but if so there is the difficulty of explaining how this electrical change, which can exert a direct influence only on the membrane itself, is able to cause fibrils in the interior of the fibre to contract. The distances involved are considerable: the fibres of skeletal muscle of the frog are commonly over 100 μ in diameter, while in Crustacea the fibres reach 0·5 mm or more. Further, A. V. HILL has shown (1949c) that the link cannot be a substance which is released at the surface membrane and reaches the fibrils by a simple diffusion process, since this would be too slow to account for the very rapid onset of heat production and of the contraction itself.

A suggestion which has occurred to many people is that it is not the membrane potential change, but the currents in the interior of the fibre, that are the immediate cause of "activation" of the contractile substance. If this were the case, there would be no difficulty in explaining the speed of the onset of contraction. But KUFFLER (1946) pointed out that contraction is produced even by a uniform depolarization of the fibre surface (e.g. by immersion in a solution with raised potassium concentration) when there should be no current flow; and STEN-KNUDSEN (1954) has shown that when a muscle is caused to contract by applied current in conditions where an action potential is not produced, the contraction is confined to the neighbourhood of the cathode, where the membrane potential is reduced, while the region midway between the electrodes, where the current density inside the fibres is a maximum, is passively stretched.

It is thus highly probable that the membrane potential change itself is the essential factor for causing activation of the contractile material, and we are left with the problem of the nature of the link between membrane and fibrils.

III. 2. *The Z line*

The *Z* line is a narrow band of high refractive index at the middle of the *I* band. It is widely, though not universally, believed to represent a more or less continuous membrane which runs across the whole fibre, uniting the fibrils to one another and to the sarcolemma. Many authors have ascribed to it a purely mechanical function, keeping the striations of the separate fibrils in register and perhaps transmitting tension in some way to the sarcolemma; the difficulties in this idea have been well stated by BENNETT (1955). But its apparent continuity

and attachment to the sarcolemma also suggested to TIEGS (1924; MATTHAEI and TIEGS, 1955) that its function might be connected with the activation of the contractile fibrils. In TIEGS's view, the action potential itself travels along the *Z* membrane, which he believes to be invariably a spiral structure; it seems clear from electrophysiological work that this cannot be the case, at any rate in normal contractions of vertebrate muscle, where the action potential is clearly a self-propagating change in the potential difference across the surface membrane. However, the possibility remains that the *Z* membrane is concerned with conveying to the myofibrils the influence of a reduction of the potential difference across the surface membrane. BENNETT (1955, p. 54), on the basis of electron microscope observations, has recently put forward the theory that an excitatory influence is conducted inwards from the sarcolemma by the sarcoplasmic reticulum and conveyed to the myofibrils at the *Z* lines (see also p. 276 below).

Dr. R. E. TAYLOR and I recently devised an experiment to test the hypothesis that the *Z* membrane is specifically concerned in the inward spread of activation (A. F. HUXLEY and TAYLOR, 1955a,b). If this is the case, then presumably it is the potential change at the attachments of *Z* membranes to the sarcolemma that counts, and no contraction would be expected to follow a potential change which involved a small area of membrane lying entirely between two adjacent *Z* line attachments. We achieved this situation by bringing a pipette with a squared-off tip, diameter about 2 μ, into contact with the surface of an isolated muscle fibre that could be observed and photographed through a polarizing microscope. The potential difference across the small area of membrane opposite the tip of the pipette could now be reduced by making the interior of the pipette electrically negative relative to the bathing fluid. The resistances at the tip of the pipette, both that of the "seal" of the tip to the fibre and that of the small area of membrane itself, are high enough for the current entering or leaving the pipette to be too small to produce appreciable changes in potential either inside the fibre or in the bathing fluid outside the pipette; this is true even if the membrane opposite the tip were to undergo an action potential.

The muscle fibre was slightly stretched, so that the *Z* lines were about 3 μ apart; the 2 μ pipette could therefore be placed either opposite a *Z* line (centre of an *I* band) or in the space between two adjacent *Z*'s (opposite an *A* band). In the former case, we found that a moderate depolarization (20–40 mV) caused a localized contraction, but in the latter case, a potential several times larger could be applied with no result at all. Further, when shortening occurred, it was confined to the *I* band opposite the pipette, and spread only a moderate distance inwards from the surface of the fibre. A contraction of this

kind is shown in Fig. 4; the pipette in this case was 4 μ in diameter, which is about the largest that can be used without overlapping more than one *Z* line, though it is too large for the space between two adjacent *Z*'s. The contraction spreads further inwards than was the case with the 2 μ pipette, but is otherwise just the same. This photograph also illustrates in passing the fact that the shortening takes place exclusively in the *I* band, the two dark *A* bands being drawn together without changing in width.

These results are entirely consistent with the hypothesis that we started from, namely that the influence of membrane depolarization which activates the contractile substance is conveyed along *Z* membranes. The experiment also shows that the resulting activation affects only the half-sarcomere on either side of the *Z* membrane in question; in Fig. 4 the contraction spreads about 10 μ inwards but does not affect the adjacent *I* bands which are only about 2 μ away. It is not actually proved that the visible *Z* membrane is the structure concerned; it may be that the activation is conveyed along some other structure near the middle of each *I* band. The anatomical evidence reviewed in the next section does however show that the *Z* membrane has the required characteristics, and it seems most probable that it is the structure concerned.

III. 3. *Continuity of the Z membrane*

The *Z* line, which is seen as a narrow band of high refractive index in a fresh muscle fibre, is also very conspicuous in isolated fibrils, whether examined by phase microscopy or in the electron microscope. It usually appears as the densest part of the fibril, as regards refractive index in the light microscope, electron scattering in unstained and unshadowed electron-microscope preparations, and thickness in shadowed fibrils under the electron microscope. There is a good deal of evidence that these dense lines in the separate fibrils are connected to one another across the muscle fibre. This evidence falls into two groups; the first is from observations on fresh muscle while the second is from examination of fixed and stained preparations.

Several very convincing pieces of evidence in the first of these classes date from the last century. KRAUSE (1869, p. 11), SCHIPILOFF and DANILEWSKY (1881) and KÖLLIKER (1888) all observed that continuous structures remained at the position of the *Z* lines when the rest of the fibrils was removed by dilute acid; this is particularly notable in the case of KÖLLIKER's observations since he was using insect fibrillar muscle, in which the fibrils, or sarcostyles, are separated by large amounts of sarcoplasm rich in granules.

In the second class are the observations of ENDERLEIN (1899) and HEIDENHAIN (1911, p. 613), who were able to stain structures uniting

the *Z* lines of adjacent fibrils even where they were separated by a broad band of sarcoplasm, and also running from the sarcolemma to the *Z* of the most superficial fibrils. This was confirmed by VON BOGA (1937), who pointed out that the fine membranes uniting the *Z*'s of adjacent fibrils stain differently from the *Z*'s in the fibrils themselves. TIEGS (1955) has also demonstrated the continuity of *Z* in both fibrillar and non-fibrillar muscles of insects.

A somewhat similar conclusion is reached by BENNETT and PORTER (1953) and BENNETT (1955) from very beautiful thin sections of muscle under the electron microscope. They show connections between the *Z*'s of adjacent fibrils and between the sarcolemma and the *Z* lines of nearby fibrils, but these connections are very much thinner than the *Z* lines within the fibrils, and are easily distorted if the fibrils are displaced longitudinally in preparing the specimens. They regard these connections as part of a "sarcoplasmic reticulum" which exists in the spaces between the fibrils, and connects with the sarcosomes: this reticulum was well described by KÖLLIKER (1888) and RETZIUS (1890) in fibres stained by procedures in which the fibrils were dissolved out or were swollen until they did not interfere with the image of the sarcoplasmic structures.

It seems legitimate to conclude that there exists in the middle of each *I* band a structure which is connected to the sarcolemma, which runs right across the fibre, and of which the corresponding *Z* lines of the individual fibrils form a part. This whole structure may perhaps be called KRAUSE's membrane, or *Z* membrane, in contradistinction to the *Z* lines in an individual fibril (VON BOGA, 1937).

The existence of a "continuous" structure in this sense must not be taken to mean that there is a complete membrane, like a cell membrane, crossing the fibre in each *I* band. The electrical evidence clearly shows that such a thing does not exist, the fibre interior having a fairly low resistance to longitudinal current (KATZ, 1948). If the *Z* membrane is of the same nature as a cell membrane, having a low permeability for ions and a high electrical resistance, it must have extensive perforations through which ions can move; if it is continuous in the sense of not being perforated, it must offer only a very slight resistance to the movement of ions.

III. 4. *Mechanism of conduction along Z membranes*

If we allow ourselves to conclude from this evidence that each *Z* membrane is concerned with conveying an influence of surface depolarization to activate the half-sacromeres which lie on either side of it, the question at once arises, what is the nature of this influence, and how is it conducted. The only clue to this that the experiments provide is the fact that the contraction does not propagate itself inwards, but

spreads for a distance which increases with the voltage applied and with the size of the pipette. The experiment does not prove conclusively that a self-maintaining inward propagation does not occur in a perfectly undamaged fibre, since the fibres had ceased to give ordinary all-or-nothing twitches by the time we managed to observe these local effects, and it is conceivable that there is normally a propagating mechanism which had by then been lost. But fibres in this state can give apparently normal twitches when the membrane is depolarized by applied current (BROWN and SICHEL, 1936; confirmed in unpublished work by Dr. R. E. TAYLOR and myself). There is therefore no need to postulate a self-propagating mechanism, and in any case our experiments show that a graded activation can spread quite a long way in. This seems to exclude such mechanisms as crystallization (HILL, 1949c) and "activated diffusion" (diffusion of a substance whose presence causes more of the same substance to be liberated) which have been suggested as mechanisms for the rapid activation of the fibre interior in response to surface-membrane potential changes, since they would be expected to be self-propagating. At the same time, the fact that the contraction did not reach nearly to the centre of the fibre would be expected on any mechanism that was not regenerative, since the pipette covered only a very small sector of the edge of the *Z* membrane, and the influence of depolarization on this small region was spread out over a broad front as it was conducted inward.

There is nothing at present to contradict the very tentative suggestion that the mechanism may be an electrical one. If the *Z* membrane contained channels whose lumen was connected to the external fluid, and whose walls had a high resistance, then the potential difference across the walls would follow that across the surface membrane of the fibre with a lag depending on the size of the channels, the capacity of their walls, etc. Rough calculations show that the lag would be very small even if the channels were as small as 100 Å diameter, and were insulated from the sarcoplasm by walls with a capacity of 1 μF/cm^2 such as is found in the membranes of many cells.

III. 5. *Longitudinal spread of activation*

There is also the question how a change spreading inwards along the *Z* membrane brings about the contractile process which presumably occurs in the nearest half-*A*-band on either side. The striking feature about this final stage of the spread of activation is that it goes no further than this: no shortening was seen in the *I* bands on either side of the one to which the pipette was applied. If diffusion of a substance from *Z* is responsible, then there must be something that limits the range of diffusion, e.g. the diffusing material might be used up in the first half of the adjacent *A* bands, or there might be an impermeable

layer at the position of *M*. The limitation of the spread of activation would however be directly explained if the change was some alteration in the actin filaments, which was conveyed along them from the *Z* line, since they terminate at or before the middle of the adjacent *A* bands. Other points which suggest that activation may be accompanied by a change in the actin filaments are mentioned on pp. 301 and 305.

III. 6. *The functional unit in striated muscle*

It is customary to take as the repeating unit of the striation pattern the sarcomere, which is defined as the region bounded by two adjacent *Z* lines. The experiments described here suggest that the functional units are centred on the *Z* lines, and not separated by them, so that the unit consists of a *Z* line with the *I* band in which it lies and the half *A* band on either side. We never saw a contraction involving one-half of an *I* band only (though our pipettes may not have been small enough to achieve this if it is possible in principle, and it is also conceivable that a contraction at one side only of the *Z* line might have been mistaken for a weak contraction involving both sides). Also, as mentioned above, we never saw any shortening of the neighbouring *I* bands such as would have occurred if contraction had taken place in both halves of each of the *A* bands adjacent to the point of stimulation. There are, however, occasional records in the literature of contraction waves in fixed preparations of insect muscle where the degree of contraction is strikingly different in the half-sarcomeres on either side of a single *Z* line (ROLLETT, 1891, fig. 10: TIEGS, 1955, fig. 39).

III. 7. *Other functions of the Z line*

It was mentioned above that there are great difficulties (BENNETT, 1955) in the idea that the *Z* membrane conveys the force of contraction to the sarcolemma, but it remains possible that the *Z* line has a mechanical function as well as being concerned in the activation of the fibrils. Within each myofibril there must be some structural link which keeps the myosin filaments alongside one another in each *A* band, and another link which keeps each set of actin filaments in register. The electron micrographs of HODGE (1955) show cross-links between the filaments of the primary array (presumably myosin) in the *A* band; it may be these that keep the myosin filaments aligned, but if so there cannot be corresponding cross-links between the actin filaments, or the sliding movement would be impossible: connections could only exist at the position of the *Z* line, where they would not interfere with the movement of the myosin filaments and their set of cross-links. It is therefore necessary to keep in mind the possibility that the *Z* line has also the function of keeping the actin filaments in alignment.

IV. A HYPOTHESIS FOR THE MECHANISM OF CONTRACTION

Most, if not all, of the hypotheses that have been put forward in recent years as explanations of muscle contraction on the molecular level have assumed that protein chains, running longitudinally in the muscle, shorten by some kind of folding or coiling. These theories have differed in the type of folding assumed, and in the nature of the forces that were supposed to produce the folding, but have been remarkably uniform in assuming that shortening takes place in each filament at a number of points in series, i.e. the decrease in length of the whole filament is the sum of the amounts of shortening that have occurred at the different points of folding along its length, while the whole tension in the filament is exerted at each of these different links. This idea appears to have been supported especially by the changes of structure that were found by ASTBURY and DICKINSON (1940) to occur when a myosin film is stretched or made to shorten, and by the appearance of continuous filaments which was suggested by the early electron-microscope studies of muscle. On the other hand it is very difficult to reconcile with the many lines of evidence, reviewed here and by HANSON and H. E. HUXLEY (1955), showing that the individual filaments do not change in length appreciably when a muscle contracts, anyhow over the greater part of its normal range.

If then we are to replace the hypothesis that shortening is generated by a filament folding at a number of places in succession, by the hypothesis that two types of filament slide past one another, how are we to suppose that this relative movement is brought about? So far, three broad suggestions have been made:

(1) The filaments move so as to increase the number of points at which some chemical interaction can take place between them (H. E. HUXLEY and HANSON, 1954).

(2) The filaments of one type are capable of small changes of length, but these take place cyclically, the attachments between the two filaments being broken and remade so that the overall result is relative movement between the filaments without appreciable shortening of either. This type of scheme, with the further suggestion that the cyclical changes might take place in the actin filaments by successive depolymerization and repolymerization, has been put forward by HANSON and H. E. HUXLEY (1955), and by AUBERT (1955).

(3) A relative force between adjacent filaments of the two kinds is generated at each of a series of points within the zone in which they overlap (A. F. HUXLEY and NIEDERGERKE, 1954; discussed also by HANSON and H. E. HUXLEY, 1955).

So far, there is nothing worthy of the name of evidence either for or against any of these three general possibilities. The second has the

advantage of bringing in some of the interesting properties of actin, while the third provides an explanation for the parallelism between the isometric tension generated in a tetanus and the width of the overlap zone, when a muscle is extended beyond its resting length (A. F. HUXLEY and NIEDERGERKE, 1954). The same authors pointed out that their scheme would account for the known correlation between narrowness of striation and speed of contraction, but this could probably also be fitted into either of the other schemes.

It is possible that high-resolution electron microscopy may help to decide between these alternatives, but it might not: some kind of structural link between the two sets of filaments is required on any of these schemes, and even if the links are seen, it may not be possible to say in which way they are acting. Another way of trying to decide between them is to follow out in detail the relationships between observable quantities (tension, heat production, etc.) that would be expected on each, and seeing whether any of the schemes can be excluded through failure to fit in with the experimental data. As I have stated them, the three types of mechanism are not specified in enough detail for making predictions which can be checked experimentally; before this can be done, a sequence of events linking the supply of chemical energy to the mechanical system must be specified, together with the factors which control the rates of the reactions. Schemes which are put forward in this amount of detail at the present time must necessarily be highly speculative, but may nevertheless be helpful both in showing how far it may be possible to go in explaining the known behaviour of muscle, and also—more important—in suggesting experiments which may exclude particular hypotheses or groups of hypotheses.

One such scheme (belonging to the third of the classes mentioned above) was worked out in some detail by the author in the summer of 1954. It fitted a number of the known properties of muscle fairly well, without many arbitrary postulates, and it therefore seems worth while to give an account of it here. It was framed primarily with reference to the "sliding" hypothesis and to the relations between load, shortening and heat production, but the chemical reactions provisionally assumed to underlie the steps of the contraction process have been chosen so as to be consistent with the main features of the behaviour of SZENT-GYÖRGYI's glycerinated muscle preparation (WEBER, 1955).

The hypothesis will first be stated in general terms, and then a mathematical treatment will be given in order to derive formulae for tension and heat production, as functions of the speed of shortening, for checking against the relationships found experimentally by A. V. HILL.

IV. 1. *Statement of the hypothesis*

The general idea is that each of the filaments of one type (provisionally assumed to be the myosin filaments) has side-pieces which can slide along the main backbone of the filament, the extent of the movement being limited by an elastic connection. These sliding members can combine temporarily with sites on adjacent actin filaments, the connections being formed spontaneously but broken only by a reaction requiring energy to be supplied from metabolic sources. The essential feature of the system is that the rates of the reactions by which the

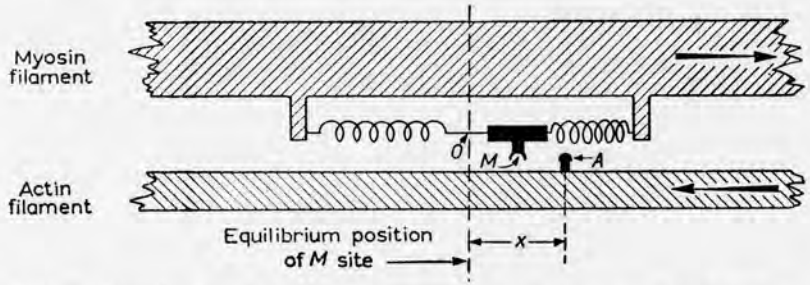

Fig. 5. Diagram illustrating the mechanism by which it is assumed that tension is generated. The part of a fibril which is shown is in the right-hand half of an *A* band, so that the actin filament is attached to a *Z* line which is out of the picture to the right. The arrows give the direction of the relative motion between the filaments when the muscle shortens.

connections are made or broken are assumed to depend on the position of the sliding member relative to the backbone of its myosin filament. This is not difficult to imagine if we suppose that the reactions are catalysed by enzymes which are fixed to the myosin filament, or perhaps actually form part of it.

The essentials of the scheme are illustrated in Fig. 5. The contractile element shown is to be thought of as lying in the right-hand half of an *A* band, so that the nearest *Z* line, to which the actin filament is attached, is off the picture to the right. During shortening, therefore, the actin filament moves to the left relative to the myosin filament. The distance of *A*, the active site on the actin filament, from *O*, the equilibrium position of the sliding element on the myosin filament, is denoted by x (positive if *A* is to the right of *O*). During steady shortening, x decreases at a constant rate. The rate constants for the reactions which make and undo the connection between *A* and *M* are denoted by f and g respectively; they depend on x in the manner shown in Fig. 6.

The system works as follows. Initially, the groups *M* and *A* are detached; *M* oscillates back and forth about its equilibrium position *O* as a result of thermal agitation. If *A* happens to be within the range

of positions where f is not zero (i.e. where the combination of M with A is catalysed), there is a chance that combination will take place; when this has happened the tension in the elastic element will be exerted on the actin thread. The region where this combination can occur is to the right of O, so that the tension in the elastic element is in the direction to help the muscle to shorten. The AM combination moves towards O as the muscle shortens; there is all the time a chance that

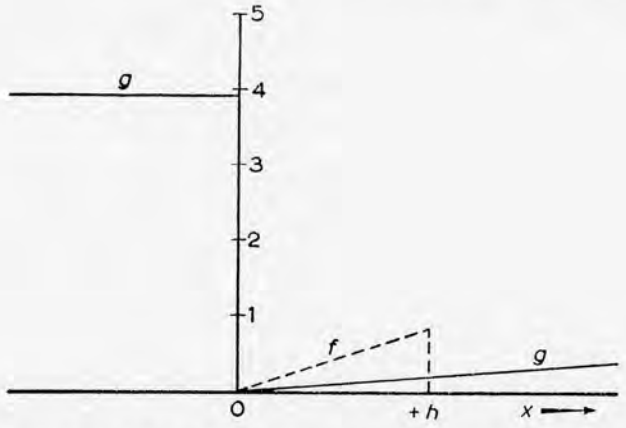

Fig. 6. Dependence of f and g on x. Dashes, f, i.e. rate constant for formation of links between actin and myosin by reaction (1). Continuous line, g, i.e. rate constant for breaking links by reaction (2). The unit of the ordinate scale is the value of $(f + g)$ at $x = h$.

the link will be broken, since g is finite everywhere (except at the point O), but the chance is small until the group passes O; g then increases greatly and the AM link is soon broken, preventing the tension in the elastic link, which is now in the reverse direction, from holding up the shortening of the muscle. At high rates of shortening, a large proportion of the links will not be broken in time to prevent a considerable resistance from being generated in this way; the speed of shortening of an unloaded muscle reaches its limit when this resistance just equals the force produced by the links to the right of O.

We have tacitly assumed that the combination of A and M took place spontaneously with the equilibrium in favour of the combined state; hence it is necessary to postulate that energy is provided in order to break the link. It would be in keeping with SZENT-GYÖRGYI's work and with recent work on glycerinated fibre models (WEBER, 1955) to suppose that this is brought about by some high-energy phosphate compound (denoted by XP) uniting with a site near A. After the A and M groups have separated, XP is split and its fragments are detached, restoring the initial conditions, except that now A is in a

position where f is zero and recombination is not catalysed and therefore does not occur. As the muscle shortens further, the A group we have been considering moves on to a region where it may combine with the next M group further to the left on the myosin chain, while the next A group to the right on the actin chain comes into the picture and is able to combine with the M group that is under discussion.

This sequence of events must be supposed to take place at a number of sites on each filament within each A band, the sites being staggered so that they come into action asynchronously as the muscle shortens. The overall tension in the muscle will therefore not fluctuate appreciably as the links are made and broken.

The reactions that have taken place during the cycle are:

$$A + M \rightarrow AM \text{ (rate constant: } f) \qquad \ldots.(1)$$

$$AM + XP \rightarrow AXP + M \text{ (rate constant: } g) \qquad \ldots.(2)$$

$$AXP \rightarrow A + X + PO_4 \qquad \ldots.(3)$$

In the resting state, reaction (1) must be prevented from occurring, so as to allow the muscle to lengthen or shorten passively. This might be achieved either by inactivating the enzyme which catalyses reaction (1), or by stopping reaction (3), which would leave the A sites blocked by the attachment of XP molecules, or by some independent change in either the A or the M groups which prevents them from uniting. For the sake of definiteness, the second of these assumptions is made here. Activation of the contractile substance then consists in causing reaction (3) to take place; it will be assumed that so long as activation persists, this reaction occurs fast enough for it not to be a limiting factor. (Some reasons for assuming provisionally that the activation change, whatever it is, affects the actin filaments and not the myosin ones, are given on pp. 278, 301, and 305). Further, it is assumed for the sake of simplicity that the back reactions are negligible in all three cases.

The reason why the rate constant g has to be given a finite value for positive as well as negative values of x is that otherwise the side-pieces that happen to be attached when activation ceases (in the sense that no more new attachments are formed) would remain attached, and the muscle would remain in a state of partial rigor instead of returning to the resting condition. This overlap of f and g causes a steady turnover of attached sites even when the muscle is stimulated isometrically; the resulting evolution of heat corresponds to HILL's "maintenance heat."

It is provisionally assumed that the sites on each filament are far enough apart for the events at one site not to be affected by the situation at nearby sites. There is no need to assume that the distance

apart of the M sites on each myosin filament is the same as that between A sites on an actin filament.

IV. 2. *Mathematical formulation*

Consider a large number of contraction sites all with A in the same position relative to O (Fig. 5), i.e. all having the same value of x. Let n be the proportion of these sites at which the M is combined with the corresponding A.

We have

$$\frac{\partial n}{\partial t} = (1 - n)f - ng$$

or

$$-v\frac{\partial n}{\partial x} = f - (f + g)n$$

where v is the velocity with which the A filament is sliding past the M filament as a result of the shortening of the muscle.

Now $v = sV/2$, where s is the sarcomere length and V is the rate of shortening in muscle lengths per second, so that

$$-\frac{sV}{2}\frac{\partial n}{\partial x} = f - (f + g)n. \qquad \ldots.(4)$$

To find the total rate at which chemical energy is being liberated, we note that the overall result of the reactions (1)–(3) is that one high-energy phosphate group is split off, liberating e ergs per contraction site in one cycle. The frequency with which A sites are presented to each M site is v/l, where l is the separation of the A sites along the actin filament. The average number of times each M site enters the cycle of reactions per second is therefore

$$\frac{v}{l}\int_{x=-\infty}^{x=\infty} f(1-n)\mathrm{d}t, \quad \text{or} \quad \frac{1}{l}\int_{-\infty}^{\infty} f(1-n)\mathrm{d}x.$$

The total rate of energy liberation per cubic centimetre of muscle, E, is therefore given by:

$$E = \frac{me}{l}\int_{-\infty}^{\infty} f(1-n)\mathrm{d}x \qquad \ldots.(5)$$

where m is the number of M sites per cubic centimetre of muscle.

To find the tension in the muscle, assume that the elastic element obeys HOOKE's law with a stiffness k dyne/cm. The average value of the work done at one myosin site as one actin site is carried past it by the shortening of the muscle is then

$$\int_{-\infty}^{\infty} nkx\,\mathrm{d}x,$$

and the corresponding average force is obtained by dividing this by l.

The total tension in the muscle is the sum of the tensions generated by all the contraction sites within one half-sarcomere; the number of these sites for a muscle of 1 cm² cross-sectional area is $ms/2$. Hence P, the tension per square centimetre, is given by:

$$P = \frac{msk}{2l}\int_{-\infty}^{\infty} nx\,\mathrm{d}x. \qquad \ldots.(6)$$

The relations (4), (5) and (6) do not depend on the particular assumptions that are being made concerning the manner in which f and g vary with x; to introduce these assumptions, we must insert the values of f and g which are indicated in Fig. 6, i.e.

$$\begin{aligned} x < 0: &\qquad f = 0 \text{ and } g = g_2; \\ 0 < x < h: &\qquad f = f_1 x/h \text{ and } g = g_1 x/h; \\ x > h: &\qquad f = 0 \text{ and } g = g_1 x/h. \end{aligned}$$

Solving equation (4) with these values, and taking V as positive (i.e. the muscle is shortening, not being stretched) we find:

$$x > h: \qquad n = 0$$

$$0 < x < h: \qquad n = \frac{f_1}{f_1 + g_1}\left(1 - \mathrm{e}^{\left(\frac{x^2}{h^2} - 1\right)\frac{\phi}{V}}\right) \qquad \ldots.(7)$$

$$\text{where } \phi = (f_1 + g_1)h/s$$

$$x < 0: \qquad n = \frac{f_1}{f_1 + g_1}\left(1 - \mathrm{e}^{-\frac{\phi}{V}}\right)\mathrm{e}^{\frac{2xg_2}{sV}}. \qquad \ldots.(8)$$

These relationships between n and x are plotted, over the whole range, in Fig. 7 for four values of V. For reasons which will appear later, $g_1/(f_1 + g_1)$ is taken as 3/16 and $g_2/(f_1 + g_1)$ as 3·919.

Inserting these formulae in equation (5) and evaluating the integral, we obtain:

$$E = me \cdot \frac{h}{2l} \cdot \frac{f_1}{f_1 + g_1}\left\{g_1 + f_1\frac{V}{\phi}\left(1 - \mathrm{e}^{-\frac{\phi}{V}}\right)\right\}. \qquad \ldots.(9)$$

When $V = 0$, this gives the "maintenance heat" rate, E_0, as

$$me \cdot \frac{h}{2l} \cdot \frac{f_1 g_1}{f_1 + g_1},$$

while the "extra rate of energy liberation" $E'(= E - E_0)$ is

$$E' = me \cdot \frac{h}{2l} \cdot \frac{f_1^2}{f_1 + g_1} \cdot \frac{V}{\phi}\left(1 - \mathrm{e}^{-\frac{\phi}{V}}\right). \qquad \ldots.(10)$$

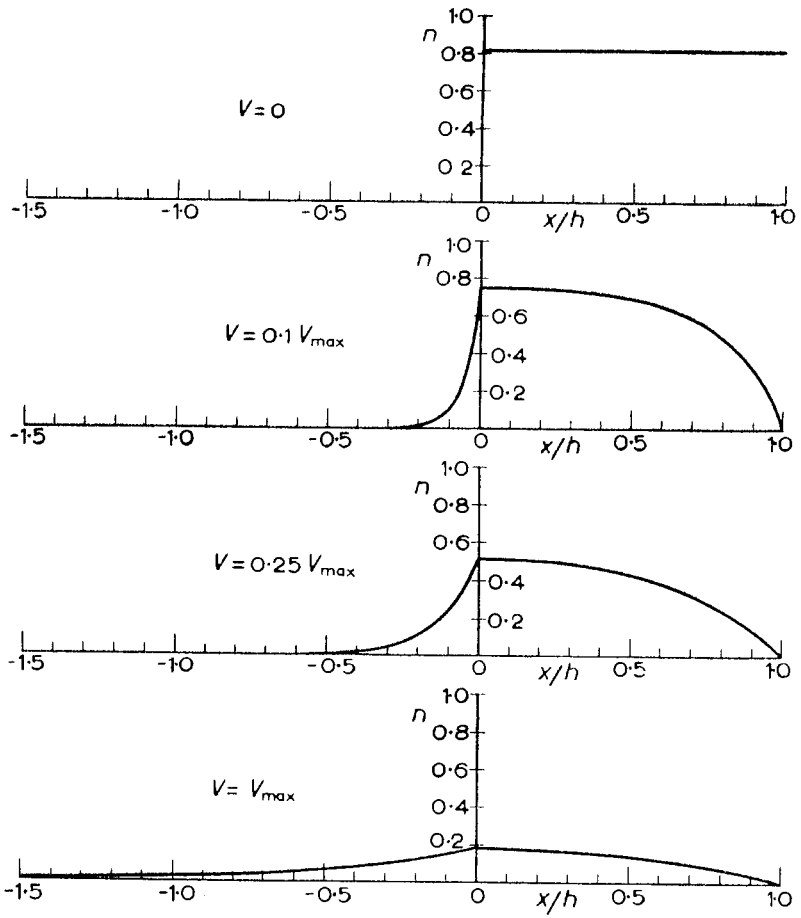

Fig. 7. Variation of n (proportion of sites at which links between actin and myosin are in existence) with x (position of A site relative to equilibrium position of M site), for the steady state in isometric contraction (*top*) and in shortening at three different speeds.

Similarly, equation (6) for the tension becomes:

$$P = \frac{msk}{2l} \cdot \frac{f_1}{f_1 + g_1} \cdot \frac{h^2}{2} \left\{ 1 - \frac{V}{\phi} \left(1 - e^{-\frac{\phi}{V}}\right) \left(1 + \frac{1}{2} \left(\frac{f_1 + g_1}{g_2} \right)^2 \frac{V}{\phi} \right) \right\}.$$

The maximum work done in a cycle at one site is $kh^2/2$: denoting this by w, we have:

$$P = \frac{msw}{2l} \cdot \frac{f_1}{f_1 + g_1} \cdot \left\{ 1 - \frac{V}{\phi} \left(1 - e^{-\frac{\phi}{V}}\right) \left(1 + \frac{1}{2} \left(\frac{f_1 + g_1}{g_2} \right)^2 \frac{V}{\phi} \right) \right\} \quad \ldots\ldots(11)$$

and the rate of doing mechanical work is

$$PV = msw \cdot \frac{hf_1}{2l} \cdot \frac{V}{\phi} \left\{ 1 - \frac{V}{\phi} \left(1 - e^{-\frac{\phi}{V}}\right) \left(1 + \frac{1}{2} \left(\frac{f_1 + g_1}{g_2} \right)^2 \frac{V}{\phi} \right) \right\}. \quad \ldots\ldots(12)$$

The rate of liberation of heat is $E - PV$.

IV. 3. *Comparison with Hill's equations*

To find out whether equations of these forms can represent the actual behaviour of muscle, we may see how closely they can be made to fit the relationships shown by A. V. Hill (1938) to be obeyed by frog muscle during tetanic stimulation at 0°C. These are:

(1) The rate of liberation of heat increases linearly with speed of shortening (constancy of shortening heat).

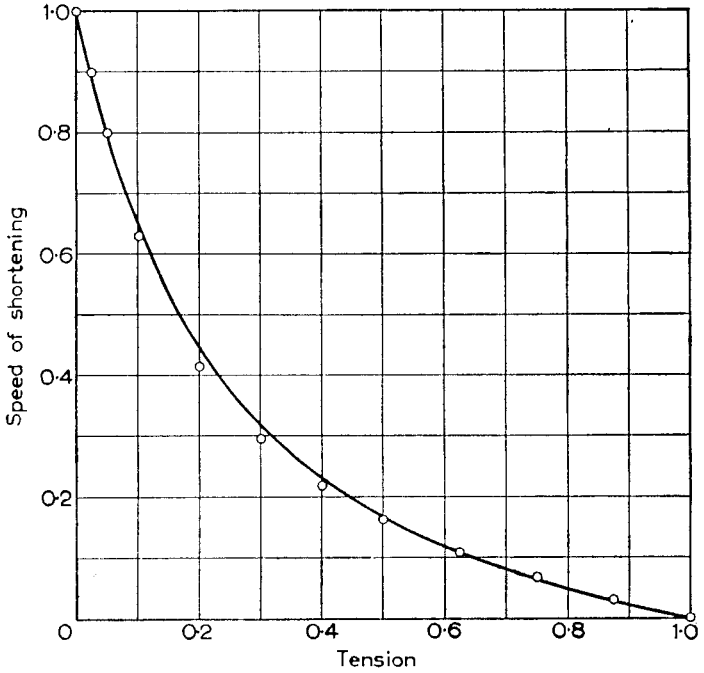

Fig. 8. Relationship between speed of shortening and tension. Continuous line: Hill's characteristic equation with $a/P_0 = \frac{1}{4}$. Circles: equation (11). Ordinate, speed of shortening as a fraction of value in unloaded tetanus; abscissa, tension as a fraction of value in isometric tetanus.

(2) The total rate of energy liberation (heat + work) increases linearly as the load is reduced below the isometric tension.

By combining these relationships Hill obtained his "characteristic equation," $(P + a)(V + b) = \text{constant}$, which he found to agree

excellently with the directly determined force-velocity curve. The constant a was found to be approximately one-quarter of P_0, the isometric tension; b is necessarily equal to a/P_0 multiplied by the speed of shortening under zero load.

Inspection of equations (9) and (12) shows that we have only two adjustable constants left for fitting our equations to HILL's relationships (with $a/P_0 = \frac{1}{4}$). They are

$$\left(\frac{wf_1 + g_1}{e f_1}\right) \quad \text{and} \quad \frac{f_1 + g_1}{g_2}.$$

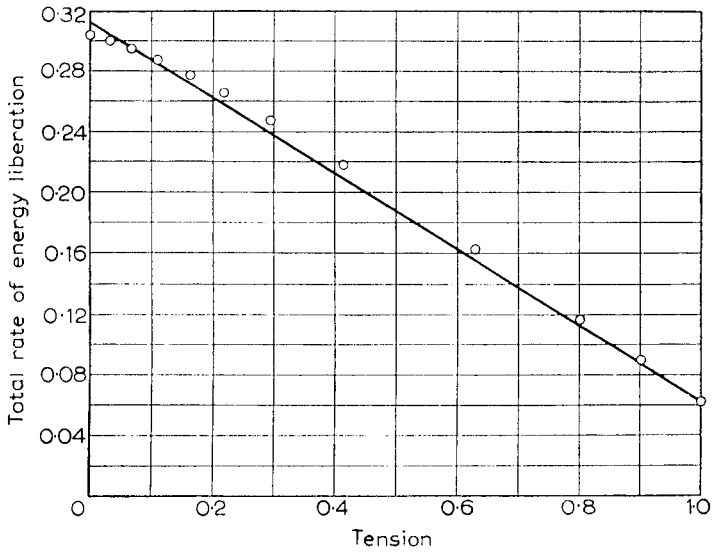

Fig. 9. Relationship between total rate of energy liberation (heat + work) and tension. Straight line, from HILL's equations with $a/P_0 = \frac{1}{4}$ and maintenance heat rate $= ab$. Circles: from equations (9) and (11). On abscissa scale, unity is P_0, the isometric tension; on ordinate scale, unity is the product P_0V_{max}.

The ratio g_1/f_1 has also to be chosen so as to give the maintenance heat rate a value corresponding to that found experimentally; HILL found that it was about equal to ab.

Trial and error showed that fair agreement could be obtained by making $w/e = 0{\cdot}75$, $g_1/(f_1 + g_1) = 3/16$, and $g_2/(f_1 + g_1) = 3{\cdot}919$. These give $V_{max} = 4{\cdot}0\,.\,\phi$, so that ϕ is equal to HILL's b; they also make the maintenance heat rate bear the correct proportion (1/16) to the product P_0V_{max}. The degree of success in matching HILL's relationships can be seen from Figs. 8, 9, 10, and 11. The deviations from hyperbolae in Figs. 8 and 11, and from straight lines in Figs. 9 and 10, are probably not much greater than the experimental error of the observations on which HILL based his relationships.

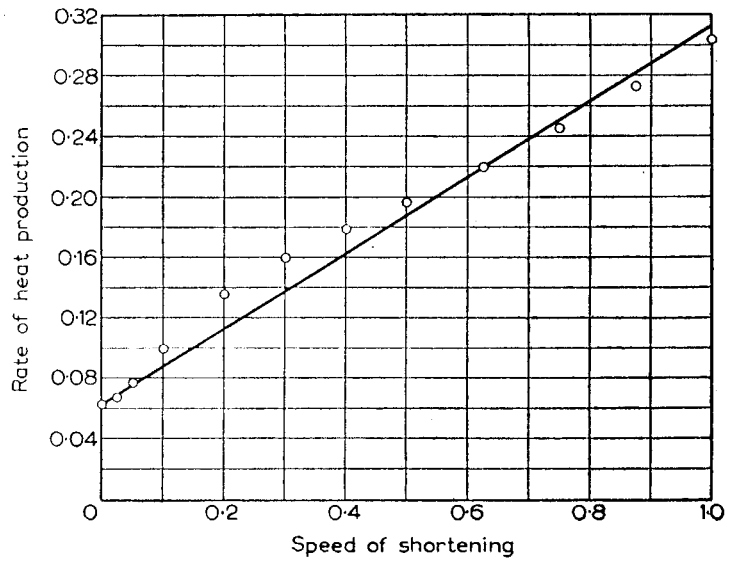

Fig. 10. Relationship between rate of heat production and speed of shortening. Straight line corresponds to a constant heat of shortening (as found by HILL) with $a/P_0 = \frac{1}{4}$ and maintenance heat rate $= ab$; circles from equations (9) and (12). Ordinate scale as in Fig. 9; unity on abscissa scale is V_{max}, the speed of shortening in an unloaded tetanus.

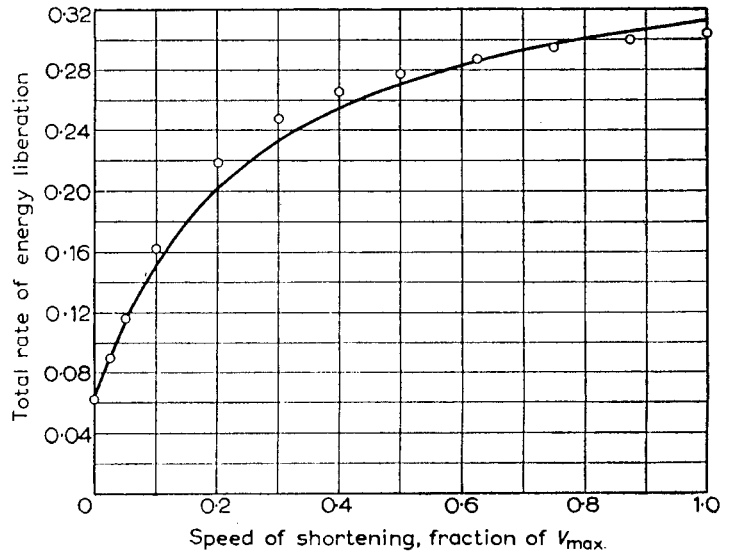

Fig. 11. Relationship between total rate of energy liberation and speed of shortening. Continuous curve (hyperbola) derived from HILL's relationships, with $a/P_0 = \frac{1}{4}$ and maintenance heat rate $= ab$; circles from equation (9). Ordinate scale as in Fig. 9.

Although only two constants were available for fitting HILL's equations, and one for fitting the amount of maintenance heat, arbitrary assumptions had been made at an earlier stage in making f and g vary with x in the ways shown in Fig. 6. One or two other forms of these relationships were also tried. With f and g constant (instead of increasing linearly with x) in the range $0 < x < h$, HILL's equations could be fitted about as well as with the system discussed here, but the ratio w/e had to be given a value of about unity. This is not plausible, as the three reactions that are postulated cannot all go spontaneously in the forward direction unless w is considerably less than e (which is assumed to be derived wholly from a change in free energy). This result was not much altered when the change-over from high f to high g was shifted from the point $x = 0$ to $x = h/5$. It can be seen intuitively that, for a given value of w/e, the efficiency will be lower in these systems with constant f than in the one developed here with f proportional to x, because during shortening a higher proportion of the attachments will be formed at small values of x (therefore contributing little to the work done) in the former system than in the latter.

Another system, within the same general framework, which is of interest, is one in which f and g vary exponentially with x. The equations are:

$x < h$: $f = f_{\max} e^{(x-h)/\lambda}$

$g = f_{\max} (1 - e^{(x-h)/\lambda})$

Force in elastic element $= p = p_{\max} \dfrac{e^{(x-h)/\lambda} - \alpha}{1 - \alpha}$

$x > h$: $f = 0$, so that no combination occurs during shortening: g and p therefore need not be specified.

The interesting point about this system is that it satisfies HILL's equations exactly. Unfortunately it gives about 5 times too high a value for the maintenance heat, because of the great overlap between the functions defining f and g. Mathematically, this can be overcome if g is allowed to become negative when x has values near to h, but this is not permissible, at any rate without extra assumptions, as it implies the spontaneous reversal of a reaction which goes in the forward direction with the release of a large amount of free energy. For this reason, I believe that it is unprofitable to search further for schemes, within the framework of the general hypothesis that is under discussion, which obey HILL's equations exactly, since this particular system is probably unique in giving an exact agreement, apart from a "family" of systems that can be obtained from it by transformations in which the ratios between f, g, and p are unchanged at each value of x, and which lead to the same relations between speed of shortening, tension, and rate of liberation of heat.

IV. 4. *Consequences of the hypothesis*

IV. 4. 1. *Lengthening of a stimulated muscle*

Several interesting results come out if one follows up the consequences of this hypothesis. The first that I shall deal with concerns the tension in the muscle when it is forcibly stretched during tetanic stimulation. HILL (1938) found that there is a discontinuity in the force-velocity curve at the point where the velocity reverses: the increase of tension, above isometric, required to produce a given small speed of lengthening is much greater than the drop in tension which allows an equal speed of shortening. KATZ (1939) investigated this further and his curves (fig. 5) show that $-\mathrm{d}P/\mathrm{d}V$, the slope of the force-velocity curve, is about six times greater for slow lengthening than for slow shortening. Our hypothesis leads to a similar discontinuity, because during slow lengthening n falls off beyond $x = h$ with a space constant equal to $(-)v/g_1$, while during shortening the corresponding space constant of the deficit of n below its steady-state value is $v/(f_1 + g_1)$, which is several times smaller. The resulting changes in tension are proportional to these space constants, but during lengthening there is also a drop in tension due to the fall of n near $x = 0$. The overall result is that the ratio of values of $\mathrm{d}P/\mathrm{d}V$ for slow lengthening and slow shortening is f_1/g_1, which we have taken as 4·33. This figure is to be compared with KATZ's value of about 6.

As the speed of lengthening is increased, n becomes appreciable at greater and greater values of x, and we are assuming that g rises in proportion. Hence, the dissociation of AM links becomes more rapid, while on the other hand there is less time for the links to be formed because of the greater speed. Together these two factors set a limit to the rise of tension; as the speed is increased indefinitely the tension approaches asymptotically a value $P_0(f_1 + g_1)/g_1$, or $5{\cdot}33P_2$ with the values taken here. This is qualitatively similar to the "give" or "slip" which happens when a muscle is stretched during a tetanus, at anything but a very low speed (HILL, 1938), but KATZ (1939) found that the load needed to cause very rapid lengthening was only $1{\cdot}8\ P_0$.

A qualitative explanation is also given for another phenomenon described by KATZ (1939, fig. 7). A muscle is tetanized and allowed to shorten under small load until it reaches a stop; then, before it has had time to develop much tension, a load about equal to the isometric tension at the shortened length is applied. The muscle is considerably stretched, and then shortens again to approach the stop asymptotically. The explanation would be that during the initial rapid shortening the total number of links in existence is small, as shown in Fig. 7 (p. 286). If the load is applied before the number of links has had time to increase, it will be more than they can hold, and the muscle will "give."

A limit to the quick stretch is presumably set by the fact that the muscle is being brought to greater lengths, at which the isometric tension of which the muscle is capable is also greater.

So far, the system has accounted well for the behaviour of a muscle that is stretched during stimulation. But as it stands it does not predict correctly the amount of heat that is liberated. ABBOTT, AUBERT and HILL (1951) found that during slow lengthening the rate of evolution of heat was increased by an amount which was less than the equivalent of the work done on the muscle; ABBOTT and AUBERT (1951) found that the total rate of evolution of heat, including the heat derived from the work done, might even be less than the maintenance heat rate during an isometric tetanus. On the system we are considering, the rate of evolution of heat (apart from mechanical work done) should increase with speed of lengthening in exactly the same way as the total rate of energy liberation increases with speed of shortening (equation 9). This discrepancy could be eliminated, at least qualitatively, if it were assumed that at the larger values of x the AM links were broken not by reaction (2) (p. 283) but by the reversal of reaction (1), which does not involve the splitting of a high-energy phosphate bond. There is no difficulty in principle about this reversal, of the kind that prevented us from assuming a reversal of reaction (2); indeed the equilibrium of reaction (1) is bound to go in favour of the dissociated state at sufficiently large values of x because of the large amount of energy needed to bring M to the position where combination can occur, and the consequent rarity of collisions between A and M.

IV. 4. 2. *Unloaded shortening*

It has been assumed tacitly that the only factors limiting the speed of shortening are the applied load and the resistance generated by those links which are still connected after x has become negative. For a given speed of shortening, the second of these terms will be proportional to the number of sites which are activated, as also is the tension generated by the contraction process. If the load is zero, therefore, the tension generated and the internal resistance to shortening will become equal at a speed of shortening which is independent of the number of sites that are active; in other words, the speed of unloaded shortening is independent of the degree of activation. In the real case, no doubt there are other kinds of resistance to shortening which do not decrease proportionately with the degree of activation, so that the speed of shortening would fall off when the degree of activation becomes very small, but nevertheless this argument does show that the independence might hold, within experimental error, over a large part of the range. This result, which would probably be true also for many other types of contractile system, suggests that

caution is necessary in interpreting the rapidity with which speed of shortening reaches its maximum after a stimulus as evidence that activation is complete equally early (HILL, 1951a; ABBOTT and RITCHIE, 1951b).

IV. 4. 3. *Activation and relaxation*

The concepts of active state and degree of activation, introduced by A. V. HILL, have been very fruitful in reducing a wide range of phenomena in muscle to dependence on a single variable. A muscle is said to be in the active state when it is capable of shortening or developing tension; during full activation it obeys HILL's characteristic equation. The degree of activation at any instant is measured by the tension which the muscle can just hold without either shortening or lengthening (HILL, 1949c). HILL and others have shown that to a large extent the time course of tension in a twitch can be explained by supposing that the degree of activation rises from zero to its maximum very quickly after the stimulus, stays at its maximum for an appreciable time, and then falls away gradually to zero. The relatively slow rise of tension, and the failure of tension to reach, in a twitch, the value that it achieves in a tetanus, are explained by the presence of a series elastic element which the contractile elements have to stretch by a finite amount in order for the tension to appear at the tendon. Relaxation is explained simply as the disappearance of this active state (HILL, 1953b).

On the hypothesis that we are discussing here, activation in this sense involves two distinct steps. The first consists in allowing reaction (3) (p. 283) to occur; this reaction is the removal of the phosphate groups which during the resting state have been preventing the A elements from combining with M. The second step is the actual formation of the A–M links. Nothing has been said so far about reaction (3) except that during a fully developed tetanus it is so fast as not to be a limiting factor. If we assume further that it is complete practically instantaneously after a stimulus, there will still be delay in the development of the degree of activation as defined by HILL, because the muscle can only hold tension in proportion to the number of AM links that have been formed, and the rate of formation of these links is set by the rate constant f which is finite and is related to the maximum speed of shortening of the muscle. The same distinction arises in relaxation: even if the degree of activation fell to zero suddenly because reaction (3) was stopped, the AM links already in existence would persist for a finite time, their number decaying with the rate constant g. So long as any of these links persisted at the end of an isometric twitch, there would still be some tension, but the muscle would not be capable of continued shortening if the load was removed because no new AM links could be formed.

Reaction (3) was chosen somewhat arbitrarily on p. 283 as the one that was inhibited during the resting state, and it was mentioned there that inhibition of reaction (1), the actual formation of the links, might equally well be the means by which relaxation was produced. Even if this was so, there would still be two steps in activation, namely the removal of this inhibition and the formation of the links: even if the first of these was instantaneous, the second would only take place with the rate constant f. Short of postulating a dual nature for the initial step, I have not been able to think of any variant of the general hypothesis which would not give two stages in both activation and relaxation.

If, then, two-stage activation and relaxation are necessary consequences of the hypothesis, it is natural to ask whether there is any experimental evidence for either or both of these phenomena. One case where such evidence exists is in the indirect flight muscles of certain insects and the tymbal muscle of cicadas, where PRINGLE (1949, 1954) has shown clearly that two distinct kinds of activation are involved. In their normal activity *in situ*, these muscles contract in an oscillatory manner at a frequency very much higher than the frequency of the action potentials in their fibres. In the cicada muscle, PRINGLE shows that a single action potential produces an active state lasting between 50 and 100 msec, which can summate with the effects of succeeding stimuli. When the muscle is attached to a lever for recording its contraction, an ordinary twitch or tetanus results, but if it is *in situ*, it performs a series of contractions at intervals of 3–10 msec so long as the active state is at a sufficient level. Each of these contractions causes the tymbal (the "drum" with which the cicada makes its song) to click from an "out" to an "in" position; about 1 msec after this contraction the tymbal clicks out again, stretching the muscle. Clearly, some kind of de-activation must have occurred as a result of the shortening, which allows the outward click to occur; this must last for only a very short time as the muscle contracts again a few milliseconds after the outward click and repeats the cycle; up to 8 of these cycles may occur within 40 msec or so after a stimulus which produces only a single action potential. On our hypothesis, the effect of the action potential might be to remove the inhibition of reaction (3) for say 50 msec, while shortening might cause dissociation of the AM links, which would then need a certain time to reform. The important point, however, is that these muscles—admittedly very specialized ones—show two quite distinct kinds of activation.

In ordinary muscle it might be expected that, if these two stages exist, they would be easier to distinguish during relaxation than at the beginning of a contraction, since it was shown above that the time constant for breaking the AM links in relaxation ($\simeq 1/g$) should

be several times longer than the time constant for forming them at activation ($\simeq 1/f$). In an isometric twitch, this two-stage relaxation would show up by the active state (detected for instance by the ability of the muscle to redevelop tension after a quick release) falling to zero while there was still considerable tension remaining. This point does not seem to have been submitted to direct experimental test, but several published results suggest that muscles do behave in this way. For example, in the contraction shown in fig. 1 (top curve) of a paper by RITCHIE and WILKIE (1955) the degree of activation has fallen to 7 per cent of its maximum value at 330 msec after the stimulus, while the tension is still 90 per cent of its value at the peak of the twitch, and 74 per cent of the isometric tetanus tension.

It also seems difficult to explain some of the quick stretch phenomena described by KATZ (1939) without distinguishing between two steps in the activation process (cf. pp. 291 and 308).

IV. 5. *Size and number of contraction sites*

IV. 5. 1. *Range of movement of side-pieces*

A rough estimate of the value of h, the largest displacement at which a side-piece can become attached to an actin filament, may be obtained as follows. On p. 288 it was shown that the quantity $\phi(= (f_1 + g_1)h/s)$ came out to be equal to HILL's b; hence

$$h = bs/(f_1 + g_1). \qquad \ldots.(13)$$

Now $g_1/(f_1 + g_1)$ was set at 3/16, in order to give the right amount of maintenance heat; hence $h = 3bs/16g_1$. b and s are known (for frog muscle at 0°C), and an estimate for g_1 can be obtained from the decay of tension at the end of an isometric twitch, accepting provisionally the interpretation given in the last section. The same curve in RITCHIE and WILKIE's paper that was referred to in that connection gives the time constant of decay of tension as about 150 msec at about 500 msec after the stimulus, when the degree of activation appears to have fallen to zero. This time constant should be of the order of $1/g_1$; this is not an exact equality because g varies with x (so that the theoretical time course is not exponential), and only reaches g_1 when $x = h$. Hence $1/g_1$ is likely to be less than 150 msec; we might take 100 msec, making $g_1 = 10\ \text{sec}^{-1}$. Now HILL (1938) found that b is about $\frac{1}{3}\ \text{sec}^{-1}$, and s is about 2·5 μ; inserting these values in the above equation we obtain

$$h = \frac{3 \times \frac{1}{3} \times 2{\cdot}5}{16 \times 10}\ \mu = 156\ \text{Å}.$$

This figure is subject to considerable uncertainties. Thus, the values taken for b and for g_1 were obtained from different frogs, and the

estimate of g_1 was in any case very rough; also the value for the ratio f_1/g_1 was obtained on the somewhat arbitrary assumptions (*a*) that the whole of the maintenance heat is derived from the breaking of AM links at values of x between 0 and h, and (*b*) that no links are broken by a reversal of reaction (1). None the less, the value is one that would fit in well with the attractive possibility that the side-pieces are placed at intervals along the filaments equal to the 415-Å period which has been observed with X-rays (Bear, 1945; H. E. Huxley, 1952, 1953b) and in the electron microscope (Hall, Jakus and Schmitt, 1946; Draper and Hodge, 1949).

IV. 5. 2. *Distance between successive A sites*

On p. 286, a formula for the tension during tetanic stimulation was derived (equation 11). Putting $V = 0$, we obtain for the isometric tetanus tension

$$P_0 = \frac{msw}{2l} \cdot \frac{f_1}{(f_1 + g_1)}. \quad \ldots.(14)$$

w was set at $\frac{3}{4}e$, and $f_1/(f_1 + g_1)$ at 13/16, so that

$$l = 0{\cdot}305\, mse/P_0. \quad \ldots.(15)$$

Values for the quantities on the right-hand side of this equation may be obtained as follows. s is $2{\cdot}5\ \mu$ or $2{\cdot}5 \times 10^{-4}$ cm; P_0 for frog muscle is about 2 kg/cm², or 2×10^6 dyne/cm². m, the number of M sites per cubic centimetre of muscle, may be provisionally identified with the number of myosin molecules in the same volume; this may be calculated from a concentration of 8 g/100 ml. and a molecular weight of 840,000 (Weber, 1950) to be $5{\cdot}7 \times 10^{16}$/c.c. If the heat of hydrolysis of the high-energy phosphate group is taken as 10 kcal/mole, then e is 7×10^{-13} erg/molecule. Inserting these values, we obtain $l = 153$ Å.

The interpretation of this result is complicated by the fact that it is close to the estimate we have just obtained for h. Equation (6), from which equations (11) and (15) were derived, assumes tacitly that l is considerably greater than h, so that each M site is always free from its last attachment to an A before the next A presents itself for combination. If l was really smaller than h, the calculation we have gone through would probably give the value of h, not of l; the relationships between force, velocity and heat production would also be modified if l was not appreciably greater than h. We may conclude either that l is equal to or smaller than h, in which case the figure we have obtained is a confirmation of the other estimate of h but the formulae for the force-velocity relation, etc. are no longer exactly appropriate, or else that l is about 150 Å and h is appreciably less, say 100 Å, which would

probably not be too small a value to conflict seriously with the earlier estimate.

IV. 5. 3. *Spacings expected on structural grounds*

Estimates of the spacings of myosin and actin molecules along their respective filaments can be obtained from the quantities of the proteins present, their molecular weights, and the arrangement and spacing of the filaments deduced from H. E. Huxley's X-ray and electron-microscope observations. These calculations have been made by Hanson and H. E. Huxley (1955, p. 253). For the myosin filaments, assuming that there are six molecules abreast (one facing each of the six actin filaments which surround the myosin filament), they find that the longitudinal spacing comes out to about 400 Å, agreeing remarkably with the well-known 415-Å period seen with X-rays and the electron microscope. Each actin filament is surrounded by three myosin filaments; assuming therefore three actin molecules abreast, they calculate a longitudinal spacing of about 130 Å. This is close to the upper limit that was obtained in the last section for the distance between successive A sites with which any one M site can combine; it would be very natural to identify this with the spacing of actin molecules along a filament.

IV. 6. *Discussion*

At the outset, it must be emphasized that the agreement which has been achieved with some aspects of the known behaviour of muscle is not to be regarded as grounds for accepting the scheme which has been put forward. There is little doubt that equally good agreement could be reached on very different sets of assumptions, all equally consistent with the structural, physical, and chemical data to which this set has been fitted. The agreement does however show that this type of mechanism deserves to be seriously considered, and that it is worth looking for direct evidence of the side-pieces, and of the localization of enzymic activity, which have been postulated.

Quite apart from the possible value of this scheme as a working hypothesis, several of the results are of more general interest. The proposed mechanism may be described as cyclic, in the sense that the number of sites in a given condition is not affected by shortening: each side-piece goes through cycles in which it combines with the actin filament by one reaction and is separated from it by another. The final states of the side-piece and of the site to which it was attached are the same as their initial states; the only changes are that the muscle has shortened, an energy-rich phosphate bond has been split and work may have been done. The mechanism may be contrasted in this respect with any of the theories that postulate folding links in series: in these

the number of links in the folded condition increases as the muscle shortens. Mechanisms based on the sliding hypothesis are not necessarily of the cyclic type: of the three classes of mechanism defined on p. 279 (2) and (3) are cyclic but (1) is not.

In a cyclic system, work may be done several times at each site during a given contraction, while in other systems net work can only be done once. If the work per cycle at one site in the first case is of the same order of magnitude as the work per link folded in the second (e.g. because both are related to the free-energy release on hydrolysis of an energy-rich phosphate bond) the number of sites per unit volume will come out smaller in the cyclic systems than in others. This is illustrated by the fact that the molecular weight of the active unit of myosin is assumed here to be 840,000, while both SZENT-GYÖRGYI (1953, p. 36) and POLISSAR (1952d), assuming non-cyclic systems, arrive at figures of around 40,000.

Another point is that several of the general questions that have been asked about muscle (for instance, whether energy-rich phosphate is split during contraction or during relaxation) may lose their meaning in connection with cyclic mechanisms. Thus, in the system developed here, phosphate is split off, by the same reaction, during both contraction and relaxation. Similarly, the question discussed by MORALES *et al.* (1955), whether contraction is produced by the combination or by the splitting of ATP, has no answer on a system of the kind discussed here: the immediate cause of tension development is the actual formation of the links between actin and myosin.

In this system, as in POLISSAR's, HILL's relations between load, speed of shortening and heat production are brought out as approximations. This is perhaps rather unsatisfying, but it does act as a reminder that theories need not be discarded simply because they do not lead exactly to the mathematical formulation that was given by HILL, so long as they fit the experimental data adequately.

A feature of some general interest is brought out by considering the second of HILL's relationships (p. 287) in a slightly different form. It is usually expressed by saying that the total extra rate of energy liberation (in excess of the maintenance heat) increases linearly as the load is reduced below the isometric tension, being equal to $b(P_0 - P)$. If we substitute for P the value given by HILL's characteristic equation, this becomes $b(P_0 + a)V/(V + b)$. Thus, as the speed of shortening increases, the total rate of energy liberation rises rapidly at first and then more slowly (cf. Fig. 11). This shows directly that the amount of chemical change per unit amount of shortening is not a constant, but falls as the speed of shortening rises. D. M. NEEDHAM (1950, pp. 48–49) drew attention to this point, concluding that individual sites were active more than once during a contraction if it were carried

out at low speed, and that, even if ATP breakdown were associated with restoration of the protein chains, it might occur during the contraction phase, bringing the sites into a condition in which they could react again, as well as during relaxation. In the system described here, the fall of energy liberation for unit shortening as the speed is increased is provided for by giving a finite (as opposed to infinite) value to the rate constant f for the formation of the links: as the speed of shortening is increased, there is a parallel rise in the chance that a pair of A and M sites will pass each other without any chemical reaction taking place.

It is natural to ask whether the mechanism proposed here for striated muscle could account also for the contraction of smooth muscle. On general grounds, it is to be expected that the mechanism is fundamentally the same in both types, so that it would be unsatisfactory to postulate for one type a mechanism that clearly cannot exist in the other. Not enough is known at present about the submicroscopic structure of smooth muscle to make a definite statement either way, but there does not seem to be anything to exclude the possibility that smooth muscle contains, in a much less orderly arrangement, filaments which are moved past one another by a mechanism similar to that proposed here for striated muscle. The absence of any marked change in the wide-angle X-ray pattern (ASTBURY, 1947) and in the strength of the intrinsic component of the birefringence (FISCHER, 1944) when smooth muscle is stretched or shortened over a wide range, do indeed suggest that the filaments move relative to one another without much internal rearrangement; and as long ago as 1936, BOZLER suggested that the mechanical behaviour of smooth muscle could be more easily explained by assuming that contraction took place by relative movement between the molecules, than by changes in their shape.

V. OTHER PHENOMENA IN MUSCLE

The hypothesis of muscular contraction set out in the preceding sections was originally developed as an attempt to fit together the available information on (1) muscle structure, (2) the relationships between shortening, tension and heat liberation, and (3) the outstanding facts concerning the interactions of actin, myosin and ATP. There are of course many other phenomena which may provide important clues to the mechanism of contraction and which will have to be explained by any theory which aims at completeness. In the following paragraphs, some of these phenomena will be discussed in relation both to the idea that length changes take place by sliding of actin and myosin filaments past one another, and to the particular hypothesis which has been developed here. Possible new interpretations of the data emerge in several cases.

V. 1. *Early changes in a twitch*

Several physical changes are known to occur very early after a stimulus is applied to a muscle, all being apparent during the latent period before the rise of tension or shortening of the muscle begins, and being complete well before the peak of the twitch. The principal ones are the following.

(1) A small drop in tension (the "latency relaxation") precedes the main rise (RAUH, 1922; SCHAEFER and GÖPFERT, 1937; SANDOW, 1944, 1947).

(2) The torsional rigidity of an isolated fibre increases (STEN-KNUDSEN, 1953).

(3) An increase in the hydrostatic pressure to which the muscle is subjected causes an increase in the tension that is subsequently developed ("alpha process," BROWN, 1934, 1936, 1941).

(4) The resistance of the muscle to passive stretch begins to rise about half way through the latent period (A. V. HILL, 1950b, 1951b).

(5) The rate of heat production rises rapidly to its maximum about half way through the latent period (A. V. HILL, 1949b, 1950a, 1953a).

(6) The amount of light diffracted by the striations decreases (D. K. HILL, 1953).

Probably all of these changes begin at about the same time, but there is a good deal of uncertainty, partly because parallel measurements have been made in only a few cases (1 and 6, D. K. HILL, 1949; 4 and 5, A. V. HILL, 1950a,b), and partly because the time resolution is not good enough in some cases (2 and 3) for precise comparisons to be made. The later part of the time course is obscured by other changes which accompany the contraction itself, except in cases 2 and 3.

V. 1. 1. *Latency relaxation*

The amplitude of the early fall in tension, and the duration of the period in which the tension is below its resting value, are both found to increase as the muscle is stretched (SANDOW, 1944; ABBOTT and RITCHIE, 1951a). A. V. HILL (1951b) has pointed out that this is difficult to explain if it is assumed that the relaxation is due to a lengthening of a structure which is in series with the contractile element and with the "series elastic element" which limits the rate of rise of tension: if the increase in the amplitude of the latency relaxation were due to increased stiffness of the series elastic component, then the rate of rise of tension ought to be increased for the same reason, and the time at which the tension curve re-crossed the baseline ought to have been unchanged. The observation would however be easily explained if the relaxation was due to the lengthening of a structure in parallel with both the contractile and the series elastic elements. This was difficult to fit in with the accepted idea that the contractile material was continuous along

the fibre even at rest; almost the only remaining possibility was the rather improbable one that the latency relaxation took place in the sarcolemma and did not involve the contractile material at all. The difficulty disappears however if the sliding model is adopted. If it is supposed that some part of the resting tension is taken by the actin filaments and the *S* filaments by which they are joined, then the latency relaxation could be the result of a lengthening of the actin filaments (Fig. 1), which could allow the elastic *S* filaments to shorten, reducing their tension. If the tension in the *S* filaments behaves at all like the total resting tension (increasing roughly exponentially with stretch), the tension drop for a given elongation of the actin filaments will increase with muscle length. The *S* filaments are not stretched by the contractile process and therefore have nothing to do with the series elastic element; there is therefore no reason to suppose that the latter becomes less compliant with increase of muscle length.

On this argument, the latency relaxation should represent a lengthening of the actin filaments. There is at present no evidence for an elastic connection from the end of each myosin filament to the adjacent *Z* line, but equally there is nothing to exclude this possibility; if such a thing existed it would be possible for lengthening of the myosin filaments to produce a drop in tension, and the latency relaxation might represent a change in them and not in the actin filaments. In either case, however, it is necessary to assume that the lengthening takes place in one type of filament independently of the other, and does not involve for instance a relative force generated between the actin and myosin filaments.

In the sequence of events which follow stimulation of a muscle, the change which underlies the latency relaxation must come earlier than the contraction itself. It is therefore probably a link in the chain of events which leads to activation of the contractile mechanism, and if it is indeed a change in the actin filaments, it is natural that the structure which conveys activation inwards from the membrane should be located in the *I* bands, as appears to be the case (p. 275).

V. 1. 2. *Increase in torsional rigidity*

STEN-KNUDSEN (1953) found that the torsional rigidity of an isolated fibre of the frog begins to increase before the main rise of tension, reaching a plateau at about one-third the contraction time and maintaining this level until well into the relaxation phase. He very naturally explained this early rise as being due to an increase in the number of cross-links between longitudinal protein chains, but this introduces a difficulty if the myosin and actin are distributed in the way shown in Fig. 1. Cross-links would be expected to be formed only in the zone of overlap, and the low rigidity of the *I* and *H* bands would prevent

any large change from being detected at the tendon ends. With the fibre at "equilibrium length", where the actin filaments of the two ends of each sarcomere are just in contact at the middle of the *A* band, STEN-KNUDSEN found a 20-fold increase of torsional stiffness, while the largest effect that could be expected from increased rigidity in the *A* bands alone is a fourfold increase, since at this muscle length the *I* band forms about a quarter of the sarcomere. It is difficult to suggest what the nature of the change may be, especially as there is no evidence at present whether it arises within the fibrils, from connections between the fibrils (e.g. the *Z* membranes) or from sarcoplasmic structures.

V. 1. 3. *The "alpha process"*

BROWN (1934, 1936, 1941) showed that the application of a pressure of a few hundred atmospheres to a muscle during the early part of an isometric twitch would cause an increase in the peak tension and in the rate of rise of tension, even though the pressure had been reduced to normal by a tenth of the contraction time. BROWN's suggestion (1941), that the high pressure acts by favouring the alteration in chemical behaviour of the muscle that results from stimulation, appears to require that the degree of activation in HILL's sense should be increased, but this appears to conflict with the evidence (HILL, 1949c; MACPHERSON and WILKIE, 1954) that activation is complete even in a twitch at atmospheric pressure. A possible alternative explanation is suggested by the sliding mechanism, together with BROWN's further observation that the application of pressure later in a twitch (e.g. near the peak) causes a *sudden* fall of tension (as well as an accelerated decay if the pressure is maintained). This suggests that increased pressure causes lengthening of either the actin or the myosin filaments (or both); if this is so, then a high pressure at the time when links are first being formed between the actin and myosin filaments will cause the filaments to become united with a greater degree of overlap, and the release of pressure will act just like a quick stretch, which is known to cause an increase in the tension subsequently developed in the twitch by helping to stretch the series elastic elements (HILL, 1949c).

V. 1. 4. *Early decrease of extensibility*

A. V. HILL (1950b, 1951b), showed that, if a muscle is stimulated while it is being stretched, an increase of tension is detectable at a much shorter interval after the stimulus than during a twitch without stretching. This effect begins at about the same time as the latency relaxation, and it has been generally assumed that both are manifestations of the same underlying process. This does not fit in with the hypothesis developed earlier in this article, since the earliest connection between the actin and myosin filaments would generate tension, and

no change involving one type of filament only should be able to alter the extensibility of the muscle; it also does not fit with the idea that the latency relaxation is due to lengthening of filaments without interaction between the two types (p. 301). An alternative is to suppose that the tension-generating process (formation of links between actin and myosin) begins at a very low rate early in the latency relaxation. The amount of tension developed would at first be masked by the relaxation, which is occurring in parallel at the same time, but when the muscle is stretched, the decreased extensibility due to the links might cause the total tension to rise instead of fall. That such an overlap is possible is shown by SANDOW's (1947) analysis of the time course of the early tension changes, in which the rate of rise of the component of tension generated by the contractile process itself was taken to be proportional to the extent of the lengthening which produces the latency relaxation, and a very good fit was obtained with the overall tension change.

V. 1. 5. *Early heat liberation*

A. V. HILL (1949a) has suggested that the heat liberated in a twitch consists only of activation heat and shortening heat, there being no component equivalent to the maintenance heat of a tetanus, which he regards as composed of the summed elements of activation heat that are liberated in response to the successive stimuli in maintaining the state of activation. In a sense, the scheme put forward here (pp. 279–284) fits in with this, in that both activation and maintenance heat must arise at least in part from the same reactions, but as far as that goes, shortening heat also arises from the same reactions. It would seem more natural, if that scheme is correct, to regard maintenance heat as something distinct from activation heat both in a twitch and in a tetanus, the activation component being associated only with the initial decrease in the number of sites blocked by combination with XP (p. 283). The rate of liberation of activation heat would then be proportional to the rate at which sites are being converted to the state in which the links between actin and myosin can be formed. The actual formation of the links is further delayed because it takes place with a finite rate constant (f, p. 281), so that the degree of activation, in the sense of the ability to hold tension, which must be proportional to the *number* of links in existence, lags by two steps behind the *rate* of liberation of activation heat. On this basis, the early maximum in the heat rate is not direct evidence that activation is complete very soon after the stimulus. The activation heat may also contain a component corresponding to the formation of the links between actin and myosin, but this component could not, on this formulation, contribute appreciably to the early peak.

V. 1. 6. *General discussion of early changes*

The discussion in the last few sections has led to postulating three steps in the activation process, apart from membrane changes and the inward spread along the Z membranes. These steps, which agree rather closely with those postulated by SANDOW (1947) in order to account for the early tension changes, are:

(1) Some changes which allows the reaction $AXP \rightarrow A + X + PO_4$ to take place (reaction 3, p. 283).

(2) The reaction $AXP \rightarrow A + X + PO_4$ itself; this reaction is accompanied by the liberation of "activation heat" and by the lengthening whose early stages show up as the latency relaxation.

(3) The formation of the links between actin and myosin by which tension or shortening is generated. The "alpha process" would correspond probably only to the earlier part of this step: as more links are formed, an increase of pressure would be progressively less able to cause the relative motion between the two sets of filaments on which it was assumed (p. 302) that the effect depends. The increase of torsional rigidity would presumably also correspond to this step.

Step (1) must proceed with a time constant of one or two milliseconds in frog muscle at 0°C, since the rate of step (2), as indicated by heat production, reaches its maximum in a time of this order (HILL, 1949b, 1953a).

The time constant of step (2) might be set equal to that of the early fall in the heat rate (after shortening heat has been deducted); from A. V. HILL's work (1949c) this appears to be roughly 25 msec. This may well be an overestimate since the activation heat may contain an element corresponding to step (3).

According to the hypothesis developed on pp. 281–288, the rate constant for step (3) is $(f + g)$. This varies according to the relative positions of the reactive sites; an average value for g was taken on p. 295 as 6·7 sec^{-1}, and $(f + g)/g$ was given the value 5·33, so that $(f + g)$ would be 35·6 sec^{-1}, corresponding to a time constant of 28 msec.

These time constants for steps (2) and (3) are about right to account for the rise of torsional rigidity (assumed to correspond to step 3) being complete at about 100 msec after the stimulus (all times refer to frog muscles at 0°C).

V. 2. *Decreased extractability of proteins*

It has been known for a long time that the fibrillar proteins of muscle are much more easily got into solution from fresh muscle than from muscle in fatigue or rigor (Deuticke-Kamp effect). At the same time a new component appears in the extracts (contractine, DUBUISSON, 1950).

BANGA and SZENT-GYÖRGYI (1943) showed that the decrease of extractability is largely explained by the union of actin and myosin, supposed to be separate in the resting fibre, to form actomyosin, but the observations of HASSELBACH (1953) suggest that other factors may also be involved. He found that a solution of ionic strength 0·6, containing pyrophosphate, will extract the myosin from minced muscle, leaving filaments presumably of actin but removing the Z lines. If the residues are broken up with a blender in the same solution, the actin is dissolved as well (HASSELBACH and SCHNEIDER, 1951); similarly, this solution dissolves both the actin and the myosin from fibrils prepared from fresh muscle (PERRY, 1955). On the other hand, fibrils prepared from muscle in rigor mortis lose only their myosin on extraction with this solution (HASSELBACH, 1953), the actin filaments and the Z lines being retained; fibrils from glycerol-extracted muscle appear to behave in the same way (HANSON and H. E. HUXLEY, 1953). Thus, both the Z lines and the actin filaments are protected by rigor from being dissolved, while the actin filaments, but not the Z lines, are preserved in muscles which has been minced but not broken up into fibrils. Clearly some change has taken place in the material of the Z line on rigor. Possibly this is enough to explain also the increased resistance to extraction of the actin filaments, or an alteration may have taken place in them too, which is an interesting possibility in connection with an earlier suggestion that the actin filaments may undergo a lengthening when the muscle is activated (p. 301).

The existence of a change in the physical properties of the Z line material further suggests that changes in this structure may conceivably be involved in some of the mechanical accompaniments of activation (e.g. the increase in torsional rigidity). On the other hand, it also suggests that rigor mortis involves definite activation of the contractile substance (since the function of the Z line appears to be to transmit activation) and not merely a union between actin and myosin resulting directly from the reduced ATP concentration. Suggestions that activation occurs in other kinds of rigor have recently been put forward on other grounds (SANDOW and SCHNEYER (1955) for iodoacetate rigor, and BARNES, DUFF and THRELFALL (1955) for dinitrophenol contractures).

V. 3. *Optical changes during a twitch*

V. 3. 1. *Scattering of light*

The early decrease in the amount of light diffracted by the striations, discovered by D. K. HILL (1949), was mentioned on p. 300. This early change is soon masked by a much larger effect, which appears to be a decrease in the amount of light scattered by the muscle, and which

follows roughly the time course of the tension (SCHAEFER and GÖPFERT, 1937; D. K. HILL, 1949, 1953). This scattering occurs almost entirely in the direction at right angles to the long axis of the fibres, and must therefore be due to longitudinal elements of the muscle structure. These are presumably (*a*) the outlines of the fibres themselves, and (*b*) the threads of sarcoplasm that lie between the groups of fibrils. If we set aside the possibility that the change depends on an alteration in the shape of the fibres, then the decrease in scattering is probably due to a decrease in the difference of refractive index between sarcoplasm and fibrils. In isolated fibres under the interference microscope, it is quite clear that the sarcoplasm has a higher refractive index than any part of the fibrils (A. F. HUXLEY and NIEDERGERKE, 1954), so that a decrease in the refractive index difference could indicate either a transfer of dissolved substances from sarcoplasm to fibrils, or of water in the opposite direction; the second seems a more likely possibility. There is no basis at present for estimating the magnitude of the shift.

V. 3. 2. *Decrease of birefringence*

It is well established that the strength of the birefringence of striated muscle falls during an isometric twitch (VON MURALT, 1932; BOZLER and COTTRELL, 1937), the amount of the fall being a maximum (about 30 per cent) if the muscle is near its natural length. This effect might also be accounted for by the shift of water from fibrils to sarcoplasm which was suggested in the last section as an explanation of the decrease in scattering. This could act in two ways: first, by concentrating the material which lies between the filaments in the fibrils, so reducing the refractive index difference which causes the form component of the birefringence, and second, by reducing any contribution, analogous to form birefringence, which may be made directly by the refractive index difference between the threads of sarcoplasm and the fibrils. If the movement of water went so far that the refractive index difference was actually reversed in either of these cases, then the form birefringence would increase again (since its strength is roughly proportional to the square of the refractive index difference); this is a possible explanation for the double humped curves that were obtained by VON MURALT.

V. 4. *Tetanus tension*

V. 4. 1. *Absolute values*

The absolute values for the tetanus tension per unit cross-sectional area that are to be found in the literature for vertebrate muscle vary over a wide range. Frog muscle at 0°C appears to give from 1·5 to 2 kg/cm² (HILL, 1938; HAJDU, 1951); at room temperature, HAJDU finds 2·5 kg/cm², while RAMSEY and STREET (1940) obtained 3·5 kg/cm²

from isolated fibres, which would correspond to about 3 kg/cm² in whole muscle when allowance is made for intercellular space. Larger values are generally quoted for mammalian muscle (e.g. 5 kg/cm² for rabbit muscle, WEBER, 1955, p. 273) but RITCHIE (1954) found only 1·5 kg/cm² in rat diaphragm strips at 37°C. It would be valuable to know whether these differences are real, or whether they are the result of some accidental difference in technique or in the arrangement of the fibres; if they are real, the question arises whether it is the number of active sites that varies, or the tension developed per site: the tension

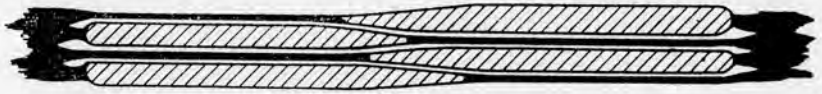

Fig. 12. Arrangement of fibres in a muscle which might give a spuriously high value for the tension per unit area. Tendon, black; muscle fibres, shaded. The tension exerted at the tendons by the fibres shown is four times the tension generated by any one fibre, while the cross-sectional area of muscle fibre at any point is only twice that of the cylindrical part of a single fibre.

per site is clearly important in developing theories of contraction (cf. p. 296). A point which does not appear to have been checked in all cases is whether the fibres of the muscle run from end to end. Even if the fibres are parallel, it would be possible for an arrangement such as is shown in Fig. 12 to produce a larger tension per unit area of the whole muscle than exists within the fibres themselves. The great majority of the fibres of the frog sartorius certainly run from end to end, but it is well known that in the longer muscles of vertebrates, a large number of fibres terminate with pointed ends within the muscle (ROLLETT, 1856; KRAUSE, 1869, pp. 2–6; ADRIAN, 1925).

A. F. HUXLEY and NIEDERGERKE (1954) pointed out that, if the tension in each filament is the sum of effects generated at a number of points in each zone of overlap between actin and myosin, then, other things being equal, a fibre with broad sarcomeres, and therefore a longer zone of overlap, would be expected to produce more tension than one with narrow sarcomeres. This would be compensated by slower contraction, since the number of contracting zones in series (double the number of sarcomeres) would be reduced in proportion. JASPER and PEZARD (1934) showed, in a comparison between different muscles of the same animal (crabs and lobsters), that speed goes with narrowness of striation, and BRENNER (1939) showed the same in a comparison between the three pairs of legs of *Dytiscus*, but I do not know of any data on absolute tension in these or similar cases. There is no comparable variation between different striated muscles of vertebrates; the striation spacing at rest length appears to be remarkably uniform at about 2·5 μ.

There does not seem to be any explanation at present for the variation of tetanic tension with temperature, nor for the failure of tension to be maintained in a constant current contracture.

V. 4. 2. *The limit to shortening in a tetanus*

The fall of active tension in a tetanus as a muscle is extended beyond its unloaded length finds a ready (though by no means proved) explanation in the decrease of the width of each zone of overlap between actin and myosin filaments, and consequent decrease in the number of sites at which tension can be developed (A. F. HUXLEY and NIEDERGERKE, 1954; this assumes that the sites produce tension in parallel). There is no correspondingly simple explanation for the fall of tension with shortening below the unloaded length. This takes the muscle into the range of lengths where the actin filaments must be shortened (perhaps crumpled at their ends); the force required to produce this deformation might be directly reducing the tension which appears at the ends of the muscle. In a tetanus which is not unduly prolonged, the extreme shortening brings the muscle just about to the point where the myosin filaments come into contact with the neighbouring *Z* lines; further shortening only occurs (at any rate in frog muscle) if the muscle is brought into the "delta state" (RAMSEY and STREET, 1940), which involves irreversible changes in the muscle. Again, it is conceivable that it is the stiffness of the myosin filaments that normally prevents further shortening. Alternatively, it may be that the degree of activation is progressively reduced as the muscle shortens; this is suggested by the experiment of KATZ (1939, fig. 6) in which he allowed a tetanized frog sartorius to shorten considerably and develop tension; he then applied a load greater than it could hold but less than the isometric tension the muscle could produce at its rest length. This load stretched the muscle beyond the point where the isometric tension should have been enough to hold it, and the muscle then shortened again to this point. This experiment suggests that the degree of activation was temporarily reduced, but there is no evidence whether this was a consequence of the shortening as such, or of the rapid stretch.

V. 5. *Distribution of other substances*

V. 5. 1. *Sarcoplasmic proteins*

It was mentioned above (p. 306) that the sarcoplasm has a higher refractive index than even the *A* bands of the fibrils, at any rate in isolated fibres from the frog. One would naturally expect the reverse, since refractive index is probably a good measure of the total concentration of solid and dissolved matter present, and the fibrils contain a large amount of actin and myosin in the form of filaments which are

absent from the sarcoplasm. The *I* bands probably contain some other material as well as actin, since the refractive index difference between *A* and *I* suggests that the total concentration in *I* is about $\frac{3}{4}$ that in *A* (A. F. HUXLEY and NIEDERGERKE, unpublished), while this ratio should be about $\frac{1}{4}$ if the *I* bands contained only actin.

Presumably either the filaments are very highly hydrated, or else the soluble proteins of the sarcoplasm are at least partially excluded from the spaces between the filaments. The second of these explanations is suggested by the observation of WEBER (1934) that a dried "myosin" thread placed in a 15 per cent myogen solution would take up five or more times its volume of water without any change of dry weight, i.e. none of the myogen entered the thread. To explain this, WEBER postulated that the micelles composing the thread must be small enough, and therefore numerous enough, to leave spaces no larger than 90 Å (based on a molecular weight of 81,000 for the main component of the myogen, with an allowance for a shell of bound water). On a simple calculation, the spaces between the filaments of fresh muscle come out to be much larger than this. In the *A* band, where both sets of filaments are present, the X-ray data of H. E. HUXLEY (1952; HANSON and H. E. HUXLEY, 1955) give the distance between centres of a myosin filament and each of its neighbouring actin filaments as 250 Å; under the electron microscope the diameters of these filaments are 110 and 40 Å respectively (H. E. HUXLEY, 1953a), so that the gap is 175 Å. For this figure to be reduced to 90 Å by hydration, the filaments would need to be increased to more than twice the diameter found with the electron microscope, and even then the situation would not be explained since the gaps are very much wider in the *H* region and in the *I* band, and they also increase when the muscle is allowed to shorten; also, such a degree of hydration would probably be too high to be consistent with the strength of the form component of the birefringence, unless perhaps the filaments are tubular as is suggested in some electron microscope photographs (H. E. HUXLEY, 1953a; HODGE, 1955). An alternative possibility is of course that the fibrils are separated from the sarcoplasm by a membrane which is impermeable to the soluble proteins, but no such structure has yet been reported from electron microscope observations; or again, "long-range forces" may be involved. The situation is altogether puzzling and deserves further investigation.

V. 5. 2. *Ultra-violet absorbing material*

The well-known measurements of CASPERSSON and THORELL (1942) show nearly all of the material with an absorption spectrum resembling that of the purines to be localized in the *I* bands. They state (without evidence) that 95 per cent of the material with this absorption consists

of the adenosine phosphates; if this is correct, their observations must mean that the concentration of ATP in free solution in the muscle fibre is very much less than the average concentration obtained by dividing the fibre volume into the total amount of ATP present. This is clearly a point of great importance for interpreting the role of ATP in contraction; it appears to be commonly assumed that all the ATP is present in free solution.

There seem to be two loopholes in CASPERSSON and THORELL's work. The first is that their measurements were made on fixed fibres, and there was no actual check that some ultra-violet absorbing material did not escape from the fibres on fixation, though their photographs certainly show that the appearance of the fibres was not noticeably changed. The second is that figures for the nucleic acid content of muscle (DAVIDSON, 1947; LESLIE, 1955) suggest that ATP may form a much smaller proportion of the absorbing material than is assumed by CASPERSSON and THORELL. The location of the ATP in the living fibre is of such theoretical importance that a check of these points might be worth while.

It is interesting that some of CASPERSSON and THORELL's photographs show the ultra-violet absorbing band to be double, so that the distribution of absorbing material corresponds to the position of the *N* bands, which are probably formed by rows of regularly aligned granules (RETZIUS, 1890), together with other sarcoplasmic structures.

CASPERSSON and THORELL's experimental findings were confirmed by ENGSTRÖM (1944), who also showed that the ash produced by microincineration at 500°C was localized in the *I* bands. He believed this ash to consist of phosphates, and to be derived from the adenylic acids and from creatine phosphate, but he did not mention as a possible source either the nucleic acids or the phospholipids; the latter appear to be concentrated in the *I* band, at any rate in mammalian muscle (DEMPSEY *et al.*, 1946).

HOAGLAND, SHANK and LAVIN (1944) also found absorption at 2537 Å, again localized in the *I* bands (HOAGLAND, 1946). Their material was fixed in formalin and sectioned in paraffin, but there is no evidence whether some of the ultra violet absorbing material had escaped.

VI. CONCLUSIONS

As a result of experimental work which has been carried out in the last three or four years, it is at last possible to give interpretations for the chief features of the striations of muscle. The following points can be regarded as very well supported, though perhaps not established beyond doubt in exactly the form stated.

(1) The *A* band does not change in width during stretch and moderate shortening.

(2) The high refractive index and birefringence which distinguish the *A* band are due to rodlets of myosin.

(3) Filaments of actin run across the *I* bands and into the *A* bands as far as the boundary of the less dense *H* region, which represents the gap between adjacent sets of actin filaments.

(4) Except in extreme shortening, changes of muscle length take place not by stretching or shortening of either of these sets of filaments, but by their sliding past one another in each zone where they overlap.

(5) The dense *Z* line which bisects the *I* band is concerned with conveying the influence of the electrical changes in the fibre membrane which accompany excitation to activate the contractile myofibrils.

The point mentioned under (1) was foreshadowed by much evidence from nineteenth-century work, while evidence for (2) continued to accumulate up to about 1940. In each case, the old (and it now appears more correct) opinion was generally discarded, on evidence which now seems inadequate but which was supported at the time by theoretical considerations. This re-emphasizes the necessity for keeping experimental data clearly in view, whatever ideas about the mechanism of contraction may be current.

These interpretations of the striation pattern are of great interest from the point of view of the nature of the contraction process. In the first place, they are sufficiently definite and detailed to play their part, alongside the biochemical and biophysical information, in the formulation of hypotheses. In the second place, they are not easily reconciled with the current idea that contraction takes place by the folding of protein chains at a number of links in series; they therefore suggest new interpretations both of the contraction process itself and of many other phenomena in muscle. The greater part of this article is devoted to exploring some of the possibilities that are opened up in this way. A suggested mechanism of contraction, formulated with reference to the idea that shortening takes place by sliding, not folding, and to the main facts of the responses of glycerinated fibre preparations to ATP, is worked out in sufficient detail to show that it can account fairly well for the mechanical and thermal behaviour of muscle. Neither this however, nor the explanations that are tentatively proposed for various other phenomena, are at present beyond the stage of acting as working hypotheses.

Note added in proof

The experiments on *local activation of a muscle fibre*, apparently through the *Z* membrane (pp. 24–26), have been extended since this article was written (A. F. HUXLEY, *Proc. Physiol. Soc.* 2–3 November, 1956). The chief new findings are:

1. In crab muscle, the sensitive region is not opposite the *Z* lines but is near to each boundary between *A* and *I*. When the membrane

is depolarized over such a point, only the adjacent half-*I*-band (from *Z* to *A*-*I* boundary) shortens.

2. The experiments on frog muscle were repeated under the interference microscope, when the *Z* line is clearly seen. It was confirmed that the sensitive region is opposite the *Z* line, and the contraction always involved both halves of the *I* band equally, even when a weak contraction was evoked by placing one edge of the pipette over the *Z* line.

3. The extent of the inward spread of the contraction in frog fibres was found to be graded according to the strength of the applied potential even when the fibre as a whole was able to respond to an ordinary stimulus with an all-or-none twitch and propagated action potential.

4. In frog fibres, the sensitivity to depolarization does not extend continuously along the line of contact between the *Z* membrane and the sarcolemma, but is restricted to spots whose separation along the perimeter of the fibre is of the order of 5 μ.

Most of the conclusions reached on pp. 27–31 are strengthened by these results as far as the striated muscle of vertebrates is concerned, but clearly some structure other than the *Z* membrane is involved in arthropods. In the latter case, the *Z* lines must have some other function (cf. p. 30), and it is likely that the *Z* lines of vertebrate muscle perform this function, whatever it may be, as well as being involved in the inward spread of activation.

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CHLOROPLAST STRUCTURE AND ENERGY CONVERSION IN PHOTOSYNTHESIS

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CONTENTS

The following abbreviations have been used in this article:
PGA, phosphoglyceric acid; ATP, adenosine triphosphate;
DPN^+, diphospho-pyridine nucleotide; TPN^+, triphospho-pyridine nucleotide;
DPNH, TPNH, the corresponding reduced forms; DNP dinitro-phenol;
FMN, flavin mononucleotide.