# Muscle Contraction: The Contraction Process

## The Scientific Basis of Medicine

**Presented by Professor Andrew Huxley FRCS, University College London.**
**Introduced by Dr Ian Gilliland.**

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**Research films by Professor Huxley in association with**
**Dr R Niedergerke; Dr R E Taylor; Dr A M Gordon.**

**Other film sequences courtesy Columbia University Educational Films.**

**Electron micrographs by L D Peachey.**

**Produced by Peter Bowen.**

**Black-and-white**
**Duration: 00:39:20:19**

**00:00:00:00**

**<Opening titles>**

**<Gilliland to camera>**

Professor Andrew Huxley is Royal Society Research Professor in the Department of Physiology of University College London. Prior to this he was the Jodrell Professor of Physiology. He will be best known to this audience as a Nobel Prize Winner in Medicine in 1963. He is a member of many distinguished foreign academies and

scientific societies and has honorary degrees from universities both in this country and abroad to mark his scientific distinction. Professor Andrew Huxley.

**<Huxley, seated, to camera>**

The voluntary muscle of a human being is of the type that's referred to as striped or striated muscle. A single muscle contains a very large number of muscle fibres of the order of a million and each of these fibres is a structure of about the size and shape of a human hair. This is a rather vague statement to make, but muscle fibres are very variable in their dimensions like a human hair, something less than a tenth of a millimetre in diameter and an indefinite number of centimetres in length. Now, each of these fibres is crossed by a series of bands which give the name striped or striated to this type of muscle. And these bands are at about 400 per millimetre length of fibre, or the equivalent is to say about 10,000 per inch. Now, these are regularly seen in stained preparations of muscle because one or other of the bands takes a particular stain more heavily than the other. But, in living muscle, the bands are clearly seen but not because one band absorbs light more than another but because of differences of refractive index. The muscle fibre is quite transparent, but the different bands have different refractive indices because the protein concentration in the different bands is different.

In order to make the bands well seen in the living fibre, we need to use a microscope which shows up refractive index differences rather than the absorption differences which are what an ordinary microscope sees. Phase contrast, of course, is a device for showing up these refractive index differences in a thin specimen, but it's not very good on a thick structure like single muscle fibre, tenth of a millimetre thick, perhaps. And an interference microscope which does rather the same trick in rather a different way is much better. And later on, I shall be showing some slides and some films of isolated, living muscle fibres photographed under an interference microscope which I built for this series of investigations.

Now, the first thing that I shall be showing in a moment is a small part of a living fibre isolated by dissection from one of the muscles in the leg of a frog. This is very similar

to the muscles of mammals. The striation pattern is substantially identical; the fibres are rather bigger in diameter, a tenth of a millimetre rather than perhaps of that size in a mammal. And these are fairly short fibres, they're about a centimetre long. Some fibres of a mammal would be of this length, some would be many centimetres. But, substantially, this is just the same as a mammalian fibre. Now, in the static picture, which I will show in a moment, the interference microscope is set up so that the high refractive index bands appear dark.

**00:04:44:00**

**<Huxley narrates over photomicrograph>**

You'll see these bands running across the fibre. The dark bands, high refractive index bands, are given the letter A to designate them, and the light bands the letter I. The sort of scale of this is that the repeat distance of the striations is about 2½ microns, one ten thousandth of an inch, and the whole fibre on this scale would be some hundreds of yards long and several feet wide.

**<Huxley to camera. Refers to slides and diagrams and narrates over them>**

Now, these bands were extensively studied in the nineteenth century, but microscopic work on them went out of fashion in about 1900, and rather little was done in the first half of this century. There were various reasons for this: one, I think, was the rise of biochemistry and it came to be realised that the really interesting things must be happening on a scale much too small to be seen in a light microscope. Another thing is that smooth muscles which just don't have these stripes are perfectly capable of contracting, and it became fashionable to argue that because smooth muscles are able to contract, therefore, these striations can't be very important. And it's remarkable that after this attitude was general, as I say, for half a century, the progress that has been made in the last twenty years has been chiefly due to a revival of interest in microscopy of striated muscle. Detailed studies of these striations, both of the light microscope and, of course, with the electron

microscope, and the discoveries that have been made, have led on to much of what is now known about how muscles work.

Now in the years 1953 / 54, the sliding filament idea was put forward and quite soon established. This was partly based on films which I shall be showing to you in a moment, but even more on work by H E Huxley and E J Hanson – electron microscopy, x-ray diffraction and extraction of proteins from the various bands. Now, Hugh Huxley's work with the electron microscope showed that the striation pattern is due to the regular arrangement of two sets of filaments. <*Diagram*> This is a diagram of the striations of filaments. The broad black bands are the A bands and the electron microscope shows that this pattern is due to a regular array of two sorts of filaments, thick and thin filaments which you see here <*next diagram*>. And when the muscle changes its length, either passively or by active contraction, these two lots of filaments slide past each other and neither filament changes its own length by any appreciable amount. <*Animated diagram*> Here you see them sliding in and being pulled out again, and this is the kind of thing that is believed, on very good evidence now, to happen whenever the muscle fibre shortens or is stretched in any way, either passive changes or active contraction.

<*To camera*> The thick filaments that you saw there are composed principally of the protein known as myosin and the thin filaments are principally composed of the protein actin. Now, the next thing I want to show is some films of living fibres contracting under the microscope. And this will be in the form of a film that was put together by the Centre for Mass Communication of Columbia University in New York. They made this when I spent a few months there in 1964. The first section will show some cinemicrographs that we taken by Dr Rolfe Niedergerke and myself in Cambridge in 1953 and 1954, again using the same interference microscope with which that static picture that I showed a short while ago was taken. Now here is this film that was made, as I say, seven years ago.

**00:09:32:13**

**<Film. Part 1: The Striation Pattern During Contraction. Film starts with Huxley to camera>**

The first four of these pieces of film were taken by Dr Rolfe Niedergerke and myself in 1953 and 1954. They all show isolated fibres from the semitendinosus muscle of the frog under an interference microscope. This instrument shows up differences of refractive index within the specimen and it can be set so as to give either a positive image, in which regions of high refractive index appear dark or else a negative image in which high refractive regions appear bright. The first piece of film shows this reversal of contrast as the setting is altered on the microscope. At first the image is negative so that the A bands appear bright because they have a high refractive index which is due to the myosin being entirely localised within them. You will then see the contrast reversing to a positive one in which the A bands appear dark. <*Film*> A bands bright changing over, A bands dark.

<*Huxley refers to diagram*> Apart from the transverse striations, you could see in that film longitudinal stripes of high refractive index; these are columns of sarcosomes or mitochondria which in frog muscle are arranged in this way in the spaces between the myofibrils. There is also a nucleus in the lower part of the field of view. This has a lower refractive index than the other components of the fibre.

Now I will show the same piece of film over again. Notice that the band on the vertical crosswire is bright at the beginning and dark at the end, that is to say it has a high refractive index and is therefore an A band. <*Film*> Band on crosswire is bright, changing over, band on crosswire is dark. Now the same piece of film for a third time. The fact that the contrast reverses, in a proper way, proves that light and dark really do correspond to refractive index differences and are not caused by some other optical properties of the band.

<*Huxley to camera*> Next pieces of film show the same muscle fibre undergoing contractions. The fibre is stimulated by a slowly increasing electric current which does not set up action potentials but causes a local contraction near the cathode. Some of the current flows like this and like this, and where it leaves the fibre, it

lowers the membrane potential below its resting value and this acts as a stimulus to the contractile material. <*Film*> Here is the contraction in negative contrast, that is to say that the bright bands are the A bands. Now the same piece of film again. Watch the dark bands, that is to say the I bands, and notice that they get narrower as contraction progresses. Now the same for a third time. This time watch the A bands which are bright. Their width does not change appreciably until a fairly extreme degree of contraction has developed.

Next we have two copies of another contraction by the same fibre, but this time, the microscope was adjusted to give positive contrast. Now the A bands show up dark because of their high refractive index. Watch the light I bands becoming narrower as the fibre contracts. The same contraction again: notice that the dark A bands keep the same width throughout almost the whole of the contraction.

<*Huxley to camera*> The natural explanation of this constancy in width of the A bands is that the myosin, the protein which gives these bands the high refractive index by which they are visible, is in the form of rodlets, parallel to the long axis of the fibre, whose length does not change when the fibre shortens or is stretched out.

The next section shows a more extreme degree of contraction. The fibre is viewed in positive contrast so that high refractive index bands are dark. At first, you will see the familiar pattern of A and I bands; the light I bands are rather narrow because the fibre is not much stretched. <*Film*> A and I bands. Now there are thin dense lines where the A bands were originally. Now a second set of dense lines in between the first. Now the same again, notice that the first set of thin dense lines appears where the A bands were. Now, and in between them, a second set of dense lines appear.

**00:15:04:12**

<*Huxley to camera*> This film was, in fact, the one that first suggested a sliding filament mechanism to us, because the formation of these dense lines, the so-called contraction bands, can be simply explained on the following basis. <*Refers to diagram*> This diagram represents the situation at the beginning of the film. The

existence of these two sets of filaments has, of course, been shown directly with the electron microscope by Dr H E Huxley. Here is the I band. As the fibre shortens, the I band becomes extremely narrow and its low refractive index becomes largely cancelled by the dense Z line at its centre. And all this structure ceases to be resolved by the light microscope and becomes invisible in the film. On the other hand, at this position, the thin filaments must either fold up at their ends or overlap, thus forming a narrow line of high refractive index at the centre of the A band. This represents the first set of narrow dense lines that we saw. With further contraction, the I band disappears altogether, and the Z line is able to show up as another thinly dense line. With the even further shortening, the ends of the thick filaments must either fold or come forward here, intensifying still further the second set of contraction bands which were formed at this position.

Now I will show the same film for the third time. <*Film*> A and I bands. First set of thin lines, and the second set of thin lines appears now. <*Huxley to camera*> This sequence of changes was not seen in every experiment. Sometimes the simple alteration of A and I bands persisted and the changeover to the pattern of thin dense lines did not occur. The explanation for this was given by the films that I will show next. These were taken by Dr A M Gordon and myself in 1962. The experimental arrangement was the same as before and the only difference was that we searched for a part of the fibre on the side facing the stimulating the cathode where the fibrils were contracting actively while it was possible that the rest of the fibre might be shortened passively. <*Huxley draws diagram on blank paper to illustrate*> The thing to watch for, the first time I show this next film, is that the fibrils near the edge of the fibre remain straight, while further into the fibre they become wavy as the contraction progresses as though the fibrils near the surface were under tension and these are being caused to buckle by being shortened passively. The film is in positive contrast, that is to say that the A bands appear dark.

<*Film*> Contraction begins, a strip of fibrils near the edge stays straight, but in the rest of the field the fibrils become wavy. Now the same again, this time watch the striations in the straight fibrils near the edge. Here you see the single set of thin

dense lines. Next time, watch the striation pattern in the left hand part of the field: A and I bands both become narrower and the thin dense line pattern does not appear. <*Huxley to camera*> Thus the contraction band pattern appears only in fibrils which are contracting actively and not in fibrils which are being pushed down passively to the same degree of shortening.

The next piece of film shows another contraction of the same kind after the setting of the microscope has been changed so as to give negative contrast, that's to say the A bands will appear bright, and in the contraction band pattern, the narrow dense lines will appear bright. <*Film*> Fibrils are going wavy on the left, but they stay straight near the edge of the fibre on the right. Same again, this time watch the striations in the straight fibrils near the edge. Here are the thin dense lines and you can see the second set. Same for a third time. Watch the striations in the wavy fibrils, A and I both become narrow without the appearance of a pattern of thin bright lines.

<*Huxley to camera*> It is still uncertain what rearrangements the filaments undergo in fibrils that are being shortened passively below their resting length, but the changes in the striation pattern, in stretched fibres and in fibrils that are contracting actively below their resting length, are all closely in accordance with what is to be expected on the sliding filament theory.

**00:20:15:01**

**<Film. Part II: The Maximum Length For Contraction. Film starts with Huxley to camera, referring to diagram beside him>**

On the sliding filament theory, one would suppose that the force of contraction is generated here where the thin filaments and the thick filaments overlap by some kind of interaction between them. If this is so, contraction ought to fail if a fibre is stretched so far that the filaments no longer overlap one another. The films in this section were taken by Dr L D Peachey and myself in 1958 in experiments designed to test this point. Current estimates of the lengths of the filaments are about 1.6 microns for the thick filaments and 2 microns for the thin. The critical length of a sarcomere above

which contraction ought to be impossible is therefore about 3.6 microns. All these films show isolated fibres from frog muscle. They are taken with an ordinary microscope at 55 frames per second so that they are being projected at rather less than half the original speed. Each piece of film is projected three times over.

This experiment is not as simple as it sounds because, even in an isolated fibre, its striation spacing is not exactly the same everywhere. The first piece of film shows a fibre undergoing an isometric tetanus. Both tendons are held stationary and the fibre is greatly stretched so that for most of its length, the striation spacing is about 4 microns at which length there is not overlap. Still, at the ends, the spacing is only about 3.1 microns where there is still a considerable amount of overlap. <*Huxley narrates over film*> Here is one end of the fibre. There is tremendous shortening when it is stimulated. Repeat the same piece of film. This shortening occurs in spite of the fact that both ends of the fibre are held stationary. Repeated a third time – very great degree of shortening.

Now the middle of the same fibre during a similar isometric tetanus. Some longitudinal movement during the contraction. Repeat that piece of film. During the contraction, there is no evident change in the striation spacing. Now the same piece of film for a third time. <*Huxley to camera*> In a contraction like this, the tension rises slowly as the end parts of the fibre shorten and stretch the middle part, although the amount of this stretch is rather small and is not obvious in the film.

Evidently, isometric contractions do not give a clear answer, so we tried isotonic ones. Here the tension is kept constant so that the ends of the fibre can shorten without altering conditions for the middle part of the fibre. The next piece of film shows a point in the middle of a fibre where the striation spacing is about 3.6 microns. <*Huxley narrates over film*> Tetanic stimulation and isotonic conditions. Same piece of film again. There are slight longitudinal movements but no general shortening. Now the same piece of film for a third time. There's no general shortening of the fibre.

**<Intertitle>**

**Striation spacing reduced from 3.6 microns to 3.4 microns**

Next, you will see the same fibre being stimulated under the same conditions except that the tension has been reduced so that the striation spacing is now 3.4 microns and the fibre shortens tremendously. <*Huxley narrates over film*> Same again. There is very great shortening. And the same piece of film for a third time.

<*Huxley to camera*> The same result is shown in a different way in the next pair of films. The whole length of the fibre is visible and its movements are made visible by pieces of thread which are stuck to it. It is held in the isotonic apparatus which allows both ends to move. First, the fibre is stretched out so that the striation spacing is about 3.6 microns for the main part of the length of the fibre, though, as before, the spacing is considerably less close to the end. <*Huxley narrates over film*> The ends of the fibre do shorten. Now the same again. Watch the markers near the middle – they don't move. Now the same for a third time. It's only the end parts of the fibre that shorten.

**<Intertitle>**

**Striation spacing near the middle of the fibre reduced from 3.6 microns to 3.4 microns**

Next we shall see the same fibre stimulated under the same conditions, except that the tension has been reduced so that initially the sarcomeres are about 3.4 microns long in the middle part of the fibre. <*Huxley narrates over film*> There is very great shortening all the way along. Now the same again. Watch particularly the markers near the middle of the fibre. Same for a third time.

<*Huxley to camera*> The series of experiments illustrated by these films showed that a fibre does not contract if its sarcomeres are stretched beyond a critical length which lies between 3.5 and 3.6 microns. Estimates made with the electron microscope of the sarcomere length above which there is no overlap range from a little below to a little above 3.6 microns, which agrees within the likely experimental error with the critical length at which contraction fails. <*End of film*>

**00:26:05:10**

**<Return to Huxley in studio. To camera>**

Well, accepting that length changes take place by this sliding movement of two sets of filaments and that force is generated by some kind of interaction between the two filaments where they overlap, the next question is – what is the nature of this interaction? The first question that we may ask ourselves is – how does the force generated by the muscle vary with the amount of overlap of the two kinds of filaments. Now some experiments to determine this were done by Dr Gordon and Dr Julian and myself in London about 6 or 7 years ago and I shall show some of their results. In these experiments, we were again using isolated living fibres from frog muscle, but we used a special device which held just a part of the length of the fibre constant so that we eliminated this complication, which was mentioned in the film, arising from the fact that in these fibres the striations are narrower at the ends than in the middles. So, we're making measurements of the tension generated by a part of a fibre within which the striation spacing is quite uniform.

**<Huxley refers to diagrams and narrates over them>**

Now, this diagram is a plot of the tension generated by the fibre against the spacing of this striation pattern. The scale here is in microns of the repeat distance of the striations – 1, 2, 3, 4 microns – and the vertical axis represents the tension generated by the part of the fibre where the striations have this particular spacing. Now, when this fibre is pulled out so that the spacing is about 3.6 microns in agreement with what you saw on the film, the tension is just about zero. And this length corresponds to the situation shown in the top one of these diagrams. Here's the thin filament which only just reaches level with the end of the thick filament. There's no overlap, correspondingly no tension. When the fibre is shorter, so that the thin filament overlaps part of the thick filament, there's a measurable tension and the tension gets bigger as the amount of overlap increases, and the maxim is reached at just this length where the thin filament overlaps all of these little projections which are seen in

the electron microscope in Hugh Huxley's work on the thick filament. So, the tension in this range, the tension increases in direct proportion to the number of these little projections that are overlapped by the thin filament. With a little more shortening, the thin filament goes beyond the part of the thick filament that carries these projections, and the tension stays exactly constant – those three points at the top. With further shortening, which is only possible by the thin filaments overlapping one another, the tension drops and it begins to drop very steeply at the length here where the thick filaments collide with the Z line, the point where the pattern begins to repeat again.

Well, the main conclusion from this is that over the part of the range of length where the filaments don't interfere with each other by collision, the tension generated is directly proportional to the number of these projections that are overlapped by the thin filaments. This left-hand side of the curve is not fully understood yet. The drop of tension is certainly partly due to mechanical interference with the shortening process, where the filaments collide with each other. But, as I shall mention in my next lecture, part of the drop in greatly shortened muscle is due to failure of the activating process to reach to the centre of the fibres.

**00:30:21:24**

**<Huxley to camera. Then refers to graphs and slides and narrates over them, interspersed with talk to camera>**

Well, that's a question of the tension generated by the fibre while its length is held constant. Now, what about the speed at which it can shorten in the other extreme condition where the load on it is zero?

<*Graph*> And that's illustrated in this graph of other results obtained by Dr Julian and myself. Again, we're plotting on the horizontal axis the spacing of the striation pattern, here going from 2 to 3 and not quite to 4 microns per repeat distance. The dashed line just repeats what the tension would be if the length were held constant, and the points that we're interested in now are these ones which show the speed of shortening of the fibre under almost zero load when it's stimulated. Just before

stimulation, speed is zero. The stimulated speed rapidly goes up to a high value and stays almost constant over a tremendous range of shortening. Here's the fibre shortening by between 30 and 40% of its length. And the speed of shortening just drops a little bit.

If we start at a more moderate length, again, the speed goes up and stays constant. So, over this range of lengths, where force at constant length increases rapidly with the amount of overlap, the speed of shortening is practically independent of the amount of overlap; if anything it goes in the opposite direction.

<*To camera*> Now, these two results suggest very strongly that in the region of overlap, there are independent force generators, or shortening generators, independent in the sense that the force produced by each of them adds together so that the total force the sum of the forces generated by the different generators within one overlap zone of one thin filament and the corresponding thick filament. And further, the fact that the rise of tension stopped when all the projections on thick filament were overlapped implies that these projections are, in fact, these independent force generators. If we think of them as independent elements shortening under no load, there's no reason why having a large number of them operating should make it shorten any faster than with just one; if there's no load, there's no way in which the different ones can help each other to shorten faster.

Now these projections, which on this evidence probably are the actual force generators, these structures are usually referred to as cross-bridges: bridges going between the thick and thin filament. This was the name given to them by Hugh Huxley who first discovered them with the electron microscope. But, A V Hill to whom most of our knowledge of muscle contraction, in many respects, mechanical and thermal aspects of contraction, which were much more thoroughly investigated by him than anyone else, now, when I mentioned this to him, he said that these ought not to be regarded as bridges; bridge is something static which remains attached at both ends. Hill, himself, in his younger days was a great athlete and he said that he thought that these things ought to be regarded as legs.

<*Refers to slide*> And if we think of them as legs, this diagram might show the situation in an isometric contraction. No shortening is allowed. The legs act as in a tug of war, generating their maximum force. But, if the filaments are allowed to slide past each other under no load, a rapid movement occurs. And next slide please. <*Next slide*> This slide illustrates what happens in that case. Now, in the tug of war situation, all the feet were on the ground at the same moment. While they're running, only a few of them are on the ground, most of them are lifted up. And one might wonder what could happen if you suddenly reapply to these filaments the force that was capable of being generated by the legs in the tug of war situation? If it was like this, the ground would slide under the feet of these legs and the thing would pull out very rapidly.

**00:35:15:00**

Now, that, in fact, is what happens when this experiment is performed on an isolated muscle fibre. <*Refers to graph*> Now, in this record, the upper trace shows the tension in one of these isolated fibres. The vertical lines are a tenth of a second apart. It's at low temperature so it's contracting fairly slowly. Repetitive stimulation begins at this point, the tension rises and comes to a plateau value. Now, this record shows the length of the fibre. Now the record is, as it were, upside down; shortening is an upward deflection of this trace. Almost no shortening under this load, just about the maximum that the fibre can produce. Now, we suddenly reduce the load to about 10% of what it was when there was no shortening and the fibre proceeds to shorten. There's a rapid jerk at first and then steady shortening at a high speed here. Then we reapply the load, almost instantaneously to what it was before, and you might expect that this would just stop the shortening. But, if you think of it as running legs from which the ground is pulled away underneath, that is actually what happens, the length increases extremely rapidly, this downward deflection is going several times faster than this speed at which the fibre is capable of shortening. So, when we reapply the load, the fibre suddenly pulls out at an enormous speed. It doesn't go on indefinitely; it recovers its ability to contract and stops the shortening process later on. But, this pull-out phenomenon, when you catch the fibre during rapid shortening and reapply a force, is a striking result.

<*To camera*> Well, the next question is – what is the real nature of these legs or cross-bridges? And on structural grounds, Dr Hugh Huxley has proposed that what's actually going on during shortening is something like what I'll show you next. Now, here again is the animated film that I showed earlier. <*Film*> This will illustrate the sliding process of the thin filaments sliding in past the thick ones. And in a moment, we shall go up to much higher magnification showing the overlap of one thick and two thin filaments <*next image*>. This represents the end of a thick filament coming in from the left and there are some of the cross-bridges, the interesting part of the myosin molecules sticking out from the thick filament. Now, this is what is thought to happen during the shortening process, each head successively detaches from and reattaches to the thin filament pulling it along.

<*To camera*> Well, in this lecture, I've been discussing the mechanism by which force is generated in striated muscle, and in the second lecture in this series, I shall be discussing the processes by which this contractile process is turned on and regulated within the cell.

**<End credits>**