Muscle Contraction: Activation of the Contractile Material

The Scientific Basis of Medicine

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Electron micrographs by L D Peachey.

Produced by Peter Bowen.

Black-and-white
Duration: 00:26:20:17

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<Opening titles>

<Huxley, seated, to camera>

Well, in my previous lecture in this series, I was discussing the contractile process itself in muscle or, at least, in the striated muscle, the kind of muscle which we have in all our own voluntary muscles. And today, I shall be talking about the process by which this contractile event is turned on. In normal muscle, contraction is initiated by the propagation of an action potential along each of the fibres of which muscle is made up. This is started by the arrival of a nerve impulse through the motor nerve to the neuromuscular junction where acetylcholine is released; there's a permeability change – membrane potential change which starts off this explosive electric event which travels to both ends of the muscle fibre.

Now, this action potential itself is a change in the potential difference across the surface membrane of the muscle fibre. <*Huxley draws on blank paper on display board beside him*> If we sketch the outline of the fibre like this, it's covered, as all cells are, by a submicroscopic cell membrane, something like 70 or 100 angstroms thick, too thin to be seen by the light microscope, and in the resting state, there's a potential difference with the inside negative relative to the outside of the fibre. When the action potential goes along, this potential difference is reversed – the inside becoming positive with relation to the outside. Now, the only place where there's a large electric field is actually in the membrane; the inside of the fibre at any moment is practically at a uniform potential, and it's only where there is a potential gradient that the electric field can do anything to any physical structure. So, it's actually in the surface membrane that the big electric field which accompanies the action potential actually exists. Well, now, some of these fibres are up to 100 microns or more across, so a myofibril at the centre of the fibre is anything up to 50 microns away from the nearest part of the surface membrane. And the question arises – how does this electric event in the surface membrane influence the myofibrils that are, well, 20 or 50 microns away?

This is not a very large distance, but if one were to suppose that the electric event in the membrane liberated some activating substance and this diffused inside, this diffusion process would take a time of around about 1 second. And it's well known from the experiments of A V Hill that the whole of the inside of one of these fibres can be brought into activity in a very much shorter time than that – a moderate number of milliseconds, 20 or 50 times less time than would be needed for diffusion, is enough to bring the middle of the fibre in to activity. So, there's a problem of how this electrical event on this surface manages in such a short time to activate the inside of each one of the fibres.

Well, since diffusion isn't fast enough, one begins to wonder about some special inward conducting mechanism, and if there were some such mechanism, one might imagine that it would go along some structure lined up with the striations of these

fibres. And thinking about this led Dr R E Taylor and myself to do the experiment which we'll show you in a minute or two.

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<Huxley refers, with indicator stick, to diagram on display board>

Now the principle of the method is shown in this diagram. This represents a longitudinal section of a part of a striated muscle fibre, again, a living fibre isolated by dissection from one of the muscles in the leg of a frog. It's lying in a bath of Ringer's solution under a microscope. We have a glass pipette, whose tip is about 1 micron across, which we can bring into contact with the surface of the fibre by means of a micromanipulator. And there's a silver wire inside the pipette to which we can apply a current. The other electrode is just in the Ringer bath, and when we close the switch here, this is connected to the negative side of the source of current, so current flows from the solution into the mouth here and out.

The potential inside the pipette is lowered because it's connected to the minus side of the battery, so the potential difference across this bit of the membrane is altered in the resting state; as we've seen, the inside is negative with respect to the outside. When we make this go negative, *<indicates pipette>* we abolish or perhaps reverse the potential difference across this bit of membrane. Now that's a change of the same kind as the change which happens during the action potential, which is the normal stimulus when a muscle fibre undergoes a twitch. But, it's localised to this small bit of membrane because, even if this bit of membrane does generate current, it's such a small area that the current is much too small to do anything appreciable anywhere else in the fibre. So, near enough, we can say that the potential in the inside of the fibre stays at its usual negative value, potential in the Ringer's solution stays at its zero level and the only thing that happens is that the potential outside this little patch is made to go negative. So, this little patch undergoes a potential change similar to an action potential but longer in duration; we put it on for about half a second and it's restricted to this small area.

<Huxley to camera>

We recorded the response of the fibre by cinephotography down a microscope and some of the pieces of film that we took are incorporated in the same film that was made by the Centre for Mass Communication at Columbia University from which I showed some excerpts in my previous lecture. Now I'll show you this part of the film.

<Film featuring Huxley speaking to camera>

The first piece of film shows an ordinary twitch fibre from the skeletal muscle of the frog being stimulated in this way. It is under a polarising microscope and the compensation is set so that the A bands appear dark. The black line in the edge of the film shows when the voltage is applied to the pipette. <*Film through polarising microscope*> You see that sometimes a contraction occurs; it never occurs when the pipette is over an A band, only when it is over an I band and it is then restricted to that single I band. Over some I bands, there is no response, but in cases like that, other experiments showed that response could be obtained from other points in the same I band at a higher or lower position of focus. There's a good response – nothing over the A band but a good response from the I band.

<*Huxley to camera*> That film was taken with the polarising microscope and the Z lines were not visible. The next piece is taken with an interference microscope in negative contrast, that's to say high refractive regions appear bright. Here the Z lines can be seen as thin bright lines in the middle of the dark I bands. The contractions of the I bands are well seen but the special point to look for in this film is that the Z lines always stay central in the I bands, even when the pipette is placed a little bit to one side. <*Film through polarising microscope*> Watch the Z line when the edge of the pipette is placed over the Z line itself like this. You will notice that the Z line always stays central within the I band, that's to say the contraction is symmetrical about the centre of the I band. You will see some contractions which spread inwards for 2 or 3 times the spacing between striations but, nevertheless, the contraction does not spread to the adjacent I bands – that contraction is still symmetrical about the centre of the I band <*end of this film*>.

00:09:59:14

<Huxley to camera and then refers to electron micrographs on display board and narrates over them>

Well, these films show that there must be some inward conducting structure at the middle of the I band in frog muscle and, of course, one wonders if there is something to be seen there in the electron microscope. Now here is a longitudinal section of a part of a fibre from frog muscle under the electron microscope and this micrograph was taken by Professor L D Peachey who is now at the University of Pennsylvania at Philadelphia. These dark bands are the A bands; the muscle is rather stretched with broad I bands. Dark line here is the Z line, and here is a place where the section has fortunately gone through a plane between contractile material above and below the plane of the section, so this is a sort of crack between different myofibrils and this part of the section shows the structures that are present between the myofibrils.

<*Next electron micrograph*> Now, the same area at higher magnification is seen here, and let's concentrate on this part which lies level with the Z line of the contractile material. Here you see that there is a continuous tube like structure transversely to the muscle at the level of the Z line, that's to say at exactly the position where the evidence from the living fibres, which I just showed on the film, shows that there must be a transverse conducting system. So, it's a very attractive idea that this tube is a structure which conducts something from the surface membrane to the inside of the fibre. And, of course, what one thinks of is that the inside of this tube is continuous with the external fluid, that the wall of that tube forms an invagination from the surface membrane of the fibre so that electric potential changes will be conducted from the surface membrane up along this tube.

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

Now, in mammals, the structures found in this kind of position are closely similar to what I've just shown you in the frog except that in each repeat of the striations there are two sets of these structures, two of these tubes with their flanking vesicles, one either side of the Z line. And the best way I can show this, I think, is in this slide which is a small part of a fibre from a mouse, which has been stained by the Golgi procedure: this is the procedure with which you're probably familiar in connection with staining of nerve cells where it has the remarkable property of staining a few cells but staining them completely. Now, it also stains intracellular canaliculi in many places, and in muscle it stains these transverse structures which you see occur in pairs and, indeed, the Z line is midway between these pairs – A band is here. So, mammalian muscle has two sets of these transverse tubular systems per sarcomere, one on either side of the Z line. And this has, of course, been shown many times with the electron microscope, and the structures are just like what I showed you in that micrograph from frog muscle. Now, this picture was published in a paper of 1902 by an Italian histologist Veratti. And, although it was well known at the time, that paper was effectively lost to science for about 50 years. <*To camera*> It was rediscovered by H S Bennett in the United States, who was one of the people who first got on to these tubular structures with the electron microscope.

So, we have these tubular structures running across fibres. Now, I suggested that it would be nice if these opened to the surface. Now, no one, I think, has yet seen clearly an opening from one of these tubules in either an adult frog muscle, which is the main experimental material, or in a mammalian muscle, but there's good experimental evidence that these tubules are, in fact, open although the openings have not been seen.

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There are two kinds of evidence for this: one is that muscles have been treated with solutions that contain particles visible under the electron microscope. If a muscle is left in a solution of this kind for some minutes and then fixed, in the electron microscope you find these particles. Ferritin molecules have been used among other markers and these are found inside these tubules but nowhere else. The other kind

of experimental approach is shown in the next slide. <*Next slide*> This shows an experiment on a living fibre, again from the frog. The experiment was done by a Japanese visitor, Dr Endo, in our laboratory a few years ago. The fibre has been immersed in a solution containing a fluorescent dye, and a few seconds before this photograph was taken, the fluorescent solution outside was washed away and replaced by plain Ringer's solution. The fibre was then illuminated with light of the wavelength which excites the fluorescence of this dye and it was photographed in the wavelength emitted by the fluorescent dye. And you see that there are these bright lines across the fibre. These are places where the fluorescent dye has entered the fibre and has not yet come out. These are, in fact, at the centres of the I bands. The dye takes a few seconds to get in and it washes out again in a few seconds time. It does not enter the main bulk of the muscle fibre, so this showing that there is an easy path from the external fluid into, again, some structure at the middle of the I band. And clearly, this is this system of tubules which is at this position in frog muscle and, as I mentioned before, exists twice-over in each repeat pattern in mammalian muscle.

<*To camera*> Now, this fact of the tubules being open at the mouth is easily seen in some animals though, as I said, not in adult frog muscle or in mammalian muscle. Now, here is another micrograph by Professor Peachey, <*photomicrograph*> showing part of a muscle from a fish. Here's one of these tubules running in, again at the level of the Z line, but here the tubule is quite clearly open at the mouth. You can see direct continuity from extracellular fluid into the mouth of this tubule which you can follow right into the fibre. And it, again, is flanked by these vesicles on the side. So there's no doubt that these tubules are effectively open, although there's some still rather puzzling what the nature of the opening is, probably a convoluted piece of tubule just inside the surface membrane in the case of mammalian and adult frog muscle.

<*To camera*> Now, clearly then, there's an electrical event spreading up these tubules from the surface membrane where the action potential occurs. And as regards to the next stage in the process, it's, well, I think, one can say it's certain that calcium ions are released from some part of this system of vesicles and pretty

certainly from these conspicuous, large vesicles which lie alongside the tubules. And, well, it's known from many experiments, on isolated myofibrils from muscle and on muscle fibres whose membrane has been destroyed so that externally applied solutions get direct to the contractile material, that calcium ions even in very low concentrations, 10^{-5} or 10^{-6} molar, are able to activate the contractile system to turn on the enzyme mechanisms which cause the movements which I discussed last time.

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Now, the muscle about which very much more is known than any other is frog muscle, in particular the sartorius muscle of the frog at nought degrees. And, in this case, it was well established by A V Hill that a single twitch, a single action potential propagating along the fibre, is sufficient to turn on the contractile material fully, as I mentioned, in a rather few milliseconds. Even a single twitch, in this frog muscle at nought degrees, generates almost as much tension as a long-lasting tetanic contraction. Now, in most mammalian muscles including human muscles, this is probably not true. The twitch is quite small, the amount of tension generated in a single twitch is only a small fraction of what the same muscle can generate in a tetanus, and in the repeated activation that occurs during tetanic contraction and in most normal voluntary contractions, the high tensions that can be produced are evidently produced by some sort of summation that goes on from the effect of one stimulus to the next.

Now, there's one aspect of this that I'd just like to say a little about. <*Huxley draws on blank paper on display board*> Last time, I mentioned this fact that the tension generated by an isolated fibre declines over a large part of the range of length; we're plotting here the length and the tension that can be produced. This is the length at which 3.6 microns per sarcomere, the length at which the filaments just don't overlap and the fibre is incapable of contracting. And here's the length where all the little contractile projections on the thick filament are overlapped by thin filament. So, over a large part of the range where this muscle is capable of working, it has this characteristic that the longer the muscle, the less tension it produces. Now this is an

unstable situation. If a little part of the muscle were shortened, it is able to generate more tension so it's more able to shorten powerfully and it will stretch other parts of the muscle.

It's an unpleasant arrangement also from the point of view of controlling the activity of muscles. If the load on a muscle drops a little so that it's able to shorten, it'll develop more tension and this is an unstable situation in which lowering the load makes the muscle produce more force, even more able to overcome the load.

Now many and perhaps all muscles have a mechanism which tends to counteract this in that stretching a muscle helps it produce more tension in a twitch. In many mammalian muscles, the curve of twitch tension against length is something of this kind. It reaches its maximum not at the place where a tetanus tension reaches its maximum but at a greater length. This factor related to the situation of the filaments is certainly present, but there's another mechanism by which stretching a fibre causes the contractile material to be more effectively turned on. And either in a twitch or in a weak voluntary contraction, in which individual fibres are activated at a rather low frequency, this factor which activates the muscle more effectively the longer it is even overcomes this negative slope over a considerable range, so that the length tension characteristic has this upward slope over a wide part of the range. This has been very nicely shown in experiments by Dr Rack at Birmingham and I think it's certainly an important feature in stabilising the contractions that are produced under normal situations. It probably acts in the same direction and it probably assists the effect of the stretch reflex, which is a mechanism, of course, utilising the spinal cord and the nervous control of the muscle to produce the same result; but if the muscle is stretched, it produces more tension in that case by activating a larger number of fibres or by producing a higher frequency of impulses to individual fibres.

So, there are these two factors: the external one working through reflex mechanisms, the stretch reflex, this one working probably through the amount of calcium that is liberated inside, which tend to make tension increase with length and which, anyhow, over part of the range, counteract this rather uncomfortable tendency which results

from the sliding filament mechanism – this tendency for a muscle to produce less tension the longer it is and correspondingly the greater the load.

So, these various mechanisms probably co-operate to produce a kind of response which is easy to control in relation to the demands that are put on a muscle during its normal activity.

<End credits>