BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

VOL. 28, NO. 1 JANUARY 1952

CONTRACTION IN THE HEART MUSCLE FIBRE*

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MUSCLE has two functions: it shortens and creates tension. The two functions are, in their essence, but one, because one and the same change will produce contraction or tension according to conditions. If the ends of the system are free, it shortens. If they are fixed, they develop tension. Tension means that the contraction would occur with a certain force.

In order to create a system which could do all this Nature had to solve a number of problems of molecular engineering which we will discuss in succession.

In order to build a system which can shorten, Nature had to use thin and long protein particles. Thin and long particles always tend to fold up, thus shorten, especially if they are very small and are exposed to the bombardment of water molecules moved by heat-agitation. The thread-like, very thin and long protein particles, out of which Nature has built the contractile matter, is "myosin."

* Presented at the 24th Graduate Fortnight of The New York Academy of Medicine, October 10, 1951.

If the myosin particle tends to fold up anyway then the first question we have to answer is not how it shortens but what keeps it straight in resting muscle. There must be repulsive forces which repel, so to say, one end of the particle from the other. Such repulsive forces are always electric coulombic charges. The myosin molecule generates its charges by dissociation. Like other proteins, it has dissociating acidic COOH, and alkaline groups. On dissociation the acidic groups acquire a negative, the alkaline groups a positive charge. The number of acidic groups is slightly greater than that of the alkaline ones and consequently the myosin molecule has a negative net-charge.

This negative net-charge repels other equally charged protein particles. Looking, however, closer into these relations we find that it is not this net negative charge which keeps other particles away. The negative charges are balanced by positive K+ ions which surround the particle. They would actually remain in the closest proximity to the negatively charged particle, would heat-agitation not drive them away, as a wind whirls up dust. It cannot drive them away altogether because this is not permitted by the electric attraction. The end-result is that the myosin particle is surrounded by a cloud of positive K+ ions, thus a positively charged atmosphere, and if another myosin particle approaches it will be these positively charged atmospheres which meet first and repel one another, and thus keep the myosin in solution. This atmosphere will also have the tendency to expand because the positive ions in it repel one another. The end result will be that not only other particles are kept away but the particles will be kept straight, extended.

How strongly this positive ionic atmosphere will repel other similar structures depends chiefly on two factors. First: the number of charges. The number of positive charges in this atmosphere is equal to the number of the net negative charges of the particle itself. Second: the thickness of this positive ionic layer. If the K-ions get very close to the particle then this latter's negative charges neutralize this outward effect. The ionic atmosphere will still be there and keep the particle straight but will not repel other particles any more, which now can approach the myosin particle. Colloidal particles always tend to approach and stick together owing to the Van der Waals cohesive forces and so, if coulombic repulsions do not drive them apart, they approach, stick together, and the final result is the formation of flocculi which rapidly settle to the bottom, i.e., precipitate.

I am not losing my way in intricacies of colloidal chemistry. These problems are most important for the contraction of the muscle fiber, be it heart or body muscle. This will be evident if we discuss the second problem of molecular engineering Nature had to solve when creating the muscle. In resting muscle the myosin particle had to be kept straight and we have seen how this is done. The next problem is, how this particle can be made to contract not at a moment's but at a thousandth's second's notice. How can all these charges be taken away within sigmas? There is one method known to all of you by which a protein can be discharged, that is precipitated (since precipitation is the expression of such discharge), and this is: by adding another specifically built protein, a precipitin. Nature keeps in muscle such a precipitin for myosin. This protein has been discovered in my laboratory and is called actin. If an actin and a myosin particle meet in presence of a physiological salt-concentration they unite to a complex, actomyosin which becomes partly discharged. This discharge is a very involved process and the ions of the solution are involved in the reaction too. Here we are interested only in the end-result. The trick, applied thus by Nature, by which it produces the sudden folding of the myosin particle at a sigma's notice is thus to keep another protein, actin, at its side. The two proteins, in resting muscle, are separated by those repulsive forces we discussed before. The repulsive and attractive forces are carefully balanced, so to say, at the razor's edge. This balance is disturbed by the wave of depolarization which travels along the membrane and is called by physiologists "excitation." As a consequence of this disturbance actin and myosin get together and interact.

The next problem which had to be solved concerns energy-relations: how to make these into a powerful machine. If our muscle lifts a weight it does work. If a system does outside work it has to lose an equivalent amount of energy itself: its free-energy content decreases. If a great amount of work has to be done, as is the case in muscle, the system must lose a great amount of free energy. There must be thus a great difference in the energy-content of the two states of actomyosin, the uncontracted, resting, high-energy state and the contracted low-energy state. Myosin itself, together with actin, is unable to produce two such states with such a great difference in energy-content. Accordingly the contraction of which actomyosin is capable is very poor, the discharge very incomplete. Here comes ATP into the picture, that amazing sub-

stance which is in the center of cell-life and its energy changes. Muscle contains ATP (adenosine-triphosphate) in relatively high concentration. It is linked to the myosin by adsorption. It is actually not myosin which unites with actin, but a myosin-ATP complex. If this complex unites with actin to actomyosin-ATP we get a new substance which has two possible states, which differ greatly in energy content and physical appearance. The one state is similar to the ATP-free actomyosin, with straight and highly charged particles while in the other state the particles are completely discharged, maximally folded. This state contains much less free energy. Every physical system tends to go into the state in which it contains the least energy, so if this actomyosin-ATP is formed in muscle under the influence of excitation it spontaneously goes over into its energy-poor contracted state. The loss of energy can be used to lift a weight, do work or develop tension.

Now comes the last engineering problem which concerns relaxation, the return of the system into its original resting state.

The first law of thermodynamics tells us that in relation to energy, we cannot get something for nothing. So if the muscle has done work, has spent free energy in its contraction, it cannot return to its high-energy resting state without paying for the expense, that is to say, without liberating an equivalent amount of chemical, bound energy. This problem is solved by Nature by smuggling, so to say, energy into the ATP molecule. When the ATP molecule is constructed and its phosphate groups are linked together energy has to be invested to establish such links. This energy is released again when the link is split. The myosin has the ability to split this link, act as ATP-ase and release the chemical bound energy which puts the energy-balance right, makes debit and credit side of the account tally again.

From the point of view of muscular contraction the metabolic apparatus is nothing but a factory for ATP or more precisely a factory of high-energy-phosphate-bonds. The metabolic apparatus releases the energy of food stuffs and makes out of it ATP or some other form of high-energy-phosphate to replace by it the high-energy-phosphate links split in muscular contraction.

You have certainly noticed I mentioned ions at every turn. They play an important role in all happenings. It is the intracellular ionic atmosphere which decides the charges, the balance of repulsive and attractive forces, equilibrium-distances, the trend of actin and myosin

to part or to get together and, last but not least, decides also the tension developed in contraction, the development of tension being also intimately connected with the distribution of charges, is governed by ions. It follows without saying that the regulation of the intracellular ionic atmosphere is one of the most important points in cell-life. At the same time it is one of the most difficult problems. What makes it so difficult is the fact that this atmosphere cannot be kept constant for it changes when the fiber is working. Every contraction, thus every heart beat, disturbs it. At rest there is a high K-concentration and a low Na concentration inside the fiber. Here, inside the fiber, the K-ions are linked to the protein by electrostatic attractions. The membrane itself is impermeable to Na. When the wave of excitement depolarizes the membrane, the membrane becomes permeable to all ions and at the same time the charge-system of actomyosin collapses, which causes the K-ions to become entirely free. They can now diffuse away through the depolarized membrane according to the gradient of their concentration and the cell loses potassium. In the next instant, however, the membrane is recharged but meanwhile the fiber has lost K and we have a new ionic balance inside with less K. It takes time until the original balance is reestablished. The next contraction, if it follows soon, finds thus a changed ionic balance. The cell is thus faced with the problem how to build its actomyosin: should this actomyosin be constructed in such a way as to be capable of exerting its maximal tension at the resting ionic balance or at the balance which is found immediately after contraction. The heart has to work with permanent rhythmicity: beat follows beat. The ionic balance depends thus on the time-interval which elapsed since the preceding one, that is it depends on the frequency. It is natural then, that the actomyosin should be built in such a way that it works well at the ionic balance which is found soon after a beat and should work poorly at the balance which will be found after a longer pause.

These relations are beautifully demonstrated by a phenomenon discovered eighty years ago by an American physiologist, Baudich. Baudich, working on the isolated frog heart, found that if the heart is arrested for some time, say for a few seconds, the first beat after this pause is weaker than the last one preceding it. If a second beat was made to follow soon it was somewhat stronger. The heart thus gradually regained its strength. The gradual increase of the height of contraction was summed up by Baudich under the name of "staircase."

We can now interpret this phenomenon and say that a beat leaves a decreased K-ion concentration behind which favors contraction. If a longer pause occurs this favorable condition deteriorates. The heart accumulates too much potassium and the beat following the longer pause finds an unfavorable ionic balance and needs a number of beats following one another in more rapid succession to re-establish the favorable charge-distribution again.

Dr. Hajdu and myself studied this phenomenon some time ago. We could fully corroborate Baudich but also found that if we replace the Ringer solution in our cannula by serum, we could stop the heart for a longer period and the next beat after this pause was just as good as the last before it. There was no staircase or only a very mild one. We were thus able to conclude that serum contains a substance which eliminates the staircase and renders the membrane relatively impermeable to the potassium thrown out during contraction, and enabled the heart to maintain the favorable condition, following contraction, for a longer period. As chemists, naturally, we wondered what this substance in serum could be. We found it to belong to the sterols. Desoxycosterone had such an action which seemed to have a high degree of specificity. Of the number of sterols tested only the closely related progesterone had a similar action, as was found in experiments in which Dr. Oscar Hechter from the Worcester Institute for Experimental Biology came to our help. We found only one other group of substances which had a similar action and this was the closely related group of digitalis. Digitoxin and all the related heart glycosides produced such an action. These substances are one of the best friends of man, which come to his help when his heart begins to fail. This indicated that the staircase has far-reaching pathological importance. When the heart is damaged it seems to be unable to keep the potassium out. Whether the heart lacks the regulating sterols which help to keep the K out or is merely unable to bind these sterols, we do not know. What we know is that we can come to its help with digitalis which keeps the ionic balance right.

When a heart becomes insufficient the pulse rate goes up. This is probably the only way in which the heart can maintain a favorable K-balance. If it would beat more slowly the pauses would be too long for the damaged permeability and, similarly to the Baudich experiment the beats would become poorer and poorer and a vicious circle would be established. The only way out is to beat fast, let one beat follow the

other in order to keep the intracellular [K] at the lower level. However, by doing so the heart finds itself, if I may say so, between the devil and the deep blue sea: by reducing the period of rest it has less time for recovery, while by extending it it develops a downgrade staircase. It is here that the balance is put right by digitalis, which allows the heart to beat slowly without losing its strength. This is probably why one of the first symptoms of a digitalis therapy is the slowing of the heart rate.

What happens in many pathological processes seems to be analogous to what we see in the perfused frog heart: the freshly isolated frog heart is well provided with the regulatory substances and develops a high tension at all frequencies. At the point at which its membrane loses its regulatory substances the staircase develops and we have to increase the frequency gradually to maintain maximal tension. Eventually the heart fails, having no time for recovery. We can now put things right by digitalis which replaces the normal regulatory substances and allows the heart to beat *slowly* and *strongly* again. Concomitant with this change there is also a change in the whole form of the heart beat. If we register it on the smoked drum, we see that the diastole develops more slowly, the descending slope of the curve becomes flatter. This indicates that actin and myosin part less readily, have a tendency to get and stay together, the ionic balance having been changed in such a way as to increase the mutual attraction between the two proteins. If this effect is exaggerated by an excessive dose of digitalis, actin and myosin will be unable to part at all and contracture develops.

To conclude, let us sum up events once more in their natural sequence. There is no essential difference between heart and body muscle as far as the mechanism of contraction itself is concerned. There is, however, an essential difference in the mode in which the excitation is generated. In the body muscle excitation is produced in the nerve-ending by messages coming from the central nervous system. The heart muscle has no such innervation and has to generate its excitation itself. This excitation, that is the depolarization of the membrane is generated at a preferential point close to the sinus, but every point is capable to do the same only at a slower rate so that the pace, under normal conditions, is dictated by the sinus node. The membrane is built in such a way that the depolarization generated here becomes self-propagatory and runs through the whole length of the fiber membrane. In its wake the distribution of charges is disturbed and brings actin and myosin together.

How the depolarization actually does this we do not know. Z. Bay and myself showed that the wave of depolarization produces an electric field inside the fiber, parallel to its axis, but we were unable to show yet conclusively that it is this "window field," which transmits the excitation of the membrane to actomyosin. The membrane, however, not only produces, propagates and transmits excitation: at the same time it also regulates by its specific permeability the intracellular ionic atmosphere which decides the whole issue. Once actin and myosin united they contract with the aid of ATP, which by its splitting and rephosphorylation causes muscle to relax. If the membrane is repolarised, ATP rephosphorylated, we have the same condition as we had in resting muscle and actomyosin dissociates into actin and myosin. The latter stretches out and the muscle is at rest again; everything is set for a new contraction. At the side of this machinery is the metabolic apparatus which replaces the split high-energy-phosphate-bonds.

In the analyses of a pathological condition the first question, which has to be answered is, which part of this complex machinery is out of order? This question has not been answered yet for most diseases of muscle tissue. The reason is evident. Our ignorance of muscle is still very dense and we do not know the single processes involved in contraction, sufficiently yet. So, for instance, we have practically no notion of the chemical mechanism of excitation, we only know that ATP here too plays a central role. With the discovery of actin and actomyosin and its amazing reactions with ATP a solid basis is created for the study of these problems which represent one of the newest and almost virgin fields of scientific inquiry.