

# On protoplasmic structure and functions.

## (A survey)

(Lecture given before the X. anniversary meeting of the Hungarian  
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BY

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With 3 plates and 2 figures.

(25.VII.40.)

### I. Introduction.

It is the tradition of this society find its pride in the honesty and ardour of the experimental work presented. Accordingly, I will celebrate this occasion of our tenth anniversary by simply putting before you a part of the work done at my laboratory this academic year. So you must not expect a survey of some well finished field; I will rather lead you into the midst of the problems that keep our minds and hands busy.

To make myself intelligible, I will have to present, along with facts, also the theories that have led us, but you are free to drop them at the exit taking with you only the solid results obtained.

Let me start with my most profound conviction that has always led my research: that Nature works only with a few basic principles, and the most varied expressions of life, like muscular contraction and excretion of urine might be but the application of the same fundamental process. This is no pure speculation. Such ideas have immediate practical consequences. If you accept them as your working hypothesis funny things might happen to you. You might start — as happened to me some years ago — with the study of the function of the adrenal cortex, be led to the study of oxidation and finish up with the isolation of a vitamin, or, as in the present case, you might start with the study of muscular contraction and finish with a virus theory. Also it will matter little to you which organ or what special function you choose for study, for you will expect that the basic processes of life will be the same in all. So you might start with the study of an organ which lends itself specially well to measurement and take muscle the function of which is of a rough mechanical nature.

### II. On the chemistry of muscular contraction.

Our work on this line had as a starting point a little experiment performed last year at Liège by BACQ, GOFFART and SZENT-GYÖRGYI<sup>1)</sup>. We took two frog muscles, perfused them with RINGER's solution containing some veratrine. We made the fluid pass through one muscle and then put it through the second. There was no other connection between the two than the fluid perfusing both. Now if the first muscle was made to contract the second muscle contracted too. Thus the liquid transmitted muscular contraction from one muscle to the other and we had, what a physiologist would call a „*humoral transmission of muscular contraction*”. The train of thought which made us perform this experiment was the following: if a muscle contracts something has to happen in it and when the muscle relaxes then the process must be reverted. What this „*something*” is, we do not know but it might be the production of a substance which makes the muscle contract, the opposite process being the disappearance of this substance.

Since contraction and relaxation take place very fast, within a few thousands of a second, it is evident that this hypothetical „*contraction-substance*” must disappear just as fast as it is formed, and we can never hope to catch it in the fluid passing through the vascular system of the muscle. According to this all attempts to produce a „*humoral transmission of muscular contraction*” had hitherto failed. One could hope to demonstrate such a humoral transmission only if one could slow down the disappearance of that hypothetical „*contraction substance*”. Now, if this slowing down would be done by means of a pharmacological agent, what would be the effect of this substance on muscular contraction? Evidently it would prolong the muscular twitch.

There is in fact a classical pharmacological agent, which has this effect: veratrine. If a muscle is treated with veratrine it will give a prolonged contraction instead of a rapid twitch, which might be explained hypothetically by the assumption that veratrine prolongs the persistence of the „*contraction-substance*”. But if this is so we might hope to catch the substance in the perfusion fluid and produce by it a contraction in a second veratrinised muscle. This, as I told you, actually happened.

The first question, any chemist would ask, is: what was this substance which transmitted contraction? The substance was potassium. With this observation the problem became most exciting, for all biochemists feel that potassium has something to do with the most basic, simple cell function: excitation, — only we do not know what. BACQ and GOFFART<sup>2, 3)</sup> showed later that a great number of very simple sea animals give the same reactions to veratrine, demonstrating in this way that veratrine attacks a basic cellular function. That this is so, and veratrine influences some basic protoplasmic reactions, is also clearly brought out by the fact that the effect of veratrine in the animal body is not limited to muscle. While it gives a prolonged contraction in muscle, in the salivary gland it gives a prolonged saliva secretion. This effect might be taken as evidence for my above statement that basic functions of the cell, in spite of their varied appearance, are the same, and muscular contraction and saliva production have the same primitive processes at their foundation.

[For me personally, the<sup>2</sup>problem became especially fascinating, for my earlier studies on metal complexes have led me to the conviction that the formation and splitting of metal complexes might be the most primitive process of life. I strongly believe that the very first primitive living molecule, formed somewhere in the ocean innumerable years ago, started its life by binding ions of its surrounding and using them as chemical tools. Now K, according to its distribution, is the most important metal atom of living Nature.

There are observations suggesting that potassium is intimately involved in muscular contraction. Muscle contains a great deal of potassium and relatively little sodium and the potassium is located, after all probability, in the contractile fibrill.

ERNST and FRIECKER<sup>4)</sup> showed that the major part of this potassium is present in the „*bound*” form and that K is „*liberated*” in contraction. ERNST has also described a most intriguing phenomenon: the „*volume-contraction of muscle*”<sup>(5)</sup>. „*Volume contraction*” means that if the muscle contracts, its total volume decreases. ERNST and KOCZKAS<sup>6)</sup> have shown that this volume contraction is a very fast process, and lasts only a few  $\sigma$ , i. e. a few thousands of a second. It is also a very early process. I mean to say that if the muscle is made to contract the first thing we can observe will be the action current and the volume contraction, both processes being followed only by the shortening of muscle.

A muscle can be looked upon as a watery solution of a certain structure. But now how can watery solution decrease in its volume? There is only one way we know of: by the formation of ions. If ions are dissolved in water they will bind water molecules, pull them together. This will lead to a decrease of the volume of the fluid. Evidently the volume-contraction of muscle

has a similar foundation. Thus this volume-contraction gives us a very accurate means for measuring the rate of production of ions within the muscle.

We can also tell which kind of ions might play a major part in this volume contraction, for the number of ions possibly formed in sufficient concentration is very limited. In fact there are only two such substances which could come into consideration: potassium and phosphate. Thus we can limit our attention to these elements.

On my return to the laboratory last autumn my first action was to secure the collaboration of E. ERNST and ask him to see what veratrine does to the volume contraction of muscle.

The experiments clearly showed that the action of veratrine was no other than to prolong volume-contraction i. e. prolong the existence of the liberated ions (plate I, fig. 1). Evidently the prolonged presence of ions is the cause of the prolonged contraction of the muscle, showing that production and presence of ions is involved in contraction while relaxation must be connected with the disappearance, i. e. binding of these ions. Further experiments of ERNST and MÓROCZ have also shown that under action of veratrine more potassium is „liberated” and also given off by the muscle to the perfusion fluid.

### III. General remarks on the shape of molecules.

As you know the muscle fibre is built up of a great number of fibrills, roughly  $1\ \mu$  in diameter, which are actually the contractile elements. Between the fibrills we find the sarcoplasm. If we make an extract of the minced muscle with a dilute saline solution the soluble proteins, about  $1/3$  of the total protein, go into the extract. The fibrils are insoluble. They are built up of a specific protein, carefully studied by EDSALL <sup>7)</sup> and EDSALL and MURALT <sup>8)</sup>. It is a globulin, but is, contrary to most other globulins, soluble only in strong, alkaline salt solutions. If the muscle is extracted with EDSALL'S salt mixture, part of the myosine goes into „solution”. This myosine is about 40—50 % of the total protein. The extracted muscle, looking at it under the microscope, has apparently retained its morphological structure but shows a strong tendency to disintegrate into transversal discs, corresponding to the former transversal striation. The muscle has thus, by the dissolution of myosine, lost its longitudinal resistance.

Myosine has, as compared to the greatest number of other proteins one very peculiar property: its molecules are fibrous, i. e. long and thin, rodlike, while most other soluble proteins are globular.

The rodshape lends very special physical properties to the molecules, which are greatly different from the physical properties of globular proteins so that the division of protein molecules into globular and fibrous molecules is very distinct.

H. STAUDINGER <sup>9)</sup> and his collaborators, who have given much attention to the highly polymer fibrous molecules, have shown that these molecules do not fold or roll up but are stretched out and behave like rods.

We can convince ourselves of the great difference of physical properties due to the rod or globular shape by comparing the qualities of a heap of marbles and wooden tooth picks. The marbles, if thrown over a heap, will readily roll apart, and show great mobility. They will be unable to form a texture or structure of any kind. We can make them into a solid lump only by glueing them together but once the single balls part there will be nothing to hold them together and they will move freely relative to each other or to the medium. They will also be unable thus to enclose a considerable quantity of water between them: to swell.

But if we throw toothpicks on a heap in a disorderly fashion they will form a three dimensional, more or less solid texture (plate I, fig. 2). We can thus see that only this rodshape is

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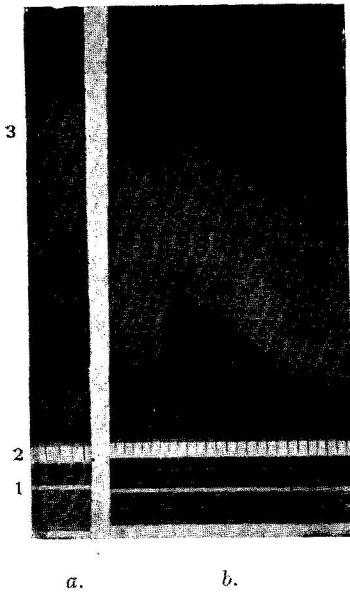


Fig. 1. Volume contraction of muscle. Single impulse. *a.* normal, *b.* veratrinised muscle (frog). 1. impulse, 2. time in seconds, 3. meniscus of fluid marking the volumecontraction. B. ERNST, unpublished experiments.

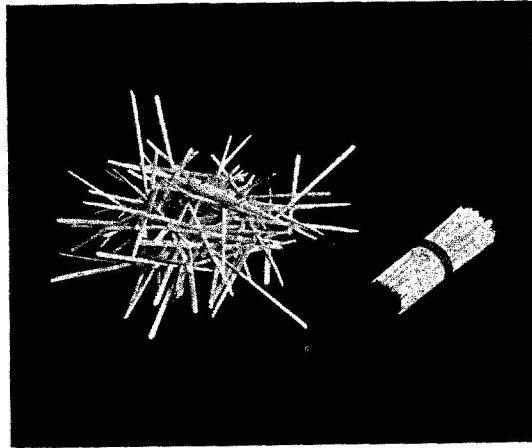


Fig. 2.



Fig. 3.

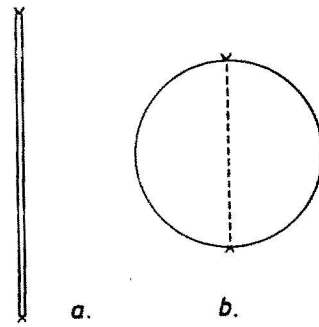


Fig. 4.

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capable of giving a solid structure. The space taken up by this structure is many fold bigger than the space actually occupied by the toothpicks themselves (plate I, fig. 2). If this structure is immersed in water the quantity of water enclosed will be very big as compared to the bulk of the toothpicks. In colloidal dimensions this would correspond to a strong ability of swelling. If the structure is disintegrated, the single long toothpicks can be moved only with difficulty through the fluid, as compared to the marbles and the single toothpicks, if not taken widely apart, will easily inhibit each other in their motion. Again, in colloidal dimensions, this means a high viscosity. Thus swelling and high viscosity at low concentration, both can be taken as indication of the rod-shape of particles.

Rod-shaped particles, if standing in a solution, have often a tendency to aggregate in such a way as to give, so to say, a scaffolding within the solvent, which resist deformation. In this way solutions of rod shaped molecules might show a certain elasticity, and an abnormally high viscosity. This is called thixotropy. This scaffolding can often be broken up by mechanical agitation whereby the fluid suddenly loses its elasticity and great viscosity. Since globular molecules are unable to give such a scaffolding thixotropy can also be taken as indication of the rod-shape of molecules.

Rod shaped particles have also the tendency to associate „end to end” forming in this way, long and thin micells. We can demonstrate this by spreading matches on a water surface. (plate I, fig. 3).

If we dry down such a colloid the long particles will entangle and form resinous, elastic masses while globular proteins give powders (STAUDINGER).

From all this we can deduce that wherever nature needs a protein with a certain mobility it will resort to the globular form. Wherever the protein will have a mechanical function, command motion of water by its swelling or give a solid structure, Nature will resort to the rod-shape.

In agreement with this we find myosine built up of long, rod-shape molecules which form still longer micells. In the muscle the molecules resp. micells are arranged coaxially, parallel to each other as shown by their double refraction and X-ray spectrum. The single micells are joined lengthwise giving the mechanical resistance of muscle to stretching. These long bundles of myosine micells can be looked upon as primitive fibrils, separated by intermicellar spaces (WEBER<sup>10</sup>). One microscopic fibril is built up of a very great number, many millions of such primitive fibrils.

The double refraction of muscle shows that within the anisotropic discs these primitive fibrils are arranged parallel to each other. In the isotropic discs the arrangement is less regular, so that we can suppose that at the borderline of the two segments the relative position of the primitive fibrils is fixed.

While the intermicellar spaces allow a lateral displacement and accumulation of water the fibrils resist stretching. Measurements of ERNST<sup>11</sup>) and others have shown that resting muscle needs a greater weight for its stretching than it is able to lift.

#### IV. Geometrical considerations about muscular contraction.

If ions are produced at certain points of a system like muscle, this must lead to a shift of water either by the osmotic activity of the ions or by their influence on the swelling of colloids. It is not a novel idea that such a shift of water might be the immediate cause of contraction. But how can a shift of water lead to a shortening of the system, to contraction?

We have agreed that the anisotropic part of the muscle fibril is built up of a great number of relatively rigid, coaxially arranged primitive fibrils, micell-bundles, the ends of which are fixed while water can be accumulated in the intermicellar spaces. Now how will the length of such a system be changed by the accumulation of water?

Let us consider this question first by taking into account geometrical considerations only and let us, to start with, simplify the problem as far as possible. Let us take, in analogy

to the primitive fibrils, two threads (plate I, fig. 4a), which can not be stretched and the ends of which are fixed but which can be pushed apart. To make it still simpler let us suppose that they can move in the plane of the paper only. Now what will happen if water is accumulated between these two threads? Since the threads do not allow stretching it is evident that they will be pushed apart. In the extreme case they will be blown up into a circle (plate I, fig. 4b) and will come to lie on the periphery of this circle and the new length of the system will be its diameter. In the extreme case the system shortens thus to  $2/\sigma = 0,64$ , i. e. 36% or 1/3. Evidently the same would happen in a three dimensional system also.

Though this simple geometrical consideration is undoubtedly correct it was desirable to demonstrate its applicability in a model-experiment. In such a model we can replace the primitive fibrils by threads of cotton wool or silk, the problem is only how to accumulate water between these threads so that it should not run out. This was done by E. ERNST, M. GERENDÁS and myself in three different ways.

In our first model (plate II, fig. 1a) threads of cotton wool were pulled through perforated celluloid discs. These discs serve to fix the threads at intervals. The space between the threads was filled with liquified 50% gelatine. After the gelatine has solidified the system was immersed in water. It was expected that the gelatine would swell and thus attract and bind water between the threads. It was found that in few days time the gelatine actually swelled up and the threads, which were parallel and straight before, now lay on the periphery of a circle and that the whole system had shortened (plate II, fig. 1b).

In our second model (plate II, fig. 2) threads of artificial silk were wetted, pulled through powdered agar-agar, receiving hereby a coating of this substance. After drying, the procedure was repeated several times to make the coating thicker. Then the threads were joined to make bundles and fixed at intervals by circular ligatures. It was expected that in this case the water will be accumulated and retained by the swelling agar-agar.

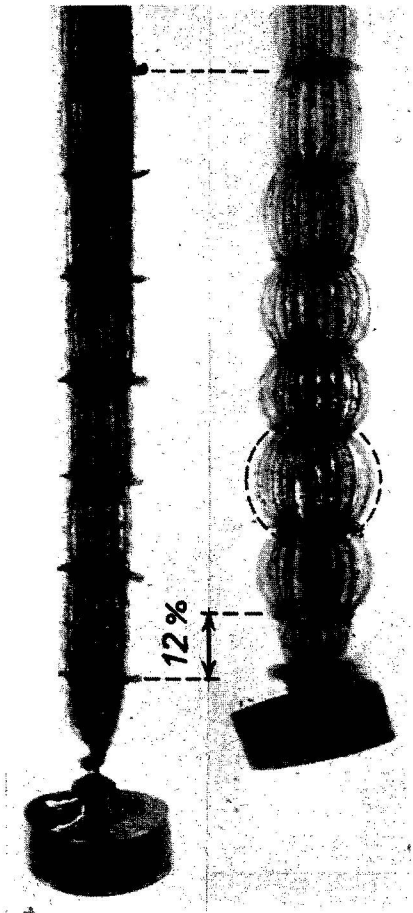
On immersing the system into water the agar quickly swelled up pushing the threads apart and lending a globular shape to the single segments. Within a few minutes the system shortened more than 10%. If the time required for equilibrium is proportional to the dimensions of the system it can be calculated that at the dimensions of the muscle fibril the shortening has to take place within a few  $\sigma$  (0,001 sec).

In a third model (plate II, fig. 3), especially fit for demonstration, a long rubber condome was pulled through a series of celluloid rings. The rings were perforated and cotton threads pulled through the holes. In this model the water is replaced by air. If blown up the structure shortened 20%.

Only after we finished playing about with these models did we find that as early as in 1897 McDougall (12) presented a theory according to which contraction of striated muscle is due to a shift of water from the sarcoplasm into the fibrils, which latter have a special structure which shortens if distended. Later E. B. Meigs (13), who advocated this theory, constructed a model almost identical with our model 3. This theory, however, found no general acceptance (O. v. Fürth 14,) partly because it admits only a shortening by 1/3 while muscle is known to shorten more than 50%, partly because at that time the results of modern X-ray studies were not yet available and we have learned only since that the muscle fibril itself is actually composed of smaller, primitive fibrillae, which structure makes such a function possible. So Bernstein (15), who discussed these theories and constructed a model very similar to that of Meigs denies any physiological importance to these considerations and calls his own model a useless toy. I will show presently how these considerations admit also a contraction of more than 1/3.

Naturally, whether muscular contraction actually has such a shift of water at its base remains to be shown. What I want to make clear is only, that if we have a structure, composed of relatively rigid fibres with fixed ends, and if water accumulates between these fibres, the structure must shorten. Such a mechanism, if applied to the muscle, easily explains many known facts. I want to mention here only two of these: the decrease of the intensity of double refraction and of the distinctness of the X-ray spectrum of muscle in isotonic contraction. Both, double refraction and X-ray spectrum persist in isometric contraction. The explanation of these facts by means of the theory presented is evident: in isotonic contraction the elements of

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a. Fig. 1. b.

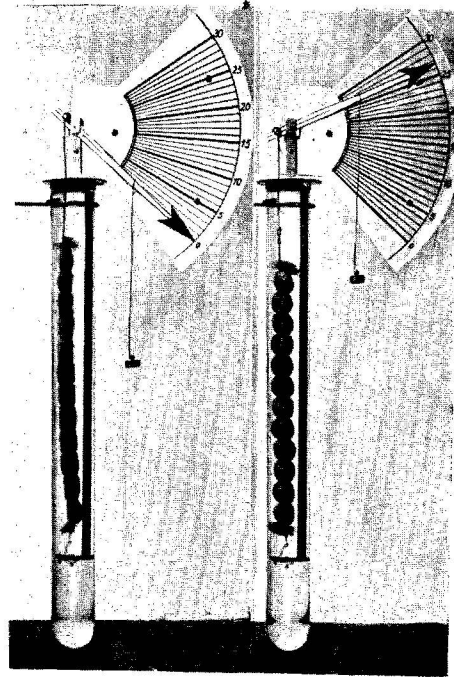


Fig. 2.

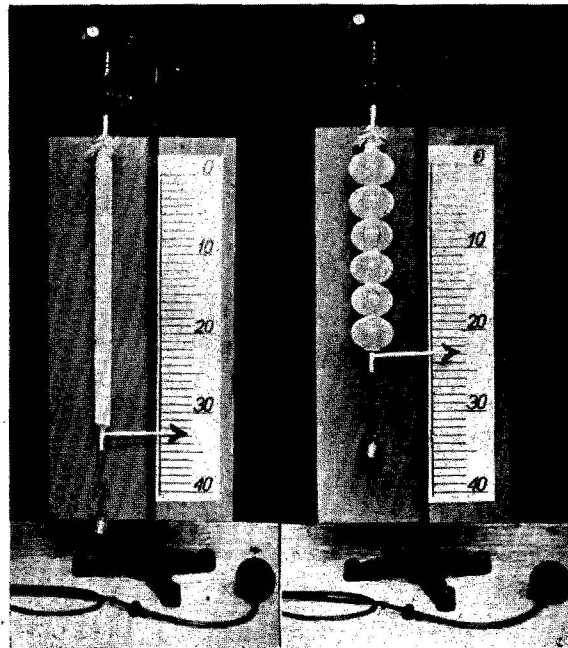


Fig. 3.

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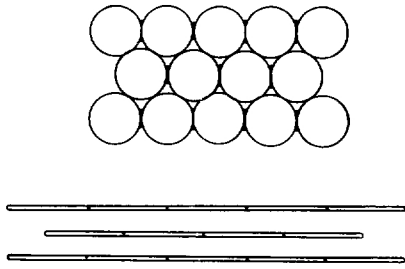


Fig. 1.

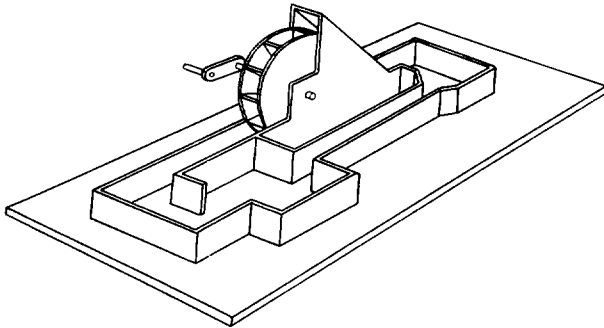


Fig. 2.



Fig. 3.

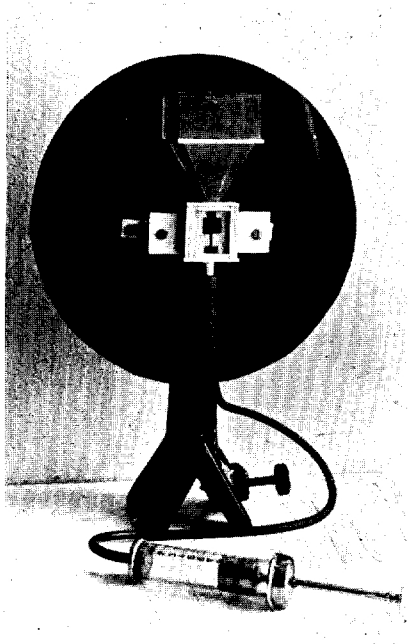


Fig. 4.

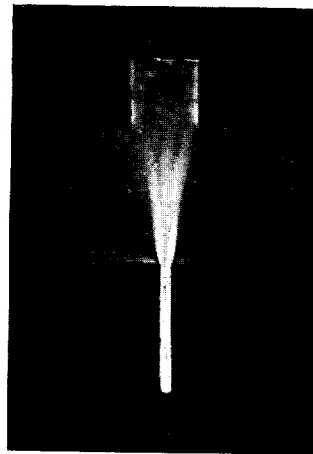


Fig. 5.

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the muscle lose their parallel arrangement while this arrangement persists in isometric contraction.

Theoretically there are different possible ways of applying the above geometrical considerations to muscle. It could be thought that the primitive fibrills stretch within the fibril through the whole length of the anisotropic Q discs, their ends being fixed at their entrance into the isotropic I disc. In this case, if water would accumulate in the intermicellary spaces the whole structure would show in its cross section an onionlike structure which is represented very schematically in fig. 1.

We could, however, equally well suppose that the spaces between the micell-bundles do not stretch from I to I disc but are much shorter. The geometrical principle could be applied to this structure also. Very schematically this is shown in fig. 2.

Finally, the same principle could be applied also to the single micells, as shown in plate III, fig. 1. In this case the rigid fibrillary structure corresponds to the C-backbone of the single myosine molecules.

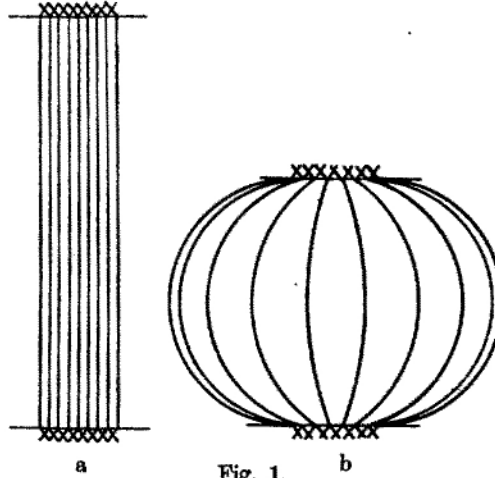


Fig. 1.

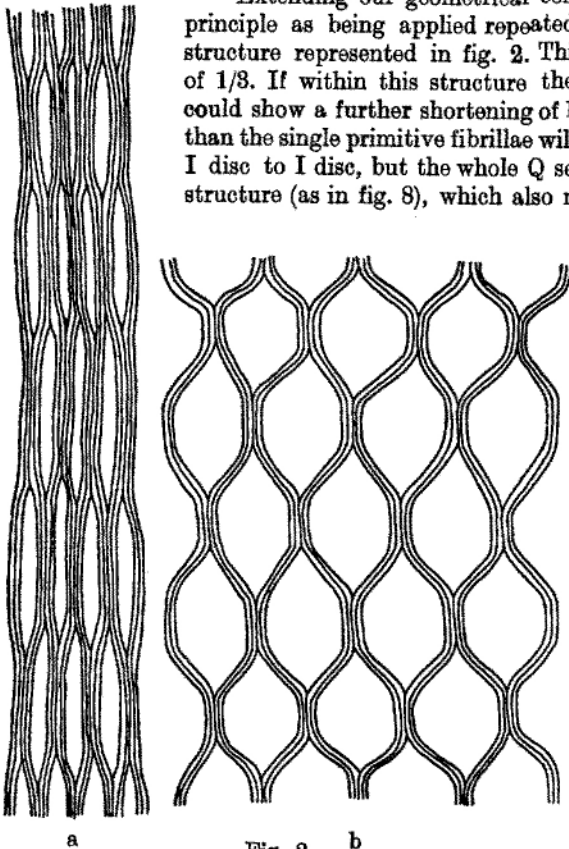


Fig. 2.

Extending our geometrical consideration we could also think of the same principle as being applied repeatedly in different dimensions. Let us take the structure represented in fig. 2. This structure could answer for a contraction of  $1/3$ . If within this structure the single micells contract also, the system could show a further shortening of  $1/3$ . But if the fibril takes up water, this way than the single primitive fibrillae will not stretch any more in a straight line from I disc to I disc, but the whole Q segment swells up and acquires an onionlike structure (as in fig. 8), which also means a further possibility of shortening.

Speculating for one more minute we can also imagine a water uptake that leads to a stretching of the fibril. Let us glance once more at fig. 2 b. If in this structure water uptake becomes excessive and breaks the lateral connections of the micell bundles the single small intermicellar spaces unite into long splits, which means lengthening. Also, if we suppose water accumulating in plate III, fig. 1 between the micells and pressing the micells together, this water accumulation will also lead to a lengthening.

Thus geometrical considerations allow us to picture a water uptake that leads to shortening and a water uptake that leads to lengthening, so it will not only be the quantity but also the location of water which matters and we could picture a shift of water even within the fibril that leads from contraction to relaxation.

I am aware that all this is only pure theoretical speculation at the moment.

But I will tell you presently about observations on myosine threads which in one case contract, when they take up water, then again relax, while still taking up water: observations, which make ones thoughts drift this way and seek for geometrical explanations.

### V. Observations on myosine threads.

H. H. WEBER<sup>16)</sup> has shown that a strong solution of myosine, if squirted in a thin stream into water, solidifies to a thread. WEBER, in his careful work, showed that many of the properties of myosine can be studied with great advantage in these threads. If the thread is stretched the myosine micells arrange themselves lengthwise and parallel to each other and assume in this way a structure, in many ways analogous to the contractile Q discs of the muscle fibrill.

Such an orientated thread can be stretched elastically and if let loose contracts to a great extent again. If the thread is dried in the stretched condition and then wetted with an alkaline salt solution it shortens and one would be inclined believe that this shortening is simply due to its elasticity resp. previous stretching and has further no interest. This is, however, not the case, since the thread, if transferred from the alkaline salt solution into water relaxes spontaneously, reaching or even exceeding its original length; if retransferred into the salt solution it contracts again, if put into water it relaxes and so the play goes on indefinitely. But you must not picture these changes as slight variations of length. The changes in length are as much as 20—25%, thus of about the same magnitude as normal contractions of muscle. The changes are quite fast too. Within two minutes the thread mostly reaches its maximum length or shortness and the most of this is done within the first minute. The rate is dependent on the diameter of the thread. The times given relate to a thread of 0,1 mm diameter. Threads of half this diameter will move about 4—8 times as fast which means that in fibrillary dimensions the whole change would take place within  $\sigma$ -s. The rate of motion depends also on the nature of the alkali.  $\text{NH}_4\text{OH}$  acts faster than  $\text{Na}_2\text{CO}_3$  or  $\text{NaOH}$ .

Now this, I think, is most remarkable. You take muscle, dissolve its contractile element, rearrange this in a way that comes the closest to its arrangement in muscle and add salts and alkali to it and it contracts. You take these elements away (i. e. put the thread into water) and it relaxes again and we know that salts and  $\text{NH}_4\text{OH}$  are formed during contraction. Naturally, I do not mean to say that by this the mechanism of muscular contraction is explained. Far from it the problem just begins. What I mean to say is that the phenomenon is most remarkable and deserves careful study and it is not only remarkable, it is amusing too.

It would be a premature attempt to try to give a physico-chemical explanation of these changes in length and at the moment I can do more than to mention a few observations.

If the thread is dried in an unstretched condition, a salt solution, instead of causing a contraction, only causes a slight lengthening. But from the observations just refered it is evident that the stretching does not condition the subsequent contraction as such, by elasticity, since the thread is able to relax and contract again. Evidently the stretching conditions this ability of contraction and relaxation by the coaxial arrangement of the myosine micells.

The changes of length are also connected with considerable changes in diameter, i. e. changes in swelling. Older physiological literature contains many attempts to explain muscular contraction by the swelling of colloids. One would thus feel tempted to explain the contraction and relaxation of the thread simply by swelling and dehydration. But the first experiment shows that things are not so simple. If a thread is put into the alkaline salt solution it swells strongly and contracts, but when it is now put into water it goes on swelling while it relaxes. The next contraction in the salt solution goes hand in hand with a strong decrease of swelling. Thus we can produce at will a contraction with simultaneous swelling or dehydration, and can produce swelling with contraction or relaxation.

But however this may be, the fact remains, that contraction and relaxation of the threads

is connected with strong changes in swelling and shifts of water and thus water must play an important rôle in the mechanism of these changes in length but evidently it is not the quantity only but also the specific localisation of water which matters. Naturally, the migration of water is, at least to a great extent, only secondary to the action of our alkaline salt solution on myosine.

It is possible to connect the single phases of the described contraction-relaxation cycle with the different ions of our salt solution. KCl in itself gives only contraction, while OH ions are necessary for the relaxation.

The action of phosphate has hitherto been studied only in relation to swelling. In this respect the action of phosphate on myosine is quite specific. While neutral salts like KCl make the myosine swell considerably only in higher concentrations (0,1 m and above) phosphate gives a strong swelling in small concentrations (0,01 m) which can actually be formed during contraction. While, within certain limits, the action of KCl is dependent on its concentration, phosphate reaches its maximal action already at 0,01 mol. and a further increase of the phosphate concentration does not entail a stronger action. Only at high concentrations (above 0,1 mol.), where the action of the kation seems to come into play, can the swelling further be increased.

## VI. The double refraction of flow. (DRF).

As globular and rod shaped proteins have such widely different physical properties it is of the greatest importance to know which group the single proteins belong to.

The classical method for establishing the dimensions of a molecule is its X-ray examination. Unfortunately this method will give results only if the particles are arranged with regularity, as in a crystal, and can not be applied to solutions.

A very simple method which allows to demonstrate the rod-shape of suspended particles is the DRF. This has the following foundation: if rod-shaped objects are floating in a streaming fluid the rods will tend to arrange themselves with their long axis parallel to the direction of flow. This is nicely demonstrated by the apparatus of M. GERENDÁS (plate III, fig. 2), a circular channel in which the water is kept in motion by a paddle wheel. If rod-shaped objects like wax threads, are floated in the moving fluid, they will arrange themselves parallel to the direction of flow in the long narrow part (plate III, fig. 3) where the velocity-gradient is the highest, the velocity of the fluid being the greatest in the middle and the smallest along the walls.

Rod shaped molecules mostly conduct light at a different velocity along their small and long axis; thus if they are arranged parallel to each other their solution will be doubly refracting. Even if the single molecules are not doubly refracting but there is a difference in the refraction index of the solvent and the solute, rod shaped particles will be doubly refracting (rod-double refraction) once arranged coaxially.

Thus, if we have a solution containing rod-shaped particles and press it through a narrow split or squirt it in a thin jet into a stagnant fluid the particles will arrange themselves coaxially and will give a double refraction. If we observe this moving fluid between crossed Nicols in the polarisation microscope the fluid will appear luminous.

All this can very conveniently be demonstrated in the chamber constructed by M. GERENDÁS (plate III, fig. 4). This chamber, made of celluloid is 6 mm deep and is covered on both sides by glass plates. The fluid is pressed, by means of a syringe, through a narrow (1 mm) split and enters a little basin. If the chamber is filled with a fluid, containing rod-shaped particles, like myosine, and is observed under the polarisation microscope, all is dark while the fluid is stagnant. As soon as the fluid is put into motion a strong luminosity appears (plate III, fig. 5), which, in the narrow split is especially strong along the wall where the velocity gradient is the biggest. The middle of the split remains dark. We can also observe the fluid entering the basin as a luminous stream.

The average position of the particles in the streaming fluid will be the resultant of different forces. The velocity gradient tends to arrange the molecules parallel to the direction of the flow while the thermic agitation tends to disarrange them. The longer the particles relatively, the

easier will they be orientated, and the better will they keep this orientation. Under the conditions of our experiment, and I want to emphasise this, only relatively big and very long particles give a strong DRF. The method gives no answer to the question whether the particles are single macro-molecules or molecule aggregates, micells.

Another factor which influences the orientation of floating particles is hydrodynamic pressure which tends to turn the particles at  $45^\circ$  to the direction of the flow. The relatively longer the particle the easier it will remain parallel to the direction of the flow. The average position of the particles can be found in the polarisation microscope by measuring the plains of the maximum luminosity or maximum darkness of the streaming fluid, by the measurement of the so-called angle of isocline which also gives an indication of the relative length of the particles.

## VII. Structure-proteins.

According to the theory presented in the previous chapters muscular contraction is due to the interaction of water ions and rod-molecules, and is a form of water transport. This takes me back to the beginning of this lecture, where I said that the most varied cellular functions might be the different applications of the selfsame simple principle. Water transport is certainly one of the most basic and primitive functions of any living organism. But, if this is so a protein fraction, analogous to myosine, should be found in any cell. Naturally we need not expect that the micells of this protein should be arranged coaxially and reveal themselves thus in the intact cell by a double refraction or X-ray spectrum as they do in muscle where this arrangement is conditioned by the mechanical function.

But even if we drop all this speculation about water transport and simply ask ourselves what we have to expect about the shape of proteins if we take into account solely the known facts about the physical properties of rod-shaped and globular molecules, we will come to the conclusion that wherever the organism wants to build up a solid structure it will resort to the rod-shape and wherever it needs a certain mobility it will apply the globular form. To the former group will belong the proteins making up the solide edifice, the morphological structure of the cell; into the latter group will belong proteins of secretions (proteins of milk, different enzymes and hormones) proteins, of transportation (serum proteins), reserve proteins (ovalbumin) and certain mobile intracellular enzymes (*e. g.* lacticodehydrogenase).

This postulate seems, however, to be in contradiction with the experience of protein chemistry. The shape of the molecules of a very great number of different animals and vegetable proteins has been studied and most of them have been found to be globular. According to this general experience we only find long, fibrous molecules in special cases only where there is a mechanical function, like in muscle (myosine), hair (keratine), collagene, fibrine, vegetable fibres etc. Rod-shaped molecules are thus looked upon at present as specific and rare exceptions conditioned by some mechanical function.

Thus the general experience of protein chemistry pleads against the correctness of our ideas. But let us see, whether research has not been misled unconsciously by some secondary factor. Let us see what proteins the physico-chemist will take if he wants to study the shape of molecules. Naturally he will select the easily accessible or extractible ones, which must be, because of their extractability, mobile, thus of globular shape. He will unconsciously leave aside the rod-shaped proteins which, by their physical nature, tend to give solid textures and are thus less readily accessible. These proteins, forming the major part of the bulk of the cell might actually be fibrous and it seems possible that fibrous molecules have been found in tissue only, having mechanical function, not because the fibrous form is limited to these tissues but because the mechanical function conditions a coaxial arrangement which makes the fibrous nature of these molecules easily recognisable by their double refraction and X-ray spectrum.

Our rough, all-day experience is in agreement with these considerations. If we mince a

tissue, let us say kidney, and extract it with water, about 1/3 of the total protein goes into solution. These easily extractible proteins are, as shown by the lack of DRF and other physical properties, globular. But in spite of the considerable loss of protein it will be impossible to find anything missing if we examine the tissue under the microscope. The whole morphological structure is still there; the globular proteins dissolved had no considerable part in the building of the morphological structure.

How can we hope now to desintegrate these structure-proteins into single micells or molecules? In analogy to myosine we can expect that these proteins will need a higher salt concentration and a certain alkalinity for their dissolution. But at the same time, knowing that the micells of these proteins, if rod-shaped, are less regularly arranged than myosine and are not separated by intermicellar spaces, we can expect that a higher salt concentration and alkalinity will be insufficient to desintegrate the structure. Since the forces, holding protein molecules together, are often „hydrogen bonds” we can hope to gain our end by adding a substance which is known to split such bonds. Such a substance is urea in high concentration, which according to WEBER and STÖVER<sup>17)</sup> also desintegrates myosine micells into molecules.

If the kidney is minced and suspended and stirred in a salt solution containing 30% urea, as done by I. BANGA and myself, the tissue particles will soon swell and the fluid itself will assume a very sticky consistence which is already an indication of the presence of rod-shaped molecules. If the tissue particles are centrifuged off and the supernatant fluid diluted with the same urea-salt solution it will be found to give an intense DRF. The angle of isocline as shown by M. GERENDÁS, also indicates very long molecules. Plate III, fig. 5 is actually the photograph of the DRF of such a solution from kidney. It is not the best picture we have obtained but was the first DRF observed. It was indeed a great excitement to see this splendid DRF in our tissue extract the first time after all these long trains of thought.

The substance, giving the DRF is a protein and behaves similarly to myosine. It can be precipitated by diluting its solution with five times its own volume of water and neutralising it. It can be redissolved in the same urea-salt solution and will give the same DRF again. It can also be pulled into threads and examined röntgenoscopically. The röntgenoscopic measurement kindly made by Prof. ST. NARAY SZABÓ, has given a periodicity in distances of 9,5, 4,6 and 2,75 Å. These data are in agreement with the data obtained on other fibrous proteins, like myosine and keratine, so that these first measurements indicate that this substance is built on the same pattern with other known fibrous proteins. In analogy with myosine I will call this protein **renosine** or in general structure protein 1. This substance makes up about one third of the total protein of the kidney.

There are two striking differences between renosine and myosine. While myosine is positively double refracting renosine shows a negative double refraction. In the former the index ellipsoid lies lengthwise, in the latter crosswise to the axis of the molecule; in other words the plane of the greater refraction lies in the long axis of the myosine molecule while in renosine the greater refraction index is found in the cross section. This is the first known example of a negatively double refracting protein.\*)

But there is also another, still more striking difference between renosine and myosine. While myosine contains no phosphorus, renosine contains phosphorus in a non-extractible form and seems to be thus a nucleoproteid. Since the cellular nucleus is microscopically still intact after the urea-salt extraction and could hardly make 1/3 of the total protein, renosine can not be of nuclear origin.

The sticky appearance of the extract is due to the thixotropy of renosine. If the thixotropy is broken up the sticky appearance vanishes giving place to a true and high viscosity, evidence of long, rod-shaped particles.

Renosine is a representative of a specific proteinfraction apparently present in all

\*) Muscle seems to contain, side by side with myosine, also a fibrous protein, analogous to renosine: a protein soluble in urea-salt only and having a negative DRF. See BANGA's subsequent paper.

tissues. If the same procedure of extraction is applied to liver, brain, nerves, mammary glands, parotis, lymphglands, whole embryos, EHRLICH or ROUS sarcoma, EHRLICH carcinoma, the same result is obtained. Only pancreas behaves differently.

After the tissue has exhaustively been extracted with the urea salt solution it is still there with one third of its total protein. Even examination under microscope does not reveal anything missing: the morphological structure is still present.

If this extracted tissue is suspended in a 30% urea-solution containing 2% NaOH (15 ml. to 1 g of tissue) and stirred for a few minutes and then heated for 5 minutes under continuous stirring to 60° C, practically the whole tissue dissolves. If cooled down again we are left with a very sticky fluid, the sticky appearance of which is again due to thixotropy. After the thixotropy is disturbed we are left with a fluid of high and true viscosity with a strong DRF.

If the solution is diluted by 5—10 times its own volume of water and then slightly acidified, the material giving the DRF precipitates and can be redissolved in alkaline urea and shows the same DRF again. I will call this fraction structure-protein II without any claim of homogeneity. Naturally, the solution must contain different substances, including the nuclear material. What is important at the moment, is that the DRF undoubtedly shows that also this most insoluble part of animal cellular material is built of rod-shaped molecules.

An objection could be raised against the content of this chapter. One could say that the molecules examined are not really rod-shaped, but they might be globular and be unfolded into rods only under action of our solvents. For this reason a series of globular proteins, like casein, lactalbumin, serum-albumin and globuline, ovalbumin, edestine, gelatine have been examined in our chamber also under action of our solvents. They gave no DRF comparable to the DRF of the described structure proteins.

### VIII. On the nature of certain viruses.

When hearing about rod-shaped molecules many of you will think at once of certain vegetable viruses, like the tobacco mosaic virus, isolated by STANLEY, which owing to their rod-shape also show a DRF.

It is now generally believed that these viruses are of exogenous origin. This belief is based chiefly on three arguments.

Firstly, it has been shown, that the tobacco mosaic virus is rod-shaped. Since it was thought that rod shaped molecules are not found in the protoplasm this was taken as evidence of their exogenous nature. But we have seen that the structural part of protoplasm is built up of rod-shaped molecules. As BANGA has shown this is also true for vegetable cells *viz.* chloroplasts. With this observation the problem arises whether these viruses are not in fact protoplasm-molecules of the plant itself: molecules, which for some reason or other attained a suspension stability and started herewith an independent existence. So it becomes necessary to review also the other arguments brought forward in favour of the exogenous nature.

The second strong argument for the exogenous nature of these viruses is the discovery of BAWDEN and PIRIE that the virus contains phosphorus, and is a nucleoproteid, thus an independent and complete organism. But I have mentioned before that the protein fraction I does contain phosphorus, and contains this element in quantities comparable to the quantity of P found in viruses. I will give, side by side, the % P content of viruses, taken from an article of STANLEY<sup>18</sup>) and the P content of structure protein I, taken from the paper of BANGA.

As the table I shows the agreement is close.

The third evidence for the exogenous nature of a virus is its immunological behaviour. The tobacco mosaic virus behaves immunologically in a different way than the protein of the tobacco leaf. At the moment I cannot disprove this argument, I can only show its weakness. For if we say: tobacco-protein, what protein do we mean? After all probability the soluble, extractible globular proteins, while the insoluble fibrous proteins probably do not come into

TABLE I.

Tobacco mosaic .....	0,48	Kidney .....	1,2
	0,39	Liver .....	0,69
	0,60		0,53
	0,45—0,55	Brain .....	1,06
<i>Aucuba</i> mosaic .....	0,51		1,1
Cucumber mosaic .....	0,55—0,60	Parotis .....	0,69
Latent mosaic of potato .....	0,51—0,58		0,53
	0,4—0,5	Mammary gland .....	2,58
Tobacco ring spot .....	3,4		1,94
Tobacco bushy stunt .....	1,3—1,5	Lung .....	0,60
Chicken tumor.....	0,7		0,67
		EHRlich Sarcoma .....	1,43
			1,3
		Chicken tumor (Rous) ....	0,55
			0,56

the picture at all. If they had been brought into solution and tested they might have given entirely different immunological reactions as compared to globular proteins.

### IX. Summary and conclusion.

To sum up, I have given you a brief survey of the experimental work done in my laboratory during the last year and have shown you that the volume-contraction of muscle is prolonged under action of veratrine (E. ERNST), which is new evidence in favour of the assumption that the production of ions has an important rôle in muscular contraction. I have also shown that under action of veratrine more potassium is liberated and given off by the contracting muscle (E. ERNST and E. MÓROCZ). I have presented geometrical considerations and models which show how a shift of water might lead to contraction. I have shown, how myosine threads are capable of contraction and relaxation under influence of ions. I have also shown that the bulk including the greatest part of the morphological structure of animal cells is made up of fibrous molecules (BANGA) and I have discussed the bearings of this finding on the virus theory.

All this work has been done with the ultimate aim to arrive at an understanding of the muscle as a machine and correlate structure, contraction, oxidation and fermentation. With this aim in sight also our earlier work on oxidation and fermentation has been continued and we tried to go on pulling the machine to bits. Thus E. and K. LAKI isolated the fumarase in crystals and identified its coenzyme. F. B. STRAUB crystallised the lactic-dehydrogenase \*) (Biochem JI., in print) and studied its kinetics. E. ANNAU made some hopeful beginnings in fat oxidation describing a new lecithine-dehydrogenase and its coenzyme.

All these experiments which I have put before you are but first steps towards our

\*) The lactic dehydrogenase, as shown by the lack of DRF, is globular (1,6% solution of the crystalline enzyme). This enzyme is readily extracted from muscle by water or weak saline, which solubility in itself is an indication of a globular nature. Fumarase and the yellow enzyme of the muscle are not extracted by water but all the same they are globular because they show no DRF (1,3% solution of the crystalline fumarase and 0,5% solution of the pure yellow enzyme, isolated by F. B. STRAUB). These two enzymes, in isolated condition, are readily soluble in water. This indicates that in the tissue they are bound to insoluble, thus fibrous proteins. Our first experiments indicate that the situation is probably similar in the case of the succinodehydrogenase and the indophenoloxydase.

distant goal, the understanding of the cell as a whole. Presenting my results has taken you to a sowing rather than to a harvesting. My one reason for taking to you about this incomplete work was to invite you to join in and share the pleasure we are having ourselves at my laboratory.

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