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北海道大学触媒研究所紀要
SOME PHYSICO-CHEMICAL PROPERTIES OF
ACTOMYOSIN-ATP SYSTEM*) **)

By

E. D. GRISHCHENKO##)

(Received June 30, 1958)

Eight years ago died the biological physico-chemist Prof. D. L.
RUBINSTEIN (4). During the last period of his life, his attention was
being attracted to the problems on the intimate mechanism of muscle
contraction. Thus, at the laboratory where Prof. D. L. RUBINSTEIN was
the chief the studies on the actomyosin-ATP system were initiated.
His untimely decease stopped them suddenly. The present author
takes a possibility to acquaint the Japanese biochemists, who have
achieved in the given field so much, with some results of these studies.
A part of experiments described below has been conducted under
Prof. D. L. RUBINSTEIN's personal leadership, and the others, having
been completed after his death, are based on his ideas, and many
experiments present a logical continuation of the same trends.

The author is citing many Russian papers: he would like to give
some information to Japanese investigators about what is done in his
country.

There are many scientists whose attention is now occupied by the
problem of conversion of the chemical energy into the mechanical one
in muscle. It is natural: the facts concerning muscle energetics are
so numerous, various and interesting (2, 9–11, 13, 24, 34–36, 41–43, 46,
47, 56, 62, 68, 82, 94, 107–109, 136, 158, 169, 170, 209, 210, 213, 218, 220,
244, 247–251), that their generalization may bring, in the near future,
to the revelation of new biological regularities. Already, we are able

*) In memory of Prof. D. L. RUBINSTEIN's 65-th birth anniversary.
**) The following abbreviations are adopted in this paper: AM, actomyosin=S-myosin=
a-myosin; MB, myosin B=natural actomyosin ; M, myosin=myosin A=L-myosin =β-
myosin; A, actin; G-A, globular A; F-A, fibrous A; ATP, adenosinetriphosphate; ATP-
ase, adenosintriphosphatase; VA, viscosimetric activity=ATP-sensitivity; DRF, double
refraction of flow; UV, ultraviolet; MW, molecular weight; τ, relative viscosity of
salt solutions of AM before ATP addition; τATP, the one after ATP addition.
##) Radiobiological Laboratory, Institute for Labour Hygiene and Occupational Diseases,
AMN, Moscow, USSR.
to postulate the following two important conclusions.

Firstly, every mechanical movement in living world is connected with the system of the contractile protein and ATP. AMs and AM-like substances reacting on ATP with specific changes of their physico-chemical properties have been discovered in all animal groups and in some low-organized plants (134, 227–231). The development of karyokinesis is also based on the interaction between appropriate structural proteins and high-energy nucleotides (104, 105, 167). Secondly, ATP seems to be the terminal accumulator of the chemical energy formed by the oxidation of nutritive matter not only in the contraction, but also in other physiological processes such as biosynthesis, bioluminescence (79, 147, 148, 208), secretory activity (91) etc. There is a distinct progress in the clearing up and in the treatment of muscle diseases 60; 61; 40a, b; 201 a, b).

PART I. PHYSICAL-CHEMICAL CHANGES IN AM PRODUCED BY ATP

The colloidal nature of AM and the changes produced in it by ATP are studied by relative, specific and anomalous viscosities, DRE, light scattering, sedimentation, diffusion, UV, infrared and visible spectra, roentgenograms, electronmicroscopy, electrophoresis and other methods. In spite of many-sided investigations, the question in what form the AM presents in solutions and suspensions is not dissolved definitely. It is caused, maybe, by the fact, that AM is composed of two components whose state depends to a great degree on external conditions. Thus, DRF of M solutions shows that the length of particles varies in wide limits 800–3500 Å according to the method of preparation, protein concentration, pH, buffer (115, 197), animal species and pathological disturbance in muscle (196). The ATP-ase activity fluctuates also sharply (203). As well known, the rapid G–F or F–G transformation of A is very susceptible to salt composition of medium (209, 210).

There is no agreement in views relating to the nature of ATP action on AM. The different authors suggest that ATP produces 1) the dissociation of AM into A and M (245), 2) the depolymerization of AM (113a), 3) the shortening of its micelles (38), 4) the combination of these effects depending on conditions (209, 210).

According to these views, the water, if mentioned, is valued only as a medium and the hydration processes, if suggested, are inter-
Some Physico-Chemical Properties of Actomyosin-ATP System

interpreted as accessory phenomena. Even such a remarkable effect as the syneresis of AM gel in the presence of ATP has not attracted the due attention, being considered to have no relation to in vivo processes (162) and to be caused by chaotic distribution of micels (209, 210).

In Prof. D. L. RUBINSTEIN's opinion, the changes of the colloidal properties of AM solutions and geles after the addition of ATP are conditioned primarily by dehydraton of the protein. The ability of swelling colloids in developing the great pressure induced D. L. RUBINSTEIN to suppose that the work performed by muscle was the one due to swelling AM. The ATP dehydrates this protein, and thus enriches it with free energy and the protein is prepared for new contraction. Some facts may be interpreted in favour of this scheme.

In this section, some experimental results on the nature of the interaction between ATP and AM are adduced, with special accentuating of the processes of the water binding and the dehydration.

Anomalous Viscosity

V. A. ENGELHARDT, M. N. LYUBIMOVA (46, 50, 51), J. NEEDHAM and others (159, 160) were the firsts who observed the physico-chemical changes in M under the influence of ATP. Shortly after them, A. Szent-Györgyi's group (209, 210) discovered the existence of AM and its ability for descending of its viscosity after the ATP addition. They investigated this reaction by means of simple OSTWALD's viscosimeter quantitatively.

However, \( \gamma \), measured by OSTWALD's viscometer, gives no idea about the delicate changes in AM micels produced by ATP. More complete information on them may be obtained by studying the so called structural or anomalous viscosity. D. L. RUBINSTEIN and M. P. PETROVA (193) investigated anomalous viscosity of rabbit MB solutions by means of a modified TSUDA's horizontal viscosimeter (231a). The apparatus consists of two graduated pipetts and a capillary between them. Therefore, the measurements may be made by flow of solution in both directions. Such a construction elevated the precision of the double records. The protein contents in MB solutions was 1-4 mg/ml. At all pressures >60-30 mm H2O, the values of \( \gamma \) remained at the same level. At lower pressures the anomaly was revealed: \( \gamma \) increased sharply with descending pressure. The ATP addition caused, as usually, a marked drop of \( \gamma \), but the strong anomaly in region of lower pressures did not disappear and behaved approximately so as in the absence of ATP.
D. L. Ruhinstein and M. P. Petrova drew a conclusion that MB particles had the form of long rods with high axial asymmetry not changed by ATP. The drop of \( \eta \) after ATP addition, hence, seems to be a result of dehydrating action of this nucleotide on the protein micels. These data are in contradiction with English authors' assumption based on measurements of anomalous viscosity by means of coaxial viscosimeter. Although they obtained practically similar results (38), they supposed that ATP produces either the shortening or the contraction of M particles. Apparently, the insufficient sensibility of their method would impel them to consider their own data with some element of doubt. For some time past, the facts against the dissociation and depolymerization of AM under ATP action are accumulated (223, 224, 226). The AM formation is an endothermic equilibric reaction. ATP shifts the balance to the direction of the decomposition of the complex, but its action is not specific, because the same action is exerted by elevating of ionic strength and by lowering of the temperature (130).

With the viscosimeter described above (193), the structural viscosity was also shown by the contractile protein of uterus, though it came to light in the region of pressures >20 mm H₂O only (31). The actin reveals also the high anomalous viscosity and the DRF. Nevertheless, its time of relaxation lets think that its particles present in solutions in the form of dimers. Therefore, the greatest effect of ATP on A seems to be due to the dissotiation of dimers into monomers (225). The marked drop of viscosity of AM is hardly caused by this process.

**Dehydration in Ultracentrifuge**

In order to establish, whether ATP changes the water binding ability of AM to a great degree, we have conducted a simple experiment estimating the water content of MB precipitate and superprecipitate after centrifugation with different rates (84). The results are summarized in Table 1. This table shows that the precipitate of AM binds the water much stronger than the superprecipitate does. Thus, for reducing the humidity to the approximately equal values (7.0 and 8.2 g/g protein) it is necessary to increase the rotation rate in the case of the precipitate 40 times as much as in the case of the superprecipitate. On the other hand, when the force, which decreases the water content of the precipitate as much as 10 times, is applied to the superprecipitate, its water content is reduced by half. Hence,
### Some Physico-Chemical Properties of Actomyosin-ATP System

**TABLE 1.** The water content of MB precipitate and superprecipitate at different rates of rotation.

<table>
<thead>
<tr>
<th>Centrifugal force G</th>
<th>Precipitate g water/g protein</th>
<th>Superprecipitate g water/g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7 x 10^4</td>
<td>72.8</td>
<td>8.2</td>
</tr>
<tr>
<td>6.9 x 10^4</td>
<td>7.0</td>
<td>3.8</td>
</tr>
</tbody>
</table>

the ATP seems to dehydrate AM not completely but to some limit.

**Vapour Pressure**

As the following step, we have decided to get some informations about the work spent by ATP for tearing away the hydrate layer of the protein. Thus the vapour pressures upon the native and the heat-denaturated MB gels with and without ATP were measured by means of an isotonoscope (32). In addition, same measurements were made on native and denaturated egg albumin, serum albumin and r-globulin (84, 192).

Whereas the vapour pressure-water content curves of albumins and r-globulin in the native and the denaturated state are nearly identical, there is a great difference between the curves of the native and the heated preparates of MB. This is caused by high hydration of native protein, denaturated precipitate binding the water much more feebly, like other proteins. ATP produces the same changes in vapour pressure and, therefore, in hydration of AM, as the heat does, but its action is reversible.

In experiments with the superprecipitate, we observed an interesting phenomenon. At some time after the beginning of measurements, the superprecipitate, which usually binds the water as weak as the other proteins, does suddenly commence to arrest the water with the strength characteristic for the native precipitate. For example, at some moment, the native superprecipitate contained 3.8 g H₂O/g and was binding the water with the strength corresponding to 98% relative vapour pressure (P/P₀), but in 1½ hour necessary for the conducting the following measurement, in spite of the same water contents (3.7 g), the vapour pressure fell to 89%, indicating the stronger water binding. We have observed such slipping of the superprecipitate curve to the precipitate one more than once. It was suggested that, in these cases
the superprecipitates turned into the precipitates owing to the decomposition of ATP fixed on the protein, free ATP being already removed by washing off the salts.

We measured $P/P_0$ of the proteins, of which water contents ranged from 4 g/g protein and more to practically zero. It stands to reason, that MB precipitate, in the course of drying under vacuum and at 25°, lost some of its properties such as solubility and enzymic activity. Nevertheless, its higher affinity to water, than of the superprecipitate, may be established even by 0.5 g H$_2$O/g protein (the influence of salt concentration on $P/P_0$ was taken into consideration).

There is no exact procedure for calculating the work of swelling by use of $P/P_0$. The theoretical formula:

$$A = \frac{RT}{M} \ln \frac{P}{P_0},$$

where $A$ is the work and $M$ is MW of liquid phase, contradicts with the results of the direct measurements. In order to have some idea about the work developed by swelling the superprecipitate, we, at will, suggested that the causes perverting the perfect curve (corresponding to the above formula) into the empirical one are similar for gelatine and other proteins. With this reason, the curve of $P/P_0$—"true" work of swelling could be drawn from literary data. The data on gelatine were used because it was the only colloid, of which $P/P_0$, the swelling pressure and the volume striction had been measured*). By means of this curve, we have converted the results of the vapour pressures over the precipitate and the superprecipitate to the corresponding swelling pressure values and have calculated the swelling work of the precipitate and the superprecipitate (83, 84). The difference is the work of the dehydration of MB gel performed by ATP. As the limits of water contents, we adopted 1 mole and 10 moles H$_2$O/100 g protein, because the sharp divergence of both curves begins approximately at the former point and the latter is the content of water in myofibrils (210). Under these conditions, the calculated work is equal to 7.5 cal/100 g MB. In other words, such a work may be produced by 100 g of the superprecipitated AM containing 18 g water in the course of binding 162 g water more after ATP has been removed.

In 1930, HILL and KUPALOV (102) stated, that the vapour pressure

*) There is an experimental formula for the relation between volume striction and swelling pressure (168 a, 217 a).
over excised muscle is practically equal to the one over water. However, this does not contradict with the hypothesis on the essential role of the swelling process in contraction, for the working elements of muscle, myofibrils, are isolated from sarcoplasm and extracellular space where the water is free.

**Indissolving Space**

In preliminary experiments, it was found that the indissolving space of the precipitate was several times more than the one of the superprecipitate, its absolute volume inversely depending on the concentration of the indicator (glucose or glycerin). These data also witness the elevation of water binding ability of AM in the absence of ATP.

**Stoichiometry of ATP-MB Interaction**

In order to prove, whether the swelling work of AM dehydrated by ATP may play an essential role in muscle contraction, it is necessary to compare this work with the minimal amount of ATP which produces the maximal physico-chemical changes in AM. As the test, we have used the viscosity of MB solutions (81, 84). The measurements were conducted as quick as possible, at 0°, because, at low temperature, the ATP produces the greatest drop of viscosity of MB solution and the rate of ATP hydrolysis is low (156). The table 2 shows the data obtained by means of four Ostwald's viscosimeters with different flow rates.

**Table 2.** Minimal ATP amounts necessary for complete lowering of $\eta$ of MB solutions.

<table>
<thead>
<tr>
<th>Viscosimeter</th>
<th>Time of flow, sec</th>
<th>Amount of 0.087% ATP solution necessary for maximal $\eta$ drop, ml</th>
<th>Amount of MB</th>
<th>Unit weight of MB interacting with 1 mole ATP, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>0.033</td>
<td>2.8 ml, 3.8 mg/ml</td>
<td>187,800</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>0.075</td>
<td>2.8 ml, 8.6 mg/ml</td>
<td>187,600</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>0.025</td>
<td>5.6 ml, 4.8 mg/ml</td>
<td>50,800</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.020</td>
<td>5.6 ml, 3.6 mg/ml</td>
<td>50,000</td>
</tr>
</tbody>
</table>

Obviously, the most reliable values are the ones obtained when the viscosity measurements are taking possibly less time. This requirement was satisfied by the viscosimeters 1 and 2 (each measure-
ment was conducted during 10–18 sec). By using the viscosimeters 3 and 4 (the time of flow is >1 min), AM seems to have time to hydrolyze several turns of ATP in spite of the temperature strictly kept at 0°.

Thus, it follows from our data that 1 mole ATP reacts with $1.88 \times 10^5$ g MB or $1.56 \times 10^5$ g M. This value is in good agreement with the results of investigation of MB and ATP stoichiometry by light scattering intensity (it is decreased by ATP) using the perfect little inert device (220, 221). The unit weight of MB is found $1.85 \times 10^5$ (rabbit skeletal muscle) and $1.8 \times 10^5$ (swine heart). There are the grounds to think (81, 84, 221) that the lower $(0.75 \times 10^5 - 1.00 \times 10^5)$ (151) and the higher $3.0 \times 10^5$ (153) values are incorrect. The MEs of other origin have some higher values: $3.9 \times 10^5$ (rapid adductor of pecten), $1.0 \times 10^6$ (swine oesophagus), $1.22 \times 10^6$ (slow adductor of pecten). The values $1.85 \times 10^5$ correspond to MW of crystalline M depolymerized by urea. It is $1.65 \times 10^5$ (224) or some more (225).

Also, Tonomura's and our results correspond to some indirect data. Kashigami and Rabinovitch (118) studied the mechanism of the spreading of M films on the salt solution surface. They concluded that ATP was bound firmly by M in relation 50 mole ATP per 1 mole protein. In the abstract of this paper, there was no information M: ATP weight ratio. Estimated by different methods, MW of M has been found to be equal to $0.85 - 0.60 \times 10^5$ (166, 204, 218). Therefore, 1 mole ATP interacts with $0.15 - 0.11 \times 10^5$ g M. In unstriated and skeletal muscles of vertebrate and invertebrate animals, the nucleotides not separable from protein have been discovered in amounts $80 - 180 \gamma$ labile P per 1 g AM (18), i.e., $3.9 - 1.72 \times 10^5$ g AM per 1 mole nucleotide.

To compare the energy brought into M by ATP, it is necessary to know the free energy change in the course of the hydrolysis of one high-energy bond of ATP. There are different values in literature: 11.5 (122), 9 (239), 8.9 (37), 7.6–7.8 (175), and 6 Cal/mole ATP (164, 165). Absolute denying the accumulation of chemical energy in phosphate bonds of ATP (76) is not well founded (83). Taking into account limit values (6 and 11.5) and above stoichiometry of MB and ATP, the protein must be enriched in free energy of 3.2–6.1 cal/100 g MB. This is the value of the same order as the work of swelling AM dehydrated by ATP in experiments with vapour pressure measurements (7.2 cal/100 g MB—see above).

Although we measured $\eta$ and $\eta_{ATP}$ with 4 different viscosimeters (the time of flow was 8, 15, 50 and 100 sec) we obtained only two values:
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1.88 x 10^6 and 0.54 x 10^6 g MB per 1 mole ATP. This may be connected with the fact (218a, 246), that, during the first 15 sec, the rate of hydrolysis decreases to the stationary level of the following 100 sec.

UV Absorption

RAVIKOVIČ, SETKINA and LEONTYEVA (173) observed in M solutions the UV absorption maxima due to the presence of the cyclic amino acids in the protein molecule: 293 (tryptophane), 278 (tryptophane and tyrosine), 286 (tyrosine), 271, 265, 259, 253.5 and 247 mμ (phenylalanine). The UV absorption by A solutions is more complete in region of longer waves than the one of M solutions. The activation of A with salts does not change its UV absorption curve. The more essential difference between UV absorption spectra of these two proteins, however, consists in the absence of phenylalanine maxima in A. This observation is in agreement with the later chemical analysis of amino acid contents in A: this amino acid is absent in A (67, 110). The preparation of MB and artificial AM (A : M=1 : 2) have the same maxima as M has. In the investigated region of UV, ATP reveals a strong absorption band with a maximum at 260 mμ which is characteristic for adenine derivatives. When UV radiation is passing through two cells containing 0.5 mg nucleotide/ml and 0.82 mg protein N/ml respectively, the nucleotide maximum at 260 mμ and the one of M disposed at 277-278 mμ do not change practically their positions: a shift of ATP maximum as little as 1.5-2 mμ to the shorter side is observed (174).

The addition of ATP to M solution causes a marked shift of the chief absorption maximum of M up to ~264 mμ. After the degradation of ATP by M-ATP-ase, the M maximum returns to its initial position at longer waves. The same effect is observed when ATP is added to AM solutions. It is remarkable that mixtures of ATP and M of low ATP-ase activity do not reveal any changes in maxima.

As the maximum at 277-278 mμ, which shifts to shorter waves by the addition of ATP, is due to the presence of tryptophane and tyrosine in M molecule, the authors (174) have concluded that these aromatic acids take part in the interaction between the protein and the nucleotide.

It is to be noted that, in their earlier investigations of spectral properties of the ATP-M system, LYUSHIMOVA and SHIPOLOV (143) have discovered no changes in positions of the absorption maxima. RAVIKO-
VICH, SETKINA and LEONTYEVA (174) supposed that the cause of this negative result consisted in imperfection of the method. If this criticism is correct, new studies have to show.

The answer to the question: what chemical groups of AM do react with ATP?—is very important for the investigation of the mechanism of muscle contraction. Hitherto, the attention of biochemists is attracted, mainly, to SH-groups. One attributes to them the paramount role in the interaction between the protein and ATP (126, 127, 128, 232, 233). But such a consideration of the problem seems to be one-sided. The complicated process of the specific enzymatic substrate degradation and of the simultaneous (or synchronous) physico-chemical change of the enzyme itself under the action of this substrate should need the exact spatial combination of several chemical groups in the molecule of protein-catalyst at once. Maybe, under these acting groups, there are SH-ones also. The fact that one ascribed to them a dominant participation in this process may be caused by the ease of their treatment with different reagents. The above experiments show that the residues of aromatic amino acids may also play a great role in the AM-ATP interaction. In this connection, it is to mention that high triptophane contents is found to be peculiar to the proteins in striated muscle and in organs of intensive physiological activity (252).

In chicken and rabbit embryos, the absorption maximum of A-fraction corresponds to 258 m\(\mu\), that is a evidence of the binding ATP by this protein. In post-natal period, the maximum shifts to 280 m\(\mu\), the A-ATP complex having been dissociated (172). This may have a distinct physiological signification because there is much evidence for the direct participation of ATP in the A transformations (152, 206, 207, 211, 212, 223). The spectrophotometric investigation (5) reveals the existences of more or less firm bond between ATP and A.

**Complexing Ability of M and A**

AM may bind not only ATP but also its protein components, M and A, form complexes with many enzymes and metabolites which take part in muscle activity. With glycogen, the absorption maximum of M shifts from 279-277 m\(\mu\) to the shorter waves by 3.5-17.5 m\(\mu\), this value depending on the nature of glycogen (171, 176, 179, 188). In this region, polysaccharides are transparent. Therefore, the shift of the maximum may be considered as the evidence for the formation of the glycogen-protein complex. This shift is maximal when the solution contains
0.5 mg protein-N and 1 mg polysaccharide/ml. Taking into account that MW of glycogen is equal about $2.5 \times 10^6$ (131), the relation between these substances should be near equimolecular. The AM and glycosides react in the same proportion (241), the anomalous viscosity of AM getting less marked (242).

The glycogen bound with AM is hydrolyzed by $\alpha$-amylase and phosphorylase more rapidly than it does in free state (178). Enzymatic and UV spectral tests show that the glycogen isolated from the muscles of alloxan diabetic rabbits and the starch do not conjugate with M (183). On the contrary, the liver glycogen of diabetic animals seems to form a firm complex with M since the AM absorption maximum does not only shift but it also disappears completely. The authors (186) suggest that diabetic glycogen should have the shortened side-chains. Then, the data of Stetten's group (205) attain particular importance. According to them, the metabolic activity of liver glycogen decreases with MW. Inversely, the larger particles of muscle glycogen are more accessible to enzymes.

Other polysaccharides and proteins also may form complexes, with 265 m$\mu$ maximum in every case. Nevertheless, muscle glycogen and M reveal this ability in the highest degree (163, 180–182, 184, 185, 187).

Thus, in vivo, M should focus on its molecule 1) ATP as an accumulator of high energy bonds (122, 164, 165, 239), 2) ATP-ase hydrolysing these bonds and so making free the energy which they enclose (46–48, 52, 136, 137, 139), 3) A that converts this energy into the mechanical work*. 4) glycogen that contains the main reserve of chemical energy. In addition, M reveals 5) ATP-desaminase activity (1, 12, 57–59, 63–66, 157, 240) which, under some conditions, may be departed from it (48, 137, 138, 140). It is possible that the deamination of ATP is one of the most important processes in muscle activity, since every contraction is accompanied by it. M is also identical with 6) cholinesterase (123, 235–237); hence, it may influence, in some way, the excitation process. Finally, 7) ATP-creatine phosphopherase is adsorbed on A (214, 215), although this bond is not always strong (142) and it is observed in A of not all animal species (4); this enzyme restores ATP decomposed during contractions at the expense of creatine phosphate and plays a distinct important part in the reversal of the physicochemical changes in AM micels (218, 220). Thus, the contractile muscle protein, AM, unites all necessary links that make possible the uninterrupted con-

*) The suggestion, that A acts only as a supporting protein (116), must be proved.

—107—
tinuous work of muscle machine from its setting in motion just to the regulation of the consumption of glycogen.

The tendency of M to form the complexes reveals also in the in vitro experiments with the substances that muscle does not contain. It has the affinity to different organic dyes and the affinity increases with the denaturation of M (28–30). The increase of dye binding may be observed in the earliest period of the dying of muscle (198). It is remarkable that some dyes (red and pink congo, benzopurpurine blue), when conjugated with M, give to this protein the same properties that A does: the dyed precipitate of M, after the ATP addition and centrifugation, engages the same volume as superprecipitated AM (6–8). To regret, the reversibility of this reaction has not been investigated. There is an information on the binding of the penicillin by A but it is not strong (80). In spite of its trend to the formation of complexes with M (120) and also A (80, 112) it seems to be immunologically (114), in the limits of species, not antigenic.

Part II. THE ACTION OF RADIANT ENERGY

As described above, the action of ATP on AM looks like the process of denaturation: the measurements of vapour pressure shows that the superprecipitate binds the water as feeble as the heated precipitate does. The problem is to determine whether the other kinds of energy than the heat produce the same effect.

Photodynamic Action

It is known that the visible light, in the presence of some dyes and oxygen, can destroy biological substrates, systems and organisms because of the photosensibilized oxydation (19, 45, 217).

When the frog gastrocnemius is radiated by visible light in the presence of erythrosine or hematoporphyrin, it contracts slowly and against the background of this irreversible contracture develops a number of single contractions (19, 113a). The same picture is observed in the case of nerveless unstriated muscle of birds' amnion (21). In the course of contracture, the affinity of muscle to basic vital dyes is increasing (135), i.e. the process of denaturation takes place here.

In our experiments, effects of visible light on M, A and MB were investigated (195). Erythrosine was used as the photo-sensibilizing dye and the electric lamp as the source of visible light. In the presence of erythrosine, the radiation produced two effects. Firstly, in the
course of lighting the viscosity of MB solutions was increased just to full gelatinization. Secondly, the protein lost its affinity to water. The first reaction was reversible: the gel was, after having been shaken, liquidized ididized and formed again if illuminated repeatedly. The loss of hydrating properties was irreversible and seemed to be the results of coagulation. In order to have some information on characteristics of the dehydration process, we centrifugated the illuminated solution for 20 min at 2800 r.p.m. If under these conditions a thin layer of transparent liquid was formed over the solution, it was decided that the dehydration took place. This transparent layer was shown to contain the protein because of his reaction to trichloracetic acid. This characteristic was reproduced very well. The reversible gelatinization and the irreversible dehydration were independent of each other. That may be seen from the quite different influence of protein concentration and the time of irradiation on these processes (Table 3).

Table 3. The minimal time of lighting necessary for gelatinization and dehydration of MB solutions (the erythrosine is present in concentration $2.3 \times 10^{-5}$ M).

<table>
<thead>
<tr>
<th>Protein concentration mg/ml</th>
<th>Gelatinization Time, minutes</th>
<th>Dehydration</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>---</td>
<td>11.5</td>
</tr>
<tr>
<td>3.8</td>
<td>8</td>
<td>9.5</td>
</tr>
<tr>
<td>3.0</td>
<td>5</td>
<td>8.25</td>
</tr>
<tr>
<td>1.5</td>
<td>20</td>
<td>66</td>
</tr>
<tr>
<td>0.75</td>
<td>Gel does not form</td>
<td>4.5</td>
</tr>
<tr>
<td>0.37</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>3.5</td>
</tr>
</tbody>
</table>

The quantity of light energy dehydrating unit amount of protein is constant and does not depend on the time of illumination and the concentration of photocatalyst. The $\eta$ of MB solution during irradiation by visible light in the presence of dye is always descending below the level reached after ATP addition ($0.015\%$).

M lighted in the presence of erythrosine loses its ATP-ase activity, its $\eta$ increases, the clots of gel are formed and, then, the complete gelatinization develops. These results are in perfect agreement with observations of Engelhardt's laboratory (49). By shaking, the M gel
does not become liquid, but forms the clumps. After centrifugation, the transparent liquid layer does not appear. M irradiated with dye also loses its AMP-deaminase activity and can not unite with A to give AM (238).

F-A, after photodynamic action, decreases its specific viscosity down to zero and ceases to form the complex with M. The irradiated A solution remains to be transparent and homogenous without precipitating during centrifugation.

Thus, A and M react to the photodynamic action of visible light by the same way: they lose their native properties irreversibly. The behaviour of their complex, AM, is more interesting. Its reaction is double: a reversible gelatinization and irreversible coagulation can take place simultaneously. The interrelation of these phenomena is so that, in the background of irreversible coagulation that reveals itself with increasing dehydration, the reversible gelatinization may be produced several times by lighting and shaking the solution in turn. We tend to think that these both reactions (coagulation and gelatinization) lay, under identical conditions, in the base of behaviour of whole muscle (see above). The similar reversible gelatinization is observed by ATP action according the scheme:

\[ \text{Viscous AM sol} \xrightarrow{+ \text{ATP}} \text{liquid AM sol} \xrightarrow{\text{shaking}} \text{AM gel} \]

The transformation of AM solution into AM gel seems to need some energy the source of which is in our case the visible light and is ATP in intact muscle. The reversible gelatinization seems to plays some physiological parts, as under appropriate conditions (lactoflavin + ascorbic acid + light), the muscle contracts reversibly without rigor contraction (20).

As shown by exact experiments (238) the above changes of AM produced by dye and visible light are the true photodynamic ones, because they are accompanied by O\textsubscript{2} consumption and do not reveal in anaerobic atmosphere. The remarkable fact is that the aromatic absorption minimum at 250 m\textmu and maximum at 276–77 m\textmu dissapear after photoxydation. We have already mentioned that this maximum, under influence of ATP, shifts to shorter waves. Both the light and ATP seem to change the aromatic groups of protein, but, in the first case, the reversible increase of the energetical level of chromatic components of M may take place (174) while, in latter, case, the break of
the rings may occur as the results of their photodynamic oxydation (238).

**Inactivation of MB by Radioactive Isotopes**

In the experiments, with visible light it is rather difficult to calculate the amount of energy absorbed by protein solutions. This would be done more easily when radioactive isotopes are used as the source of energy. If they are radiating $\beta$-particles, only their energy is absorbed by solution completely. The mechanism of the action of ionizing radiation must be the same as the one of photodynamic action. Electrons moving in water, according to present views, cause the formation of free radicals and hyperoxydes such as $\text{HO, HO}_2, \text{H}_2\text{O}_2, \text{H}_2\text{O}$, and so on. That is why they should stimulate oxydative processes (74, 111, 216).

In our first experiments, we investigated the action of Ca$^{4+}$ on muscle extracts (85). Ca$^{4+}$ emits electrons of relatively low energy (0.254 Me V), $\gamma$-radiation being absent. Its half-life is 163.2 days (161). The concentration of Ca$^{4+}$ should be as high as possible. But our Ca$^{4+}$Cl-solution contained the stable component, CaCl$_2$, in a great concentration. Therefore, the AM solutions would contain 325 mM Ca. In such a high concentration, CaCl$_2$ produces an immediate precipitation and denaturation of protein. Nevertheless, we could abolish the hurting action of Ca$^{4+}$ by addition of equimolar quantity of EDTA (see below).

Fig. 1 shows that, after 27 days of experiment, $\gamma_{\text{ATP}}$ decreased in some degree, i.e., contents of MB is a little lower at the end of experiment than in the first day. But this fall of $\gamma_{\text{ATP}}$ is equal in all three series (K; K+Ca+EDTA; Ca+Ca$^{4+}$+EDTA). Therefor, it is not caused by Ca, EDTA or Ca$^{4+}$. After 27 days, Ca$^{4+}$-extract reveals a higher $\gamma$ than extract with stable Ca and EDTA only. This difference is detectable already the next day after addition of Ca and Ca$^{4+}$. At the 27th day, the flakes of protein could be discovered in Ca$^{4+}$ extract. Thus, some analogy may be stated between the action of ATP or visible light, in the presence of photodynamic dye (reversible gelatinization, irreversible dehydration) and the action of Ca$^{4+}$ (the increase of $\gamma$, the formation of flakes). However, in the latter case, both actions are not very distinct, although a large quantity of energy has been absorbed by extract during 27 days. This energy is 400 cal/100 g MB, being about hundred times as much as the one that ATP is imparting to MB by maximal changes of physico-chemical properties in
Fig. 1. The Ca and Ca⁴⁵ action upon the changes of γ (open columns), γ_{ATP} (full columns) and VA (the curves above the columns: ×—control, ○—Ca, △—Ca⁴⁵).

Salt muscle extract; ~0.5 M KCl; 0.1 M carbonate-acetate buffer; pH 6.8; volume 6 ml; initial MB contents 60 mg; 325 mM CaCl₂; 325 mM Na₂ EDTA; initial total radioactivity 0.91 mC; 4°; γ and γ_{ATP} have been measured at room temperature (20–25°C).

We have suggested that the slight affect of Ca⁴⁵ may be explained with the low energy of β-particles emitted by this isotope. To prove this assumption, we have investigated effects of a more powerful isotope, P³² (86). Its emitted electrons have the energy, 1.708 MeV. The experiments have been conducted with muscle extracts and MB solutions. In a set of experiments with MB, the incubation time was varied at the constant P³² contents and, in other set, the P³² concentration was changed at the same duration of experiment. It has been found that P³² provokes the fall of γ both in muscle extracts and MB solu-

*) The irradiated energy has been calculated according to the formula: 

\[ E_r = 6.8 \times 10^{-7} AET(1-e^{-\theta/T}) \]

where \( E_r \) is the radiated energy (cal), \( A \)—the initial total radioactivity in solution (mC), \( E \)—the maximal energy of emitted electrons (MeV), \( T \)—half-life of isotopes (sec), \( \theta \) —the duration of experiment (sec).

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tions. This effect depends on the emitted energy \( (E_r) \), being the stronger the greater the energy has been absorbed. It is interesting to note that the concavity of the VA-\( E_r \) curve of muscle extracts is directed downwards and the one of MB solutions upwards (Fig. 2). That means that, in the former case, the first portion of \( \beta \)-particles produces the smaller effect than the following one; with MB, an opposite picture occurs. The extrapolation of VA-\( E_r \) curves to zero VA shows that the complete inactivation of MB solutions is caused by the energy 12,000 cal/100 g protein, the energy 3,000 cal/100 g protein being enough to suppress VA of muscle extracts. It is possible that, in muscle extracts,

Fig. 2. The action of \(^{31}\text{P}\)-\( \beta \)-radiation energy on VA of muscle salt extracts (I) and MB solutions (II).

\( \times \) — muscle salt extracts.

\( \circ \) — MB solutions.

• — the inactivation of guinea pig serum complement depending on the dose of irradiation with accelerated electrons (94a).
the factor might be present that decreased the AM stability to irradiation. Such an assumption is also corroborated by the fact that the repeated ATP addition to a irradiated preparates decreases VA of muscle extracts to a greater degree than of AM solutions.

In some cases, the ATP-ase activity was determined. The result was unexpected: the irradiation does not influence it though, as known, (39, 253), the oxydation of M involves the inhibition of ATP-ase. Maybe, the preservation of ATP-ase activity is only apparent. In our experiments \( Q_p \) values both of the control and the irradiated MB were rather low. According to Garay and Guha, ATP, if dissolved in irradiated water decreases its ability to be attacked by ATP-ase (73). If it is true, our results seem to mean not that the irradiated MB-ATP-ase remains normal, but that the control one can not develop its true activity. Anyhow, this explanation is to be considered only as a possible one.

Thus, ATP, visible light in presences of dye, and \( \beta \)-radiation of low and relatively high energy reveal some general signs in their action on MB. But at the same time, they differ with each other significantly. ATP dehydrates AM reversible and, after having been hydrolysed by AM, it may cause the reversible gelatinization or the increase of thixotropy of the protein solution. Under the action of ATP, the ATP-ase may be inhibited reversibly. That occurs when the appropriate regulating systems are present such as some cations, Marsh-Bendall factor (14, 15, 146)\(^*\) and so on. But usually the MB-ATP-ase activity is preserved. Under photodynamic action, MB forms a gel reversibly and is dehydrated and loses its ATP-ase activity irreversibly. Small doses of ionizing radiation stabilize \( \gamma \) and even can cause some increase of it. The energy, 400 cal/100 g protein also gives the first signs of denaturation in the form of light flakes. A more massive irradiation (12,000 cal/100 g) dehydrates MB irreversibly (\( \gamma \) falls down to \( \gamma_{\text{ATP}} \)), but it is possible that ATP-ase is not destroyed. Thus, the different kinds of radiation may imitate all properties of ATP as the factor influencing AM except the ability to dehydrate MB reversibly. These facts may mean that the physico-chemical changes in MB micels produced by ATP seem to need some energy which is originated from high-energy bonds of muscle ATP. Then, the return of protein to its initial state must be accompanied with the release of free energy.

\(^*\) The nature of Marsh-Bendall relaxing factor is not known (168) though, some time, it has been assumed to be myokynase (16).

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that may execute the mechanical work. X-rays denaturate M. Polysaccharides giving the complexes with M (see above) increase the stability of M toward the X-radiation (129).

It is remarkable that physiological properties of very different proteins is inactivated to the same degree by equal doses of radiation. This may be concluded from the exact coincidence of the inactivation curves of complement (94a) and MB (Fig. 2).

PART III. THE BEHAVIOUR OF CONTRACTILE PROTEINS IN THE PRESENCE OF SOME METALLIC CATIONS

While ATP supplies the chemical energy which can be transformed into the mechanical one, and AM functionates in the capacity of an actor-catalyst realizing this transformation, the metallic cations regulate the activity of the whole system. The intensity and the direction of their action depend on their nature and valency. In intact tissue, monovalent cations, Na and K, differ in mode of their action on other elements of contractile apparatus, or it is difficult to understand the significance of mysterious Na-pump. But I suggest that their cardinal role is to create some background for normal activity probably by means of maintaining the necessary ionic strength. The main regulatory function belongs preferably to the bivalent cations, Ca and Mg, because of their ability to change, at small concentrations, the physical and chemical properties of proteins. Their proportion seems to determine the direction, intensity and scale of physiological processes, that may be ascribed, to some degree, to the difference of their dissociation constants of their complexes with metabolites. At last, polivalent and heavy metals, probably, do not take part in the reactions of muscle activity. When they are not included into the molecule of any enzyme organically, they will disturb the function of contractile protein as soon as their concentration increases above the normal level.

In this part, some observations relating to these three groups of metals are described and a possible interpretation on their effects is given.

The Action of High Na and K Concentrations

The author planned to study the action of Na\textsuperscript{2+}Cl on VA of MB. Our preparate of Na\textsuperscript{2+}Cl contained much stable component. That was why, we, in preliminary experiments, studied the action of NaCl on
salt muscle extracts and MB solutions. With increasing the concentration of NaCl up to 1 M (+0.3 M KCl in all tests), $\gamma$ falls rather abruptly; at 1-2 M NaCl, it does not change to any significant degree, and, then, $\gamma$ does rise suddenly again. As $\gamma_{ATP}$ decreases only in the region 0-0.3 M NaCl and, then, remains at the same level; VA is near 100% at $[\text{NaCl}] \leq 0.5$ M, as low as 40-50% at 1-2 M, but at 2.26 M VA is as high as 57% and reaches up the normal value, 97%, at 2.72 M NaCl. In 24 hours $\gamma_{ATP}$ values return to the original $\gamma$. MB solutions shows the similar behaviour. In experiments with KCl, $\gamma$ and VA decrease more sharply with increase of the concentration than in NaCl experiments. There is no return to high values at higher concentrations of KCl. At unchanged salt concentration (2.3 M) and varied Na : K ratio VA oscillates in narrow limits, 10-35%, increasing with increased Na proportion and forming a plateau (20%) at Na : K = 0.4-2.6.

The described behaviour of VA at different concentrations of Na and K could be explained in the manner that metallic cations take part in the formation of the hydrate space around AM micells; that is, at the Na : K ratio which causes the displacement of K for Na, $\gamma$ and VA have to increase because of the higher hydration of Na$^+$. The Combined Action of Ca$^{2+}$ and EDTA

EDTA in the concentration of 6 mM increases $\gamma$ of salt extracts of muscle, but it does not influence $\gamma_{ATP}$, and, thus, elevates VA (85). At 12 and 24 mM EDTA, the lowering of $\gamma$ under the action of ATP is not complete. At $\geq 60$ mM EDTA, ATP produces no drop in the least, i.e., it can not dehydrate AM. These observations are in good agreement with the data of Tonomura's laboratory (218-221). These authors found that EDTA, at the concentrations $> 5$ mM, prevented the decrease of light scattering in MB solutions produced by ATP.

As known (90), EDTA, at some concentrations, elevates ATP-ase activity of heart homogenates, but, above some concentration, it inhibits the ATP-ase activity. The reaction of EDTA with MB is analogous: its ATP-ase activity is optimal at 0.1-10 mM EDTA (22, 72). Since CaCl$_2$-EDTA does not effect the ATP-ase (72) it may be concluded that the activation and inhibition of ATP-ase by EDTA is associated with the binding of bivalent cations. Thus, EDTA, at the appropriate concentrations, may prevent the action of M on ATP and, inversely the action of ATP on AM.

In the presence of high concentrations of CaCl$_2$ (63-325 mM), VA,
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Fig. 3. The influence of different ratios CaCl₂ : EDTA on \( \tau \) of muscle salt extracts. Ordinate—\( \tau \) (the time of flow of extract; the time of flow of a solution containing appropriate amounts of CaCl₂ and EDTA), abscissa—the EDTA concentration in logarithm scale. CaCl₂ concentrations are noted over every curve. The broken line below is \( \tau_{\text{ATP}} \) (it is the same at all tests). The upper broken line is the control \( \tau \) (in absence of Ca and EDTA). The arrows below note the points where the EDTA concentrations are equimolar to CaCl₂ ones.

\( \gamma \) and the native state of muscle extracts may be preserved during a long period (1 month) by means of the simultaneous addition of equivalent amount of Na₂-EDTA. CaCl₂ without Na₂-EDTA produces an immediate denaturation of protein (35). The correlation between VA and Ca\(^{2+}\) : EDTA ratio, even at as high Ca concentration as 325 mM, is strongly pronounced (Fig. 3). It may be interpreted as an evidence for the obligatory participation Ca\(^{2+}\) in the intimate mechanism of ATP action on AM. It is possible that Ca\(^{2+}\) itself dehydrates AM whereas the ATP plays an auxiliary part. Probably because of low dissociation constant of the complex Ca-ATP (8.7 \( \times \) 10\(^{-5}\)) (40) ATP releases Ca\(^{2+}\) of any nonionogenic form. Ca\(^{2+}\) displaces K\(^+\) or Na\(^+\) in the appropriate centers on AM and dehydrates this protein. ATP, being bound with Ca\(^{2+}\), softens the dehydrating action of this cation and, therefore, it is reversible. According to this view, it may be easy to understand why moving off the divalent cations by means of cation exchange resin causes the gelatinization of liquid muscle suspension (93).

Many investigators studying muscle models are persuaded that the formation of M-ATP complex causes the contraction of muscle. However, Bozler (23–27) thinks that his experimental data contradict this view. According to his scheme, Ca activates M-ATP-ase, stimulates the ATP hydrolysis and, therefore, the contraction of muscle. This

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hypothesis is in some agreement with our idea that AM dehydrated by ATP can swell (and, thus, the muscle can contract) only after the ATP has been decomposed. Ca seems, indeed, to take part in the ATP hydrolysis but, in addition, it causes the dehydration of AM, i.e., according to our suggestion it promotes the muscle relaxation. This may be its chief function. If Ca would keep its dehydrating ability but lose the one to activate ATP-ase, one should observe a protracted dehydration of AM, normal $\gamma_{\text{ATP}}$ or, muscle relaxation, if the fiber structure is not destroyed. Such seems to be the mechanism of relaxation of glycerinated muscle fibers under the action of EDTA because this process is possible in the presence of Mg and ATP (or ITP) only, at equivalent Mg:EDTA ratios (243). By the change of Mg concentration, the relaxation may be produced due to the substrate inhibition of ATP hydrolysis (75).

Decrease fo VA fo MB Solutions by Cu

If Cu$^{2+}$ and MB present in the solution in the ratio 17 mole Cu: 1.88 x 10$^5$ g protein no drop of VA may be discovered, at least, in 24 hours after ATP addition (81). M is more sensitive towards heavy metals than MB. Ag$^+$ suppresses M-ATP-ase activity at the concentration 10$^{-7}$ M, the protein contents being equal to 0.1 mg ml (50). The corresponding Ag: M ratio is 2 mole Ag: 1.74 x 10$^5$ g protein. This value is comparable with the effectiveness of ATP. Thus, A, being complexed with M, seems to increase the stability of M-ATP-ase to heavy metals. At the ratio 34 mole Cu: 1.88 x 10$^5$ g protein, MB reacts on the ATP addition by usual fall of $\gamma$ down to $\gamma_{\text{ATP}}$ but irreversibly: $\gamma_{\text{ATP}}$ does not return to $\gamma$. It may depend on the ATP-ase inactivation, or on the inhibition of creatinephoshokinase that is suggested to be a necessary component in the process of reversal of AM, under ATP action, from physically changed active state to the initial one (218, 220). At the ratio 68 mole Cu: 1.88 x 10$^5$ g MB, the protein undergoes the irreversible coagulation (81). Our experiments with Cu may be interpreted as the evidence of the participation of metallic cations in the AM dehydration under the influence of ATP. The action of cation should be the stronger the less its hydrate sphere is.

Anisometric Contraction of AM Threads

The muscle activity is determined by two interconditioned properties of this tissue, i.e. ability to contract and to carry out the me-
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mechanical work. As the whole organ, the muscle contracts anisometrically: it shortens and thicken. What is doing AM during the contraction of muscle is not known distinctly. Many investigators deduce that this protein, when muscle contracts, should also contract, this contraction being, in the obligatory way, anisometric. This view stimulated many experiments that had the purpose to get, at all costs, such a behaviour of AM that it would resemble, in this relation, the intact muscle. The AM threads seem to be the most suitable form of the protein to realize this intention. Anisodimesional muscle contraction is suggested to be possible only because of the exact longitudinal orientation of all M micels, the isometrical contraction of AM threads being due to the chaotic disposition of their protein molecules. Under ATP action, they only decrease their volume as much as 30 times, equally contracting in three directions of space. They can produce some mechanical work, but it is negligible (109) and is not to take into consideration. Therefore, the problem of the creation of the anisodimensionally contracted AM model might be reduced to the orientation of protein micels in it. Many authors try to achieve a success solving of this problem.

It is supposed that the only way to put the micels of AM threads in order is the stretching. The threads prepared by usual method are very fragile and break at any attempt to stretch them. When denaturated with the salts of heavy metals, they become elastic but of course lose their enzymic activity and ability to change their physico-chemical properties under the ATP action. In order to avoid these difficulties the threads were dried slightly. They became more extensible and revealed ATP-ase activity. The similar result was obtained by partial denaturation of threads with sufficiently diluted solution of zinc sulfate.

In Rubinsteins's laboratory, the more success was achieved. Toriko- 
chinka (222) treated the MB threads with 1 mM CuSO₄, stretched them 3-3.5 times as long as the original and, in stretched state, reactivated with cysteine solution. After the weight has been removed the threads remain 2.5 times as long as in initial state. Such threads possess ATP-ase activity and, after the elevation of KCl concentration up to 0.2 M, response to the ATP addition with anisometric contraction and with the drop of double refraction.

The creation of AM model of contractile mechanism of muscle seemed to be realized partly. It only remained to communicate to these threads the ability to do mechanical work during the contraction

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under ATP influence. But the thing is that nobody could do it.

From our point of view, the efforts of this kind are quite vain. Indeed, if we suppose that the muscle does work because of the swelling of AM dehydrated by ATP, then could exist no model, that would do any work during the process of dehydration of these micels under the action of ATP even if its micels may be oriented. The present author wonders why RUBINSTEIN concentrated his attention on the construction of anisodimensionally contracting AM model that would be able to do mechanical work: such a model is impossible from the view of RUBINSTEIN's own idea that the source of the mechanical work of muscle might be the process of protein hydration.

Recently, the fictitious nature of the advances in modelling the anisometrically contracting AM threads is shown: regardless of the method of preparation (film threads, drying, partial inactivation with heavy metal, reactivation with cysteine), the oriented micels in such threads are completely denaturated and the ATP-ase activity is revealed only by native chaotically disposed micels (113). The work that AM threads do under the action of strong acid solutions (222) is insignificant and seems to have no connection with their contraction after ATP addition. This unnatural contraction seems to belong to the same series of phenomena as the contraction of glycerinated AM threads and muscle fibers in the presence of TOULER's reagent, CuCl₂, or heat (154-156).

**Activating Action of Cu²⁺ on MB-ATP-ase and Muscle Excitability of a Mollusc**

RUBINSTEIN and TORBOCHKINA (194) investigated the action of Cu²⁺ on the ATP-ase activity of the protein extracted with 0.6 M KCl, pH 7, from the sexual pouth of *Helix pomatia* and on the excitability of this organ. They ran across an interesting fact. Cu inhibits ATP-ase at 2.5 mM, but produces an opposite affect at 1.4 mM: ATP-ase activity doubles, whereas the one of rabbit M, under these conditions, disappears completely. ATP, at the concentrations >0.4 mM, decreases the excitability of sexual pouch muscle and increases it at 0.05-0.3 mM. There is a correlation between ATP-ase activity and the threshold of excitability. If ATP-ase is activated, the threshold decreases and, inversely, if ATP-ase is inhibited (at high CuSO₄ concentrations, or in the presence of cysteine), the excitability is also suppressed. This correlation between actions of ATP and Cu, and between ATP-ase activity and
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excitability may be explicable by the fact that M reveals acetylcholinesterase activity (see above). Some snails are known to contain, as a normal constituent, the Cu-proteid, hemocyanin in their hemolymph. We think that, in these invertebrates, Cu activates ATP-ase in the same manner as Ca does in the muscles of vertebrates. The affect produced by a cation seems to be determined by its dehydrating power rather than by its qualitative peculiarity.

The State of ATP-AM System by Lead Intoxication

The ability of heavy metals to denaturate the proteins, including muscle ones, has been well investigated. But there is no information about what happens with AM in vivo during the intoxication with heavy metal. At the same time, it is established very well that the pathological state of this kind is often accompanied by marked disturbance of muscle activity. That is why we investigated the changes in the contractile proteins in course of chronic poisoning of rats with Pb (88, 89).

In the control group, the contents of muscle and heart proteins extracted with Wheelen's solution decreased with the age (Table 4). VA of muscle extracts, during this time, is changing inconsiderably whereas VA of heart extracts is increasing as if in order to compensate the loss of the protein. The incorporation of methionine-S35 both into the tissue and the contractile proteins of muscle and heart at the 10th day is more intensive than at 50th and 125th days of experiment.

The lead intoxication brings some changes into this picture. The contents of extracted protein is low at 10th day of poisoning, but, at

<table>
<thead>
<tr>
<th>The day of poisoning</th>
<th>Heart</th>
<th></th>
<th>Muscle</th>
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<tbody>
<tr>
<td></td>
<td>MB, %</td>
<td>VA, %</td>
<td>MB, %</td>
</tr>
<tr>
<td>Control</td>
<td>Poisoning</td>
<td>Control</td>
<td>Poisoning</td>
</tr>
<tr>
<td>The 10th</td>
<td>6.75</td>
<td>5.92</td>
<td>22</td>
</tr>
<tr>
<td>The 50th</td>
<td>5.12</td>
<td>5.58</td>
<td>25</td>
</tr>
<tr>
<td>The 125th</td>
<td>4.99</td>
<td>6.13</td>
<td>49</td>
</tr>
</tbody>
</table>

*) To spare place the standard deviations are not adduced. The differences between values are statistically reliable; 3-8 animals have been used in every experiment.

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50th and 125th day, it is much higher than in the control group. During the first days of poisoning, the labelled methionine incorporates into muscle and heart tissues slower than in the later phase. At the same time, the incorporation of radio-S\textsuperscript{35} into MB is about the same as at 50th and 125th days. At this periods, it is also equal in the control and poisoned groups. VA of heart extracts is not changed considerably at the 10th but decreases markedly at 50th and 125th days. The opposite relation is observed with muscle extracts: VA is lower than the control values at the 10th day, but it is equal to them at the 50th and 125th days.

These and other data (basoerythrocytes, body weight, organ: body weight ratios, Hb contents) let to divide the process of chronic lead intoxication into three rather well separated periods. In the beginning, a transient suppression of physiological activity is observed since the organism has not been ready to defend itself against the harmful agent. The contents of contractile proteins in muscle tissue declines because of slackening of their synthease, that is shown by the decrease of methionine-S\textsuperscript{35} incorporation. It is known that the intensity of renewal of muscle proteins is also low at other patologies such as atherosclerosis (124) and diabet (70). The inhibition of AM synthesis may be responsible for the muscle weakness that is one of the symptoms by saturnism (234).

In the second period of poisoning the mobilization of protective and compensatory mechanisms conditions the restoration of functions of organism. The formation of complexing agents that transform the heavy metal into the nonionogenic form is naturally assumed to be one of these mechanisms. The other way to neutralize the injuring action of Pb could be the intensive increation of the substances stimulating the protein synthesis and so compensating its inhibition caused by poison. It may be to mention that the growth hormone injected to rats and mice causes the increase of methionine-S\textsuperscript{35} incorporation into muscle proteins as much as 70\% (71). The forced synthesis has to be accompanied by the intensification of oxydative processes. Indeed, there is an information that the patients intoxicated by Pb consume more \textit{O}_2 than healthy persons (202). In the end, the compensatory mechanisms become insufficient, the metabolism is disturbed and the animal dies.

The acute lead intoxication caused by intraperitoneal injection of 1.4 ml of 1\% lead acetate (9 mg Pb) is accompanied by marked depression.
of the incorporation of radiomethionine into muscle and heart MB. But the MB contents of these tissues does not decrease significantly during 8 days after Pb introduction. At the same time, VA does already decrease in 24 hours. VA of heart extracts restores as soon as in 48 hours, but VA of muscle ones, at the 8th day, remains as low as in 24 hours after poisoning. It is probably that VA drop both in chronic and acute experiments is the result of the direct action of Pb as a heavy metal on AM. Such an action has been investigated with other heavy metals in the relation to M (17, 139), with Pb in the relation to intact muscle (33).

CONCLUDING REMARKS

In the above parts, I have tried to put in order some experimental facts relating to the AM-ATP-cations system, mainly, those obtained by Russian authors. Now, I would like to share some considerations of more abstract kind.

On the possible significance of swelling processes in muscle contraction. I have already noted (82) the excessive bent of many recent investigators for the mechano-kinetic schemes of folding, curling of supercontracting M chains that are drawn to explain the mechanism of muscle contraction.

The swelling is always accompanied by volume striction of the system liquid + colloid, heat production, vapour pressure lowering of liquid component, pressure and mechanical work developed by swelling colloid (119). The contracting muscle reveals analogous phenomena: it strains, does mechanical work, produces the heat and its volume diminishes.

If the muscle work is supposed to be done because of the swelling of AM dehydrated by ATP, then, the following values of energy for the same amount of M should be equal to each other: 1) by heat production, mechanical work and volume striction of the twich; 2) by heat production, volume striction, swelling pressure and vapour pressure of the swelling AM; 3) by minimal amount of ATP producing maximal reversible change of physico-chemical properties of AM; 4) by ATP decrease in muscle during a twitch, and so on. Some of these values have been calculated on the basis of literary data (Table 5).

Taking into account a rather low precision of measurements taken for calculations and the fact that they all have been obtained quite
TABLE 5. The energy of single contraction calculated by different ways, cal/100 g M

<table>
<thead>
<tr>
<th>The value used for calculation, and references</th>
<th>Muscle</th>
<th>Actomyosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical work of skeletal muscle (103)</td>
<td>4.1</td>
<td>—</td>
</tr>
<tr>
<td>Mechanical work of heart in vivo (92)</td>
<td>9.2</td>
<td>—</td>
</tr>
<tr>
<td>Heat production (96)</td>
<td>5.1</td>
<td>—</td>
</tr>
<tr>
<td>Volume striction (53)</td>
<td>7.9-42.3</td>
<td>—</td>
</tr>
<tr>
<td>Vapour pressure (102, 192)</td>
<td>0</td>
<td>9.0</td>
</tr>
<tr>
<td>Amount of ATP (81, 133, 144, 145, 218, 220, 224)</td>
<td>4.1- 5.3</td>
<td>5.5-6.44</td>
</tr>
</tbody>
</table>

Independently, these values may be considered to be in good agreement with each other. This is to be considered as the evidence, although a indirect one, in favour of the intimate participation of processes of AM swelling and dehydration in muscle contraction. The high upper value calculated from volume striction seems to be connected with the fact that the swelling of muscle proteins during contraction, this process being distinctly real (55), is responsible only to a part of the volume striction. The other part of volume striction may depend on chemical reactions (149) and K ionization (137). Naturally, the vapour pressure over the intact muscle can not change since the water of intercellular space does not take part in the contraction.

An exemplary scheme of muscle contraction with participation of swelling process. The scheme of concrete hypothetic mechanism of muscle contraction that would take into account the swelling pressure as the source of muscle work and, simultaneously, the microstructure of muscle fiber may be outlined on the basis of the following more or less well established facts: 1) myofibrils run in the fiber without breaking off (189); 2) discs A and I blend during contraction (95); 3) ATP dehydrates AM; 4) dehydrated AM can swell and do mechaniacl work. Then, the contraction of muscle fiber may be pictured, for example, as follows (Fig. 4).

Myofibrils run along in fibers but, qualitatively, they are different in discs A and I. They are built of some elastic matter in I discs and of AM in discs A. In relaxed fibers, the myofibrils are drown together, and the A and I discs are separated distinctly. AM-ATP-ase in A discs is inhibited by corresponding ratio of bivalent cations, MARSH-BENDALL factor and other regulatory systems. Therefore, the ATP is present and
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Fig. 4. One of the possible schemes of muscle contraction based on the swelling of actomyosin dehydrated by ATP.

prevents the swelling of myofibrils. Nerve impulse, in the end, shifts this proportion so that ATP-ase is activated. ATP in A disc degradates, AM swells for account of the water that moves from the I discs into the A ones. A discs thicken, elastic elements of myofibrils in I discs shrink and shorten, muscle fiber contracts and thickens. However, soluble ATP-ase that is present in the water of I discs dehydrates AM again. The return to the initial, relaxed state may occurs owing to either the elasticity of sarcolemma that has been strained by swelling AM, or the elasticity of membranes M and Z, or the straightening of elastic elements in I discs, or, rather, the simultaneous action of all these forces. Heat production and volume striction, according to this scheme, have to reveal in contracting phase, and they do so indeed.

It would be mentioned that the author does not pretend the scheme given above is quite correct in its details or on the whole. He would only like to show with it that theory of muscle contraction considering the AM swelling as the source of muscle does not contradict with the modern knowledge of muscle histology, biophysics and biochemistry, and, then, should be taken into account equally with other hypotheses that make use of different principles.

On the investigations to be done. The proof of a hypothesis that sug-
gests the AM swelling to be the source of mechanical force of muscle
may be realized in some directions.

Firstly, the additional experiments must be done in order to estimate
the energetical significance of the swelling and dehydration of AM.
With this purpose, 1) volume striction of AM solutions or suspensions
under the influence of ATP, 2) the heat production of the physico-
chemical interaction between ATP and AM, 3) the insoluble space
with and without ATP, best of all, for the labeled glycerin taken in
different concentrations, 4) water contents of the precipitate and the
superprecipitate after the centrifugation at different rates of rotation
and other values must be measured.

Secondly, it is desirable to try to construct a model where AM
after having been dehydrated by ATP could do some work periodically,
about like shown in Fig. 4. The main difficulty seems to consist in the
preparation of solid semipermeable plat which would easily pass the
water and would not be penetrated by AM.

It is also difficult to find the conditions under which the AM dehy-
drated by ATP would swell. As known, the removal of ATP only is
not sufficient to swell the superprecipitate: the increase of ionic
strength is also necessary, that, of course, is undesirable if the purpose
is the repeated reversal of the process.

A few words on carnosine-anserine fraction. Recently, the data have
been published that, during the single contraction, the carnosine phos-
phate is decomposed. This compound was synthesized and studied in
SEVERIN'S laboratory (150, 200, 201). I am far from FLECKENSTEIN'S
negativism (68) and Hill's scepticism (67) in the relation to ATP as
the source of energy making AM able to do mechanical work: the
changes produced by ATP in AM are too voluminous. It is doubtful
that ATP would be inferior to carnosine phosphate in the respect of
being the terminal accumulator of the energy of oxydation of nutritive
substances. But, at the present the function of carnosine is not disso-
vered completely. It influences a few reactions of phospho-carbohydrate
metabolism (199) but, obviously, this is not its essential role. In the
muscles of mammalia, its contents is approximately equal to those of
ATP. In contradiction to ATP, it is a base. It would seem important to
study its relation to ATP in a more detailed manner; it is not un-
probable that carnosine phosphate is the substance that binds ATP
and so makes it inactive; and that the carnosine fraction takes part
in the interphosphorylation between AM and ATP, and so on.
SUMMARY

In the present review, the evidences are considered to support the idea that the mechanical work of the muscle during the contraction may be the one due to the swelling of actomyosin (AM) dehydrated by ATP and so enriched in free energy. On this base a possible microstructural picture of muscle contraction is adduced. Some other aspects of the ATP-AM interaction are also discussed.

References

10. X. Aubert, Le Couplage Énergétique de la Contraction Musculaire, Bruxelles (1956).
11. X. Aubert, J. Physiologie 48, 105 (1956).
24. E. Bozler, Experientia 9, 1 (1953).
25. E. Bozler, J. Gener. Physiol. 37, 63 (1953).
45. B. B. Efimov, Usp. eksper. biologii 1, 382 (1922).
53. E. Ernst, Pflüger's Archiv. 299, 613 (1925).
54. E. Ernst, Pflüger’s Archiv. 218, 137 (1927).
55. E. Ernst and K. Czimber, Pflüger's Archiv. 228, 683 (1931).
59. D. Federman, Usp. biol. khimii 1, 216 (1950).
61. D. Federman, Vitaminy 1, 225 (1953).
Some Physico-Chemical Properties of Actomyosin-ATP System

85. E. D. GRISHCHENKO, Biokhimiya, in press.
86. E. D. GRISHCHENKO, Vopr. med. khimii, in press.
87. E. D. GRISHCHENKO, Usp. sovr. biol., in press.
88. E. D. GRISHCHENKO and V. V. NIKITENKO, Vopr. med. khimii 2, 328 (1956).
89. E. D. GRISHCHENKO and V. V. NIKITENKO, Farmakol. i toksikol. 21, N 1, 81 (1958).
108. I. I. Ivanov, Usp. biol. khimii 1, 179 (1950).
122. A. F. Kotelnikova, Usp. biol. khimii 1, 332 (1950).
129. A. M. Kusin and E. G. Plyshevskaya, Biophysika 1, 141 (1956).
133. R. A. Lawrie, J. Physiol. 121, 275 (1953).
Some Physico-Chemical Properties of Actomyosin-ATP System

141. M. N. LYUBIMOVA and D. PEVSNER, Biokhimiya 6, 178 (1941).
143. M. N. LYUBIMOVA and M. S. SHIPALOV, Biokhimiya 5, 144 (1940).
144. E. LUNDSGAARD, Biochim. Ztschr. 217, 162 (1930).
149. O. MEYERHOF and K. HARTMANN, Naturwissenschaften 21, 661 (1933).
180. E. L. ROSENFELD, Usp. sovrem. biol. 36, 179 (1953).
189. G. I. ROSKIN, Usp. sovrem. biol. 34, 268 (1952).
198. O. SCHEIBE, and M. EDER, Acta Histochem. 3, 6 (1956).
199. S. E. SEVERIN, Usp. biol. khimii 2, 335 (1954).
Some Physico-Chemical Properties of Actomyosin-AT System

222. L. I. Torbochkina, Diss., Moscow (1952).
238. T. V. Venkstern, Biochimija 18, 97 (1953).
240. M. M. Vorobyeva, Vopr. med. khimii 1, N 1-2, 311 (1949).
249. N. N. YAKOVLEV, Usp. sovrem. biol. 27, 257 (1949).
250. N. N. YAKOVLEV, Studies on the Biochemistry of Sport, Moscow (1955).