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THE RELATION OF TEMPERATURE AND MUSCULAR CONTRACTION

BY L. VARGA
FROM THE INSTITUTE OF BIOCHEMISTRY, UNIVERSITY OF BUDAPEST
(PRESENTED BY A. SZENT-GYÖRGYI. — RECEIVED 18 DECEMBER 1945)

Rabbit. As has been shown in this laboratory, muscular contraction is due to the contraction of a complex protein, actomyosin. Actomyosin can be prepared *in vitro* from actin and myosin and can be brought to contraction by the addition of certain metals and ATP. This contraction can be regarded as the transition of the protein into a new, more stable modification in which the protein particles are shortened. If rabbits actomyosin or muscle is used, this transition does not take place at 0° which suggests that the transition of relaxed into contracted actomyosin is an equilibrium reaction, dependent on temperature, and the failure to contract at 0° is due to the fact that the equilibrium, at this temperature, is shifted towards the relaxed form.

The object of this research was to ascertain the equilibrium constants at different temperatures. In an actomyosin gel it is evident that there must be a certain relation between the number of contracted particles and the extent of contraction. If the system consisted of perfectly oriented actomyosin particles, conclusions could be drawn from the shortening on the relative number of uncontracted to contracted particles. As will be shown later this supposition, which formed the base of this research, was fully born out by the observations.

METHODS

Rabbit psoas was used as material. This muscle is built of long parallel-running fibres. The animal was killed by decapitation, rapidly skinned, eviscerated and cooled in ice water then all the muscles except the psoas were cut out. The vertebral column was cut above the XI dorsal vertebra. In this way, the body is cut into two, the psoas remaining on the lower part which was placed into a big volume of 0° distilled water. Here the preparation was left for 24 hours, the water being exchanged twice. During this time the ATP, present in the muscle, is entirely washed

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out and the muscle does not contract any more if frozen and thawed or cut. Than a small and long strip was cut out, parallel to the fibres. The piece was placed on the table of the freezing microtome in such a way that the fibres lay parallel to its surface. Then the muscle was cut into slices, each slice consisting of one sheet of fibres, running along the whole length of the slice. Since all the slices had the same dimensions it is sufficient to acertain their lenght once. The slices were placed into distilled water and then transferred into the ATP-salt-solution of different temperature, in which the muscle contracted. The length was measured under a microscope by means of an ocular-micrometer. Maximum contraction was reached in 5—10 minutes. The most reproducible results were obtained between 4—16° C.

The ATP-salt-solution had the following composition:

Distilled water	0,8 ml.
M KCl	0,1 ml.
1,2% ATP	0,1 ml.

Constant temperature was established by means of Höppler's thermostat, a small chamber (Fig. 1.) being perfused with its water. The bottom of this celluloid-chamber was made of glass. The ATP-saltsolution was placed in the test-tube. After temperature-equilibrium was reached the slice was transferred into it by means of a glass rod. The whole chamber was put under the microscope.

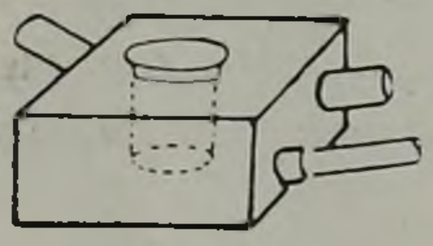

fig. 1.

The results are summed up in Fig. 2. where the percentage contraction is coordinated with the temperature.

As shown by the figures, the extent of contraction depends on temperature. White there is no contraction at all at 0°. above 16 the

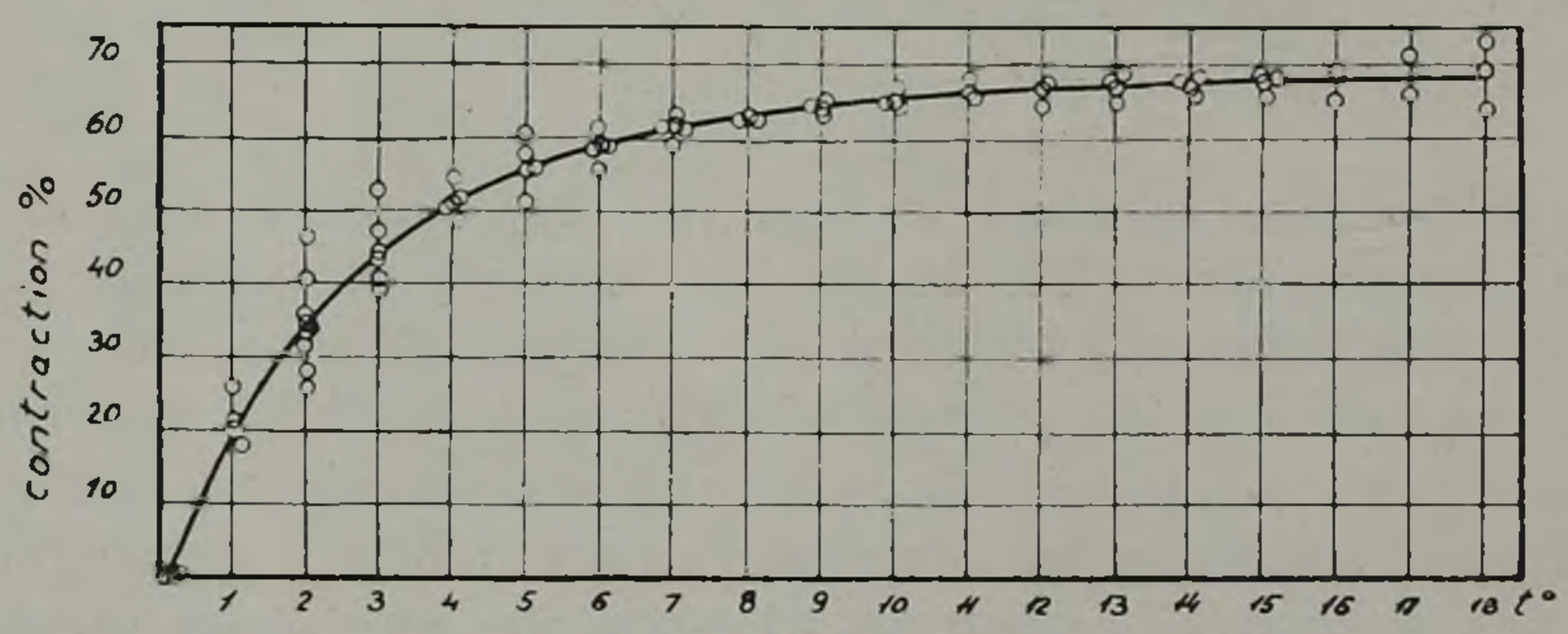

fig. 2.

contraction approaches 68—70% asymptotically. If we suppose that at this maximal contraction all, (100%) of the actomyosin particles are contracted, while at 0° none (0%) is contracted, then we can easily calcu-

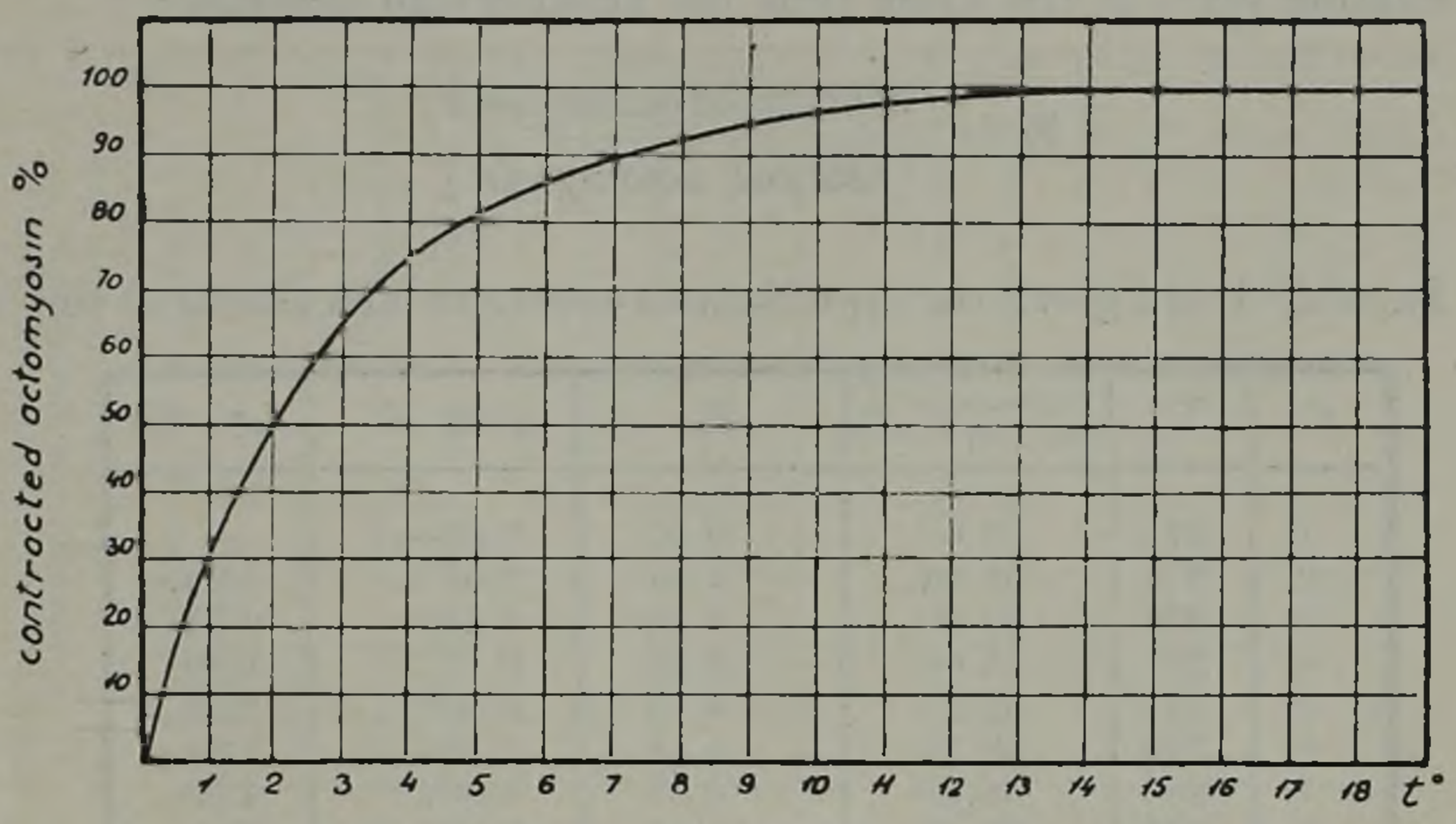

fig. 3.

late from figure 2. the % of contracted and uncontraced particles at any temperature. Fig. 3 shows the result of these calculations. In this Fig. the temperature is coordinated with the % of contracted particles. So, for

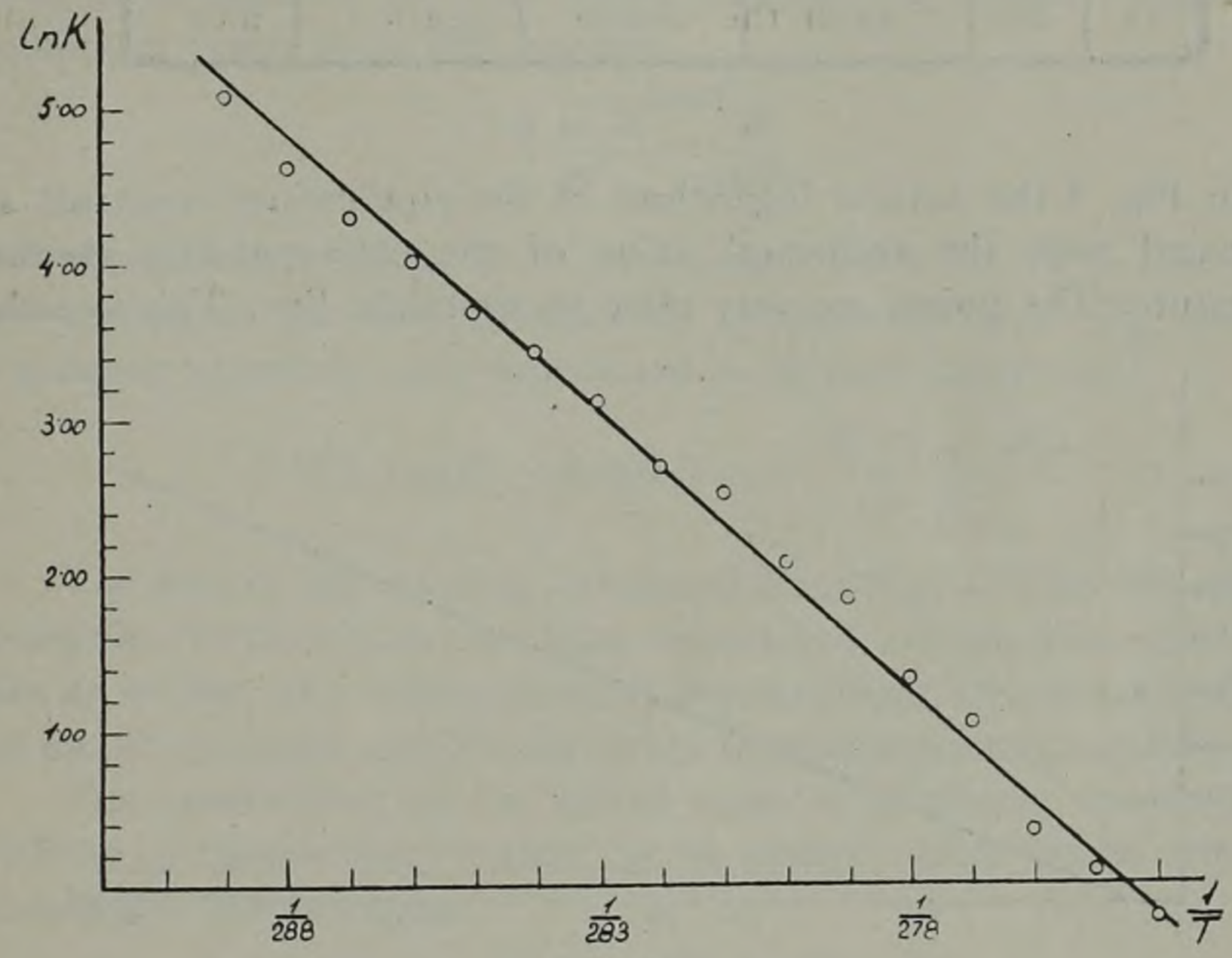

fig. 4.

instance, according to Fig. 2. at 5° the contraction was 55,7% of the total length. If we call the maximal contraction 100%, 100% of the actomyosin particles being contracted, then according to Fig. 3. at 5° 82% of the particles must have been in contracted and 18% in relaxed state. This relation gives at the same time the equilibrium constant:

$$K = \frac{[\,contracted\ actomyosin\,]}{[\,relaxed\ \ actomyosin\,]}$$

In table 1. are given the equilibrium constants calculated in this way.

t°	T°	Contract. in %, max (68) = 100	K.	Log. K.	Ln. K.
1	274	30.66	0.43	0.634—1	—
2	275	51.00	1.04	0.017	0.039
3	276	64.00	1.69	0.230	0.53
4	277	75.00	3.00	0.477	1.09
5	278	82.00	4.55	0.658	1.52
6	279	86.10	6.19	0.792	1.82
7	280	88.99	8.07	0.907	2.09
8	281	92.00	11.05	1.060	2.45
9	282	93.72	14.79	1.170	2.69
10	283	95.73	22.40	1.351	3.12
11	284	96.50	30.55	1.485	3.42
12	285	97.44	38.11	1.581	3.65
13	286	98.19	54.33	1.735	4.00
14	287	98.63	72.11	1.858	4.28
15	288	98.99	98.86	1.995	4.60
16	289	99.40	165.00	2.218	5.05

Table 1.

In Fig. 4 the natural logarithms of the equilibrium constants are coordinated with the reciprocal value of the corresponding absolute temperature. The points are very close to a straight line. This is rather

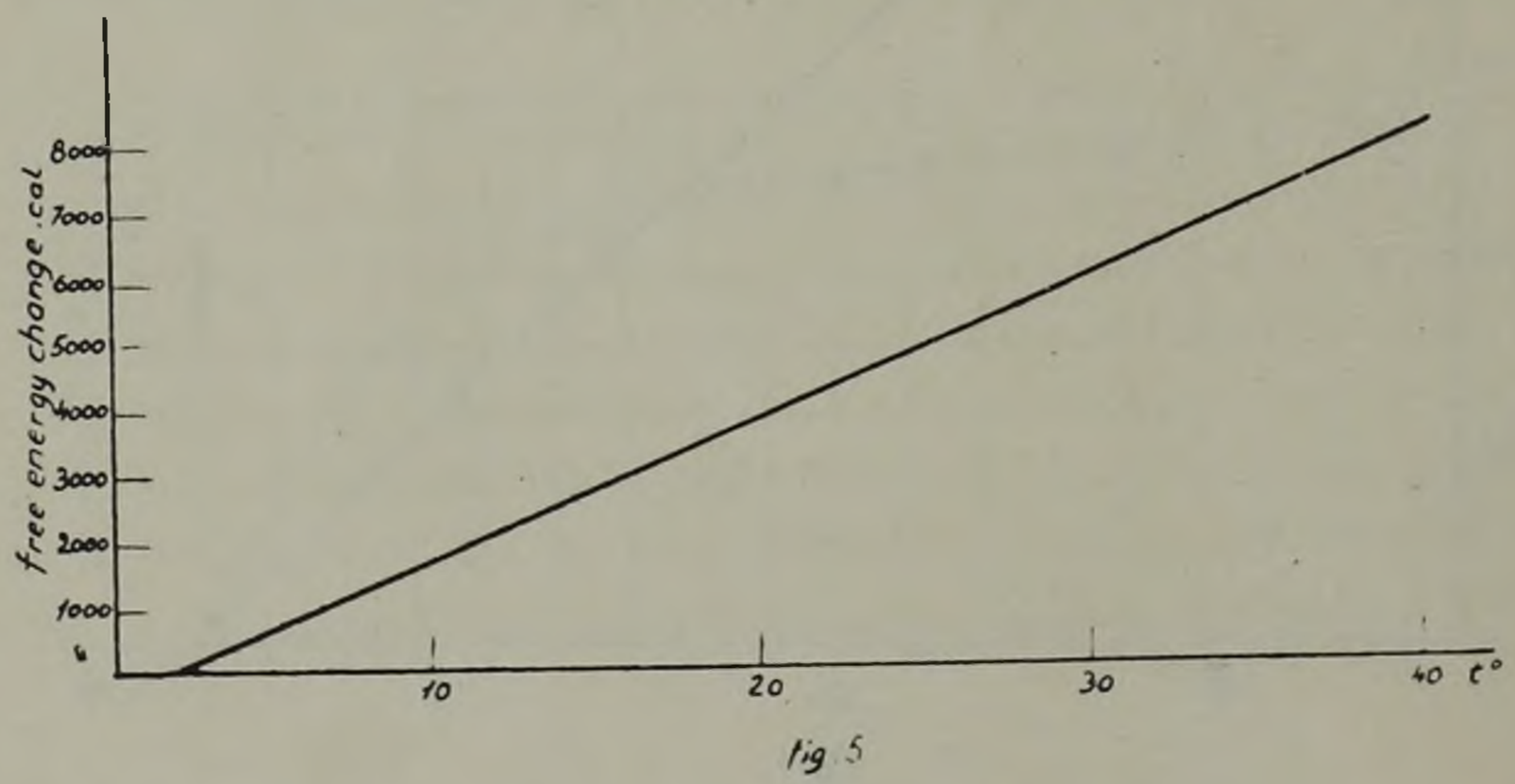

fig 5

important, because such a straight line could be obtained only if our supposition was correct, that conclusions can be drawn from contraction on the relative number of contracted particles. At the same time this involves that there is no partial contraction of single particles, they either contract

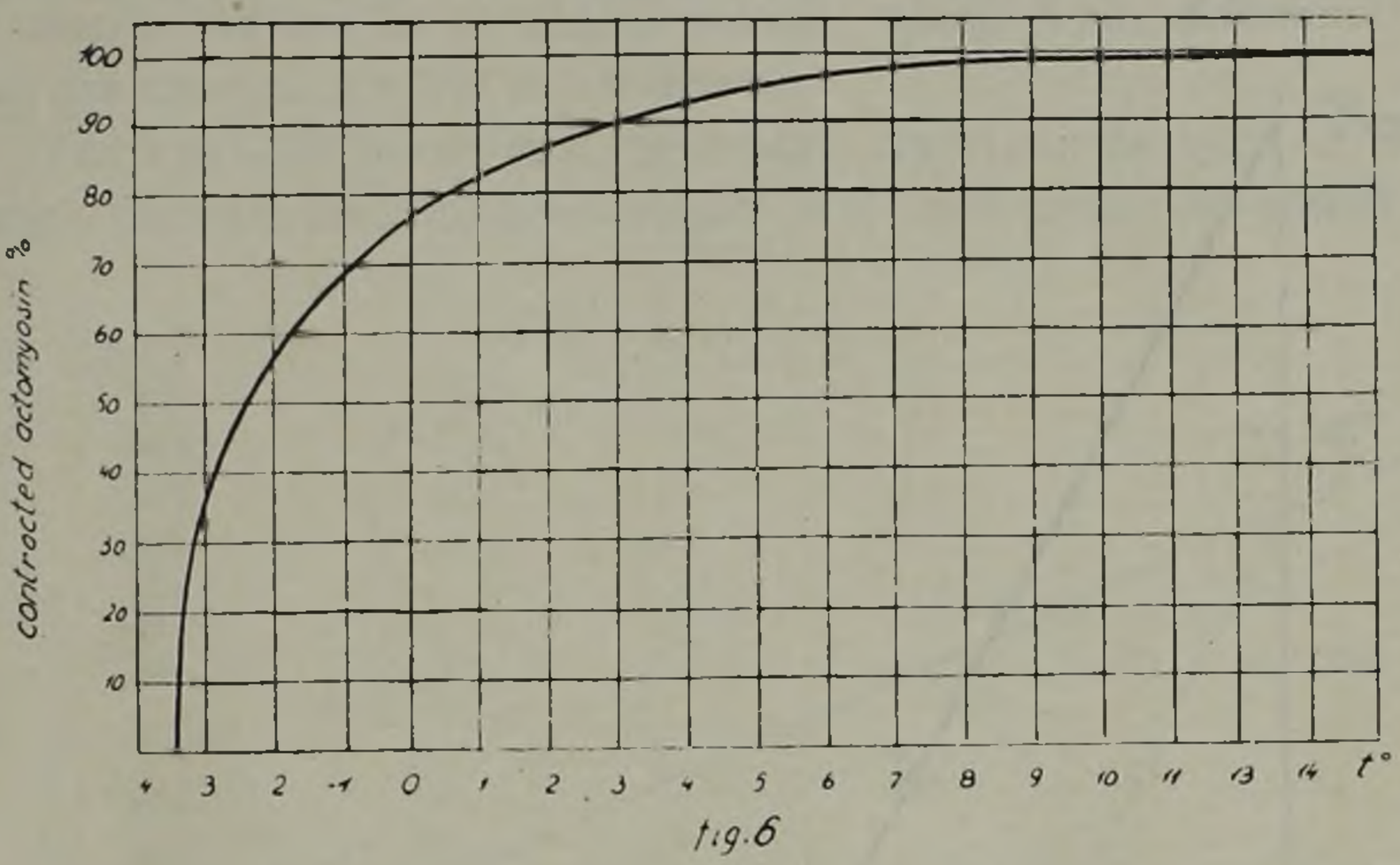

fig.6

or not, but if they contract they contract maximally; contraction and relaxation are two distinct states of the contractile substance.

The dependence of the the equilibrium constant on temperature allows us to calculate the heat of the reaction. Van't Hoff's equation of equilibrium reactions is well known:

$$\frac{d\ ln\ K}{dT} = \frac{W}{RT^2}$$

(W = heat of the reaction, R = gas constant 1,986 cal., T = abs. temp.)

Integrated for the difference of temperature $T_1 - T_2$, suiteably for numeric calculation and transferred to decimal logarithm:

$$2{\cdot}303\ (LogK_2 - LogK_1) = -\frac{W\ (T_2 - T_1)}{R\ .\ T_2 T_1}$$

The heat of the reaction, calculated from Fig. 3. is 56.000 cal. The actomyosin, while it goes over from the relaxed into the contracted state, takes up 56.000 cal., cooling down its surrounding. This value is close to that found by Anson and Mirsky for the reversible denaturation of trypsin.

The contraction can be looked upon as allotropic transformation and from its equilibrium constant the fre energy change can be calculated according to the equation:

$$\Delta F = -RT\ ln\ K$$

Fig. 5 shows the free energy change, thus calculated, at different temperatures, extrapolated till 40°. According to this figure the free energy change at 37°, is 7000 cal.

Frog. The foregoing measurements on rabbits muscle are based on the fact that the actomyosin of this animal does not contract at 0° but is

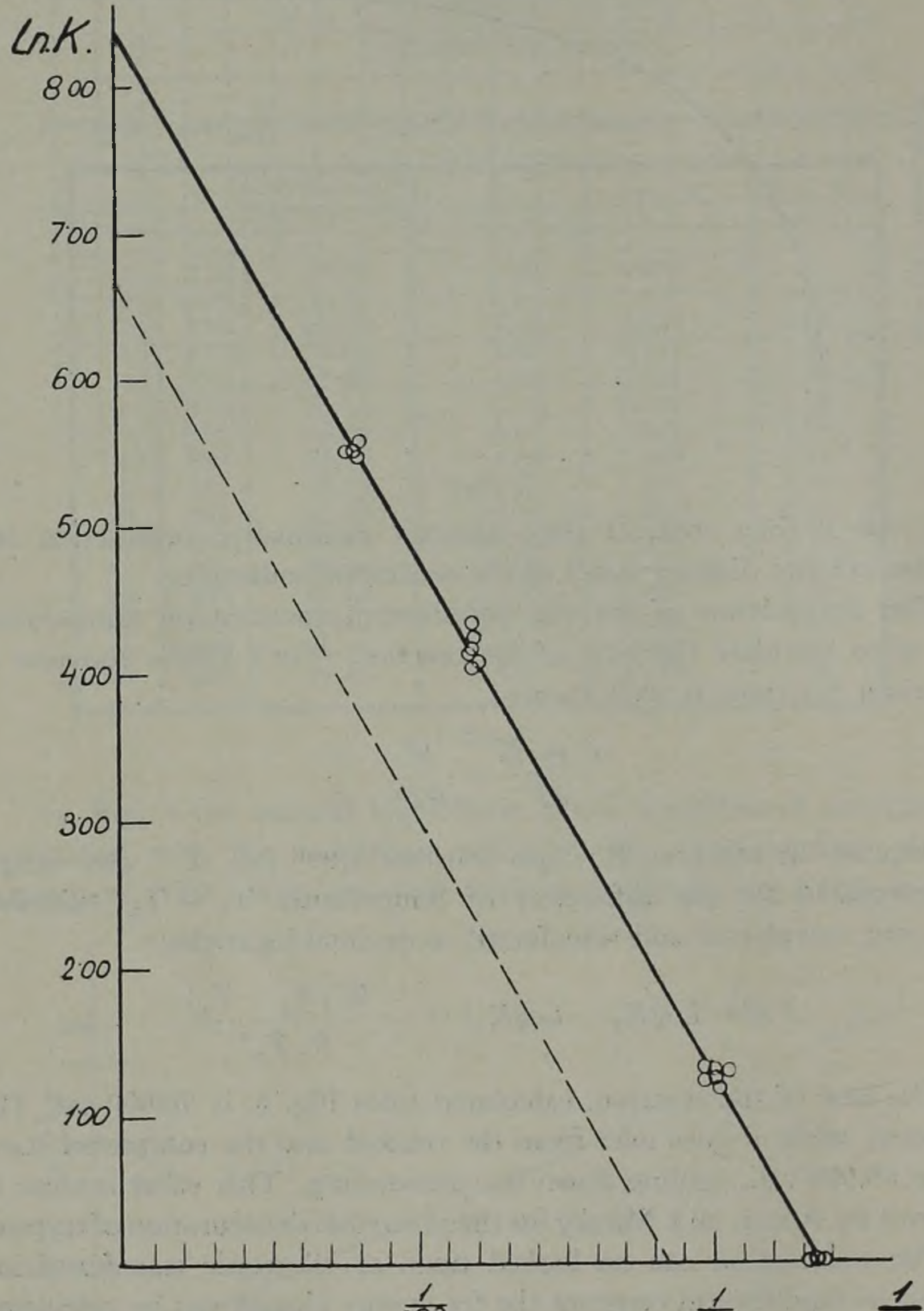

fig. 7.

capable of maximal contraction at 16° C. This, however, cannot be true for all animals, because certain cold-blooded species live under such conditions that they have still to move at 0°. So, for instance, the frog is capable of rapid movements, at this temperature, especially if its muscles are directly stimulated. It seemed interesting to know in what way the frog-muscle differed from rabbits muscle, which is the difference which allows the animal to move in ice-water.

The methods, used with the rabbit, could not be used in the case of frogs, because the muscles contract at 0° and cannot be cooled below

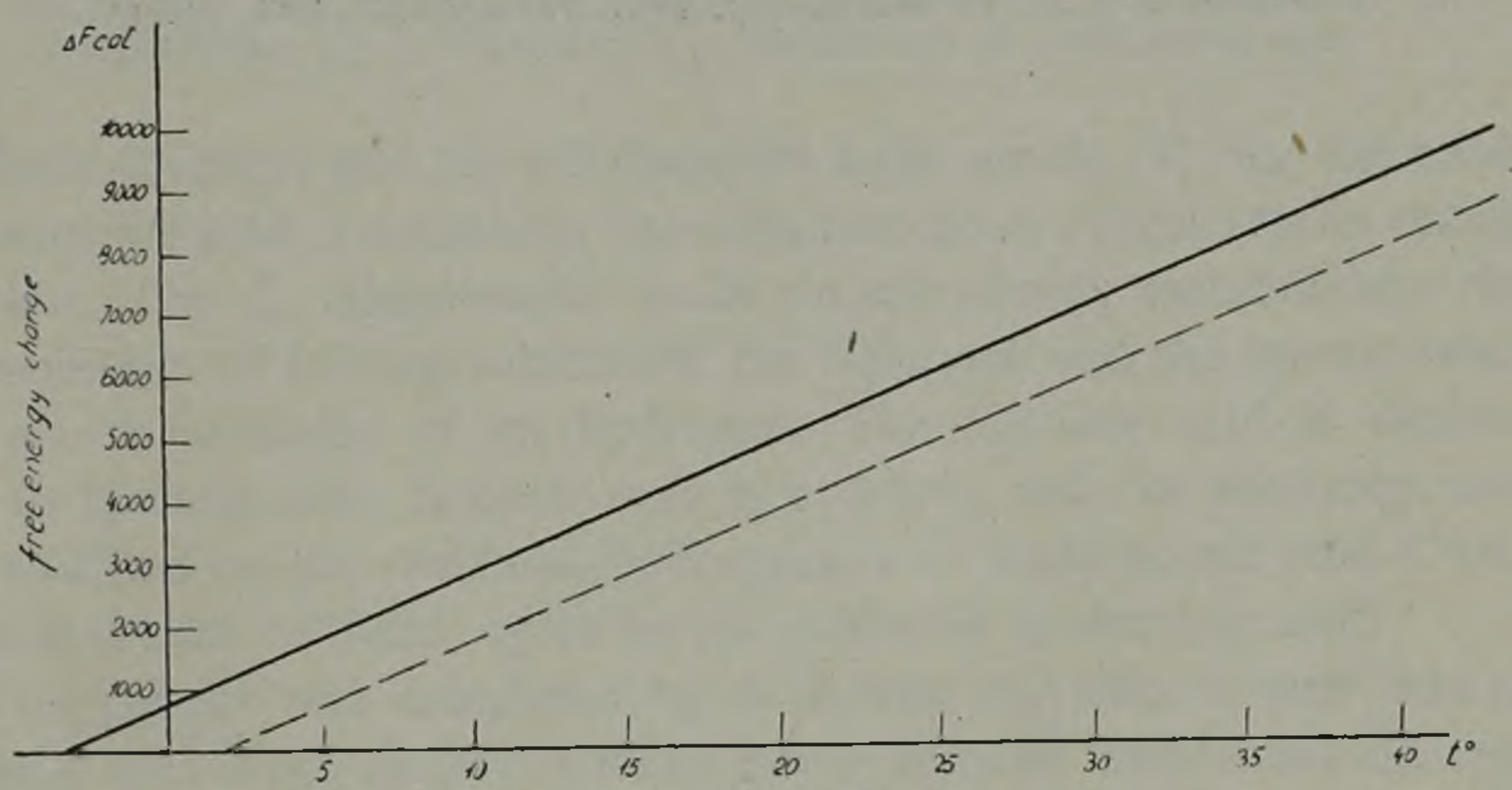

fig. 8

his temperature, because they freez. The method was thus modified in the folloing way: the sartorius of the decapitated animal was prepared free from the other surrounding muscles. Then it was frozen with dry ice. The frozen muscle was cut into narrow and thin strips, the lenght of which was measured. Then the strips, still frozen, were placed into distilled water of varied but constant temperature. On thawing the muscle contracts maximally under action of its own ATP. Maximal contraction was 70%, which was thus called „100% contraction". The calculation of the results was effected as described on the foregoing pages.

The result of these experiments is summed up in the figures 6, 7 and 8 which correspond to the figures 3, 4 and 5. The corresponding values for the rabbit are given in these figures with broken lines.

As will be seen there is no considerable difference in the heat of the reaction, which was found to be —53.500 cal in the frog as compared to the 56.000 cal in the rabbit. The difference between the two muscles lies in the difference in the free-energy change, which is bigger in frog, allowing the muscle to contract at 0° C. Extrapolated for 37° the free-

energy change was found to be 7000 cal in the rabbit, while in the frog it amounted to 8500 cal.

SUMMARY

The transition of relaxed into contracted actomyosin is an equilibrium-reaction. Equilibrium-constants were measured and the heat of the reaction and the free-energy change calculated for the rabbit and the frog.

LITERATURE

M. L. Anson and A. E. Mirsky, *J. gen. Physiol.* **17**, 393, 1934.

OBSERVATIONS ON THE OXIDATION OF SUCCINIC ACID

BY N. A. BIRÓ AND A. SZENT-GYÖRGYI JUN.

FROM THE BIOCHEMICAL INSTITUTE, UNIVERSITY OF BUDAPEST

(PRESENTED BY G. MANSFELD. — RECEIVED 18 DECEMBER 1945)

Szent-Györgyi and his collaborators have shown (1) that the oxidation of succinic acid is a catalytic function and plays a basic rôle in cellular respiration. The C_4 dicarboxylic acids are not merely metabolytes: they are transmitters of hydrogen between the substrate and the system which brings about oxidation of its hydrogen. The succinic acid is oxidised hereby to fumaric acid, fumaric acid is hydrated and the resulting malic acid is oxidised by the malico-dehydrogenase to oxalo-acetic acid. Oxalo-acetic acid is then reduced again by the substrate to succinic acid.

This picture was completed by A. Krebs (2), who showed that the oxaloacetic acid is not simply reduced but is coupled with acetic aldehyde to citric acid according to the reaction of Knoop and Martius (3). The citric acid is oxidised by the citric-oxidase to succinic acid. Lately H. Breusch (4) produced evidence showing that it is not only the acetic aldehyde, the product of triose-oxidation, which couples with oxaloacetic acid, but this acid is instrumental in the β oxidation of the higher fatty acids also.

As early as 1910 Batelli and Stern (5) have shown, that both oxidases, the succinic and citric oxidase are bound to the insoluble matter of the cell. A special name, „oxidon" was suggested to distinguish these catalysts from soluble enzymes.

The extensive research of this laboratory showed (6) that muscle fibril is built of two proteins, actin and myosin, which unite to a water-insoluble complex, the contractile actomyosin. No other insoluble proteins are known to take part in the building of the fibril, the insoluble matter of which is accounted for by the presence of actin and myosin. On the other hand, it is the fibril which makes the greatest part of the insoluble part of the cell. The question arose, what is the relation of the succinic- and citric-acid-oxidase to actomyosin? Is there any intimate relation and if so what is its meaning?

The present paper deals with the relation of the succinoxidase to actomyosin. It is known from the research of this laboratory that the physical state of actomyosin greatly depends on the nature and concentration of the ions and the ATP present (6). As first approach, the authors tried to answer the question whether the oxidation of succinic acid is influenced by ions and ATP in a similar way as the physical state of actomyosin. Such influence would show a close relation between actomyosin and succinoxidase, while the lack of such influence would definitely show the independence of these systems.

This problem has a very profound biological significance. Actomyosin, as contractile matter, is an energy-consuming system. Succinoxidase is an energy-producing system. This paper may thus be looked upon as an approach to the problem of the relation of energy-production and energy-consumption and the transference of chemical energy into mechanical work.

EXPERIMENTAL

Rabbit muscle was used as experimental material. The animal was decapitated, rapidly skinned, eviscerated and dipped in ice-cold water for a few minutes; then the muscles were cut out, packed in ice for a short while, then minced on a cooled mincer with a sieve plates with holes of 2 mm diameter. The brei was suspended in 20 vols of ice-cooled metal-free water. The suspension was stirred gently for ten minutes, filtered through a cloth, pressed out, then re-suspended and washed as at first. The washed muscle had 8,5—10% dry weight. The washed muscle was kept at 0° C; the respiration-experiments were performed 3—7 hours after the death of the animal. Experience of this laboratory shows that actomyosin is a very labile substance and its properties rapidly change on storage. The authors find that, contrary to the data of litterature, succinic acid-oxidase is a fairly labile system.

Respiration was measured in the Warburg respirometer. The final volume of the reaction-mixture was 4 ml. The muscle-brei was weighed on an analytical balance. The succinic acid (1,2 mg) was neutralised with KOH to pH 7. ATP was added as K-salt from the side-vessel after temperature equilibrium was established. Temp. 37° C. Readings were made every 10 Min.

In most experiments 300 mg. of muscle were used. At the smallest K-concentrations 150 mg. were used in order to reduce the quantity of the ATP and succinic acid and herewith the concentration of K. In calculating the K-concentration the K, used for the neutralisation of the succinate and the ATP, were taken into account. KCl was added to bring the K-concentration to the desired level.

RESULTS

The main results are summed up in the Fig. 1. The curves do not represent the actual readings: they sum up the result of different experiments. The actual readings are given in the tables. The curves could not be extended further towards the smaller K-concentration because the quantity of ATP and succinate — both present as K-salt —, made a further reduction of the K-concentration impossible.

The K-curve shows that succinic acid oxidation is limited to a rather narrow range of K-concentration, lying between 0—0,2 *M*. The

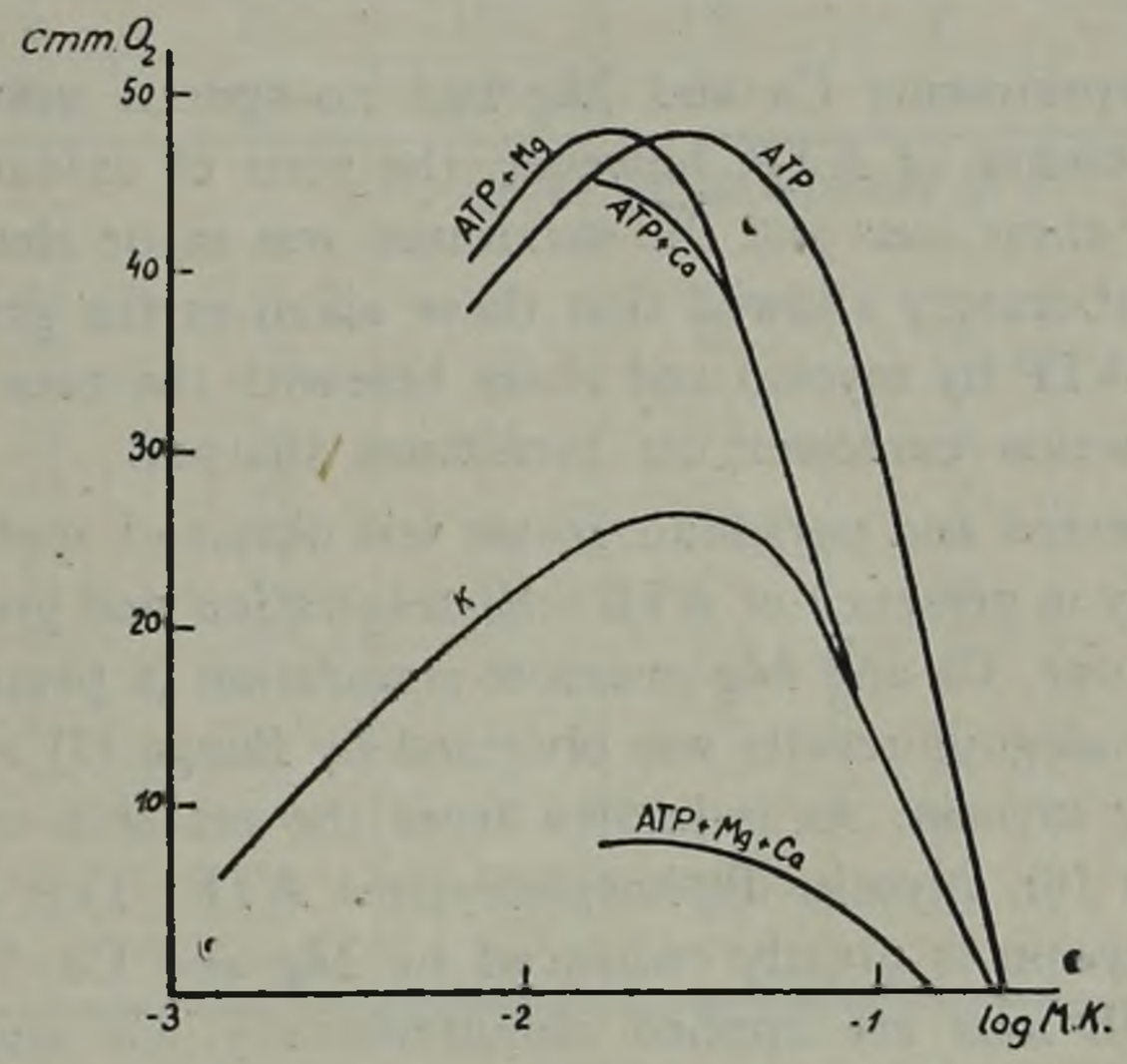

Fig. 1.

Oxygen uptake of 300 mg of washed muscle at varied K-concentration in absence and presence of 0,125% ATP, 0.005 *M* $MgCl_2$, 0,005 *M* $CaCl_2$, 1,2 mg succinate. Abscissa : log *M* K present. Ordinate : cmm 0_2 absorbed.

oxidation has a maximum at 0,02—0,05 *M*, which coincides with the maximum of the precipitation of myosin and actomyosin by K. As shown in this laboratory, myosin precipitates maximally in presence of 0,025 *M*, actomyosin in presence of 0,05 *M* KCl (6).

This dependence of succinic acid oxidation on the K-concentration is rather striking. The question presents itself, why it has not been observed before? The probable reason is this: the colloidal effects of K on myosin and actomyosin are not specific and are duplicated by other alkali ions, like Na. In respiration experiments, succinate was used mostly as K or Na-salt, and this way the alkali metal ions, necessary for the

oxidation process, were introduced. As solvent mostly „distilled water" was used which gave the impression that the presence of salt is dispensable.

The curve, marked with „ATP" shows that in presence of this nucleotide the respiration is greatly increased. Experiments of this laboratory showed, that ATP very greatly increases the salt-precipitation of actomyosin (6) and causes contraction. It could be thought the ATP did not activate the oxidation of succinic acid in our experiment, but some different system. The experiments, however, showed that, in absence of succinate, ATP in itself, did not increase the respiration of our washed muscle.

In our experiments Ca and Mg had no special influence on the K-curve. In presence of ATP, however, the zone of oxidation was narrowed down by these ions and the maximum was made sharper. Experiments of this laboratory showed that these alkali-earths greatly increase the binding of ATP by myosin and make herewith the zone of precipitation and contraction narrower, its maximum sharper.

An unexpected and paradoxic result was obtained applying Mg and Ca simultaneosly in presence of ATP: the respiration was greatly reduced, though, one by one, Ca and Mg promote respiration in presence of ATP. A thoroughly analogous results was obtained by Banga (7) with the splitting of ATP by myosin. As is known from the research of Engelhardt and Ljubimowa (8), myosin dephosphorylates ATP. This phosphatase-action of actomyosin is greatly enhanced by Mg and Ca, but is greatly inhibited if both ions are applied simultaneously. Ca and Mg, given simultaneously, inhibit respiration in absence of ATP too, but the respiration, in this case being less intense, the effect is less marked.

All these results show that there is a very close analogy in the behaviour of myosin, actomyosin and succinic-acid oxidation. This suggests

TABLE I.

O_2-uptake in presence of different KCl-concentrations.

A) 150 mg. muscle-brei, 0,3 mg succinate.

M K	0_2-uptake in 30 minutes in μl	
	I.	II.
0,0012	9	2
0,0025	20	8
0,0050	21	14
0,0100	21	12

that succinic oxidase and actomyosin, the contractile substance, are closely connected, if not identical. Naturally, succinoxidase is not a substance but a system of substances. Whether all members of this system are subject to the above regularity, remains to be shown.

B) 600, respectively 300 mg muscle, 1,2 mg succinate.

	I.	II.	III.	IV.	V.	VI.	VII.
mg muscle	600	600	300	300	300	300	300
M K	0_2-uptake in 30 minutes in μl						
0,005	35	—	—	—	—	—	—
0,010	43	—	—	16	—	—	—
0,015	50	—	—	—	40	23	35
0,020	50	—	—	—	—	—	—
0,025	58	36	32	15	34	23	34
0,030	45	40	—	—	—	—	—
0,035	69	39	—	—	—	—	—
0,040	51	38	26	—	26	22	39
0,045	—	—	29	—	—	—	—
0,050	—	—	23	—	—	—	27
0,060	—	—	—	—	—	—	32
0,065	—	—	—	—	29	26	—
0,075	—	—	18	—	—	—	—
0,100	—	—	—	6	17	—	20
0,150	—	—	3	—	—	—	7
0,200	—	—	—	0	—	—	0

TABLE II.

Effect of simultaneous action of 0,005 M $MgCl_2$ and 0,005 M $CaCl_2$ on the 0_2-uptake at varied KCl-concentration.

300 mg muscle-brei, 1,2 mg succinate.

M K	I.		II.	
	—	Ca+Mg	—	Ca+Mg
	0_2-uptake in 30 minutes in μl			
0,016	13	8	24	14
0,025	21	8	37	12
0,040	14	7	23	14
0,065	17	0	20	9
0,100	6	1	20	15

TABLE III.

Effect of ATP on respiration at varied KCl-concentration.

(The numbers with indices relate to 150 mg muscle. In these experiments these smaller quantities of muscle were used in order to enable us to use smaller quantities of ATP and succinate and establish, herewith, lower K-concentrations. The result were multiplied by two, thus calculated for 300 mg muscle.)

300 mg muscle-brei, 1,2 mg succinate.

M K	I.		II.		III.		IV.		V.	
	—	5 mg ATP	—	5 mg ATP	—	5 mg ATP	—	5 mg ATP	—	5 mg ATP
0,007	6[1]	9[1]	—	—	—	—	—	—	—	13[2]
0,0125	16	32	—	—	—	—	—	—	—	19[2]
0,016	—	—	13	29	23	45	35	40	—	—
0,025	15	23	24	28	23	43	34	59	11[3]	25[3]
0,040	—	—	14	28	22	42	39	68	—	—
0,050	—	22	—	—	—	—	27	44	—	—
0,065	—	—	—	—	20	37	32	67	—	—
0,100	6	11	12	38	—	—	20	33	—	—
0,150	—	—	—	—	—	—	7	14	—	—
0,200	0	6	—	—	—	—	0	4	—	—
	0_2-uptake in 30 minutes in μl									

TABLE IV.

Effect of 0,005 M Mg in presence of 5 mg ATP a varied KCl concentration.

300 mg muscle-brei, 1,2 mg succinate.

M K	I.			II.			III.		
	—	ATP	Mg+ ATP	—	ATP	Mg+ ATP	—	ATP	Mg+ ATP
	0_2-uptake in 30 minutes in μl								
0,006	—	—	24[1]	—	—	—	—	—	—
0,012	18[1]	—	34[1]	—	—	—	—	—	—
0,016	—	—	—	23	45	58	35	40	53
0,025	—	—	—	23	43	42	34	59	54
0,040	—	—	—	22	42	41	39	68	51
0,065	—	—	—	26	41	18	32	67	42
0,100	—	—	—	—	—	—	20	33	19
0,150	—	—	—	—	—	—	7	14	10
0,200	—	—	—	—	—	—	0	4	7

[1] 150 mg muscle-brei, 2,5 mg ATP, 0,3 mg succinate, value calculated for 300 mg.

[1] 150 mg muscle-brei, 2,5 mg ATP.
[2] 150 mg muscle-brei, 0,3 mg succinate.
[3] 150 mg muscle-brei, 5 mg ATP, 0,3 mg succinate.

TABLE V.

Effect of 0,005 M $CaCl_2$ in presence of 5 mg ATP at varied KCl concentration.
300 mg muscle-brei, 1,2 mg succinate.

M K	—	ATP	Ca + ATP
	0_2-uptake in 30 minutes in μl		
0,016	35	40	43
0,025	34	59	48
0,040	39	69	39
0,050	27	44	38
0,065	32	67	37
0,150	7	14	19
0,200	0	4	1

TABLE VI.

Simultaneous action of 0,005 M $MgCl_2$ and 0,005 M $CaCl_2$ in presence of 5 mg ATP at varied KCl-concentration.

300 mg muscle-brei, 1,2 mg succinate.

M K	I.		II.	
	ATP	Ca+Mg +ATP	ATP	Ca+Mg +ATP
	0_2-uptake in 30 minutes in μl			
0,016	29	5	52	22
0,025	24	8	58	18
0,040	14	3	44	15
0,065	22	0	31	15
0,100	—	—	17	6

SUMMARY

The activity of the succinoxidase is greatly dependent on the presence of ions and ATP. The oxidase is most active under conditions in which the contractile matter is maximally precipitated or contracted.

LITTERATURE

1. A. Szent-Györgyi: *Nobel-Vortrag.* Stockholm, (1938).
2. H. A. Krebs and W. A. Johnson: *Enzymologia* **4.** 148 (1937).
3. F. Knoop and C. Martius: *Z. physiol. Chemie* **242,** 104 (1937).
4. F. L. Breusch: *Science* **97.** 491, (1937).
5. K. Batelli and L. Stern: *Soc. Biol.* **74.** 212, (1913).
6. A. Szent-Györgyi: *Studies on Muscle,* Suppl. XXV. of the *Acta Physiologica Scandinavica.* Vol. **9.** *1945.*
7. I. Banga: *Studies from the Institute of Medical Chemistry,* University Szeged. **3,** 1943.

OBSERVATIONS ON MUSCLE FIBRES

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Mammalian muscle or actomyosin does not contract at 0° C, so if muscle is minced at this temperature a brei is obtained consisting of uncontracted fibres. If this brei is suspended in distilled water of 0° and then brought to room-temperature the fibres remain uncontracted, as shown by Th. Erdős (1). If, however, the fibres are suspended in a dilute KCl-solution, say in 0,02—0,10 *M* KCl, the fibres contract energetically as the fluid warms up. Erdős obtained contraction up to 0,2 *M*. At 0,25 *M* or higher concentration there was no contraction at all, except on addition of ATP, in which case contraction was obtained up to 0,4 *M* KCl.

Repeating these experiments the author used a mincer with a sieve plate with holes of 2 mm diameter. The rabbit was decapitated, quickly skinned, eviscerated, dipped for a few minutes into ice-water. Then the muscles were cut out, preference being given to the more fascia-free deep muscles of the back. The muscles were packed for a few minutes in ice and then minced in a cooled mincer. The brei was suspended in 0° KCl solutions of different concentration. The suspension was kept at 0° for five minutes and then brought to room temperature; the contraction was observed under the microscope. The result was the following: from 0—0,02 *M* KCl there is no contraction. From 0,02—0,05 there is an intermediary zone in which the results are not quite uniform. Not all fibres contract and contraction reaches varying degrees. From 0,05—0,14 *M* KCl contraction is maximal. All fibres contract to $^1/_3$—$^1/_5$ of their original length. Between 0,16—0,2 again there is an intermediary zone and from 0,20 upwards there is no contraction at all. There seems to be a turning-point at 0,16 *M*, the partial contraction above this limit being due to the imperfection of the technique.

The concentration 0,16 *M* is of especial interest for the physiologist since it is the concentration of „physiological saline". Mammalian tissues

are in osmotic equilibrium with this fluid. Inside the cell or fibre there must be thus a solution of similar concentration. Accordingly, it was found in the experiment that our muscle fibres did not contract in Ringer's solution, but contracted if this latter was diluted with no more than 10% water, which shows that this physiological saline is just beyond the contracting limit. We can thus draw the conclusion that the contractile system is built in such a way that at the ionic concentrations of the fibre it is just in relaxed condition, and a slight dilution of the medium suffices to elicit maximal contraction. If the Ringer is diluted with 0,16 *M* KCl instead of with water, there is no contraction. The slight and irregular contraction, which can be observed in undiluted Ringer, is due to the imperfection of the method and shows how close we are to the contracting level.

Addition of 0,002 *M* $MgCl_2$ or 0,005 *M* $CaCl_2$ to the KCl-solutions does not alter appreciably the above results with KCl.

Evidently, the contraction of fibres in 0,05—0,14 *M* KCl is due to the action of the ATP present. The freshly minced muscle contains this substance in rather high concentration. 1 g of freshly minced rabbit-muscle contains 2,5 mg ATP. The contraction-limit of this ATP-actomyosin system is thus just below the ionic concentration of the fibre, at which concentration there is no contraction; a slight dilution is sufficient to bring the system below the limit and herewith into the contracted state.

A rather striking and unexpected effect is obtained if ATP is added to the saline solution. In this case maximal contraction is obtained up to 0,45 *M* KCl. At 0,48 *M* the contraction becomes less regular and above 0,5 *M* there is no contraction. At this concentration, 0,5 *M*, the actomyosin system disintegrates and the myosin dissolves. For ATP, added from without, the contraction limit lies thus at 0,48 *M*, while for the ATP present the limit is 0,16 *M*.

The cause of this difference could be sought in a difference of the actomyosin, in the difference of the ATP or in the modifying action of the accompanying substances. The first possibility could be excluded, because the actomyosin is the same in both cases. Inquiry about modifying action of accompanying substances remained negative and so it had to be supposed that there is a difference between the ATP present in the resting muscle and the ATP added from without and obtained by preparation.

It lay at hand to suppose that ATP is present in muscle in an inactive form which being very labile, transforms during preparation into the active modification. The author tried thus to extract inactive ATP, applying very careful methods of exctraction. The methods were the following:

1. The freshly minced muscle was precipitated with alcohol, quickly treated with acetone and aether, rapidly dried and quickly extracted with 0° distilled water.

2. The freshly minced muscle was rapidly coagulated by heat and the juice used as „ATP-solution".

3. The freshly minced muscle was extracted for 15 minutes with equal amounts of distilled water at 0° for 15 minutes, squeezed out and the juice employed.

All these experiments gave a uniform result: the solutions elicited maximal contraction of muscle fibres up to 0,45 *M* KCl. The author was thus unable to extract inactive ATP.

It seemed desirable to obtain information about the concentration of ATP which is necessary to elicit contraction. To see the response of actomyosin to ATP, the freshly minced muscle was suspended in 20 vol. of 0° distilled water, stirred gently for ten minutes, filtered through a cloth, the muscle residue pressed out, suspended once more and separated again. This „washed muscle" does not contract spontaneously, but contracts if suspended in dilute KCl and ATP is added, as shown by Th. Erdős (see 1). The experiment shows that maximal contraction is obtained up to 0,45 *M* KCl and that at least 0,1 mg ATP per ml is needed to obtain vigorous contraction.

That washing did not materially alter the reaction of the actomyosin to the ATP is shown by the fact, that unwashed, but unexcitable muscle behaved in the same way. In these experiments the leg of the freshly killed rabbit was cut off and kept at room temperature still it did not respond to electrical stimulation. As a rule, this took 5—6 hours. As soon as the muscles became unexcitable the leg was cooled in ice, the muscles cut out, minced and their reactions to KCl and ATP observed in the way described. Here, too, contraction was obtained up to 0,45 *M* KCl and it was found that 0,1 mg ATP per ml is necessary to elicit it. This limit of 0,1 mg is in agreement with the extensive experience of this laboratory with actomyosin suspensions *in vitro*. It has been shown that ATP is bound by myosin and that a certain degree of ATP-saturation of the actomyosin is necessary to obtain energetic contraction. The necessary saturation is reached if the actomyosin is in equilibrium with an ATP-solution containing 0,1 mg ATP per ml.

A very striking and unexpected result was obtained if the same experiments were repeated with excitable muscle. The mince was suspended in 0,25—0,3 *M* KCl (in which there is no spontaneous contraction). Then ATP was added in varying concentration. It was found that 0,0005 mg, thus half a γ per ml was sufficient to elicit maximal contraction.

This result cannot be explained by a direct action of the ATP added, its concentration being 200 times lower than is necessary to bring the actomyosin of the fibre into contraction. The result can be explained only by supposing that by the ATP added the ATP, present in the muscle in high concentration, is brought into action.

It seemed interesting to repeat some of these experiments with frog-muscle for the following reason. Mammalian muscle is in osmotic equilibrium with 0,9% NaCl or 0,155 *M* KCl. This is just the contracting limit, as has been shown on the previous pages. Frog-muscle is in equilibrium with 0,6% NaCl. If the experiments reported have any bearing on the physiology of muscle and the close proximity of the ionic concentration of physiological saline and the contraction-limit are not a mere coincidence, then we have to expect that the contraction limit in the frog will be at 0,6% NaCl or 0,1 *M* KCl.

Unfortunately, the methods used with rabbit-muscle could not be used without any modification because frog muscle contracts at 0° C and is very excitable. The author succeeded in a few cases to arrive at results in the following way; the fresh and uncontracted muscle was carefully cooled to about —3° C. Then, by means of needles, fibres were isolated and placed into KCl solutions cooled to 0° or below. A few minutes later the fluid was gently warmed and contraction observed under the microscope. It was found to be at 0,1 *M*. The results were less sharp as with rabbits but still definite.

The author also tried to perfuse the frogs leg with Ringer and diluted Ringer. In this case the osmotic equilibria are established much slower and the muscle has more time to adapt to new conditions. It was found that if the frog-Ringer was diluted with 20% of water and the muscle stimulated with single induction-shocks, the twitch was prolonged and the curves obtained had a flat top revealing a tendency of the muscle to go over into contracture.

The last series of experiments, the author wants to report, relates to the action of poisons, known to cause contracture of muscle. The results, briefly summed up, are the following: if the freshly minced and excitable rabbits-muscle is suspended in a 0,25 *M* KCl and physostigmine is added in a concentration of $1 : 10^7$, or veratrin in $1 : 10^6$, quinine or caffeine in $1 : 10^4$, maximal contraction is obtained. Acetylcholine and nicotine were inactive. On unexcitable muscle all the poisons were found to be inactive. It may thus be concluded that the poisons do not act directly on the actomyosin, causing immediately its contraction, but they activate, in some way, ATP present and put herewith in action the normal apparatus of excitation.

SUMMARY

At the ionic concentration of muscle the ATP present is unable to cause contraction. It causes contraction if the ionic concentration is decreased to a small extent ($10^0/_0$). Very small amounts of ATP (0,5 γ per ml) suffice to elicite contraction. Since these quantities of ATP are too small to cause contraction by themselves, it must be supposed that they act by activating the ATP, present in muscle in inactive condition. Physostigmine, veratrine, quinine and caffeine cause contraction in isolated fibres by activating the ATP and putting in action herewith the normal apparatus of excitation.

Post scripta. After this paper has been closed down a letter came to the author's notice, written by Fr. Buchthal, A. Deutsch and G. S. Knappeis to the Editor of *Nature* (**153**, 774, 1944). In this letter the writers state to have obtained twitch-like contraction in isolated muscle fibres on addition of very small amounts of ATP. They conclude that the ATP, in resting muscle, must be present in an inactive modification or must be separated from the contractile matter.

LITERATURE

A. Szent-Györgyi: *Studies on Muscle,* Suppl. XXV of the *Acta Physiologica Scandinavica,* 9,—1945.

ON THE FIXATION OF ATP BY METAL-MYOSINATES

BY V. SZ. HERMANN

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I. Banga has studied the adsorption of ATP by myosin (1) and showed that myosin is capable of binding ATP. One of her two curves is reproduced in the curve 1 of Fig. 1. In this Fig. the abscissa shows the quantity of ATP present, the ordinate the number of moles of ATP adsorbed by a unit-weight (UW) of myosin. As UW 17,600 g was taken irregardless whether such units actually exist or not. It can be seen that the quantity of ATP adsorbed increases proportionately to the concentration of ATP. Banga also noted that KCl enhanced the adsorption though at this point no reproducible results were obtained. At that time the great lability of myosin was not known yet.

It was the object of this present research to repeat and extend Banga's experiments with possibly fresh material. Banga used highly purified myosin, free of actin and recrystallised twice. Her experiments showed that actin did not alter the adsorptive properties of myosin towards ATP or metals. This experience permitted to use less pure but fresher material, obtained by simpler and faster methods of preparation. The author thus traded in purity for freshness and dispensed with recrystallisation. The myosin was crystallised (1), washed with dilute KCl, dialysed over night and then subjected to experiment. This way the experiment could be performed 24 hours after the death of the animal as compared to the 48 hours in the experiments of Banga.

5 ml of the dialysed myosin, containing 100 mg protein, was pipetted into 20 ml of ATP solution of varying concentration. Temp. 0° C. The mixture was agitated for two minutes and then 80 ml of methyl-alcohol was added, the precipitating myosin separated, treated and analysed as described by Banga. Special experiments showed that at room-temperature the adsorption was but slightly intenser. Higher temperatures had to be avoided with regard to the great lability of the protein and its strong enzymic activity.

The result of such an experiment is reproduced in the curve 2 of Fig. 1. As the curve shows, a straight line was obtained, similarly to Banga. The two curves run almost parallel, have thus the same gradient, the only difference being that the curve 2 is higher, i. e. the quantity of ATP bound bigger. In presence of 0,2 *M* KCl (curve 3 Fig. 1) the curve is still higher but retains its shape and gradient.

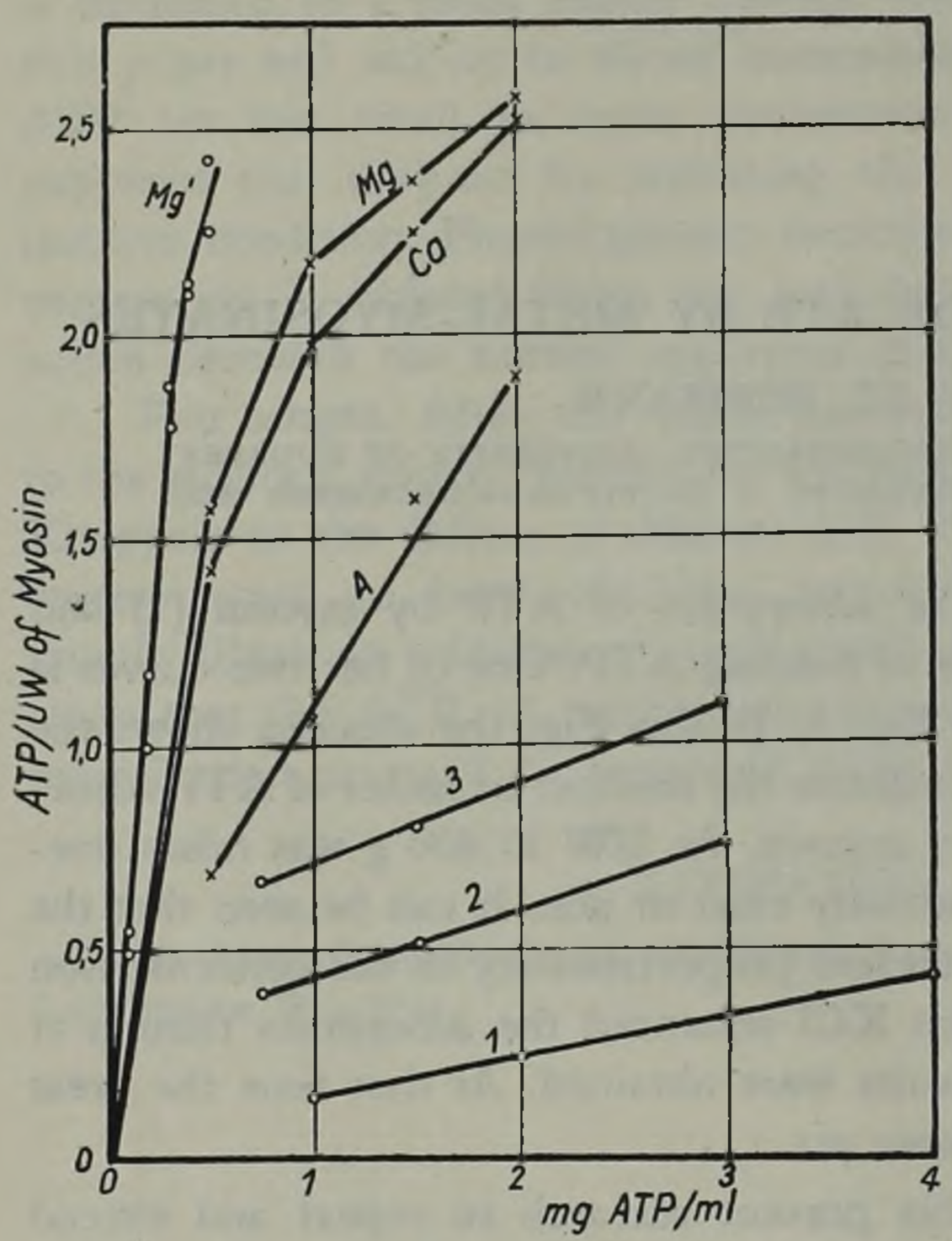

Fig. 1.

This experiment was extended and the ATP-adsorption was measured in presence of 0,17% ATP at varying KCl-concentration. The result is reproduced in the upper curve of Fig. 2. In the lower curve is given the quantity of adsorbed K (in equivalent weights), as calculated from the results of Banga. This lower curve relates to the right-hand-side ordinate. Unfortunately the ATP-adsorption could not be measured at the lowest K-concentrations because ATP itself is a K-salt. Its K-concentration was taken into account.

As can be seen, the two curves of Fig. 2 run parallel which means that the quantity of ATP adsorbed is a function of the adsorbed K. This makes it probable that in entire absence of K myosin would not bind ATP at all, which conclusion was supported by later experiments (see below). This result can easily be understood, the metal-free myosin (at neutral reaction) and the ATP both being anions.

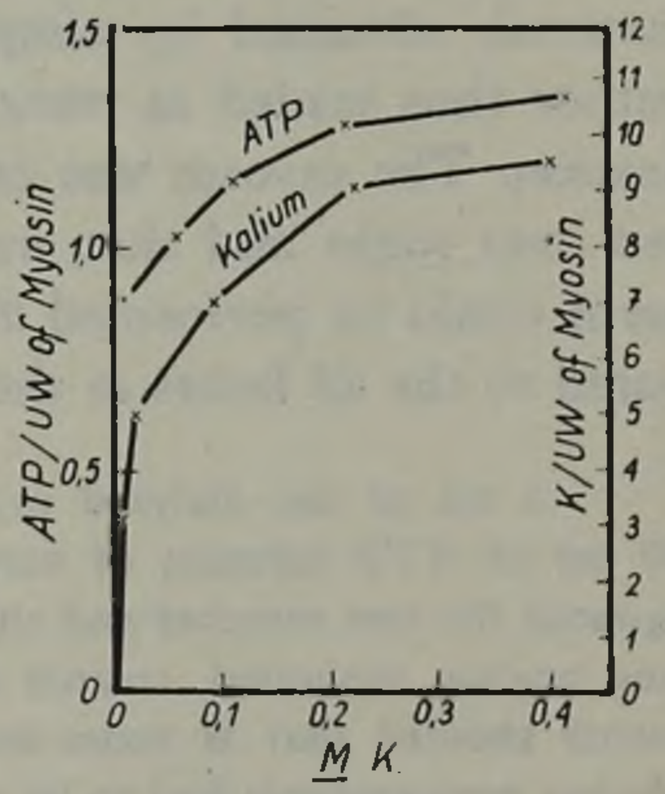

Fig. 2.

All these curves of ATP-adsorption were unsatisfactory for one reason. Experience of

this laboratory showed that ATP, in muscle, is completely bound to myosin, which conclusion is very strongly supported by Rózsa's preceding paper. Our adsorption-curves did not explain such a strong fixation: they were too low and too flat. This suggested that our myosin might have been damaged during the cours of preparation, the method employed being still too slow. For this reason the author tried to go one step further in trading in purity for freshness, simplifying and shortening her method.

The muscle was extracted with 0,6 *M* KCl for 10 Min. at 0° C, 3 ml of KCl being used for every g of muscle. Then, for every 3 ml of salt 12 ml of ice-cold water were added, the suspension filtered through a cloth and then diluted further with 18 ml of water. The precipitating myosin was separated on the centrifuge, suspended in cold 0,025 *M* KCl, centrifuged, dissolved in 0,5 *M* KCl. The myosin-content was estimated and quantities of the solution, containing 50 mg protein, were pipetted into 40 ml of a 0° solution of ATP and KCl. The mixture was stirred gently for two minutes, 4 vols. of methyl-alcohol added and the precipitating myosin treated as above.

This method of preparation is not only very fast, allowing to use the myosin two hours after the death of the animal; the method is careful too, because the myosin was most of the time in contact with ATP which stabilizes it. Moreover the myosin was in touch with KCl all the time. In salt-free condition myosin is more labile.

The ATP-adsorption of such a myosin preparation is reproduced in the curve 4 of Fig. 1. 0,2 *M* KCl was present in all samples. As can be seen the curve is still linear but is very steep.

The question arose whether this very strong ATP adsorption was due to a greater binding power for K, which, in a secondary way, induced a higher ATP adsorption, or else did the careful treatment of the protein increase directly its ATP-fixation. To decide this question the whole extraction of myosin was omitted and the minced muscle was simply washed, being suspended for ten minutes in 20 vols. of 0° distilled water. The washed mince was then suspended in KCl-solutions of varied concentration for twenty- minutes at 0° and then the bound K estimated according to the procedure of Banga (1). In this way the muscle was subjected to experiment half an hour after the death of the animal. The K-fixation was of the same order as that found by Banga, supposing that $^1/_3$ of the dry weight of the muscle washed was myosin.

This shows that the very high ATP-fixation in the curve 4 of Fig. 1 was not due to a correspondingly high K-adsorption. The two qualities, the K- and ATP-fixation-power of myosin are two more or less independent qualities. The latter is much more labile and the K-fixation suffers considerably later as the ATP-fixation. A very fresh and carefully treated

myosin is capable of binding considerably more ATP for the same amount of K adsorbed, than a somewhat damaged protein. The K-fixation drops on storage at 0° and pH 7 considerably in 48 hours; the ATP-fixation is damaged within a few hours.

Though this curve 4 of Fig. 1 was very much steeper than the previous ones, it was still unsatisfactory. In search of improvement, the experiment was repeated in presence of Ca and Mg, all samples containing besides KCl 0,001 *M* $CaCl_2$ or $MgCl_2$. The results are given in the two curves „Ca" and „Mg" of Fig. 1. „Mg" gives the result of a similar experiment with Mg, the curve itself giving the average between two parallels.

The curves show that the bivalent ions make the curve very much steeper, the adsorption much intenser. They also change the shape of the

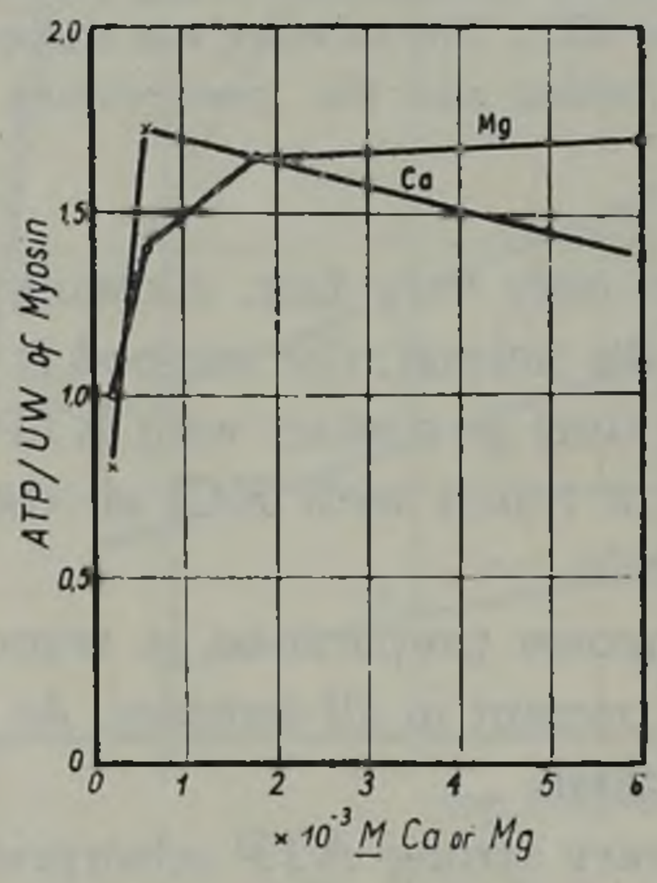

Fig. 3.

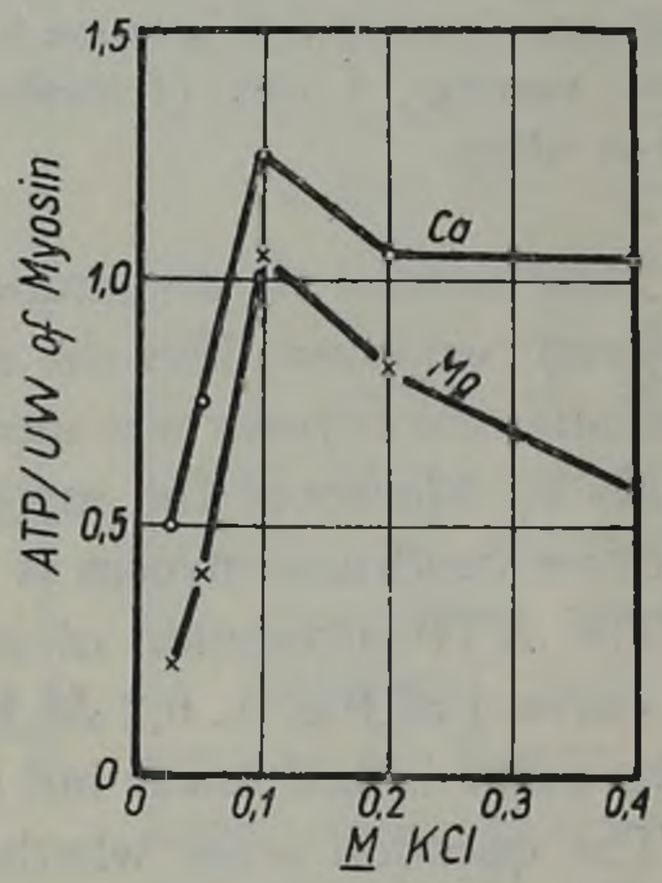

Fig. 4.

curve from straight to hyperbolic. The question arose, whether this greatly increased adsorption was due to the additive action of K and Ca or Mg or had the alkali-earths such an action by themselves, or else did alkali and alkali-earths sensitise the protein for their mutual action? To decide this question the following two experiments were performed: in the first experiment the KCl-concentration was kept constant at 0,2 *M* and the Ca and Mg-concentration was varied, while in the second experiment the Ca and Mg-concentration was kept constant, the total concentration being 0,0005 *M*, and the KCl was varied. The ATP-concentration was constant in both experiments, being 0,017% in the first and 0,019% in the second. The result of the first experiment is reproduced in the Fig. 3 and shows that maximum of adsorption is reached at a rather low concentration of the bivalent ions. The result of the second experiment is reproduced in Fig. 4 and shows that maximum of adsorption is reached

at a rather low K-concentration, 0,1 *M*; the curve, extrapolated towards 0, shows that in absence of K there is no ATP-adsorption at all. At the low ATP-concentrations employed, the Ca and Mg-myosinate, formed in presence of 0,0005 *M* Ca or Mg and containing probably 4 equivalents of Ca or 3 equivalents of Mg, does not bind the nucleotide at all. Ca and Mg, however, very greatly sensitise the system towards the action of K, which causes a very intense adsorption of the nucleotide by these Ca- or Mg-myosinates. The effect of both ions, Ca and Mg, were similar, that of Mg being somewhat weaker, corresponding to its somewhat weaker adsorption by myosin.

In Fig. 5 the same experiment is repeated more in detail. 0,0005 *M* $CaCl_2$ was added to all samples and 0,02% ATP. Part of the ATP was

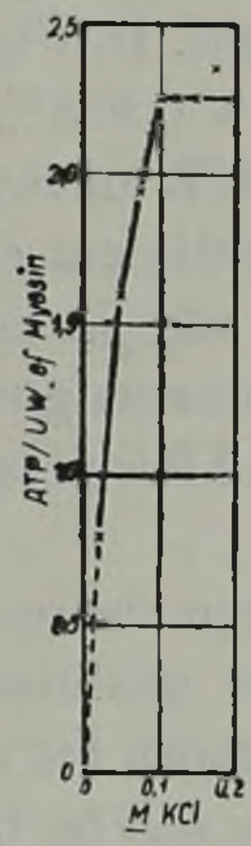

Fig. 5.

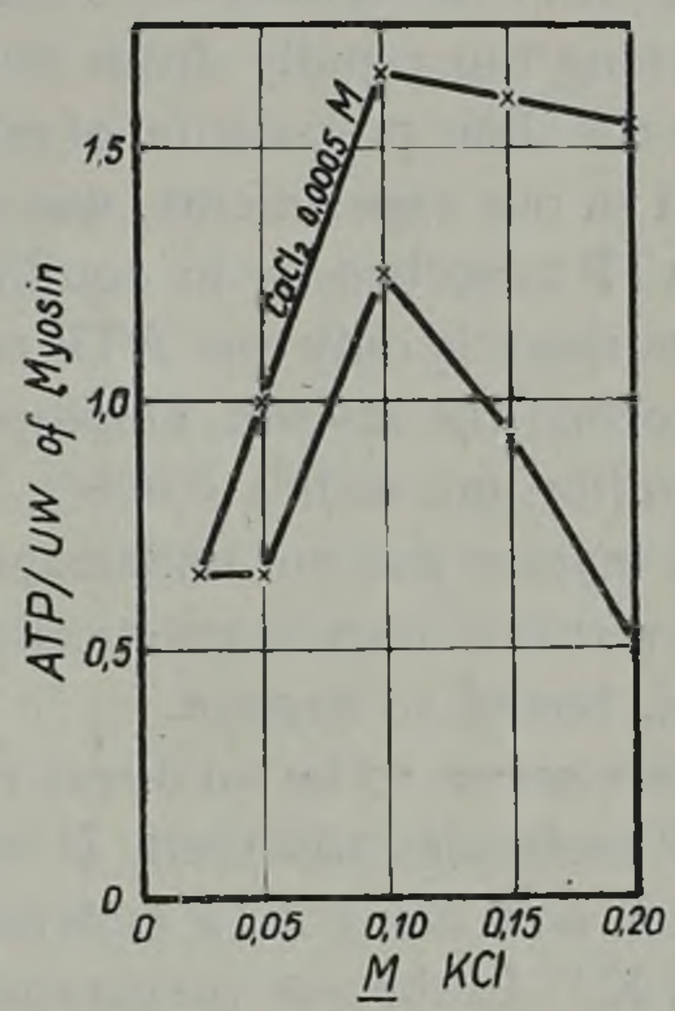

Fig. 6.

adsorbed so its actual concentration was still lower. As the curve shows, the adsorption is exceedingly strong and the curve rises rapidly with increasing K-concentration, to reach maximum with a sharp break at 0,1 *M* K. If the curve is extrapolated towards 0 K (broken line), it cuts the ordinate at 0 showing that in entire absence of K there would be no adsorption at all. The adsorbed Ca in itself, causes no ATP-adsorption at all. A similar experiment is reproduced in Fig. 6. Here, side by side, the experiment was performed with and without Ca. In both cases a maximum is reached at 0,1 *M* K. The maximum, in presence of Ca, is considerably higher and is stable, the values remaining practically unchanged with a further increase of the K-concentration. In absence of Ca the maximum is equally at 0,1 *M* K, showing that this maximum is not conditio-

ned by the Ca but must be explained by some special relation of the myosin and the K.

In all experiments (Fig. 4, 5, 6) the maximum is reached with a sharp break at 0,1 *M* K. This result is most remarkable in the light of the chemical composition of muscle. M. Dubuisson (2) has shown that cross-striated muscle contains 0,105 *M* K, calculated for 77% of water. A small part of this K (about 0,02 *M*) must be adsorbed to myosin, so that the actual concentration must be somewhat lower, but the fibre contains also some Na, and, as shown by Erdős, Na acts in the same way as K. So taking everything together the concentration of alkali ions must be about 0,1 *M*, at which concentration the adsorption maximum is just reached. Any dilution of the ionic milieu must entail thus a liberation of adsorbed ATP.

The ATP-adsorption in a fresh myosin preparation is thus exceedingly strong but rapidly drops on storage. There are reasons to believe that even the short preparation of myosin is not indifferent and the myosin, employed in our experiments, was not undamaged. In Fig. 5 the myosin, with 2 ATP adsorbed, is in equilibrium whith a 0,015% ATP solution. In muscle there is only one ATP molecule per UW of myosin. With one ATP adsorbed the myosin, employed in the experiment of Fig. 5, would be in equilibrium with a 0,008% ATP. If we take in account that our extracted myosin was not undamaged, the experiments give a full support to the view that in muscle practically the whole ATP is present in adsorbed condition, bound to myosin.

There seems to be no direct relation between the number of K-ions and ATP molecules adsorbed. It depends on the qualities of the myosin preparation how many ATP molecules it will adsorb for a given number of bound K^+. In the one preparation two ATP-s will be bound for every four K^+, while in the other but one. It may be concluded herefrom that the single K ions do not take part individually in the fixation of the ATP but set up a more or less diffuse positive electric field and it will depend on the adsorptive qualities of the preparation how much ATP it is capable of binding in this field. Naturally, we must suppose that the difference in the adsorptive qualities of our preparations is due only to the more or less grave damage done to them during preparation and that *in vivo* the adsorptive power, under normal conditions, is always maximal.

SUMMARY

ATP is very strongly bound by fresh myosin. The ATP adsorption very rapidly declines on storage and is greatly dependent on the (K^+). Ca and Mg in themselves induce, at low ATP-concentration, no adsorption but greatly sensitise the myosin towards the action of K. In absence of K there is no ATP-adsorption under conditions of these experiments.

LITERATURE

1. A. Szent-Györgyi. *Acta physiologica Scandinavica*, 9, 1945, Suppl. XXV.
2. M. Dubuisson. *Arch. Internat. Physiol.* 52, 439, 1942.

ON CONDITIONS IN RESTING MUSCLE AND THE NATURE OF EXITATION

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(RECEIVED 18 DECEMBER 1945)

The series of foregoing papers from the Biochemical Institute Budapest is the continuation of research on muscle, begun at Szeged and discontinued nearly two years ago. This research has been resumed lately and its first results are presented in this volume. In the light of these newer results some of the earlier views have to be reconsidered.

In our earlier papers (1) the view was held that resting muscle contains its actomyosin in dissociated form; more exactly, it contains no actomyosin at all but actin and myosin, side by side. This view was based mainly on the experience that actomyosin, in presence of ATP, cannot exist in relaxed form: it either contracts or dissociates. The paper by Varga fully supports this view. Since muscle contains ATP in a rather big concentration Varga's paper seems to exclude the presence of actomyosin in resting muscle. At the same time, however, Rózsa's paper shows that ATP, in muscle, is present in an inactive state. If ATP, in muscle, is inactive, then our conclusion about the state of actomyosin loses its validity and it might be as well that muscle contains undissociated and uncontracted actomyosin. In the authors opinion Rózsa's results directly prove that actomyosin, in muscle, is undissociated. Rózsa showed that the addition of a very small amount of ATP causes the contraction of muscle fibres. If actomyosin had been present in dissociated condition it could not have contracted or else we would have to suppose that ATP causes association first, which is contrary to the extensive experience of this laboratory.

In his earlier paper (1) the author held the view that actomyosin, in resting muscle, must be dissociated, also because actomyosin, at the concentration in which it is present in muscle, must form a very stiff gel, the stiffness of which is incompatible with the softness of muscle. He did not take into consideration the division of this

colloidal mass into very thin threads, fibrils, nor the possible hydrating (softening) action of the ATP present.

The great mechanical strenghth of muscle, its great resistance to stretching and tearing also suggests the presence of actomyosin. Both actin and myosin have a very low and normal viscosity and do not in themselves, form elastic gels. The high viscosity and viscosity-anomaly of actomyosin may explain the strength of muscle. Viscostity begins to rise strongly in an anomal way above 0,2%. Actomyosin is present in the fibril in about 33% concentration. These conclusions, however, loose much of their validity by the fact that the myosin, as we know it in its extracted state, and the myosin, as it is present in muscle, are different substances. The actin-compound of myosin dissociates and dissolves *in vitro* above 0,1 *M* KCl if ATP and a small concentration of Mg is present. The muscle, however, contracts up to 0,45 *M* KCl, as shown by Erdős and Rózsa. There are thus forces in muscle, holding the myosin particles together, which are not present *in vitro* in the extracted myosin, and a high KCl-concentration is needed to break them. These links maintain the structure of the fibril, a structure no more present in the extracted myosin.

If we suppose that muscle contains inactive ATP and admit herewith the possibility of its containing undissociated but uncontracted actomyosin, then we have to reconsider another point.

Banga (1) has shown that the phosphatase-activity of dissociated actomyosin (free myosin) is greatly inhibited, that of undissociated actomyosin is greatly enhanced by Mg. Muscle contains considerable amounts of Mg. Believing, at that time, that resting muscle contains dissociated, contracted muscle undissociated actomyosin, it was thought that the observation of Banga explained the fact that resting muscle does not split ATP, while contracted muscle does.

Conditions in the test tube and in the muscle fibre differ in several points. For the *in vitro* conditions the experience still holds that, in presence of ATP, actomyosin is either contracted or dissociated. Thus whenever Banga had undissociated actomyosin, she always had contracted actomyosin (ATP being always present in her experiments). Her observation could thus be formulated equally well by saying that the phosphatase-activity of uncontracted actomyosin is inhibited, that of contracted actomyosin is enhanced by Mg. This way the action of Mg may still account for the low phosphatase activity of resting muscle and for the high phosphatase activity of muscle in contraction.

The next question, which presents itself is that about the nature of this „inactive ATP" of resting muscle and the mechanism of its inactivation. The comparision of the papers of Rózsa and Hermann throws some light on this point.

Resting muscle is in osmotic equilibrium with „physiological" saline, 0,9% or 0,155 *M* NaCl, or Ringer's solution. Dubuissons (2) analysis shows that in this state muscle contains 0,105 *M* K (as calculated

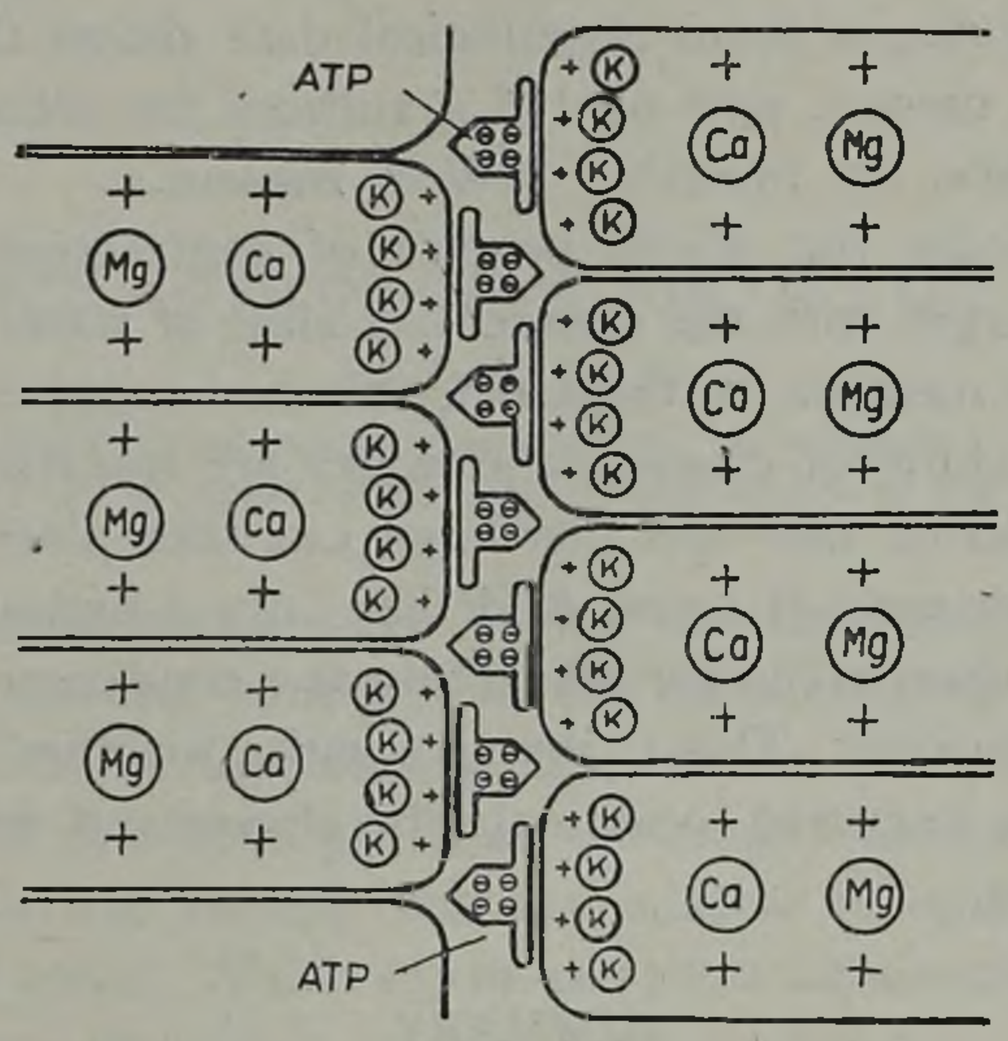
for 77% water). It is difficult to make quite exact statements about the actual concentration of K, because a small part (about 0,02 *M*) of the K present must be adsorbed to myosin. But on the other hand, muscle contains also some Na and this acts (as shown by Erdős), as if more K would be present. Taking everything together the actual concentration must be about 0,1 *M*. According to the results of Rózsa, at this ionic concentration ATP is inactive, while Hermann has shown that at this $[K^+]$ ATP, in presence of Ca and Mg, is maximally adsorbed to myosin. Rózsa has shown, that ATP becomes active if the ionic concentration is decreased while Hermann has shown, that ATP is liberated, if the ionic concentrations are lowered. Inactive ATP means thus adsorbed ATP, activation of ATP means release of ATP. Whether adsorbed ATP is inactive because it is transformed into an inactive modification, stable in the adsorbed state only, or adsorbed ATP is inactive simply because is it is held adsorbed in such a way that its active pyrophosphate group is not in touch with the protein, we do not know. The latter assumption is the simpler but at the moment it is equally possible that the ATP is activated by its release because it is trasformed into an active modification, or that it is activated simply because the liberated ATP molecules have a random distribution and may hit the protein with their active group.

We may try now to picture a myosin-particle in muscle. Muscle conatins 8% of myosin (H. H. Weeber, 3). If we calculate 17,600 g as unit-weight (UW), 8% is 0,005 *M*. Muscle contains, according to the experience of this laboratory, 0,25% round 0,005 *M* ATP. This ATP, as shown by Hermann, is adsorbed to myosin. It can hardly be a coincidence that there is just 1 molecule of ATP for every unit of myosin while experience shows that actomyosin reaches maximal reactivity if its ATP-saturation is such that one ATP is bound per UW of myosin.

According to Dubuisson muscle contains round 0,005 *M* Ca. As shown by Banga this is very strongly bound by myosin, so every UW of myosin will contain 1 Ca. Muscle contains also Mg, which is adsorbed also fairly strongly by myosin. According to Dubuisson muscle contains round 0,01 *M* Mg. If every UW of myosin binds one Mg, this leaves 0,005 *M* in solution. According to the measurements of Banga we have to expect that a myosin, containing one Ca and one Mg will be in equilibrium with a 0,005 *M* Mg-solution.

According to the results of Banga, if myosin is brought in touch with salts, the five first equvalent of metal will be bound very strongly. They will, we may say, built in deeper into the protein structure as the subsequent ones. The binding of bivalent ions is especially strong, that of K considerably looser. The Ca and Mg, bound by the myosin in muscle,

are thus fixed strongly and neutralise the original negative charge of the protein. The Ca-Mg-myosinate will bind K; according to the curves of Banga, three or four K will be bound in presence of 0,1 *M* K. These K-ions will be bound more superficially and held more loosely and



will readily be given off on dilution. As shown by Hermann, the Ca-Mg-myosinate is enabled to bind ATP by the K adsorbed. In the resting muscle the positive charges of the adsorbed K will be instrumental in binding the one ATP molecule with its negative charges. Fig. 1 may symbolize these conditions. The drawing-pins at the end of the myosin-unit symbolize ATP molecules with their negatively charged active groups pointing away from the protein into the small amount of intermicellar water present. The flat head of the drawing-pins correspond to the purine configuration.

Though such pictures must always be taken with a grain of salt, this picture makes it easy to immagine how an electric shock may cause contraction in such a system. A suddenly applied electric field may tear off a few K^+, which will entail the sudden release of ATP molecules. The released ATP molecules, hitting the myosin particles with their active group, will cause its discharge and herewith dehydration which, on its turn, entails contraction (1). Naturally, the contraction of a few myosin units will not lead to visible contraction, but it is easy to see how this contraction may become self-propagating. Banga has shown that if the actomyosin particle contracts it gives off its water and not the ions adsorbed. The liberation of hydrate-water will cause a sudden dilution of the small quantity of intermicellar water present. (The intermicellar

water in the fibril occupies only about $^1/_3$ of the total volume.) As shown by Hermann and Rózsa, even a 10% dilution of the ions present suffices to cause liberation and herewith activation of ATP. Relaxation may be the reverse process: if one of the micels resumes its positive charge, takes up hydrate water and the process starts in the opposite direction. Approximate calculation from physilogical data shows that the splitting of one ATP per myosin unit of 10^6 g suffices for relaxation. 10^6 g is, according to Weber, the probable MW of myosin.

In this picture the whole process of contraction and relaxation is the play of charges with the consecutive shift of water. The protein is more or less but a medium for these reactions and supplies the mechanism by whicht these shifts of charges and water are translated into motion.

It is remarkable that this discharge can take place in presence of actin only. In absence of actin, ATP has only a hydrating effect, thus only increase the charge, the particle acting as a condenser charged negatively as well as positively. This is the case with „inactive" adsorbed ATP also, which can be expected to increase the charge and herewith the hydration of myosin.

SUMMARY

The structure of myosin in resting muscle is discussed and a theory of excitation is given.

LITERATURE

1. A. Szent-Györgyi, Suppl. XXV, *Acta Physiol. Scand.* 9, 1945.
2. M. Dubuisson, *Arch. Internat. Physiol.* 52, 439, 1942.
3. H. H. Weber, *Naturwiss.* 27, 33, 1939.

THE COMBINED ACTION OF K AND Na ON ACTOMYOSIN

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Banga' s experiments have shown that the adsorption of Mg and Ca on myosin does not sum up. If, for instance, from a 0,012 *M* Mg-solution, or a 0,006 *M* Ca-solution, myosin adsorps 5—5 equivalents per unit weight (UW = 17,600 g), from a solution containing 0,012 *M* Mg and 0,006 *M* Ca, the myosin will not adsorb 10 equivalents, but only 5 equivalents of metal. Which of the metals is adsorbed depends on their relative affinity to myosin.

It seemed desirable to know how K and Na behave in this respect. Having no dependable method for the estimation of Na, the author has tried to answer this question in an indirect way. As shown in this laboratory, in presence of ATP, actomyosin either contracts or dissociates, depending on the salt-concentration. So, for instance, the actomyosin, used in this experiment, prepared from actin and myosin, still contracted in presence of 0,16 *M* KCl and dissociated in 0,17 *M* KCl. If, in the above experiment, KCl was replaced by NaCl, the same limits were obtained, 0,16 *M* for contraction and 0,17 *M* for dissociation. As shown by Banga, this effect, contraction or dissociation, is a function of the quantity of K adsorbed by the myosin. Na has thus the same affinity to myosin as K. If, instead of NaCl or KCl the isomolar mixture of both was used, the limit of contraction and dissociation remained unchanged, as calculated for the total molarity. The addition of NaCl to a KCl solution has thus the same effect as if more KCl had been added and the actions sum up; the myosin seems not to distinguish beetween K and Na.

Ca and Mg, though being very similar in their chemical reactions, have, in certain respects, an antagonistic action on actomyosin. As has been mentioned, the actomyosin, employed in these experiments, dissociated in presence of 0,06% ATP at 0,17 *M* KCl or above, while it contracted at 0,16 *M* or below. If 0.0005 *M* $MgCl_2$ was added, the limit was lowered

to 0,12—0,13 *M* KCl. On the other hand $CaCl_2$, added in 0.0005 *M* concentration, raised the limit to 0,20—0,22 *M*. If both alkali-earths were added simultaneously, the limit went down to 0,12—0,13 *M* as if no Ca had been present at all. Possibly the antagonistic effect of these ions in Melzers narcosis can be explained by some analogous activity of both ions.

A szerkesztésért Mansfeld Géza, a kiadásért Szent-Györgyi Albert felelős.

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