ON THE REVERSIBILITY OF THE BIOCHEMICAL REACTIONS OF MUSCULAR CONTRACTION DURING THE ABSORPTION OF NEGATIVE WORK*

Hans G.MANNHERZ**
Institut für Zellphysiologie, Ruhr-Universität Bochum, Germany

Received 11 September 1970

1. Introduction

Muscle produces mechanical work by splitting ATP into ADP and P_i, i.e. at the expense of chemical energy. The consumption of chemical energy and the production of mechanical work are located closely together on the so-called cross-bridge, the junction of two proteins: myosin and actin. These proteins fulfil simultaneously structural and enzymological functions. Enzymologically, the actomyosin catalyses a biochemical reaction leading to a steady state of mechanical work out-put and consumption of chemical energy. The question therefore arises whether mechanical work absorbed by the muscle may cause the biochemical reactions to proceed in reverse.

This investigation was stimulated by results obtained some time ago when glycerinated fibre bundles of insect flight muscle (as well as from rabbit psoas muscle) were sinusoidally stretched and released at temperatures ranging from 1 to 6°. Under these conditions, a suppression of the ATP-splitting rate during negative work was observed [6], whereas at temperatures around 20° the ATP-splitting rate was raised proportionally to the positive work performed by the same fibre bundle [9, 10]. Similar effects of stretch on the ATP-splitting rate or the rate of heat production of live muscle had been reported by Davis, Aubert, Abbott and Hubert, and Maréchal [4, 2, 1, 7].

2. Materials and methods

Giant tropical water bugs (*Lethocerus maximus*) were kindly supplied by Dr. F.Bennett (Trinidad). The dorsal longitudinal muscle was dissected, extracted in glycerol and stored for several months before use as described previously [10]. Bundles of about 4 to 7 fibres were glued to the shaft of a RCA 5734 force transducer valve and to the arm of a Goodman VP 47 vibrator driven by a Hewlett Packard 330A function generator in order to impose sinusoidal stretch and release on the muscle fibre. Length and tension signals were displayed on a 502A Tectronic Dual Beam oscilloscope, as originally developed in Pringle's Laboratory [8].

The incubation solution contained (mM): 10 Na azide, 5 $MgCl_2$, 1 to 2 ATP, 20 tris-maleate-buffer pH 6.9, 30 KCl, 4 Ca EGTA (pCa about 5). This standard incubation solution was supplemented with varying amounts of ^{32}P-orthophosphate. In order to detect ATP-^{32}P, formed 10 mM glucose and a small amount of crystalline hexokinase were added during incubation. Aliquots of 20 or 50 µl were taken at given intervals and separated by paper chromatography according to Bandurski [3]. The glucose-6-phosphate was located on the paper and the whole chromatogram was cut into strips of 1.5 cm and counted in a toluene cocktail with a Beckman Ls 100 Liquid Scintillation Spectrometer.

** Present address: Max-Planck-Institut für medizinische Forschung, Abteilung Biophysik, 69 Heidelberg, Jahnstr. 29, Germany.

3. Results and discussion

Fig. 1a,b illustrates an experiment with a fibre bundle of 6 fibres which was sinusoidally stretched and released with a frequency of 1 cps and with an amplitude of 2% L_0 at 9°, thus imposing negative work on the fibre. The graphs give cpm of ^{32}P along the developed paper chromatogram. The first peak represents orthophosphate (given in 10^3 cpm), the second the cpm ^{32}P found in glucose-6-phosphate, the position of which is marked. Aliquots were taken after 0, 30, 150, 300 min and 20 hr incubation and from a control solution incubated under the same conditions but without a fibre bundle.

Fig. 1a,b shows a time-dependent increase of ^{32}P incorporation in glucose-6-phosphate. After 20 hr incubation, the ^{32}P incorporation in glucose-6-phosphate reaches 0.016% of the total ^{32}P-orthophosphate in solution. The amount of the added ^{32}P-labelled orthophosphate was 100×10^{-9} moles. Calculated for standard fibre length (1 cm) and a single fibre, this gives a ^{32}P incorporation into glucose-6-phosphate of 90×10^{-15} mole per minute. From experiments under similar conditions it is known that the ATP-splitting rate is suppressed from about 45×10^{-12} mole/cm fibre/min under isometric contraction down to about 33×10^{-12} mole/cm fibre/min, when a similar amount of work is absorbed. 90×10^{-15} mole/cm fibre/min $^{32}P_i$-exchange with ATP would amount to a net $^{32}P_i$-exchange with ATP of about 0.75% of the ATP saved by the suppression of the ATP-splitting rate. This figure certainly is the lower limit of the total $^{32}P_i$-ex-

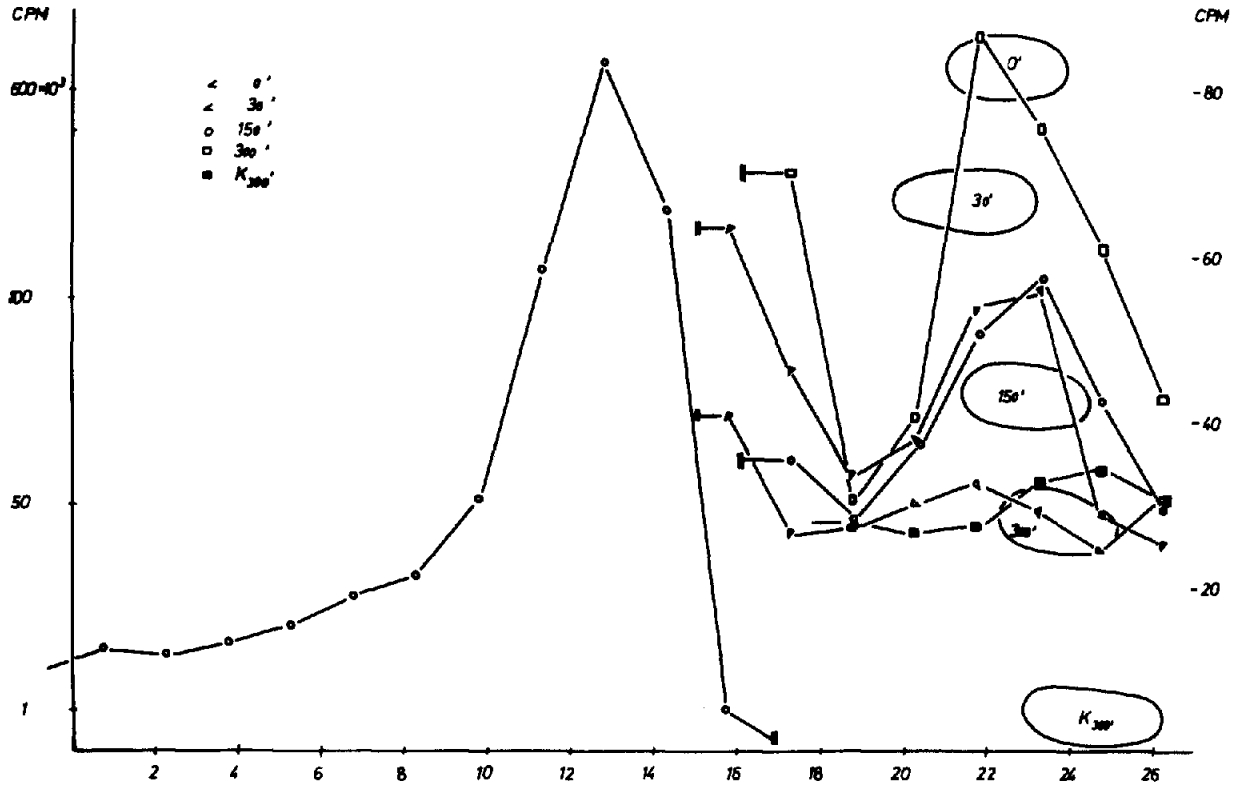


Fig. 1a. Incorporation of $^{32}P_i$ into glucose-6-phosphate during continuous absorption of work done *on* glycerinated muscle fibres, which were sinusoidally stretched and released at 1 cps (see Methods). Cpm along the one-dimensional chromatogram (abscissa: cm-distance from start; location of G-6-P_i marked by circles). Duration of negative work and incubation in ATP-salt solution containing $^{32}P_i$ (min): △ 0, ▲ 30, ○ 150, □ 300, ■ control solution after 300 min incubation. T = 10°.

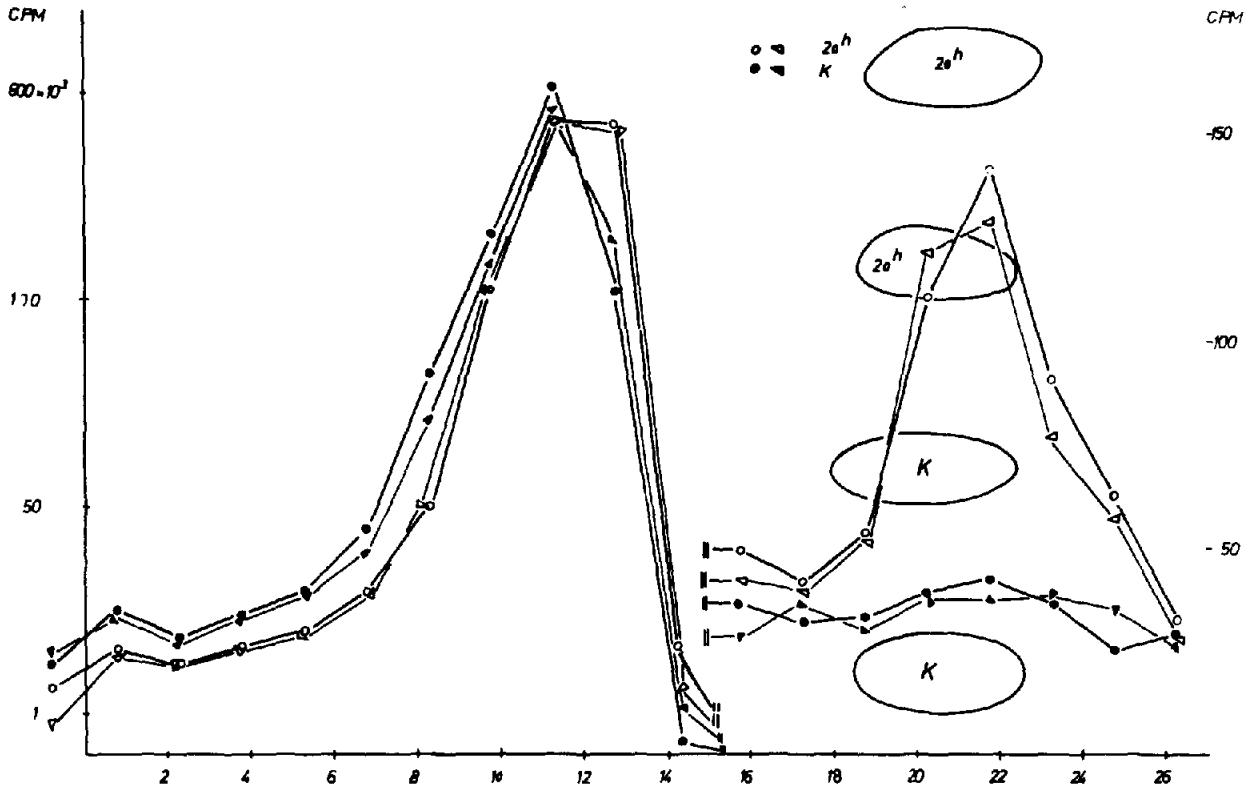
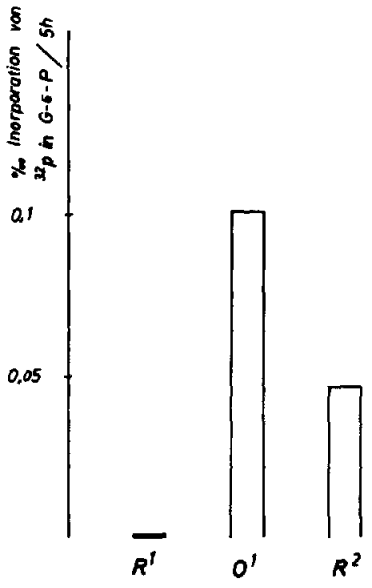


Fig. 1b. Same experiment, but after 20 hr incubation. △ and ○ fibre bundle, ▲ and ● control solution.



change with ATP occurring within the myofibril as the ^{32}P-γ-ATP has to cover a considerable distance when diffusing out of the fibre. On its way out, the probability of it being split again by the myofibrils is considerably greater than of it being metabolised by the hexokinase, since the concentration gradient of ATP outside to ATP inside the fibre is about 0.7 mM [6].

The extent to which $^{32}P_i$-exchange with ATP by glycerinated fibres may be due to contaminating enzymes will be further investigated. This possibility, however, seems to be rather unlikely because of the following two observations: first, muscle homogenate (1 to 3 mg/ml) prepared from a large number of glycerinated muscle fibres did not show a $^{32}P_i$-incor-

Fig. 2. Experiment similar to that of fig. 1. Incorporation of $^{32}P_i$ into glucose-6-phosphate as a % of total $^{32}P_i$ in solution. R^1 and R^2: isometric contraction, O^1: during the absorption of negative work.

poration into glucose-6-phosphate when incubated with a similar solution at room temperature for several hours; second, fig. 2 demonstrates the mechanical effect on the rate of the $^{32}P_i$-exchange with ATP. A fibre bundle of 5 fibres is alternatively subjected to periods of isometric contraction and negative work absorption (for 5 hr each), and the $^{32}P_i$-incorporation into glucose-6-phosphate is estimated. A correlation of increased $^{32}P_i$-incorporation into glucose-6-phosphate and the absorption of negative work by the fibre bundle is clearly shown, thus strongly indicating reversibility of the biochemical reactions of muscular contraction at some not yet precisely defined stage of the chemomechanical energy transformation.

Acknowledgements

I am greatly indebted to Mrs. B.Poeschmann for her expert technical assistance and to Prof. J.C.Rüegg for vauable criticism throughout the course of this work, and especially to Dr. K.C.Holmes for reading the manuscript and for his comments.

Supported by Grant No. RU 154/3 of the Deutsche Forschungsgemeinschaft.

References

[1] B.C.Abbott and X.M.Aubert, Proc. Roy. Soc. London Ser. B 139 (1951) 104.
[2] X.M.Aubert, Le Couplage Energétique de la Contraction Musculaire, ed. Arscia (Bruxelles, 1956) p. 315.
[3] R.S.Bandurski and B.Axelrod, J. Biol. Chem. 193 (1951) 405.
[4] R.E.Davies, Nature 199 (1963) 1068.
[5] B.R.Jewell and J.C.Rüegg, Proc. Roy. Soc. London Ser. B 164 (1966) 428.
[6] H.G.Mannherz, Arch. Ges. Physiol. 303 (1968) 230.
[7] G.Maréchal, Le Métabolisme de la Phosphorylcréatine et de l'Adenosine Triphosphate durant la Contraction Musculaire, ed. Arscia (Bruxelles, 1964) p. 184.
[8] J.W.S.Pringle, Insect Flight (Cambridge University Press, 1957).
[9] J.C.Rüegg and R.T.Tregear, Proc. Roy. Soc. London Ser. B 165 (1966) 497.
[10] G.J.Steiger and J.C.Rüegg, Arch. Ges. Physiol. 307 (1969) 1.