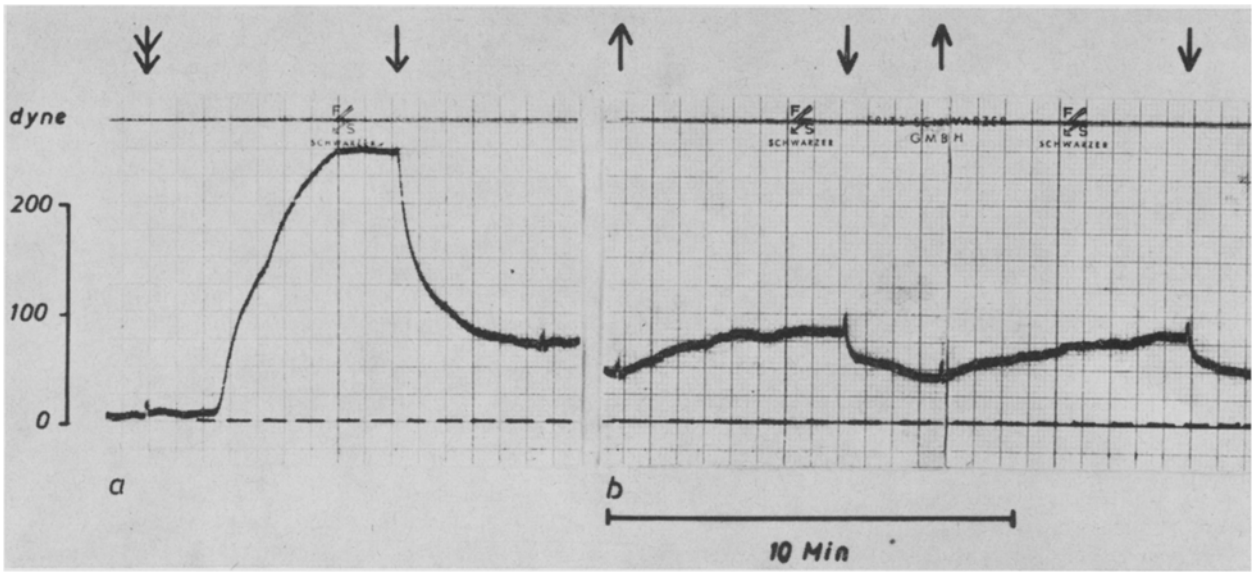

Contraction cycles without ATP. a) A bundle of 8 fibres (DLM) suspended in ATP relaxing solution is transferred (at ↕) into ATP-free rigor solution (pH = 6.5, 22 °C) in order to produce rigor contraction. At ↓ (incomplete) relaxation is induced by immersing the preparation into PP-relaxing solution containing 0.5 m*M* Mg-pyrophosphate in place of ATP. b) 20 min later the fibres exhibit a resting tension of about 7 dynes (cf. Table, column 2). Note that repeatable contraction-relaxation cycles can be induced by removal (↑) and readdition (↓) of Mg-pyrophosphate.

Conclusions. The experiments just described show that it is possible to generate stiffness and a force comparable to the active tension (up to 5 dynes per fibre) merely by removing a plasticizer (pyrophosphate), i.e. without the presence or splitting of a nucleoside triphosphate. Since this effect can be reversed, it may be concluded that force is generated because of the spontaneous formation of actin-myosin linkages after removal of the actin-myosin dissociating agent, and that energy (supplied by the binding of pyrophosphate) is required to break the linkage and induce relaxation. These experiments must, of course, be considered in conjunction with morphological data[15] suggesting that myosin cross-bridge orientation does undergo a reversible change after addition of pyrophosphate to and removal from ATP-free rigor solution. It will now be investigated whether the energy transformations caused by pyrophosphate can be used to obtain mechanical work-cycles, and whether it is reversible in the sense that chemical energy (free energy of dilution) can be transformed into mechanical energy and vice versa: such processes might be indeed analogous to those occurring in contracting polyacrilic acid gels[16, 17].

Zusammenfassung. Durch Zugabe und durch Auswaschen von Mg-Pyrophosphat (0,5 m*M*) oder Mg-Tripoly-Phosphat (1 m*M*) konnten (in Abwesenheit von ATP) in glyzerinextrahierten Fasern von fibrillären Insektenmuskeln (*Lethocerus maximus*) reversible Kontraktionszyklen und Änderungen des Dehnungswiderstandes bewirkt werden.

H. J. Kuhn, H. Schröder and J. C. Rüegg[18]

Department of Cell Physiology, Ruhr-Universität Bochum, D-463 Bochum-Querenburg (Germany),
23 December 1971.

[15] G. Beinbrech, H. J. Kuhn and J. C. Rüegg, in press.
[16] W. Kuhn, G. Ebner, H. J. Kuhn and D. H. Walters, Helv. chim. Acta *44*, 325 (1961).
[17] W. G. Pohl, H. J. Kuhn and W. Kuhn, Z. Naturforsch. *21a*, 756 (1966).
[18] Supported by Grant No. Ru 154/b of the Deutsche Forschungsgemeinschaft. The excellent technical assistance of Mrs. Helgard Jung is gratefully acknowledged.

Electron Microscope and Optical Diffraction Studies on Glycerol-Extracted Insect Flight Muscle Fibres Relaxed by Pyrophosphate[1]

During the contraction-relaxation cycle of muscle, ATP has two functions: a plasticizing function (dissociation of actomyosin into actin and myosin), and the provision of energy by splitting. Results of Taylor et al.[2] suggest that the energy liberated by ATP splitting is used to bring back the detached myosin heads (= cross bridges) into the relaxed, rectangular position[3]. If this suggestion is correct, the angled cross bridges of a muscle in rigor should detach from the actin filaments without a change of conformation by the action of pyrophosphate (PP) which is known to imitate only the plasticizing action of ATP[4].

To test this hypothesis, the rigor state of glycerinated fibres of the dorsolongitudinal flight muscles of *Lethocerus spec.*[5] was used as a starting condition[6]. To be sure that no ATP was left in the fibre, the rigor solution (50 m*M* KCl, 20 m*M* histidine, pH 6.5, 10 m*M* NaN_3) was changed several times. When the fibres were transferred to the

PP-solution (50 m*M* KCl, 20 m*M* histidine, pH 6.5, 5 m*M* $MgCl_2$, 5 m*M* $Na_4P_2O_7$, 10 m*M* NaN_3) the tension dropped down approximately to the relaxation level. Thereafter the fibres were fixed in glutaraldehyde and OsO_4 solutions and prepared for electron microscopy. Optical diffraction patterns of electron micrographs from thick sections[7] were photographed in a Polaron diffractometer. The preparation procedures for electron microscopy caused similar shrinkage artifacts as described by REEDY et al.[8]. For the sake of clarity, the discussion of the optical diffraction patterns will be simplified by neglecting these artifacts and using the values measured by X-ray diffraction[8].

The optical transform of a relaxed sarcomere is characterized by a strong 146 Å layer line and shows only weak periods of 388 and 194 Å (Figure 1). According to REEDY[7], the 146 Å layer line is caused by successive cross bridge pairs repeating at axial intervals of 146 Å and azimuthal intervals of approximately 60°. The diffraction pattern of a sarcomere fixed in rigor is dominated by strong layer lines at 388 and 194 Å and shows only a very weak period of 146 Å (Figure 3). This reduction of the 146 Å period during rigor contraction has been associated[7] with the angling of cross bridges: In the rigor state, the actin end of one bridge very often overlaps the myosin end of the next and thereby weakens severely the transverse lattice planes repeating at 146 Å. On the other hand, the periods of 388 and 194 Å should be intensified by the attachment of cross bridges at the actin filaments, occurring in periods of 388 Å in the plane of sectioning. This period is demonstrated in Figure 4. The micrograph shows a 'thin' longitudinal section through a part of a sarcomere with a single actin filament layer. Actin ends of cross bridges are attached to the actin filaments in regular distances. If the micrograph is sighted perpendicular to the filaments at a glancing angle to the page[7] lines can be observed crossing the filament axis and consisting of myosin heads arranged one after the other. The disappearance of these lines during relaxation of a fibre by ATP solution probably causes the weakening of the 388 and 194 Å periods in optical transforms and admits of the appearance of the 146 Å layer line mentioned above.

Figure 2 shows an optical transform of a sarcomere fixed in PP solution. The striking difference to the pattern of a rigor muscle (Figure 3) is the appearance of a strong 146 Å period, typical for ATP relaxed sarcomeres (Figure 1). The difference between sarcomeres relaxed in PP solution and in ATP solution consists of stronger layer lines at 388 and 194 Å, the intensity of which can be greater or less, but causes a pattern similar to that of a rigor muscle. These findings suggest that the optical transforms of PP relaxed sarcomeres combine in some way the attributes of ATP relaxed and rigor fibres. These results would fit rather well the hypothesis advanced in the introduction. The myosin heads could be detached from the actin filaments resulting in the appearance of the 146 Å layer lines as stated above, whereas the periods of 388 and 194 Å could be intensified by the angled position of the cross bridges which were not able to return into rectangular position because of the lack of energy.

However, regarding electron micrographs of PP relaxed sarcomeres, little evidence could be found that this interpretation is correct. In this respect, a second artifact caused by the fixation medium is of interest: during the fixation with glutaraldehyde, detached cross bridges of ATP relaxed fibres seem to be reattached to the actin filaments[8]. Thus the detached and possibly angled cross bridges of the PP relaxed sarcomeres likewise should be attached to the actin filaments during the fixation procedures. In this case, similar lines crossing the filament axis as demonstrated in Figure 4 should be observed in Figure 5. However, the artifactual reattachment of the myosin heads to the actin filaments failed to restore a periodicity. The lateral projections of the actin filaments in Figure 5 demonstrate the attachment of myosin heads but obviously at irregular distances. The periodicity could be restored if the PP-relaxed fibres were transferred from PP solution back into rigor solution before fixation (Figure 4). This transfer has been associated with an increase of tension[9] suggesting a movement of cross bridges into the rigor position of Figure 4.

[1] Supported by a short term fellowship of the EMBO to one of us (G.B.).

[2] E. W. TAYLOR, R. W. LYMN and G. MOLL, Biochemistry *9*, 2984 (1970).
[3] H. G. MANNHERZ and H. SCHIRMER, Chemie in unserer Zeit *4*, 165 (1970).
[4] L. B. NANNINGA, Biochim. biophys. Acta *82*, 507 (1964).
[5] B. R. JEWELL and J. C. RÜEGG, Proc. R. Soc. B. *164*, 428 (1966).
[6] D. C. S. WHITE, J. Physiol., Lond. *208*, 583 (1970).
[7] M. K. REEDY, Am. Zool. *7*, 465 (1967).
[8] M. K. REEDY, K. C. HOLMES and R. T. TREGEAR, Nature, Lond. *207*, 1267 (1965).
[9] H. J. KUHN, H. SCHRÖDER and J. C. RÜEGG, Experientia, in press.

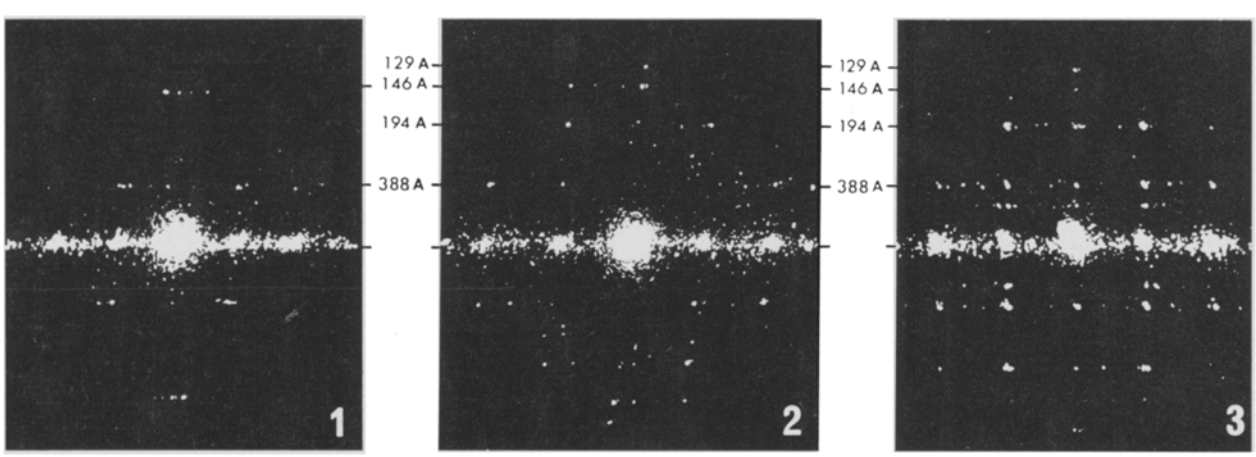

Fig. 1–3. Optical transforms of sarcomeres fixed in ATP- (1), Mg-pyrophosphate- (2) and rigor solution. The values of the layer lines are converted into those measured by X-ray diffraction[8] (details see text).

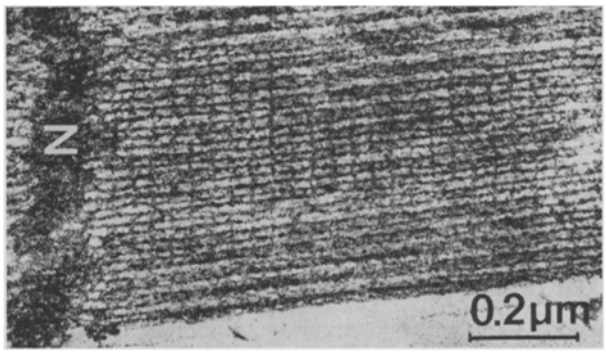

Fig. 4. 'Thin' longitudinal section through a fibre which had developed tension in a rigor solution, was relaxed by Mg-pyrophosphate, transferred back into the rigor solution and fixed there. The center of the micrograph shows a single layer of actin filaments with lateral projections consisting of the actin ends of cross bridges. If the micrograph is sighted at a glancing angle perpendicular to the filaments, the projections form lines with regular distances. (Z = Z-line).

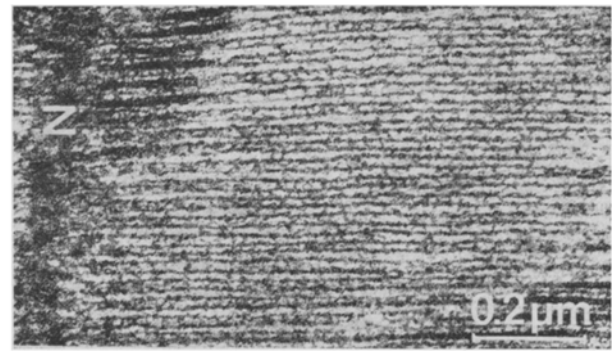

Fig. 5. 'Thin' longitudinal section through a fibre fixed after relaxation by Mg-pyrophosphate, showing a single layer of actin filaments. The lateral projections of the filaments are attached in irregular distances (Z = Z-line).

Until now the results have been discussed as if all cross bridges behaved in the same manner during PP relaxation. However, it is not yet clear if this simplification is permitted. Regarding single actin-myosin layers of PP-relaxed fibres, most of the micrographs indeed show similar orientation of the myosin heads to that of fibres relaxed with ATP[7,8]. On some micrographs, however, the cross bridges appear to prefer an angled position. Further experiments, together with X-ray diffraction controls (in conjunction with Mrs. PHYLLIS ARMITAGE and Dr. R. T. TREGEAR, Oxford) will be necessary to exclude the possibility that artifactual cross bridge movements are happening during the fixation procedures. But, in any case, the present experiments suggest that PP is capable of inducing a reversible change of cross bridge configuration[10].

Zusammenfassung. Elektronenmikroskopische Aufnahmen von einfachen Filament-Lagen Mg-Pyrophosphat-erschlaffter Fasern der dorsolongitudinalen Flugmuskulatur von *Lethocerus spec.* gleichen weitgehend dem Bild ATP-erschlaffter Muskeln. Optische Transformationen scheinen dagegen die Charakteristika von ATP-erschlafften Fasern und solchen, die sich im Rigor mortis befinden, zu kombinieren. Die Überführung der Fasern aus einer Mg-Pyrophosphat- in eine Rigor-Lösung stellte das Rigor-Muster wieder her.

G. BEINBRECH, H. J. KUHN and J. C. RÜEGG

Department of Cell Physiology, Ruhr-University of Bochum, Postfach 2148 D–463 Bochum (Germany), 5 January 1971.

[10] Acknowledgment. We are greatly indebted to Dr. LOUISE N. JOHNSON, P. EAGLE and L. J. STUMPP, Oxford, Dept. of Zoology, for their help with the optical diffraction measurements, to Mrs. HILTRUD GÜLKER for her expert technical assistance and to Prof. PRINGLE and Dr. R. T. TREGEAR for their hospitality and many helpful discussions and criticism.

3′-5′- cAMP and Lysosome Membrane Labilization

In our preliminary experiments[1] it was suggested that 3′–5′-cAMP produces an endogenous activation of phospholipase A enzyme in tissue homogenate. The aim of the following experiments would be to investigate the effect of exogenous 3′–5′-cAMP on the ultrastructure of lysosome membrane prepared from rat liver tissue, studying the release of lysosomal enzymes: acid phosphatases and β-glucuronidases from lysosomes.

The labilization of the lysosome membrane and an increased level of lysosomal enzymes in extralysosomal space indirectely indicates a substantial transformation of lysosomal membrane structure, presumably the predominance of micellar configuration[2].

Material and methods. Male rats of the Wistar strain were used; 7 g of the chilled liver were weighed out and lysosomes were prepared according to WEISSMANN[3].

The following procedure was a modification of KARLSSON's techniques[4]. The lysosome-rich sediment obtained after washing with sucrose was resuspended in 10.0 ml of ice-cold salt phosphate buffer solution (pH 7.4)[5] containing 0.25 *M* sucrose and 5 g human serum albumin (resuspended sediment). In order to determine the total activity of lysosomal enzymes per ml of suspension, 0.7 ml of the suspension was mixed with 2.3 ml of the above mentioned sucrose – HSA buffer with added Triton X-100 (final concentration 0.15%).

[1] S. IMRE, Abstr. Congress of Hung. Phys. Soc. (Ed. J. Salanki; Budapest 1971).
[2] J. A. LUCY, Br. med. Bull. *24*, 127 (1968).
[3] G. WEISSMANN and L. THOMAS, J. exp. Med. *116*, 433 (1962).
[4] H.-O. KARLSSON, Experientia *25*, 1290 (1969).
[5] B. MOSINGER and M. VAUGHAN, Biochem. biophys. Acta *144*, 569 (1967).