tions of America, Inc., The Life Insurance Medical Research Fund and the Massachusetts Heart Association. Preliminary reports of this work have been presented[1,2].

This work was carried out during the tenure by J. G. of an Established Investigatorship of the American Heart Association, Inc.

REFERENCES

1 J. GERGELY, *Ann. N.Y. Acad. Sci.*, 72 (1959) 538.
2 J. GERGELY, F. N. BRIGGS AND G. KALDOR, *4th Intern. Congr. Biochem., Vienna, 1958, Abstr. Communs.*, p. 83.
3 B. B. MARSH, *Nature*, 167 (1951) 1065.
4 H. PORTZEHL, *Biochim. Biophys. Acta*, 26 (1957) 373.
5 F. N. BRIGGS, G. KALDOR AND J. GERGELY, *Biochim. Biophys. Acta*, 34 (1959) 211.
6 S. V. PERRY, *Biochem. J.*, 51 (1952) 495.
7 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
8 J. R. BENDALL, *Nature*, 181 (1958) 1188.
9 L. LORAND, J. MOLNAR AND C. MOOS, *Conference on the Chemistry of Muscular Contraction, Tokyo, 1957*, Igaku Shoin Ltd., p. 85; *Biol. Bull.*, 113 (1957) 323.
10 S. EBASHI, *Arch. Biochem. Biophys.*, 76 (1958) 410.
11 H. KUMAGAI, S. EBASHI AND F. TAKEDA, *Nature*, 176 (1955) 166.
12 G. KALDOR AND J. GERGELY, *Arch. Biochem. Biophys.*, in the press.
13 H. H. WEBER, *Ann. N. Y. Acad. Sci.*, in the press.

PARTICIPATION OF A DIALYZABLE COFACTOR IN THE RELAXING FACTOR SYSTEM OF MUSCLE

III. SUBSTITUTION OF PYROPHOSPHATE FOR THE COFACTOR

G. KALDOR* AND J. GERGELY

Cardiac Biochemistry Research Laboratory, Departments of Medicine, Massachusetts General Hospital and Harvard Medical School,

AND F. NORMAN BRIGGS**

Department of Pharmacology, Harvard Medical School, Boston, Mass. (U.S.A.)

(Received October 4th, 1958)

SUMMARY

2 m*M* pyrophosphate restores the capacity of muscle granules sedimented at 35,000 × *g* to relax muscle fibres and inhibit myofibrillar adenosinetriphosphatase. These effects are reversed by Ca^{++}; pyrophosphate, thus, can replace the dialyzable cofactor described in the preceding two communications[7,8]. Pyrophosphatase destroys the activating effect of pyrophosphate but not that of the cofactor present in the 35,000 × *g*-supernatant, rendering it unlikely that the cofactor is identical with pyrophosphate.

The following abbreviations are used: ATP, adenosinetriphosphate; PP, pyrophosphate; PPase, pyrophosphatase; ATPase, adenosinetriphosphatase; RFS, relaxing factor system; Tris, tris(hydroxymethyl)aminomethane.

* Present address: McArdle Memorial Laboratory, University of Wisconsin, Madison, Wisc.
** Present address: Department of Physiology, Tufts University School of Medicine, Boston, Mass.

References p. 227.

INTRODUCTION

BENDALL[3], working with glycerinated fibre bundles, found that 1–2 m*M* PP caused relaxation in the presence of ATP. He later suggested that the RFS might function by producing PP[4]. A similar suggestion has recently been made by LORAND *et al.*[5] in connection with their work on PEP relaxation, but no direct evidence has been put forward for the participation of PP in the relaxation brought about by the RFS. BOZLER AND PRINCE[6] observed that the spontaneous relaxation in ATP of fresh glycerinated fibre bundles was accelerated by PP in concentrations as low as 0.4 m*M*.

It will be shown in this paper that the dialyzable cofactor of the RFS[7,8] could be replaced by PP, although the cofactor was found not to be identical with PP.

MATERIALS AND METHODS

All the preparations and determinations were carried out as described in the preceding two communications[7,8], using sucrose for the preparation of the 35,000 × *g*-granules. Crystalline pyrophosphatase was obtained through the courtesy of Dr. KUNITZ of the Rockefeller Institute, New York.

RESULTS

The effect of PP and muscle granules sedimented at 35,000 × *g* on the ATP induced tension of glycerinated single fibres is illustrated in Fig. 1a. 2 m*M* PP was without effect on tension. Addition of granules, in a concentration in which they were ineffective by themselves, produced a good relaxation which was completely reversed by Ca^{++}. In the experiment illustrated in Fig. 1b PPase was added first, and its

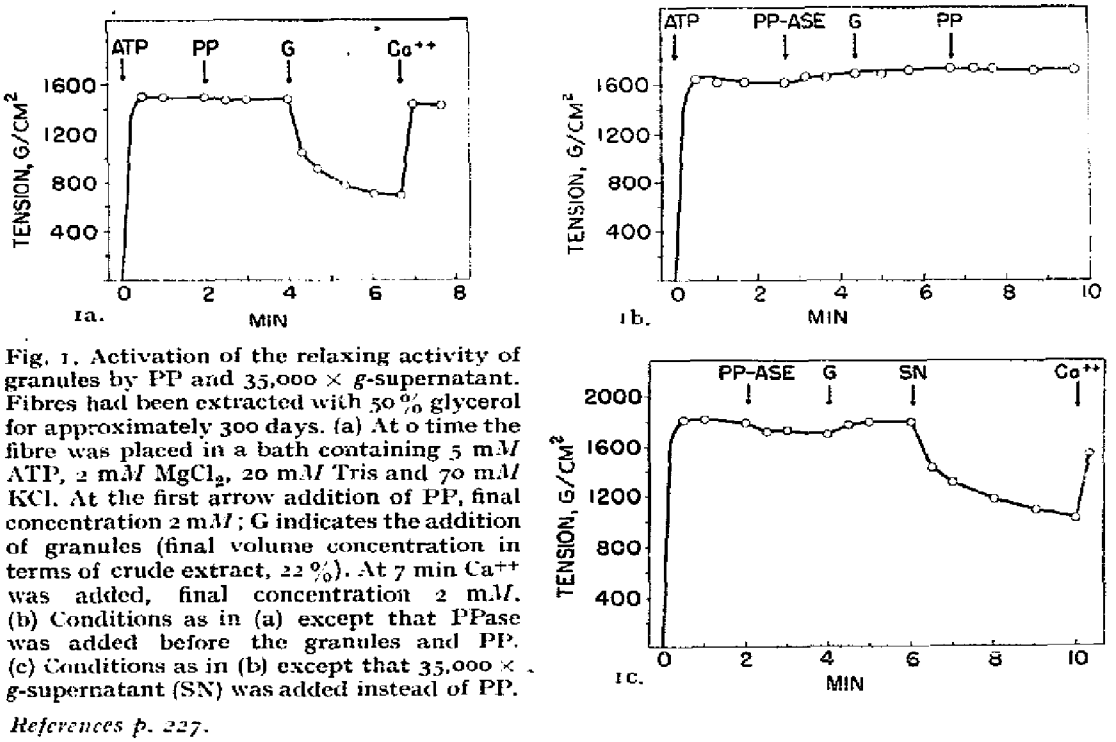

Fig. 1. Activation of the relaxing activity of granules by PP and 35,000 × *g*-supernatant. Fibres had been extracted with 50% glycerol for approximately 300 days. (a) At 0 time the fibre was placed in a bath containing 5 m*M* ATP, 2 m*M* $MgCl_2$, 20 m*M* Tris and 70 m*M* KCl. At the first arrow addition of PP, final concentration 2 m*M*; G indicates the addition of granules (final volume concentration in terms of crude extract, 22%). At 7 min Ca^{++} was added, final concentration 2 m*M*. (b) Conditions as in (a) except that PPase was added before the granules and PP. (c) Conditions as in (b) except that 35,000 × *g*-supernatant (SN) was added instead of PP.

References p. 227.

presence completely prevented the relaxation that would have been produced by granules and PP. Addition of PPase to a fibre relaxed by PP and granules reverses the relaxation (not shown in the Figs.).

If the 35,000 × *g*-supernatant was used, instead of PP, with granules, addition of PPase was without influence on the relaxation, although it was reversed in the usual manner by Ca (Fig. 1c). This experiment would exclude the identity of the cofactor in the supernatant with PP.

PP could replace the dialyzable cofactor in the inhibition of myofibrillar ATPase (Fig. 2). Granules in a sufficiently low concentration produce only a small inhibition, but addition of 2 m*M* PP led to an almost maximal depression of ATPase. 2 m*M* PP in the absence of granules was without effect on the myofibrillar ATPase.

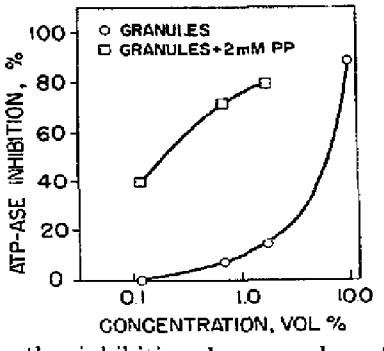

Fig. 2. The effect of PP on the inhibition by granules of myofibrillar ATPase. Abcissa: concentration of granules in terms of an equivalent volume of crude extract. ○, granules; □, granules + 2 m*M* PP.

DISCUSSION

The result that PP can replace the dialyzable cofactor in the RFS furnishes an explanation of the earlier observations of BENDALL[3] and of BOZLER AND PRINCE[6] that PP in low concentrations relaxes glycerinated fibre bundles in the presence of ATP. Presumably their fibres contained the granular component of the relaxing factor but were deficient in the dialyzable cofactor. PORTZEHL's finding[9] that even higher concentrations, 15 m*M*, of PP failed to affect the tension produced by ATP are probably due to the fact that the experiments were done with thoroughly washed fibres devoid of the RFS.

Both the PP effects observed by BENDALL and by BOZLER AND PRINCE and the PP effect when replacing the cofactor were inhibited by Ca^{++}.

In view of our results it is tempting to speculate that PP is somehow involved in muscle relaxation. The failure of PPase to counteract the cofactor makes a direct participation unlikely, and the adsorption of the cofactor on charcoal[7,8] is also at variance with the behavior of PP.

Although PP and the cofactor do not seem to be identical, the PP effect may provide useful clues to the mechanism of the RFS, since the cofactor and PP may have some features in common. It is not unreasonable to suppose that the cofactor and PP both act on the granules, perhaps by stimulating the release of some substance which is ultimately responsible for relaxation or by maintaining their structural and functional integrity. Elucidation of this problem must await further work, including isolation and characterization of the cofactor.

References p. 227.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Heart Institute, U. S. Public Health Service (H-1166 (C5) (C6), H-2205, H-3250), the Muscular Dystrophy Associations of America, Inc., The Life Insurance Medical Research Fund and the Massachusetts Heart Association. Preliminary reports of this work have been presented[1,2].

This work was carried out during the tenure by J. G. of an Established Investigatorship of the American Heart Association, Inc.

REFERENCES

1 J. GERGELY, *Ann. N.Y. Acad. Sci.*, 72 (1959) 538.
2 J. GERGELY, F. N. BRIGGS AND G. KALDOR, *4th Intern. Congr. Biochem., Vienna, 1958, Abstr. Communs.*, p. 83.
3 J. R. BENDALL, *Nature*, 172 (1953) 586.
4 J. R. BENDALL, *Proc. Roy. Soc. (London)*, *B*, 142 (1954) 409.
5 L. LORAND, J. MOLNAR AND C. MOOS, *Conference on the Chemistry of Muscular Contraction, Tokyo, 1957*, Igaku Shoin Ltd., p. 85.
6 E. BOZLER AND J. T. PRINCE, *J. Gen. Physiol.*, 37 (1954) 53.
7 F. N. BRIGGS, G. KALDOR AND J. GERGELY, *Biochim. Biophys. Acta*, 34 (1959) 211.
8 J. GERGELY, G. KALDOR AND F. N. BRIGGS, *Biochim. Biophys. Acta*, 34 (1959) 218.
9 H. PORTZEHL, *Z. Naturforsch.*, 7b (1952) 1; also cited by H. H. WEBER, *Z. Elektrochem.*, 55 (1951) 511.

STUDIES ON THE MECHANISM OF FATTY ACID SYNTHESIS

VI. SPECTROPHOTOMETRIC ASSAY AND STOICHIOMETRY OF FATTY ACID SYNTHESIS

SALIH J. WAKIL, EDWARD B. TITCHENER AND DAVID M. GIBSON

Institute for Enzyme Research, University of Wisconsin, Madison, Wisc. (U.S.A.)

(Received October 4th, 1958)

SUMMARY

A spectrophotometric assay for the synthesis of long-chain fatty acids from acetyl CoA which depends upon the oxidation of TPNH has been described. The oxidation of TPNH requires all the components of the system and correlates very well with the incorporation of radioactivity from [1-^{14}C]acetyl CoA to palmitate.

The stoichiometry of the fatty acid synthesis has been studied and the results for the synthesis of palmitic acid may be expressed by the following equation:

$$8\ \text{AcCoA} + 16\ \text{ATP} + 16\ \text{TPNH} \xrightarrow[\text{HCO}_3^-,\ \text{Mn}^{++}]{\text{R}_{1g}\text{ and }\text{R}_{2g}} \text{palmitic acid} + 16\ \text{ADP} + 16\ \text{P}_i + 16\ \text{TPN} + 8\ \text{CoA}$$

The following abbreviations were used: AcCoA, acetyl coenzyme A; CoA, coenzyme A; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide; DPN and DPNH, oxidized and reduced diphosphopyridine; ATP, adenosine triphosphate; ADP, adenosine diphosphate; P_i, orthophosphate; and R_{1g} and R_{2g}, the two enzyme preparations referred to in ref. 4.

References p. 233.