kinase was sonicated in the presence of a very low concentration of [^{14}C]ATP and a large concentration of phosphocreatine. Incorporation of the [^{14}C]ADP into the F-actin and splitting of the phosphocreatine were followed at the same time. If the unmasked ADP were rephosphorylated before dissociating from protein, the phosphocreatine splitting should take place without accompanying the incorporation. However, this was not realized, both reactions proceeding at approximately the same rate under the vibration (Fig. 3). We must consider, then, that, in our sonic experiments, the rephosphorylation reaction takes place in the solvent, although HAYASHI AND ROSENBLUTH[7] have recently suggested that, in the case of G-ADP-actin, the ADP bound on the actin can be directly rephosphorylated by the creatine kinase system.

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SC 2255

The effect of sonic vibration on myosin and actomyosin adenosine triphosphate activities

In this communication investigations are reported of the effect of sonic vibration on myosin and actomyosin ATPase activities (EC 3.6.1.3) made in connection with studies of sonically activated F-actin ATPase[1–3]. Sonication of sample solutions was made in the same apparatus as used in our actin studies.

When the sonic generator was driven, the bulk temperature of solutions in the container rose about 2° higher than that of circulating water in the 1st min and then reached a constant value within an error of $\pm 0.5°$. To obtain a constant temperature during reactions under sonication, sample solutions were sonicated for 1 min in the absence of ATP and then ATP was added to the vibrating solutions. The constant bulk temperature of sample solutions was used as a measure of reaction temperature. Practically the same ATPase activity was found for myosin whether it was placed in the sonic field or not, while for F-actin ATPase an appreciable sonic activation was observed (Table I).

Under suitable conditions, actomyosin solutions undergo clearing followed by superprecipitation by the addition of ATP. The ATP splitting takes place slowly during the clearing phase and increases in speed with the superprecipitation[4–6]. If ATP is added to the same actomyosin placed in the sonic field, the ATP splitting

TABLE I

THE EFFECT OF SONIC VIBRATION ON F-ACTIN AND MYOSIN ATPase ACTIVITIES

0.15 M KCl, 0.02 M Tris–maleate (pH 7.0), 2 mM $MgCl_2$ or $CaCl_2$, and 1 mM ATP. Reaction temperature, 20°. ATPase activity is expressed in mμmoles/min/mg protein.

Treatment	*F-actin*		*Myosin*	
	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}
Untreated	0	0	11	440
3 min sonication			12	390
Under sonication	0.45	0.52	12	490

proceeds at the slow speed observed during the clearing phase in the static experiment and does not increase in speed (Fig. 1). Sonic vibration seems to inhibit the superprecipitation of actomyosin.

If a system composed of F-actin, myosin, creatine kinase (EC 2.7.3.2) and phosphocreatine does not contain free nucleotide, little if any phosphocreatine splitting takes place[7,8]. This indicates that in the F-actin–myosin complex the bound ADP of F-actin is still non-reactive to the ATP-regenerating system. If, on the other hand, the same composite system is subjected to vibration, a markedly accelerated phosphocreatine splitting takes place (Fig. 2). This acceleration can not be attributed to diffusion nor to irreversible liberation of the bound ADP which might result from the sonication. After sonication, the bound ADP of F-actin becomes dissociable and reactive to the ATP-regenerating system[2]. The rephosphorylation reaction is considered to take place when the ADP dissociates from the actin[2]. The question then

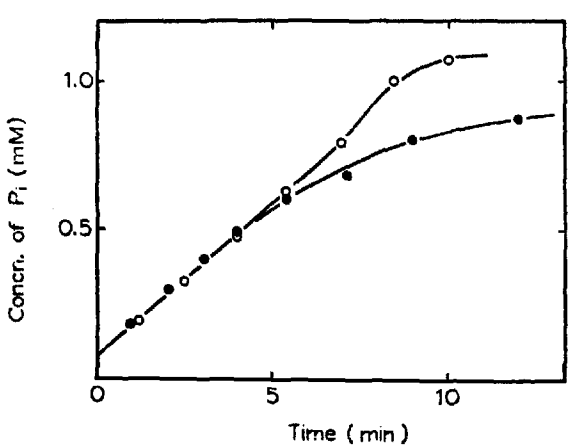

Fig. 1. The effect of sonic vibration on the ATPase activity of actomyosin. Reaction mixture contained 1.0 mg/ml myosin, 0.25 mg/ml actin, 0.08 M KCl, 0.02 M Tris–maleate (pH 7.2), 1 mM $MgCl_2$ and 1 mM added ATP. Reaction temperature, 20°. ○, untreated; ●, sonicated.

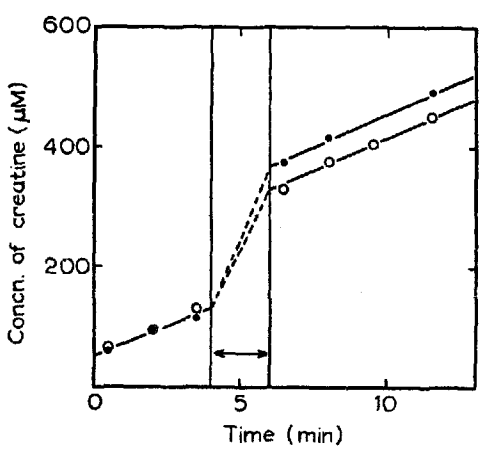

Fig. 2. Sonically accelerated splitting of phosphocreatine in a system composed of F-actin, myosin, creatine kinase. After washing a mixture of 40 mg F-actin and 100 mg myosin 3 times with 0.08 M KCl, 100 mg creatine kinase was added. Neglecting loss of protein during the washing, the reaction mixture contained 0.5 mg/ml actin, 1.25 mg/ml myosin and 1.25 mg/ml creatine kinase. 0.08 M KCl, 0.03 M Tris–maleate (pH 7.0), 1 mM $MgCl_2$ or $CaCl_2$. Added phosphocreatine, 1 mM. The reaction mixture was subjected to the vibration during the period indicated by the arrows. Temperature, 18–20°. ●, in the presence of Mg^{2+}; ○, in the presence of Ca^{2+}.

arises: Which constituent of the complex splits the ATP, the myosin or the actin? We have no direct information on this problem. However, taking into account the fact that the complex does not seem to suffer any irreversible change after the sonication, it seems more probable that the ATP splitting is caused by the (F-)actin ATPase. If this is assumed to be the case, it follows that the accelerated phosphocreatine splitting during the sonication is a consequence of the increased rate of production of the ADP available to the creatine kinase system. As one possibility, we can expect that sonically activated F-actin ATPase itself is very much activated under influence of myosin. Its activity must be more than 100 times greater than that of the pure F-actin under the same conditions.

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Association of gluten proteins in solution

The presence of association–dissociation phenomena in solution has been well established for the complex seed proteins of the peanut[1,2], soyabean[3,4], and jack bean (urease[5]). This communication reports evidence for the existence of an aggregation equilibrium in solutions of a wheat protein fraction.

The acetic acid-soluble proteins of an Australian wheat (Gabo) flour, extracted by the method of COATES AND SIMMONDS[6], were chromatographed on a column of CM-cellulose according to the procedure described previously[7], but on a larger scale. The contents of the tubes corresponding to the required fraction (fraction B) were combined and subjected to the same chromatographic procedure several times, until traces of adjacent peaks could no longer be detected. Chromatographic patterns of fraction B and the parent acetic acid extract are compared in Fig. 1.

Fraction B dissolved readily and completely in the solvent used for ultracentrifuge experiments (0.09 M NaCl, 0.01 M sodium acetate, 1.0 M dimethylformamide, pH adjusted to 4.1 with acetic acid) to give a clear solution, which was then dialysed against the same buffer at 4° for 24 h. Lower concentrations were prepared by weight dilution of the stock solution, the concentration of which was determined colorimetrically[8]. Sedimentation-velocity experiments were performed at 59 780