TABLE I

EFFECT OF DNP ON ACTOMYOSIN ATPASE AT VARIOUS IONIC STRENGTHS

No.	*I*	*Activity without DNP µmol P/mg protein/min*	*Acceleration by DNP*
1	0.050	0.88	−9
2	0.068	0.98	−2
3	0.088	1.00	+9
4	0.150	1.03	+23
5	0.300	0.60	+112

In No. 1: Actomyosin, 0.045 mg protein/ml; KCl, 0.011 M; $CaCl_2$, $5 \cdot 10^{-3}$ M; K-ATP, 10^{-3} M; glyoxaline-Cl pH 7.0, 0.0163 M; K-DNP, $4 \cdot 10^{-3}$ M (or equivalent KCl). In Nos. 2–5, I was raised by further addition of KCl. 5 min, 25°. Activity in absence of added Ca^{++}: 0.05 (I = 0.05), 0.22 (I = 0.30).

The deaminase activity of one of our L-myosin preparations was tested under the following conditions (*cf.* ref.[11]): 0.08 mg protein/ml; 0.04 M succinate buffer, pH 5.5; 0.02 M $CaCl_2$; $3 \cdot 10^{-3}$ M adenosine-5′-phosphate; 15 min at 25°. NH_3 was determined by microdiffusion (CONWAY[12]) followed by Nesslerisation. Q_{NH_3} (ref.[11]) was 1260, and unchanged by DNP ($2.5 \cdot 10^{-3}$ to $1.25 \cdot 10^{-4}$ M).

To summarise, with both mitochondria[6] and myosin DNP accelerates the ATPase activity and this effect is abolished by PMA; in neither system does DNP accelerate the ITPase activity. On the other hand, Ag^{++} in low concentrations greatly accelerates mitochondrial ATPase but only inhibits myosin ATPase[13]. Actomyosin at high ionic strengths behaves towards DNP and PMA similarly to myosin.

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The stimulation of the adenosinetriphosphatase activities of myofibrils and L-myosin by 2:4-dinitrophenol

In the course of studies on the metabolism of adenosinetriphosphate (ATP) by intracellular components of skeletal muscle[1,2,3] it was noted that whereas the adenosinetriphosphatase (ATPase) activity of freshly prepared pigeon breast muscle mitochondria was markedly stimulated by 10^{-4} M 2:4-dinitrophenol (DNP), this substance in concentrations ranging from 10^{-4} to $5 \cdot 10^{-3}$ M failed to increase the ATPase activity of rabbit myofibrils. As this latter observation did not appear to be consistent with WEBSTER'S[4] finding that myosin ATPase was stimulated by DNP, further investigations were carried out to discover why this difference in behaviour should exist between the purified enzyme and myosin occurring naturally as actomyosin in the myofibril.

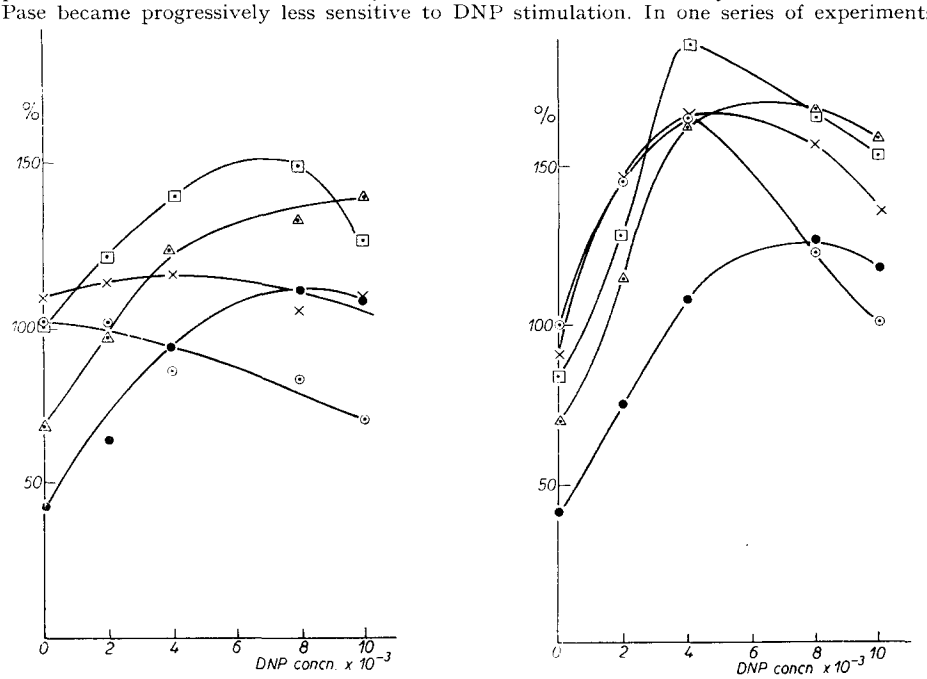
In our standard incubation medium (containing 0.005 *M* $CaCl_2$, 0.05 *M*-trishydroxymethylaminomethane (TRIS) buffer, pH 7.4, and 0.005 *M*-NaATP), $2 \cdot 10^{-3}$ *M* DNP had little effect at 20° C on the ATPase of rabbit myofibrils prepared as described by PERRY[5], although inhibition became progressively greater as the DNP concentration was increased to 10^{-2} *M*. DNP stimulated the ATPase activity of the myofibrils, however, when the ionic strength of the incubation medium was increased (Fig. 1a). In the presence of added 0.05 *M* KCl little stimulation was obtained, but at higher concentrations the effect was quite marked. For example when 0.2 *M* KCl was added to the incubation medium $6 \cdot 10^{-3}$ *M*-DNP induced 130% increase in activity.

With Mg^{++} as the activating cation the myofibrillar ATPase falls rapidly as the ionic strength increases, with the result that in the presence of 0.2 *M* KCl activity is very low indeed[6]. At all concentrations of KCl within this range, $2 \cdot 10^{-3}$ *M*-DNP caused slight inhibition of the Mg^{++}-activated ATPase.

When tested under similar conditions to those used for the myofibril experiments, Ca^{++}-activated L-myosin[7] ATPase was found to be almost maximally stimulated by DNP in the absence of added KCl (Fig. 1b).

NaCl was as equally effective as KCl in promoting the stimulation of the myofibrillar ATPase by DNP, but for a given DNP concentration K_2SO_4 induced maximal stimulation at lower concentrations. In general it could be said that whereas the myosin ATPase was almost fully stimulated by DNP at the ionic strength of the standard incubation medium, higher ionic strengths were necessary to achieve stimulation of the ATPase activity of myofibrils. Preliminary experiments suggested that under the conditions for DNP stimulation of myofibrillar ATPase activity at 20° C, the enzyme is partly in solution. The presence of the ATPase in the sol form was not by itself a prerequisite for DNP stimulation, however, because in an incubation medium at 0° C containing added 0.2 *M* KCl, 65% of the myofibrillar ATPase was in solution and DNP induced inhibition rather than stimulation (see later).

Experiments carried out on synthetic actomyosin made from purified L-myosin and actin[8] preparations showed that when increasing amounts of actin were added to myosin, the Ca^{++}-activated ATPase became progressively less sensitive to DNP stimulation. In one series of experiments in



(a) Myofibrils (b) Myosin

Fig. 1. Effect of DNP and KCl on the Ca^{++}-activated ATPases of rabbit myofibrils and L-myosin. Incubations carried out for 10 min at 20° C in the standard incubation medium to which the following additions were made:

⊙——⊙ nil, ⊡——⊡ 0.1 *M*-KCl ●——● 0.4 *M*-KCl. ×——× 0.05 *M*-KCl △——△ 0.2 *M*-KCl

The reaction was started by the addition of the enzyme. Activity, measured as inorganic phosphate, is represented as a percentage of that obtained in the absence of both DNP and added KCl.

the standard incubation medium, myosin ATPase was stimulated 100% by $5 \cdot 10^{-3}$ *M*-DNP, but the addition of increasing amounts of actin reduced this stimulation progressively until when the myosin to actin ratio was 3.2 to 1, stimulation was only 5%.

Mn^{++} also activated the myofibrillar ATPase but like Mg^{++} this activation fell off rapidly as the ionic strength of the incubation medium increased. These ions differed in their effects, however, in that $5 \cdot 10^{-3}$ DNP stimulated the Mn^{++}-activated myofibrillar ATPase both in the presence and absence of added KCl (0.2 *M*).

The extent of stimulation of the myofibrillar ATPase by DNP when Ca^{++} was the activating ion was independent of the concentrations of enzyme, or of substrate in the range 10^{-3} to 10^{-2} *M*. In general the concentration of DNP required to bring about maximum stimulation increased with rising ionic strength although the maximum rates of ATP hydrolysis tended to fall off with increasing ionic strength. The effect of DNP was shown to be reversible by exposing myofibrils to $5 \cdot 10^{-3}$ *M*-DNP in an incubation medium which induced stimulation and then dialysing exhaustively against a solution containing 0.039 *M*-borate buffer pH 7.1 and 0.025 *M*-KCl. On further addition of DNP the ATPase of the dialysed myofibrils was stimulated in a manner similar to that of untreated myofibrils.

Low concentrations of phenylmercuric acetate (PMA) also stimulated the Ca^{++}-activated ATPase of myofibrils in the presence of KCl. For example in one experiment when the incubation medium contained 0.125 *M*-KCl, $1.25 \cdot 10^{-3}$ *M* PMA stimulated the ATPase activity 115%; higher concentrations of PMA produced inhibition.

Similar stimulation effects with PMA and DNP on myosin ATPase are reported by Drs. G. D. GREVILLE AND D. M. NEEDHAM in an accompanying communication[9].

The experiments described above were carried out at 20° C, but if incubations were carried out in the same medium at 0° C marked differences were observed in the stimulation of myosin and myofibrillar ATPases by DNP, and in the activation of the myofibrillar ATPase by Mg^{++}.

The effects were obtained in both TRIS and glyoxaline buffers of pH 7.3 and 7.0 respectively (measured at 20° C) and were independent of the pH changes resulting from cooling these buffers from 20° – 10° C.

They may be summarised as follows:

1. For a given ionic strength the stimulating effect of DNP is much reduced at 0° C. Addition of DNP (10^{-3} – 10^{-2} *M*) did not induce any appreciable stimulation of the Ca^{++}-activated myofibrillar ATPase until the concentration of added KCl was raised above 0.4 *M*, and whereas 50% stimulation could be obtained with $4 \cdot 10^{-3}$ *M*-DNP in the presence of 0.8 *M*-KCl, the stimulated activity was lower than that shown by the myofibrils in the absence of added KCl and DNP.

2. At 0° C the differences between the effect of DNP on the myosin and myofibrillar ATPases were not so great as those observed at 20° C. No stimulation of myosin was obtained in absence of added KCl and in general the stimulations obtained at higher ionic strength by DNP, expressed as percentages of activity in the absence of DNP, were much lower than those obtained at 20° C.

3. Whereas in the absence of DNP the pattern of Ca^{++} activation of the myofibrillar ATPase was similar at 0° C and 20° C, Mg^{++} activation was considerably modified at the lower temperature. At 0° C in the absence of added KCl Mg^{++} inhibits at low concentrations (*circa* 0.001 *M*) and activates only very slightly if at all in the range 0.005 to 0.01 *M*.

These investigations, which are now being extended, indicate that there are basic similarities in the action of DNP on myosin ATPase and on the mitochondrial ATPase systems which have been previously studied[2,10,11], although in the latter system stimulation is obtained at lower DNP concentrations.

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