the isolated RNA[8] was subjected to alkaline hydrolysis yielding 2'(3') mononucleotides which were separated chromatographically[9]. Pools of non-radioactive 5' UMP and orotic acid were added to the RNA hydrolysate before chromatography to localize any radioactivity arising from contamination by these substances, but they remained unlabeled. The results (Table I) show that the 2'(3') uridylic acid obtained from the RNA was labeled and it may be concluded that an acid-soluble 5'-nucleotide, or a derivative thereof was incorporated into a diester linkage in a molecule with the properties of RNA. The reaction probably results in the labeling of at least one type of RNA molecule. These conclusions are further supported by the studies with diesterase reported herewith[6]. In this reaction system, the specific activity of the RNA begins to decline after 45 minutes incubation and the proximal precursor is unknown. The studies by OCHOA *et al.*[2,3] offer possible explanations which are being tested in both instances.

TABLE I

RADIOACTIVITY OF NUCLEOTIDES OBTAINED BY ALKALINE HYDROLYSIS OF CYTOPLASMIC RNA

A 20% homogenate of rat liver in 0.25 *M* sucrose was centrifuged 10' at 600 *g* and the pellet was washed once with an equal volume of sucrose which was then combined with the first supernatant fraction to give the "cytoplasmic fraction". Fifteen ml of this preparation were mixed with an equal volume of a reaction supplement consisting of sucrose, 0.25 *M*, fumarate, 0.008 *M*, pyruvate and glutamate, 0.02 *M* each, $MgCl_2$, 0.006 *M*, and inorganic phosphate 0.02 *M*. All acidic compounds were in the form of the potassium salts and the supplement was adjusted to pH 7.2, aft r the orotic acid or 5' UMP were added. All components were at 0° until the incubation at 30° was begun, with air in the gas phase. Supplements of ATP, fructose and ribose-5-phosphate had no beneficial effect and ATP inhibited the incorporation. The reaction was stopped with perchloric acid at 20'.

Added precursor	*Acid-soluble fraction*		*RNA*
	cpm/μM precursor	*cpm/μA 5' UPM**	*cpm/μM 2' (3') UMP***
3 μ*M* Orotic acid-6-^{14}C	$1.5 \cdot 10^6$	342,000	88
25 μ*M* 5' UMP-4-^{14}C	$0.1 \cdot 10^6$	70,000	53

* Dilution of added precursor results from endogenous 5' UMP and equilibrating metabolites.
** The radioactivity of the other 3 nucleotides and of added 5' UMP and orotic acid was negligible.

VAN R. POTTER
L. I. HECHT
E. HERBERT

McArdle Memorial Laboratory, The Medical School, University of Wisconsin, Madison, Wis. (U.S.A.)

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Effects of 2:4-dinitrophenol and other agents on the nucleoside triphosphatase activities of L-myosin

Recent work has shown that DNP*[1,2] and PMA[1] increase the ATPase and decrease the ITPase velocity of Ca^{++}-activated L-myosin. We now report observations on other nucleoside-5'-triphosphatase activities of this enzyme.

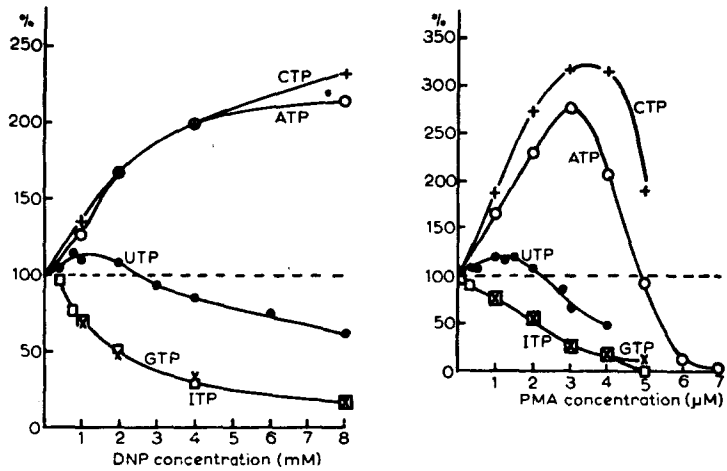
Thrice-precipitated L-myosin was prepared from rabbit muscle[3]. Activity was measured by incubating the enzyme (0.10 mg/ml) for 5 min at 25° with 0.05 *M* aminotris(hydroxymethyl)-methane chloride, pH 7.5, 2 m*M* substrate, and other additions as noted below. Unless otherwise

* Abbreviations: ATP, CTP, GTP, ITP, UTP, adenosine, cytidine, guanosine, inosine and uridine 5'-triphosphates; DNP, 2:4-dinitrophenol; EDTA, ethylenediaminetetraacetic acid; PMA, phenylmercuric acetate.

stated, reaction was started by addition of enzyme. Production of inorganic phosphate was determined by the method of FISKE AND SUBBAROW. Table I and Fig. 3 give, in absolute measure, the activities of myosin with the different substrates* under various experimental conditions. In Figs. 1 and 2 the activities with each substrate in the presence of DNP and PMA respectively are shown as percentages of the activity in absence of these two agents.

Effect of DNP (Fig. 1). This was studied in the presence of 5 m*M* $CaCl_2$ and 0.1 *M* KCl.

Effect of PMA (Fig. 2). 5 m*M* $CaCl_2$ was again added, but with 0.4 *M* KCl, because the percentage stimulation of ATPase activity due to PMA increases with the KCl concentration[1]. The enzyme was kept for 6 min at 25° in complete incubation medium containing PMA but no substrate, and hydrolysis begun by addition of the latter; pre-incubation of enzyme in the presence of PMA augments the stimulation by this agent.



Figs. 1 and 2. Effect of DNP and PMA on the rate of hydrolysis of various substrates by Ca^{++}-activated L-myosin. The ordinates represent enzymic activity expressed as a percentage of that in absence of DNP or PMA.

TABLE I

ENZYMIC ACTIVITY OF L-MYOSIN (μmol. P/mg PROTEIN/min) AT 25° (pH 7.5)

	0.1 *M* KCl 5 m*M* $CaCl_2$	0.4 *M* KCl* 5 m*M* $CaCl_2$	0.05 *M* KCl —	0.65 *M* KCl —	0.65 *M* KCl 10^{-4} *M* EDTA	0.65 *M* KCl 10^{-3} *M* EDTA	0.65 *M* KCl 10^{-2} *M* EDTA
ATP	0.84	0.42	0.07	0.28	1.30	1.88	1.54
CTP	0.50	0.23	0.02	0.08	0.22	0.51	0.43
UTP	1.89	1.00	0.04	0.09	0.08	0.28	0.18
ITP	1.79	1.66	0.04	0.08	0.12	0.10	0.07
GTP	1.04	1.20	0.07	0.07	0.05	0.04	0.03

* Reaction started by addition of substrate 6 min after enzyme.

It is seen that both DNP and PMA caused strong stimulation with ATP and CTP as substrates, slight stimulation with UTP, and only inhibition with ITP and GTP. It may be significant that increasing the KCl concentration from 0.1 to 0.4 *M* about halved the Ca^{++}-stimulated activity with ATP, CTP and UTP, but did not materially affect that with ITP and GTP (Table I, columns 1 and 2).

Effect of EDTA (Table I). Another substance affecting nucleoside triphosphatase activity is the chelating agent EDTA, which stimulates ATPase activity of KCl-activated L-myosin and actomyosin[4,5]; the ITPase and UTPase activities of actomyosin are inhibited[5]. Experiments with this agent were done with 0.65 *M* KCl and no Ca^{++} in the medium. It is clear (column 6) that, with L-myosin, the effects of EDTA roughly paralleled those with DNP and PMA (Figs. 1 and 2), acceleration being greatest with ATP and CTP, small with UTP, and negligible or absent with ITP and GTP.

* Commercial products were used (CTP, UTP, Pabst; ITP, Sigma; ATP, GTP, Pabst and Sigma).

Effect of NH_4Cl (Fig. 3). KIELLEY AND BRADLEY[6] reported that NH_4Cl is somewhat more effective than KCl in promoting activation of myosin by EDTA with ATP as substrate, and much more effective than KCl with 6-hydroxy substituted substrates. We find that with ATP, ITP, UTP and CTP, but not GTP, NH_4Cl is a potent activator even in absence of EDTA (00.5 *M* KCl was present throughout). One notes, however, that, with respect to substrates, there is no parallelism here with the effects of DNP, PMA or EDTA. BANGA[7] found that with actomyosin, NH_4Cl was inferior even to KCl as activator of ATPase. With L-myosin, however, we find that maximal activation of ATPase by NH_4^+ was as great as that produced by Ca^{++}, but much higher concentrations of NH_4^+ were required.

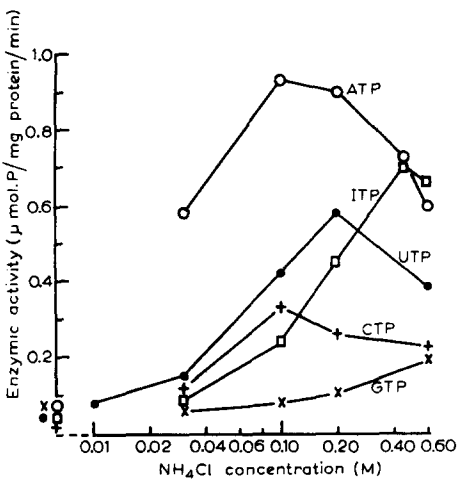


Fig. 3. Activation of L-myosin by NH_4Cl, with various substrates.

The experiments reported herein were done with a fixed reaction time*, at one pH value (at which enzymic activity is low[8]), with a single substrate concentration, and at one Ca^{++} level (optimal for ATP). With these reservations, the results indicate that, under the conditions described for each, DNP, PMA and EDTA all cause a large stimulation of activity with ATP and CTP, a smaller one with UTP, and none (or inhibition) with ITP and GTP. The actions of these three agents, under their appropriate conditions, may have something in common. Possibly they affect enzyme velocity by modifying some interaction, dependent on the 6-substitution, between the purine and pyrimidine moieties of the substrates and myosin. The 6-amino compounds ATP and CTP behave similarly, but the difference between UTP and the other two 6-hydroxy compounds requires explanation. Inosine and guanosine chelate internally with metals whereas uridine, like adenosine and cytidine, cannot[9]. (ATP and CTP may possibly form chelates by linkage of the 6-substituents and terminal phosphates with metal.) The order ATP > UTP > ITP found for stimulation by DNP, PMA and EDTA applies also to BLUM's[10] values for $1/K_m$. Since DNP accelerates with ATP and not ITP, and the enzyme appears to be saturated with ATP under our conditions, the effect of DNP is unlikely to be solely on K_m. The Bethesda workers[4,11] have studied the possibility that EDTA chelates with Mg bound to myosin. DNP might also act by chelation; however, a phenol which cannot chelate, pentachlorophenol (optimal concentration $2 \cdot 10^{-4} M$), also accelerates the ATPase activity of L-myosin.

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Department of Biochemistry, University of Cambridge (England) G. D. GREVILLE

Department of Physiological Chemistry, The Johns Hopkins School of Medicine, Baltimore, Md. (U.S.A.) EDWARD REICH**

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* Acceleration of ATPase activity by DNP and sub-optimal concentrations of PMA is constant throughout the incubation period.

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