DEPHOSPHORYLATION OF NUCLEOTIDES
BY INSECT FLIGHT MUSCLE

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Recent studies have demonstrated that nucleotide triphosphates, other than ATP, function in biological systems in a manner analogous to that of ATP. These purine and pyrimidine ribotides are now known to serve as coenzymes in the biosynthesis and interconversion of hexose phosphates (1–3), in the formation of phospholipides (4), and as phosphate donors or acceptors in carboxylation reactions (5, 6). Additional functions, yet to be uncovered, are probable in view of recent discoveries of the widespread occurrence of these nucleotides (7, 8). In rabbit skeletal muscle UTP and GTP have been found, each in concentrations as high as 2 to 3 per cent of the total nucleotide fraction. CTP is also present, although in smaller quantities. Moreover, in other experiments with rabbit muscle preparations, both UTP and CTP were able to replace ATP, mole for mole, in eliciting a contractile response, but GTP caused only reduced rates of tension development (9). These observations suggest that in the living system each of the nucleotides may have one or more specific roles and at the same time they emphasize the need for further study to ascertain the precise physiological significance of each in the over-all metabolism of muscle.

The importance of nucleotides in the intermediate metabolism of mammalian tissues is paralleled in insects. ATP from insects has been isolated and found to be identical with that of other organisms (10, 11). Further examination of the phosphorylated intermediates in flight muscle of houseflies indicates that approximately 12 per cent of the nucleotide fraction has bases other than adenine. Also, it has been established that insect muscle mitochondria (sarcosomes) can synthesize energy-rich phosphate bonds such as are found in ATP (12–14) and that this compound serves to provide the ultimate energy necessary for the contractile processes as well as for

1 The following abbreviations are used: ATP, ADP, and AMP for adenosine tri-, di-, and monophosphates; CTP and CDP for cytidine tri- and diphosphates; GTP, GDP, and GMP for guanosine tri-, di-, and monophosphates; ITP, IDP, and IMP for inosine tri-, di-, and monophosphates; UTP, UDP, and UMP for uridine tri-, di-, and monophosphates; P for inorganic phosphate; Tris for tris(hydroxymethyl)aminomethane; and DNP for 2,4-dinitrophenol. Unless otherwise indicated, the nucleotide phosphates are in the 5' position.

2 Sacktor, B., in preparation.
other metabolic reactions which take place in the muscle (15–18). It has recently been observed, however, that, during oxidative phosphorylation, uridine and inosine ribotides in addition to ADP are phosphorylated and that sarcosomes possess nucleotide transphosphorylating and dephosphorylating enzymes (19). The present study continues these latter observations and describes some of the characteristics of the nucleotide dephosphorylating enzymes in the mitochondrial fraction of insect flight muscle, together with the distribution and nature of such enzymes in other fractions.

**EXPERIMENTAL**

_Fractionation Procedures—_Houseflies, _Musca domestica_, of mixed sexes from 4 to 7 days old were used. These were reared and maintained by the procedure described previously (20). Flight muscle mitochondria were isolated by the technique already detailed (12, 15). The sarcoplasm and particulate fractions were obtained as explained earlier (21). The particulate fraction consists of muscle fibrils and sarcosomes.

_Materials_—ATP, AMP, UTP, UMP, and CTP were purchased from the Pabst Laboratories and ADP, A-3'-MP, ITP, IDP, IMP, GTP, and hexokinase (practical type III) from the Sigma Chemical Company. U-(2',3')-MP and G-(2',3')-MP were obtained from the Schwarz Laboratories, Inc., and U-(2',3'),5'-DP was prepared and kindly furnished by Dr. H. G. Khorana (22).

_Methods_—Nucleotide dephosphorylation activity was assayed essentially as described in earlier experiments for ATPase activity (12). Reaction mixtures were incubated at room temperature (22–25°C). The reaction was stopped by the addition of 0.2 ml. of cold 50 per cent trichloroacetic acid. Experimental mixtures were accompanied by non-incubated controls, identical in composition, to which the acid was added immediately before addition of the enzyme. P values determined at this zero time were subtracted from all subsequent values. P was measured by the method of Fiske and Subbarow (23). Protein, in mitochondrial suspensions and other fractions, was determined by the method of Lowry _et al._ (24).

**Results**

_Substrate Specificity and Rates of Hydrolysis_—The specificity of the dephosphorylating mechanism in flight muscle mitochondria is demonstrated in Table I and in Fig. 1. It is apparent that, in addition to their previously known ATPase activity (15, 25), sarcosomes will hydrolyze ITP, GTP, UTP, and CTP. P is also liberated from ADP and IDP, but their monophosphates, AMP and IMP, are not dephosphorylated. As shown in Fig. 1, GTP is like ATP and ITP in that only the two labile phosphates are removed. In marked contrast to these purine ribotides are the pyrimi-
dine ribotides, UTP and CTP, from which all three phosphates are released. Table I also reveals that insect mitochondria catalyze the dephosphorylation of 5'-nucleotides only, for the sarcosomes were inactive with U-(2',3')-MP, G-(2',3')-MP, and A-3'-MP. Under our experimental conditions, however, the amount of P liberated from U-(2',3'),5'-DP was of question-

**Table I**

*Substrate Specificity and Relative Rate of Nucleotide Dephosphorylation by Insect Flight Muscle Mitochondria*

The reaction mixture contained the following components: Tris buffer, pH 7.4, 30 μmoles; MgCl₂, 1 μmole; nucleotide, 2.5 μmoles; 0.05 ml. of mitochondrial suspension (approximately 0.7 mg. of protein); and 1.5 per cent KCl to make the final volume 1.0 ml. Incubation time, 1 hour. Each datum is based on two to three replicates with different enzyme preparations.

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>P hydrolyzed in 1st hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>100*</td>
</tr>
<tr>
<td>ADP</td>
<td>25</td>
</tr>
<tr>
<td>AMP</td>
<td>0</td>
</tr>
<tr>
<td>A-3'-MP</td>
<td>0</td>
</tr>
<tr>
<td>ITP</td>
<td>56</td>
</tr>
<tr>
<td>IDP</td>
<td>8</td>
</tr>
<tr>
<td>IMP</td>
<td>0</td>
</tr>
<tr>
<td>GTP</td>
<td>65</td>
</tr>
<tr>
<td>G-(2',3')-MP</td>
<td>0</td>
</tr>
<tr>
<td>UTP</td>
<td>22</td>
</tr>
<tr>
<td>UMP</td>
<td>6</td>
</tr>
<tr>
<td>U-(2',3'),5'-DP</td>
<td>1</td>
</tr>
<tr>
<td>U-(2',3')-MP</td>
<td>0</td>
</tr>
<tr>
<td>CTP</td>
<td>6</td>
</tr>
</tbody>
</table>

* The value of 100 for ATP corresponds to a rate of 1.90 μmoles of P hydrolyzed per hour per mg. of protein.

It is also known from earlier studies that hexose phosphates, glycerophosphates, and inorganic pyrophosphate are not suitable substrates for the mitochondrial dephosphorylating enzymes (15).

Table I also shows the relative rate of nucleotide dephosphorylation. The sarcosomes are most active towards ATP, but appreciable rates are also obtained with GTP and ITP, both of which are dephosphorylated at about the same rate. On the other hand, the velocity of hydrolysis with UTP and in particular with CTP is considerably less. P is liberated from
ADP at a rate 25 per cent of that from ATP. ADP, however, is about 3 times as active as IDP. Apparently, the presence of P on the (2′,3′) position in U-(2′,3′),5′-DP markedly reduces the hydrolysis of P from the 5′ position.

Direct Dephosphorylation of Nucleoside Triphosphates—Additional experiments indicate that the P liberated from the five nucleoside triphosphates is due to direct dephosphorylation of these nucleotides rather than to the action of nucleoside diphosphokinase followed by ATPase. Nucleoside diphosphokinase described from yeast and vertebrate muscle (26, 27) mediates the reactions as follows:

(1) \[ \text{ITP} + \text{ADP} = \text{IDP} + \text{ATP} \]

(2) \[ \text{UTP} + \text{ADP} = \text{UDP} + \text{ATP} \]

Since catalytic quantities of ADP may have been present either as a constituent of the isolated mitochondria or as an impurity in commercial nucleotide samples, it is possible that ATP would be synthesized if diphosphokinase occurred in insect mitochondria. Whether this enzyme is present here or not, the insignificance of its reaction in the nucleotide dephosphorylation activities observed in these experiments was demonstrated in the following manner. The glucose-hexokinase system was combined with

Fig. 1. Time-course of nucleotide hydrolysis by insect flight muscle mitochondria. The reaction mixture contained the following components: Tris buffer, pH 7.4, 140 \( \mu \text{moles} \); \( \text{MgCl}_2 \), 4 \( \mu \text{moles} \); nucleotide, 10 \( \mu \text{moles} \); and 2.0 ml. of mitochondrial suspension. Total volume, 4.0 ml. The protein content per ml. of mitochondrial suspension was 20.5 mg. for ATP, ITP, and UTP, 14.0 mg. for GTP, and 26.6 mg. for CTP. 0.2 ml. aliquots of the reaction mixture were withdrawn for each point. Horizontal broken lines represent, from top to bottom, total P, acid-labile P, and first P for each nucleotide.
either the ITP or the UTP dephosphorylation system. Thus, if ATP were formed by nucleoside diphosphokinase activity, then no P would be liberated. Conversely, if the dephosphorylation of ITP and UTP were unaffected by the addition of this trapping system, then ATP would not be formed, nor would it be a prerequisite for the hydrolysis of the other nucleotides. It was found that as much P is liberated from ITP and UTP in the presence of glucose-hexokinase as in its absence. These results establish the fact that mitochondrial enzymes dephosphorylate the nucleoside triphosphates directly.

Number of Enzymes Involved—Since it is now known that insect mitochondrial preparations can catalyze the dephosphorylation of five nucleoside triphosphates, information was sought as to the number of enzymes involved. Evidence bearing on this problem was obtained by experiments with mixed substrates.

Fig. 2 shows the relationship between ATP concentration and ATPase activity. As determined by the method of least squares, $K_s$, the Mi-
Michaelis-Menten constant, is \(3.17 \times 10^{-3} \, \text{m}\). Fig. 2 also shows that the addition of ADP (1.3 \(\mu\)moles) diminishes the rate of evolution of P from ATP. Analysis of the data according to procedure of Lineweaver and Burk (28) reveals that ADP satisfies the requirements for a competitive inhibitor of ATPase and that \(K_I = 4.43 \times 10^{-4}\). In contrast to the action of ADP, IDP (2.2 \(\mu\)moles) is without significant effect on the dephosphorylation of ATP. However, an interpretation of our results with combinations of ATP plus ITP is not as obvious as for mixtures of ATP with either ADP or IDP. The addition of ITP (2.0 \(\mu\)moles) to several different concentrations of ATP resulted in an increase in the rate of P formation over what was obtained with that concentration of ATP alone, but the dephosphorylation velocity of the combination was less than the additive rate obtained from the action of the mitochondria on the two substrates separately. The magnitude of this increase in P, owing to the addition of ITP to ATP, was approximately constant throughout a wide range of ATP concentrations, even though at low ATP concentrations ATPase was far from saturated with its substrate and the ITP concentration used was below its \(K_s\) for ITP dephosphorylation.

The relationship between ITP concentration and ITP dephosphorylation is shown in Table II. A Lineweaver-Burk analysis of the data gave a \(K_s\) value of \(2.85 \times 10^{-3} \, \text{M}\). In analogy to the situation with ATP, in which the dephosphorylation product competitively inhibits dephosphorylation, with ITP as substrate the product IDP is a competitive inhibitor. The dissociation constant, \(K_I\), of this inhibitor-enzyme complex is \(1.59 \times 10^{-3}\). The dephosphorylation mechanisms of ATP and ITP differ, however, for IDP has little effect on ATPase, whereas ADP is an extremely effective

### Table II

<table>
<thead>
<tr>
<th>ITP concentration (mM)</th>
<th>Activity, (\mu)moles P hydrolyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ITP alone</strong></td>
</tr>
<tr>
<td>0.8</td>
<td>0.19</td>
</tr>
<tr>
<td>1.2</td>
<td>0.32</td>
</tr>
<tr>
<td>2.0</td>
<td>0.42</td>
</tr>
<tr>
<td>2.8</td>
<td>0.53</td>
</tr>
<tr>
<td>4.0</td>
<td>0.71</td>
</tr>
</tbody>
</table>
inhibitor of ITP hydrolysis. As shown in Table III, the inhibition of ITPase by ADP is proportional to the ADP concentration. At concentrations of $5.3 \times 10^{-5}$ M and less, ADP satisfies the requirements for a competitive inhibitor, and $K_I$ is approximately $1.65 \times 10^{-5}$. At higher concentrations, ADP appears to act as a non-competitive inhibitor. The

TABLE III

Inhibition of ITP Dephosphorylation by ADP

The components of the system were as indicated in Table II.

<table>
<thead>
<tr>
<th>ITP concentration</th>
<th>Activity, μmole P hydrolyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td>No ADP</td>
</tr>
<tr>
<td>2.0</td>
<td>0.53</td>
</tr>
<tr>
<td>2.8</td>
<td>0.59</td>
</tr>
<tr>
<td>4.0</td>
<td>0.70</td>
</tr>
<tr>
<td>7.0</td>
<td>0.89</td>
</tr>
<tr>
<td>10.0</td>
<td>0.94</td>
</tr>
</tbody>
</table>

TABLE IV

Inhibition of GTP Dephosphorylation by ADP and IDP

The reaction mixture contained 30 μmoles of Tris buffer (pH 7.4), 1.0 μmole of MgCl₂, 5 μmoles of GTP, ADP, or IDP as indicated, 0.2 ml. of mitochondrial suspension (2.8 mg. of protein), and 1.5 per cent KCl to bring the final volume to 1.0 ml, 8 minute incubation period. Corrections were made for dephosphorylation of ADP and IDP in the experiments with combined nucleotides.

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Activity, μmole P hydrolyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTP</td>
<td>0.40</td>
</tr>
<tr>
<td>&quot; + ADP, 1.33 mM</td>
<td>0.10</td>
</tr>
<tr>
<td>&quot; + &quot; , 0.13 &quot;</td>
<td>0.13</td>
</tr>
<tr>
<td>&quot; + &quot; , 0.013 &quot;</td>
<td>0.28</td>
</tr>
<tr>
<td>&quot; + IDP, 2.2 mM</td>
<td>0.18</td>
</tr>
</tbody>
</table>

dephosphorylation of relatively large amounts of ITP can be completely inhibited by $1.3 \times 10^{-3}$ M ADP.

Table IV shows that ADP and IDP also inhibit the dephosphorylation of GTP. With this substrate also, ADP is again a more effective inhibitor than is IDP. It is also apparent that the inhibition of GTPase is a function of ADP concentration but that the effect of ADP on GTPase is different from its action on ITPase. Low ADP concentrations ($1.3 \times 10^{-5}$ M) inhibit both GTP and ITP hydrolysis to the same extent. On the other hand, though high ADP concentrations ($1.3 \times 10^{-3}$ M) completely block P
release from ITP, substantial P is still liberated from GTP under these conditions. GTPase is like ITPase in that both are inhibited by IDP. However, GTPase is different from ATPase since ATPase is not inhibited by IDP.

The use of mixed nucleotides further substantiated our finding that the mechanism of pyrimidine ribotide dephosphorylation is distinct from that of purine ribotide breakdown. We observed that neither ADP \((1.3 \times 10^{-3} \text{ M})\) nor IDP \((2.2 \times 10^{-3} \text{ M})\) inhibited the dephosphorylation of UTP \((7.5 \times 10^{-3} \text{ M})\).

Stimulation of Nucleotide Dephosphorylation by Dinitrophenol—The catalytic effect of DNP on mammalian mitochondrial ATPase is well known

<table>
<thead>
<tr>
<th>DNP (m \times 10^{-4})</th>
<th>ATP</th>
<th>ITP</th>
<th>GTP</th>
<th>UTP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1.25</td>
<td>1.23</td>
<td>1.82</td>
<td>1.38</td>
<td>1.36</td>
</tr>
<tr>
<td>6.25</td>
<td>1.83</td>
<td>1.81</td>
<td>1.84</td>
<td>1.86</td>
</tr>
<tr>
<td>12.50</td>
<td>2.34</td>
<td>2.10</td>
<td>2.16</td>
<td>2.12</td>
</tr>
</tbody>
</table>

In Table V it is shown that the ATPase from insect flight muscle sarcosomes is also stimulated by this uncoupling agent, and that it enhances the mitochondrial dephosphorylation of ITP, GTP, and UTP, as well as of ATP. It is also clear from Table V that \(m \times 10^{-3}\) DNP caused an increase of about 100 per cent in rate of P release and that the dephosphorylation of each of the nucleoside triphosphates was stimulated to the same extent by a given concentration of DNP. These observations indicate that one of the characteristic properties of mitochondria, latent ATPase activity \((31)\), can now be extended to include the other nucleoside triphosphates.

**Metal Ion Activation**—Previous experiments with insect flight muscle demonstrated that mitochondrial ATPase is activated by Mg and Mn, whereas Ca is slightly inhibitory \((15)\). The effect of these metal ions on the dephosphorylation of other nucleoside triphosphates has been exam-
ined and the results are shown in Table VI. In general, the hydrolyses of
the four nucleotides, ATP, ITP, GTP, and UTP, are similar in that Mg
and Mn stimulate and Ca does not. Mn appears to be a more potent
activator of nucleotide breakdown than is Mg. Some distinctions in the
response of each of the dephosphorylating enzymes to these ions may be
noted, for Ca (1 X 10^{-3} M) markedly inhibits P liberation from ITP and
GTP, whereas it reduces ATPase activity only slightly.

**Table VI**

**Distribution of Nucleotide Dephosphorylation Activity in Insect Flight Muscle and Effect of Bivalent Cations**

The reaction mixtures contained 30 μmoles of Tris buffer, pH 7.4. In determina-
tions with mitochondria and myofibrils plus mitochondria, 1.0 μmole of metal ion
and 0.1 ml. of enzyme were used, and with sarcoplasm 10 μmoles of metal ion and 0.3
ml. of enzyme were used. The nucleotide content was 1.5 μmoles, except for ATP,
with which 2.5 μmoles were used. The final volume was made to 1.0 ml. with 1.5
per cent KCl. The values are given in micromoles of inorganic phosphate hydro-
lyzed per 10 minutes per mg. of protein.

<table>
<thead>
<tr>
<th>Muscle fraction</th>
<th>Nucleotide</th>
<th>Bivalent cation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>ATP</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>ITP</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>GTP</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>UTP</td>
<td>0.04</td>
</tr>
<tr>
<td>Myofibril + mitochondria</td>
<td>ATP</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>ITP</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>GTP</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>UTP</td>
<td>0.07</td>
</tr>
<tr>
<td>Sarcoplasm</td>
<td>ATP</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>ITP</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>GTP</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>UTP</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Nucleotide Dephosphorylating Enzymes in Other Fractions of Flight Muscle**

In an earlier study it was found that the housefly flight muscle possesses
three distinct mechanisms for ATP hydrolysis and that these are associ-
ated, respectively, with the myofibrillar, mitochondrial, and sarcoplasmic
components of the muscle (15). Similar cellular fractions were procured
for the present experiments and were examined for the distribution and
metal activation of enzymes that dephosphorylate other nucleotides. The
results shown in Table VI demonstrate that P is liberated from ITP, GTP,
and UTP, as well as from ATP, by enzymes present in the fibrils and sarco-
plasm as well as in mitochondria. The fibrillar fraction contains enzymes
which are stimulated by Mg, Mn, and Ca. This fraction, however, includes both mitochondria and muscle fibrils. Thus, the activation by Mg and Mn could be attributed to the nucleotide dephosphorylases in these mitochondria, whereas it becomes evident that insect muscle fibrils must possess Ca-activated enzymes that hydrolyze ITP, GTP, and UTP in addition to ATP.

Examination of Table VI also reveals that, although the sarcoplasm contains enzymes that dephosphorylate all the nucleotides, specific differences in the metal ion activation of these enzymes are obvious. In this muscle fraction, the hydrolysis of ATP is stimulated by Mg and Mn but inhibited by Ca. Ca also inhibits the dephosphorylation of the other nucleotides. However, in contrast to the activation of ATP hydrolysis by both Mg and Mn, the liberation of P from ITP is stimulated only by Mg. Furthermore, the dephosphorylation of GTP and UTP is actually inhibited by Mn and with these two nucleotides Mg gives only a slight activation. These results provide additional evidence for the presence in insect flight muscle of a multitude of enzymes capable of hydrolyzing nucleotides and, at the same time, direct attention to the complexity as well as to the individuality of their enzymatic properties.

**DISCUSSION**

The data given demonstrate that mitochondria isolated from the flight muscle of houseflies hydrolyze the nucleoside triphosphates, ATP, ITP, GTP, UTP, and CTP. Our results show also that, irrespective of the substrate, the enzymes concerned have several properties in common. First, appreciable increases in their activity can be elicited by DNP, and this suggests that these enzymes exist in sarcosomes in a partially latent state. Also, both Mn and Mg activate dephosphorylation, the former being the more effective. In all cases Ca is inhibitory, although the magnitude of this inhibition is dependent on the particular nucleotide hydrolyzed. Furthermore, all the nucleotides that are dephosphorylated by these enzymes have phosphate groups on the 5' hydroxyl.

Despite such similarities, other evidence demonstrates the individual character of these enzymes. Purine ribotides are dephosphorylated in a manner distinctly different from that of pyrimidine ribotides. With ATP, ITP, and GTP, only the two labile phosphates are released. In contrast, all three phosphates of UTP and CTP are hydrolyzed. Moreover, the liberation of P from purine ribotriphosphates is inhibited by one or both of their corresponding diphosphates, ADP and IDP. These diphosphates have no effect on the hydrolysis of UTP.

Our results with mixtures of adenosine and inosine nucleotides are in reasonable agreement with similar experiments in which a specific ATPase
found in liver mitochondria (32) or a water-soluble ATPase found in vertebrate and locust muscle (33, 34) was used. With fly muscle mitochondria, as well as with these other preparations, ADP inhibits ATPase; both ADP and IDP inhibit ITPase, ADP being the more effective; and the rate of P release from a combination of ATP and ITP is less than the rate computed from the action of the preparation on the two substrates separately. From such evidence, Goldberg and Gilmour (33) concluded that a single enzyme is responsible for the hydrolysis of ATP and ITP. Our data do not support this concept for the following reasons. First, IDP has little or no effect on ATPase, yet it inhibits ITPase and GTPase. Furthermore, although the rate of P liberation from a combination of ATP and ITP is less than the additive rate of each substrate separately, such results do not necessarily argue against the presence of two separate enzymes, an ATPase and an ITPase. Examination of our data on this point (Fig. 2) reveals that the addition of ITP to several different concentrations of ATP results in an increase in the rate of P formation over what is obtained with that concentration of ATP alone. The magnitude of this increase is approximately constant throughout a wide range of ATP concentrations, even though at low ATP concentrations both ATPase and ITPase are far from saturated with their respective substrates. With these experimental conditions, if a single enzyme is responsible for the dephosphorylation of both nucleotides, one would expect considerably more ITP breakdown when it is in combination with a low ATP concentration than with a high ATP level. This does not occur. Our results as well as those of Goldberg and Gilmour (33) can be explained more readily not by an interaction between substrates ATP and ITP for a single enzyme but rather by an inhibition of ITPase by ADP, the product of ATPase.

Other data also support the viewpoint that there is in mitochondria a specific ATPase as well as an ITPase. ADP inhibits hydrolysis of both ATP and ITP, and one can show that the diphosphate acts as a competitive inhibitor. However, if a single enzyme dephosphorylates both substrates, then $K_i$ values for the inhibitor-enzyme complexes should be identical, since values of $K_i$ for ITP and ATP are approximately equal. Again, this does not agree with our findings, for the dissociation constant of the ADP-ATPase complex is about 30 times greater than for the ADP-ITPase complex. In light of this new evidence it seems best to conclude that there is a specific enzyme (or enzymes) for the dephosphorylation of each of the purine nucleoside triphosphates. (It is not yet shown that the enzyme for CTP is different from that for UTP, which is different from the three purine enzymes.)

Since sarcosomes catalyze the dephosphorylation of five nucleoside triphosphates, the question arises as to the number of enzymes responsible
for the hydrolysis of each of these nucleotides. This is of considerable interest since it was clearly demonstrated previously that the liberation of both labile phosphates from ATP by these mitochondria occurs through the combined action of two enzymes, a specific ATPase and an adenylate kinase (15). Unfortunately, our present data do not permit a similarly definitive answer with respect to the other nucleoside triphosphates. However, recent observations by others are useful in pointing out the pathways which may play a role in our preparations.

It has been shown above that nucleoside diphosphokinase is not a factor in the dephosphorylation activities observed in these experiments. This indicates that there is a direct hydrolysis of each nucleoside triphosphate to its corresponding diphosphate. We also know that ADP is metabolized further by the adenylate kinase reaction (35),

\[
2\text{ADP} = \text{ATP} + \text{AMP}
\]

Recently, other enzymes have been found, in yeast and liver, which, although not identical with adenylate kinase, are analogous to it (36, 37). These catalyze the transformation of other nucleoside diphosphates as follows:

\[
2\text{UDP} = \text{UTP} + \text{UMP}
\]

\[
2\text{GDP} = \text{GTP} + \text{GMP}
\]

In addition to these schemes, Plaut (38) found an enzyme (or enzymes) from liver mitochondria which converts the diphosphates IDP, UDP, and GDP, to their corresponding monophosphates by direct hydrolysis of the terminal phosphate. However, neither ADP nor GDP acts as a substrate for this enzyme. It is apparent that either this nucleoside diphosphatase or the adenylate kinase type of enzymes in conjunction with the specific nucleoside triphosphatases found here may account for the dephosphorylation of the two labile phosphates from all triphosphates, except CTP. The complete hydrolysis of the pyrimidine nucleotides to their respective nucleosides cannot be attributed to these mechanisms and this suggests that insect mitochondria possess a previously undescribed enzyme (or enzymes) which hydrolyzes pyrimidine ribomonophosphates.

Gilmour claimed that the Mg-activated ATP dephosphorylation activity in fly mitochondria is an apyrase, presumably because two labile phosphates are released from ATP and ITP and because IDP is not a substrate for adenylate kinase (25). It is obvious that these criteria are not sufficient reasons for such a classification, since (a) IDP could be dephosphorylated by an IDPase; (b) five nucleotide triphosphates are hydrolyzed and three phosphates are liberated from the pyrimidine ribotriphosphates; and (c) the conversion of ADP to ATP by adenylate kinase is a prerequisite for
the production of P from ADP (15). All the evidence cited above is consistent with the conclusion that these mitochondria have a specific nucleoside triphosphatase for each of the nucleoside triphosphates.

Our findings that myofibrils possess Ca-activated enzymes that dephosphorylate nucleoside triphosphates other than ATP supplement the reports of Ranney (9), Kielley et al. (39), and Blum (40), who showed that these nucleotides can elicit a contractile response from a model muscle system or are dephosphorylated by myosin preparations. These observations, plus the fact that these nucleotides also occur in muscle (7), suggest that more than one nucleotide may react with the contractile elements in vivo and that a reexamination of the physiological role of nucleotides in muscle contraction is warranted.

**SUMMARY**

Insect flight muscle mitochondria dephosphorylate the triphosphates of adenosine, guanosine, inosine, uridine, and cytidine. The rates of hydrolysis for these nucleotides are in the order listed above. Mitochondria liberate the two labile phosphates from the purine nucleoside triphosphates. In contrast, all three phosphates are released from the pyrimidine analogues. Magnesium and manganese activate dephosphorylation, manganese being the more potent of the two. Calcium is inhibitory. Dinitrophenol stimulates the hydrolysis of all triphosphonucleotides to the same extent.

Adenosine and inosine diphosphates are competitive inhibitors of their respective triphosphatases. Adenosine diphosphate is also an extremely effective inhibitor of inosinetriphosphatase and guanosinetriphosphatase. Inosine diphosphate has no effect on adenosinetriphosphatase, but it inhibits guanosinetriphosphatase. Neither purine ribodiphosphate inhibits uridinetriphosphatase. The significance of these observations is discussed and it seems best to conclude that there is in mitochondria a specific nucleoside triphosphatase for each of the nucleoside triphosphates.

In addition to the mitochondria, other portions of the flight muscle, myofibrils and sarcoplasm, can hydrolyze the triphosphates of adenosine, inosine, guanosine, and uridine. The enzymes in the sarcoplasm are activated by magnesium and inhibited by calcium. The effect of manganese depends on the nucleotide being dephosphorylated. The dephosphorylation of these four nucleoside triphosphates by the myofibrils is activated by calcium.

**BIBLIOGRAPHY**

DEPHOSPHORYLATION OF NUCLEOTIDES BY INSECT FLIGHT MUSCLE
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