THE ENTHALPY CHANGE OF ADENOSINE TRIPHOSPHATE HYDROLYSIS*

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Because the conversion of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and orthophosphate (P) is a part of so many biological energy transfers, the thermodynamics of this conversion is of special biological importance. For some time, the free energy change and the enthalpy change associated with this process have been thought to be large compared to the corresponding quantities for other organic phosphate hydrolyses, and this circumstance has led to the popular idea that ATP is an "energy-rich" compound. In the present paper we submit measurements of the enthalpy change of ATP hydrolysis which in magnitude are considerably smaller than those hitherto reported for ATP, but which are consistent with recent measurements on simple polyphosphates. Together with parallel developments in free energy measurement, our results suggest a major quantitative revision of ATP energetics; such a revision, however, does not threaten the energy-donor role of ATP.

If a quantity of ATP hydrolyzes to equilibrium in a medium of maintained acidity (H⁺), so that the final total concentrations of participants are (ATP), (ADP), and (P), the relation

\[ e^{-\Delta F^o / RT} = \frac{(ADP)(P)}{(ATP)(H_2O)} \]

defines what has come to be called the "standard free energy of ATP

* An earlier phase of this study is being reported elsewhere by one of us (R. J. P.) and Professor J. M. Sturtevant. The calorimeter used in this study will be separately described by its inventors, Dr. T. H. Benzinger and Dr. C. Kitzinger.

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‡ The views expressed in this article are those of the authors and do not necessarily reflect the opinions of the Navy Department or of the Naval Service at large.

1 Here and elsewhere the conventional assumption is made that the activity of water in the solutions considered is unity. Also, since activity coefficients are not explicitly included, equilibrium constants, "standard" free energy changes, and enthalpy changes are "apparent" (this commonly used adjective is unfortunate because, of course, the quantities are real enough under the conditions measured); i.e., they differ from the corresponding "thermodynamic" values by terms involving the
HEAT OF ATP HYDROLYSIS

dehphosphorylation," *viz.* $\Delta F^\circ$. Corresponding to this quantity there is also an "enthalpy of ATP dephosphorylation," $\Delta H$, which in principle is derivable from $\Delta F^\circ$ by the Gibbs-Helmholtz relation

$$\frac{\partial \left( \frac{\Delta F^\circ}{T} \right)}{\partial \left( \frac{1}{T} \right)} = \Delta H$$

(2)

Until recently, both $-\Delta F^\circ$ and $-\Delta H$ have been considered to be of the order of 10 to 12 kilocalories per mole (1-3).

ATP, ADP, and P are all polybasic acids, so that $\Delta F^\circ$ and $\Delta H$, as defined above, clearly *depend upon* $[H^+]$. In order to examine this dependence, and in order to relate these quantities to better established thermodynamic conventions, we consider the four equilibrium constants ($K_1, K_2, K_3,$ and $L$) involved in the accompanying over-all dephosphorylation process.

$$L \quad \text{ATP}_4^- + H_2O \rightleftharpoons \text{ADP}_2^- + P^2^- +$$

$$H^+ \quad \text{ADP}_2^- \rightleftharpoons H^+ \quad K_2$$

$$K_1 \quad \text{ATP}_3^- \rightleftharpoons H^+ \quad P^- \quad K_3$$

In terms of the quantities defined implicitly by Equation 3 one may show (4, 5) that

$$e^{-\Delta F^\circ/RT} = \frac{L(1 + K_2^*) \left( 1 + \frac{1}{K_3^*} \right)}{1 + \frac{1}{K_1^*}}$$

(4)

and by applying Equation 2, that, for $\text{pH} = -\log [H^+]$,

$$\Delta H(\text{pH}) = \Delta H_L + \left( \frac{1}{1 + K_1^*} \right) \Delta H_1 + \left( \frac{K_2^*}{1 + K_2^*} \right) \Delta H_2 - \left( \frac{1}{1 + K_2^*} \right) \Delta H_3$$

(5)

where

$$K_i^* = \frac{K_i}{[H^+]}; \quad i = 1, 2, 3$$

(6)

activity coefficients. These corrective terms do not vanish save at infinite dilution, but, as will presently be shown in the case of enthalpy changes, the corrective terms do not appear to be very large.
ΔH_L is the enthalpy change of the L process (Equation 3) and ΔH_i, etc., are the heats of ionization. Equation 5 shows that ΔH(pH), the enthalpy change associated with converting 1 mole of ATP, in ionization equilibrium at acidity (H^+), into 1 mole each of ADP and P, in ionization equilibrium at acidity (H^+), not only contains the heat of hydrolysis per se (ΔH_L), but also contains contributions from the acid ionizations of the various participants. In these accessory contributions, the coefficients of the heats of ionization (ΔH_1, ΔH_2, ΔH_3) must therefore be the moles of protons produced by each ionization per mole of ATP hydrolyzed. Since the acidity is constant, whatever device maintains the pH must remove, per mole of ATP hydrolyzed, a number of moles of protons,

\[ \varphi_H(pH) = \left( \frac{1}{1 + K_1^*} \right) + \left( \frac{K_2^*}{1 + K_2^*} \right) - \left( \frac{1}{1 + K_3^*} \right) \]  

(7)

If the pH-maintaining device is a buffer system (B),

\[ BH^+ \rightleftharpoons B + H^+ \]  

(8)

then, per mole of ATP hydrolyzed, \( \varphi_H \) moles of B must be converted into \( \varphi_H \) moles of \( BH^+ \), so that, per mole of ATP hydrolyzed, the system as a whole suffers an observable enthalpy change,

\[ \Delta H_{obs} = \Delta H - \varphi_H \Delta H^{(ioniz)} \]  

(9)

where \( \Delta H^{(ioniz)} \) is the heat of ionization of the particular buffer system employed. In the present work we have measured \( \Delta H_{obs}(pH 8) \) and \( - \Delta H^{(ioniz)} \), for various buffer systems, by direct calorimetry, and we have measured \( \varphi_H(pH 8) \) by compensatory titration with base in unbuffered reaction systems (see the accompanying paper by Bernhard (6)). A pH of 8 was selected because this pH is high enough to effect certain theoretical simplifications, and myosin, the enzyme used to catalyze the hydrolysis, is both stable and effective at this pH.

**EXPERIMENTAL**

*Enzyme*—Myosin was prepared from rabbits according to a slight modification of the Weber-Edsall procedure (7); its ATPase properties have been characterized elsewhere (8). Calorimetric measurements were made with myosin that had been precipitated at least four times. Such preparations removed only the terminal phosphate groups from ATP. Residual phosphatase activity, with products ADP and P as substrates, amounted to less than 1 per cent per hour.

*Substrates*—Sodium ATP was supplied by Pabst (Lots 116 and 117) and “crystalline” sodium ATP by Sigma (Lot 44-94). Further purification was considered to be unnecessary, since enzyme kinetic measurements...
with these compounds after treatment with Dowex 1 according to the procedure of Cohn and Carter (9) were quantitatively indistinguishable from those with untreated preparations. The sodium ADP and sodium inosine triphosphate (ITP) were supplied by Pabst and Sigma, respectively, and were used without further purification.

**Calorimetry**—For this study the calorimeter was checked electrically, as well as by measurement of the heat of formation of water (10). Checks of other sorts were made by measuring reaction heats for which reliable values could be found in the literature (the heat of dilution of KCl (11), HCl (11), urea (11), etc.). Only glass or Lucite reaction vessels were employed. Enzymatic runs were carried out in “equicompartmented” vessels containing (before mixing) equal volumes (5 to 8 ml.) of enzyme and substrate solutions; heats of neutralization were measured in “drop” vessels containing 0.03 to 0.10 ml. of one solution and 10 to 20 ml. of the other. The total heat developed in the reactions studied was from 6 to 400 milli-calories. Control experiments measuring heats of dilution indicated that non-reproducible, exothermic physical effects with accompanying heats of the order of 1 millicalorie introduce a non-random uncertainty in the calorimetry. The instrument was operated in a room maintained at 20° ± 2°. We are indebted to Dr. T. H. Benzinger and Dr. C. Kitzinger for making the thermal measurements.

**Analyses**—Orthophosphate was assayed by the Fiske-Subbarow method (12) after deproteinization with trichloroacetic acid. Deamination of adenine nucleotide was measured by differential spectrophotometry with the Beckman model DU spectrophotometer (13). In the presence of phosphate buffer, dephosphorylation of ATP was verified by the hexokinase-glucose-6-phosphate dehydrogenase method of Kornberg (14). Acid production upon ATP hydrolysis was measured electrotitrimetrically (15, 16).

**Conditions of Measurement**—The dephosphorylation reaction was studied at ionic strengths ranging from 0.05 to 0.7. Three buffers were employed: tris(hydroxymethyl)aminomethane (Tris), glycyglycine, and phosphate. In some experiments Tris and phosphate were used together. The pH of the dephosphorylating system was in every case maintained at 8.0 ± 0.1. Most of the hydrolyses were carried out at 20° ± 2°. In the early part of the study, 0.001 M Ca++ was present in the reaction mixture to activate the myosin. This was later discontinued to avoid the possibility of Ca++ complexes with reactants or products or both (17).

**Calorimetric Runs**—Equal volumes of substrate and enzyme solutions, both in the same electrolyte and at the same pH (±0.02), were placed in one vessel of the calorimeter. The calorimetric tare contained either water or enzyme solution and buffer or water. After thermal equilibration (1 to 3 hours), the reactants were mixed and the reaction was allowed to
run to completion. Analysis for orthophosphate was then performed on the products of the reaction. In some runs the products were not analyzed directly, but the phosphate value was taken from a parallel experiment with the same solutions. When orthophosphate buffer was employed, it was assumed that the ATP hydrolyzed to the same extent as in Tris. This assumption was supported by the finding that, according to the Kornberg method for nucleoside triphosphate, no more than 2 per cent ATP remained after treatment of ATP with myosin in the presence of orthophosphate buffer. The concentrations of phosphate used as buffer were insufficient to affect the extent of the reaction; however, the rate of hydrolysis in phosphate was significantly slower than in Tris when the ratio of orthophosphate to initial ATP was 100:1.

**Results**

*ATP Hydrolysis in Tris Buffer, 0.6 m KCl*—The system studied most extensively was the hydrolysis of ATP by myosin in 0.6 m KCl and 0.1 m Tris buffer (Table I). When Ca++ is used to activate the ATPase, the mean value of $\Delta H_{\text{obs}}(\text{pH 8})$ is $-16.4$ kilocalories per mole ($\sigma$, 0.3 kilocalorie per mole). Without Ca++ (and with no added phosphate) the mean is $-16.2$ kilocalories per mole ($\sigma$, 0.7 kilocalorie per mole). Since the difference of means, 0.2 kilocalorie per mole, is not statistically significant, there is no reason to think that, at the concentrations involved, complex formation between calcium and ATP, ADP, or P has any appreciable effect on the heat of hydrolysis at pH 8. The dispersion in data from measurements with Ca++ is less, probably because such experiments end sooner, and the thermal stability of the calorimeter is therefore taxed less.

"Equivalence Ratio," $\varphi_H$—Equations 7 and 8 show that, in order to calculate the contribution of buffer neutralization to $\Delta H_{\text{obs}}(\text{pH})$, one must know $\varphi_H(\text{pH})$, the number of H$^+$ ions produced per mole of ATP hydrolyzed, at the given pH, ionic strength, and concentration of specific ions (if any). That $\varphi_H$ depends on such variables has been reported (16, 17); therefore it was necessary to measure $\varphi_H(\text{pH 8})$ in 0.05 m KCl and 0.60 m KCl. This measurement must be made with some precision, for the heat of neutraliza-

2 With each orthophosphate analysis there was, of course, the corresponding blank containing myosin (inactivated by trichloroacetic acid) and ATP. However, since the sample is made acidic by the trichloroacetic acid, and even more so during the color development phase of the Fiske-Subbarow procedure, there is hydrolysis of ATP (the hydrolysis of ADP under similar conditions is much slower) in the blank during the analysis, resulting in an excessive blank value. Under our conditions this would result, according to unpublished observations of S. A. Bernhard, in an error of about 4 per cent. We have therefore corrected our orthophosphate assays for hydrolysis of the blank.
TABLE I

<table>
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<th>Myosin No. and concentration</th>
<th>$n_{\text{ATP}} = \Delta P$</th>
<th>$h$</th>
<th>$\Delta H_{\text{obs}} = \frac{h}{n_{\text{ATP}}}$</th>
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<td>15.5</td>
<td>-259</td>
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Mean ........................................................ -16.4

σ ............................................................. 0.3

With $10^{-3}$ M Ca$^{++}$

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<th>gm. per 100 ml.</th>
<th>µmoles</th>
<th>millicalories</th>
<th>kilocalories per mole</th>
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<tr>
<td>37</td>
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<td>3.95</td>
<td>-61</td>
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<tr>
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</tr>
<tr>
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<tr>
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<tr>
<td>48</td>
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Mean ........................................................ -16.2

σ ............................................................. 0.7

No Ca$^{++}$

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<th>millicalories</th>
<th>kilocalories per mole</th>
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</thead>
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<td>2.74</td>
<td>-42.7</td>
</tr>
</tbody>
</table>

Mean ........................................................ -16.2

σ ............................................................. 0.7

* In these experiments it is assumed that $n_{\text{ATP}}$ moles of ATP originally present in the system are completely hydrolyzed, producing an equivalent orthophosphate increment, $\Delta P$, and thereby generating $h$ millicalories of heat.

In "amine" buffers, such as Tris, $\Delta H^{(\text{ion})}$ is of the order of 10 kilocalories per mole; hence a 1 per cent error in $\varphi_H$ introduces an error of 100 calories per mole in $\Delta H$. $\varphi_H$ was calculated from several simultaneous measurements during
the course of ATP hydrolysis of (a) the amount of orthophosphate produced and (b) the amount of base which had to be added to the unbuffered system in order to maintain the initial pH, viz. 8.00. These paired data were plotted against one another with time as a parameter. The slope of the best straight line through these points was taken as $\varphi_H$. At the temperatures of the calorimetry, with 0.1 to 0.2 g.m. of myosin per 100 ml. and initially $10^{-3}$ M ATP, and in either 0.05 M KCl or 0.60 M KCl, with or without $10^{-3}$ M Ca++, $\varphi_H$(pH 8.00) was found to be 1.00. This value is about 1 per cent higher than that reported by Green and Mommaerts (16) for the intermediate ionic strength, 0.15 M.

**Heat of Ionization of Tris Buffer in 0.6 M KCl**—Measurements of this quantity were conducted in "drop" vessels in order to minimize effects arising from heats of dilution. The main compartment of the experimental vessel was filled with 15 to 20 ml. of Tris-KCl solution at pH 8.00; 0.03 to 0.10 ml., accurately measured, of HCl-KCl solution was placed in the drop compartment. Since the KCl concentration was the same in both liquids, there was no heat of dilution of KCl on mixing.³ The total heat absorbed ($h$) in such an experiment is

$$h = -n_{HCl}\Delta H^{(ion)}_{Tris} + n_{HCl}\Delta H^{(dil)}_{HCl} + n_{Tris}\Delta H^{(dil)}_{Tris}$$

(10)

where the $n$ values represent the mole numbers involved in the experiment, $\Delta H^{(ion)}_{Tris}$ is the heat of ionization of Tris buffer, and $\Delta H^{(dil)}$ is the molar heat of dilution of the designated species in passing from the unmixed to the mixed concentration. By mixing a drop of 0.60 M KCl with 0.10 M Tris buffer in 0.60 M KCl it was found that $n_{Tris}\Delta H^{(dil)}_{Tris}$ is negligible. In water, $\Delta H^{(dil)}_{HCl}$, in passing from 0.10 M to infinite dilution, is $-150$ calories per mole (18); for the dilution in 0.60 M KCl under discussion here, $|\Delta H^{(dil)}_{HCl}|$ is almost certainly less than 150 calories per mole, since the change in the ionic environment of the HCl ions as the dilution proceeds (this change being the principal source of the heat of dilution) is less than that when the dilution is carried out in water.⁴ For these reasons it has been

³ Such a heat of dilution could lead to a large relative error even though in absolute magnitude it is only a few millicalories. For example, if one measures the heat exchanged when 0.04 ml. of water is mixed with 20 ml. of 0.60 M KCl and divides by the number of moles of HCl which would normally have been in the drop of water (the HCl concentration employed is 0.10 M), the result is more than 1 kilocalorie per mole of "acid." This is so despite the fact that KCl is diluted only 1:500, because the total quantity of KCl is 2000 times the quantity of HCl.

⁴ Direct experimental measurement of the heat of dilution of 4 $\mu$M HCl, from $10^{-1}$ to $2 \times 10^{-4}$ M, in 0.60 M KCl was attempted. Results ranging from $-300$ to $-600$ calories per mole were obtained. In these experiments, however, the total heat generated (1 to 2.5 millicalories) was within the range of secondary effects in the calorimeter, and the results cannot be considered reliable.
assumed (Equation 10) that \( \Delta H_{\text{Tris}}^{(\text{ioniz})} = h/n_{\text{HCl}} \). In 0.6 M KCl and 0.1 M Tris, \( \Delta H_{\text{Tris}}^{(\text{ioniz})} = 11.6 \pm 0.1 \) kilocalories per mole (\( \sigma, 0.2 \) kilocalorie per mole). Inclusion of 0.1 to 0.2 gm. per 100 ml. of myosin did not affect this result (\( \Delta H_{\text{Tris}}^{(\text{ioniz})} = 11.6 \) kilocalories per mole; \( \sigma, 0.1 \) kilocalorie per mole). By application of the Gibbs-Helmholtz equation under similar experimental conditions, Bernhard (6) finds \( \Delta H_{\text{Tris}}^{(\text{ioniz})} = 11.7 \pm 0.2 \) kilocalories per mole, a value in excellent agreement with our calorimetric measurement and therefore with our approximation to Equation 10. Bernhard (6) has further shown that, in the range 5--50°, \( \Delta H_{\text{Tris}}^{(\text{ioniz})} \) (and therefore the contribution of buffer neutralization to \( \Delta H_{\text{obs}} \)) is strictly independent of the temperature. It is likewise insensitive to ionic strength; for example, in 0.15 M KCl and 0.05 M Tris, \( \Delta H_{\text{Tris}}^{(\text{ioniz})} = 11.8 \) kilocalories per mole (\( \sigma, 0.2 \) kilocalorie per mole). Inclusion of 0.1 gm. per 100 ml. of myosin in two additional experiments at the lower ionic strength had no appreciable effect (\( \Delta H_{\text{Tris}}^{(\text{ioniz})} = 11.6 \) and 11.2 kilocalories per mole).

Heat of Hydrolysis of ATP in 0.6 M KCl—At pH 8.00, \( K_{\text{eq}} \gg 1 \); thus the value of \( \varphi_H \) is 1.00, (ATP) \( \rightarrow [\text{ATP}^+] \), (ADP) \( \rightarrow [\text{ADP}^+] \), (P) \( \rightarrow [\text{P}^+] \), and the reaction represented by Equation 3 reduces to

\[
\text{H}_2\text{O} + \text{ATP}^+ \rightleftharpoons \text{ADP}^+ + \text{P}^+ + \text{H}^+ \tag{3'}
\]

Equation 9 thus becomes

\[
\Delta H(\text{pH 8}) = \Delta H_{\text{obs}}(\text{pH 8}) + \Delta H_{\text{ioniz}} \tag{9'}
\]

where \( \Delta H(\text{pH 8}) \) is the enthalpy change for Equation 3’. From the foregoing data we therefore conclude that at pH 8.00, in 0.6 M KCl,

\[
\Delta H(\text{pH 8}) = (-16.3 \pm 0.2) + (11.6 \pm 0.1) = -4.7 \pm 0.2 \text{ kilocalories per mole}
\]

In addition to uncertainty due to dispersion of the measurements, there is an uncertainty of about 1 per cent in the phosphate determination and a corresponding possible error of 0.3 kilocalorie per mole in \( \Delta H(\text{pH 8}) \). The non-statistical uncertainty in the thermal measurement corresponds to 0.2 kilocalorie per mole. The value of \( \Delta H(\text{pH 8}) \) is therefore considered to be -4.7 kilocalories per mole with a maximal uncertainty of 0.7 kilocalorie per mole.

ATP Hydrolysis in Glycyglycine Buffer and 0.6 M KCl—In two pairs of experiments with 0.6 M KCl and \( 10^{-3} \) M Ca\(^{++} \), in which Tris was replaced by 0.1 M glycyglycine, it was found that \( -\Delta H_{\text{gly-gly}}^{(\text{ioniz})} = -11.5 \) kilocalories per mole, and that \( \Delta H_{\text{obs}}(\text{pH 8}) = -16.1 \) kilocalories per mole. Since \( \varphi_H = 1.00 \), it follows that \( \Delta H(\text{pH 8}) = -4.7 \) kilocalories per mole. This result is in good agreement with that found by using Tris as a buffer.

\(^5\) The uncertainty interval is taken as the standard error of the mean, \( \sigma/\sqrt{N} \), where \( N \) is the number of experiments.
ATP Hydrolysis in Orthophosphate Buffer and 0.6 M KCl—When Tris or glycylglycine is used as a buffer, the neutralization of the proton (Equation 3') accounts for 70 per cent of the measured heat; thus an uncertainty of 1 per cent in $\Delta H_{\text{obs}}$ corresponds to an uncertainty of 3 per cent in $\Delta H$ (pH 8). This handicap can be reduced by employing a buffer with as small a $\Delta H^{(\text{ioniz})}$ as possible. Furthermore, it is necessary to prove that the only interaction of the buffer is proton association; this can be done by showing that $\Delta H$(pH 8) is independent of the chemical nature of the buffer employed. Although at pH 8.00 orthophosphate is not the most effective buffer, it does, under the conditions employed here (about 20°; see Bernhard (6)), satisfy the criteria of having a relatively small heat of ionization and of being chemically quite different from an “amine buffer.” However, since the $pK$ of $\text{H}_2\text{PO}_4^-$ in 0.6 M KCl is about 6.7 (6), the histidine residues of myosin can measurably contribute to the buffering capacity of a solution of pH 8 which is 0.6 M KCl, 0.05 M orthophosphate, and 0.1 gm. per 100 ml. in myosin ($2 \times 10^{-4}$ M histidine (19)). Such an effect was observed (though somewhat variably); i.e., the heat of neutralization in phosphate buffer differed from that in phosphate buffer and myosin. For this reason calorimetric measurements were made in pairs; i.e., a neutralization followed each hydrolysis. The neutralization heats were measured in phosphate-myosin systems of the same composition as that used in measuring the hydrolyses. Because the temperatures employed in the two measurements could not be made precisely the same, a temperature correction (of the neutralization heat) was made, based on the fact that, for the temperature range in question, $-d(\Delta H_P^{(\text{ioniz})})/dt = 320$ calories per mole degree (6). From Table II it follows that in orthophosphate buffer

$$\Delta H$(pH 8) = -4.75 ± 0.1 kilocalories per mole

Heat of Hydrolysis of ATP in Presence of Products of Reaction—Several experiments were performed in 0.6 M KCl and 0.1 M Tris, with either orthophosphate or ADP present as well as ATP. Neither 0.4 mM ADP nor 50 mM P affects significantly the dephosphorylation heat of initially 0.5 mM ATP, with about 0.1 gm. of myosin per 100 ml. at pH 8.00.

Heat of Hydrolysis of Inosine Triphosphate—To exclude the possibility that the foregoing conclusions are complicated by a side reaction involving

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6 With the concentrations of ATP and orthophosphate used in these experiments the pH change during hydrolysis was, however, less than 0.1 pH unit.

7 As is well known, the quantitative behavior of different physical properties varies slightly from preparation to preparation. Whether in the present instance this is due to variations in the effective concentration or $pK$ value of imidazole groups, or to some different phenomenon, we cannot tell.

8 In a solution 0.05 M in orthophosphate and 0.1 M in Tris, pH 8.00, the heat of neutralization is essentially that of Tris.
heat of ATP hydrolysis, and to test the hypothesis that the energetics of ATP dephosphorylation are exclusively a function of the triphosphate structure, at least when the purine ring is uncharged as it is in ATP (20) and ITP at pH 8.00, measurements were made of $\Delta H(pH \ 8)$ for ITP. This quantity proved to be $-16.2$ kilocalories per mole of ITP ($\sigma$, 0.5 kilocalorie per mole), i.e. essentially the same as $\Delta H(pH \ 8)$ for ATP, as would be expected in the absence of deaminase activity and of ring-triphosphate interactions.

### Table II

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Myosin</th>
<th>$n_{ATP}$</th>
<th>$h$</th>
<th>$\Delta H_{obs}$</th>
<th>Temperature</th>
<th>$n_{HCl}$</th>
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<th>$\Delta H_{obs}$ + $\Delta H_{P^{(1+1)}}$</th>
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<td>°C.</td>
<td>gm. per 100 mlt.</td>
<td>µmoles</td>
<td>mili-calories</td>
<td>kilocalories per mole</td>
<td>°C.</td>
<td>µmoles</td>
<td>mili-calories</td>
<td>kilocalories per mole</td>
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<td>0.28</td>
<td>6.83</td>
<td>-45.9</td>
<td>-6.7</td>
<td>19.4</td>
<td>4.47</td>
<td>-8.7</td>
<td>-1.94</td>
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<td>0.28</td>
<td>6.93</td>
<td>-46.2</td>
<td>-6.7</td>
<td>19.4</td>
<td>4.52</td>
<td>-8.9</td>
<td>-1.97</td>
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<tr>
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<td>-43.6</td>
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<td>18.3</td>
<td>4.52</td>
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<td>-1.94</td>
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<td>18.7</td>
<td>4.57</td>
<td>-8.95</td>
<td>-1.96</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* The HCl is in 0.6 M KCl.
† Corrected to the temperature of the corresponding hydrolysis run. This correction is valid if most of the measured heat is due to neutralization of phosphate. Since in a series of eight measurements of the $\Delta H_{P^{(1+1)}}$ in the absence of myosin (average temperature, 20°) we obtained 1.8 kilocalories per mole, we conclude that the thermal contribution of this myosin preparation (No. 48) to $\Delta H_{P^{(1+1)}}$ is insignificant and that the correction is valid.
‡ For the neutralization measurement the HCl was added to the products remaining after the measurements of the heat of hydrolysis.

**Effect of Ionic Strength upon $\Delta H(pH \ 8)$**—A reference medium of high ionic strength was chosen for this study in order to make the conclusions applicable to previous work from our Laboratory (myosin is easily soluble only at high ionic strength). It is, however, important to know whether the value of $\Delta H(pH \ 8)$ is appreciably different at the lower ionic strength (about 0.28) thought (21) to obtain in vivo, or at the ionic strength employed by earlier workers (22, 23); accordingly, several experiments were conducted with ATP as the substrate and precipitated myosin as the en-
zyme (Table III). At 0.15 M KCl the value of $\Delta H_{\text{obs}}$(pH 8) is not significantly different from that at 0.60 M KCl. As shown above, $\Delta H_{\text{Tris}}^{(\text{ionic})}$ is also the same at 0.15 M KCl as at 0.60 M KCl (this is to be expected because there is no change of charge in the neutralization reaction). Data (Table III) taken in 0.01 M KCl should be regarded as qualitative. The reduction in concentration of enzyme-activating $K^+$ (24) reduces the dephosphorylation rate below that required for the best calorimetry; moreover, $\Delta H_{\text{Tris}}^{(\text{ionic})}$ under these conditions was measured only semiquantitatively as 11.5 ± 0.5 kilocalories per mole. Despite the uncertainties at the lowest ionic strength studied, however, it seems quite safe to say that reducing the ionic strength by a factor of 10 (from what it is in 0.60 M KCl and 0.1 M buffer) does not change $\Delta H$(pH 8) by more than 1 kilocalorie per mole.

Table III
Effect of Ionic Strength on Enthalpy Change upon Hydrolysis of ATP
Common solvent, 0.05 M Tris, pH 8.00.

<table>
<thead>
<tr>
<th>KCl $M$</th>
<th>Myosin No. and concentration</th>
<th>$n_{\text{ATP}}$ = $\Delta P$</th>
<th>$h$</th>
<th>$\Delta H_{\text{obs}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>46 gm. per 100 ml. 0.10</td>
<td>6.4</td>
<td>-106</td>
<td>-16.6</td>
</tr>
<tr>
<td>0.15</td>
<td>46 gm. per 100 ml. 0.10</td>
<td>6.5</td>
<td>-105</td>
<td>-16.2</td>
</tr>
<tr>
<td>0.01*</td>
<td>48 gm. per 100 ml. 0.10</td>
<td>3.60</td>
<td>-56</td>
<td>-15.6</td>
</tr>
<tr>
<td>0.01</td>
<td>48 gm. per 100 ml. 0.14</td>
<td>6.7</td>
<td>-102</td>
<td>-15.2</td>
</tr>
</tbody>
</table>

* This experiment was interrupted before the substrate was completely hydrolyzed.

Evidence against Presence of Side Reactions—The foregoing calculations of $\Delta H$(pH 8) rest on the assumption that the only net reaction which occurs when enzyme and substrate are mixed is the splitting of the terminal phosphate group from the triphosphate structure of the substrate. The following evidence supports this assumption: (1) The ratio of orthophosphate produced (as measured by chemical analysis) to amount of adenine-containing substances (e.g., ADP, adenosine-5-monophosphate) measured spectrophotometrically was 1:1. With concentrations of myosin and ATP such that $\Delta P$(adenine) reached 0.95 in 10 to 20 minutes, the subsequent liberation of phosphate was less than 1 per cent per hour. (2) For a given amount of ATP dephosphorylated at pH 8.00, the amount of H$^+$ produced was that calculated on the basis of the independently measured pK' values of ATP, ADP, and P. (3) No deamination of the adenine ring could be detected spectrophotometrically (therefore not more than 3 per cent deamination could have occurred). (4) With either ATP or ITP, the same $\Delta H$(pH 8) was found. (5) When ADP was used as a substrate, the heat
liberated per mole of adenine-containing substances was 4 per cent of that found with an equivalent amount of ATP. In all probability, this heat was due to an ATP impurity in the ADP preparation. (6) There was no correlation between $\Delta H_{\text{obs}}(\text{pH 8})$ and the ratio of myosin concentration to ATP concentration, as there might have been if myosin were not functioning as a true catalyst (see Morales et al. (25) for discussion); moreover, available evidence (26, 27) indicates that the concentration of myosin ATPase sites was never more than 2 per cent of the initial concentration of ATP. Preaddition of products ADP and P does not change the $\Delta H_{\text{obs}}$ (pH 8) for ATP.

**DISCUSSION**

Comparison of $\Delta H_L$ with $\Delta F^\circ_L$—Hitherto, the large value ascribed to $-\Delta H$ has been considered “consistent” with the large value (10 to 12 kilocalories per mole) originally (1) ascribed to $-\Delta F^\circ$, and, in general, with the supposed “high energy” character of ATP. Recent estimates of $-\Delta F^\circ$, however, are significantly less than 12 kilocalories per mole (Table IV; (3, 25, 29, 30)), but interestingly enough they are “consistent” with the much reduced$^{10}$ value of $-\Delta H$ reported here. This general downward revision of the magnitudes of $\Delta F^\circ$ and $\Delta H$ is equally necessary in considering the free energy and enthalpy changes associated with the splitting process as such, i.e. the quantities $\Delta F^\circ_L$ and $\Delta H_L$. In Table IV we have recorded $\Delta F^\circ_L$ by “dissecting” from recent estimates of $\Delta F^\circ$ the contributions of ionization (Equation 4; (4, 5)). It is seen that the results range from $-5.9$ to $-8.6$ kilocalories per mole. An estimate can also be made of

$^9$ There is, of course, no thermodynamic reason for $\Delta F^\circ$ to resemble $\Delta H$, but there has been claimed (28) an empirical regularity in the entropy (8) change of pyrophosphate bond hydrolysis, viz. that $T\Delta S \approx 1$ kilocalorie.

$^{10}$ The inference (3), $\Delta H \approx -12$ kilocalories per mole, follows from earlier work of Meyerhof and Lohmann (22) and of Ohlmeyer (23) only if the dismutation of ADP is thermoneutral, as yet an unproved assertion. In neither earlier investigation was buffer heat considered. If the “muscle extract” which Meyerhof and Lohmann used as buffer at pH 8 was, in effect, a histidyl buffer, then correction of their result by $\Delta H_{\text{hist}} \approx +7$ kilocalories per mole leads to good agreement with our result. Ohlmeyer, however, employed citrate buffer at pH 3.7, and neither the buffer neutralization correction nor the pH difference reconciles his result with ours. Dr. S. A. Bernhard has investigated by titrimetry the possibility that another ionization may complicate measurements at pH 3.7. In support of the interpretation of Alberty et al. (20), he showed that the next to the largest pK values for ATP and adenosine-5-monophosphate refer to ring ionizations (since these are abolished by conversion to the inosine structure), and, also, that these ionizations are probably of an $-\text{NH}_2$ rather than of an $-\text{NH}_3^+$ group (6 position) because the accompanying heats are small. The last fact, however, excluded ring ionization as the explanation of our difference with the Ohlmeyer result.
At pH 8 Equation 5 reduces\(^1\) to
\[
\Delta H = \Delta H_L + \Delta H_2
\]
(5')
since the \(K_i^* \gg 1\) (20). The heat, \(\Delta H_2\), can be estimated from titration data (20) to be less than 0.3 kilocalorie per mole; thus within the accuracy of our measurement, \(\Delta H_L = \Delta H(\text{pH 8}) = -4.7\) kilocalories per mole. This value, paired with the estimate, \(\Delta F^o_L \cong -6.0\) kilocalories per mole, of Table IV, suggests that quantitatively the "high energy" character of

**Table IV**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Bibliographic reference No.</th>
<th>Present calculations*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corresponding (-\Delta F^o_L)</td>
<td>(-\Delta F^o_L, 20^\circ)</td>
</tr>
<tr>
<td>Temperature</td>
<td>pH</td>
<td>kilocalories per mole</td>
</tr>
<tr>
<td>10.5</td>
<td>20</td>
<td>7.8</td>
</tr>
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<tr>
<td>7.0</td>
<td>37</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* \(-\Delta F^o_L\) is calculated from the reported \(-\Delta F^o\) by Equation 4, by using for the pK values (assumed temperature-independent) of ADP and ATP 6.4 and 6.5, respectively (20), as recently revised, and for that of orthophosphate the values from Bernhard (6). This procedure neglects some ionic strength effects. Variations of \(\Delta F^o_L\) with temperature have been calculated, assuming that \(\Delta H_L = -5\) kilocalories per mole. The original Burton-Krebs estimate of \(-\Delta F^o\) (29) has recently (30) been revised downward to the cited value. The original Levintow-Meister (31) and Morales (5) calculations were based on a glutamate-aspartate analogy, which, when corrected (25) by the difference of pK values of the two acids, gives the cited value of \(-\Delta F^o\). ATP may be generally overrated. Also of interest is the comparison of \(\Delta F^o_L \cong -6\) kilocalories per mole with a theoretical estimate (4) of the electrostatic contribution to the free energy of hydrolysis, viz. 5 to 6 kilocalories per mole.

**Comparison of \(\Delta H\) (ATP) with Heats of Hydrolysis of Other Polyphosphates**—The value for \(\Delta H\) of ATP reported above is in reasonable agreement with those reported for analogous substances. For the hydrolysis of pyrophosphate (PP) in orthophosphate buffer\(^12\) (pH 7.3, temperature

\(^1\) Furthermore, the same reduction leads to \(e^{-\Delta F^o/LRT} = LK_2/[H^+] = [\text{ADP}^3-][\text{P}^4-]/[\text{ATP}^7][\text{H}_2\text{O}]\), which means that \(L_2\) is the equilibrium constant for Equation 3'. This reduction shows the connection between the general Equations 3 and 4 and the commonly employed approximation of Equation 3'.

\(^12\) Since at pH 7.3 the predominant species of PP and P are PP\(^{5-}\), PP\(^{4-}\), and P\(^-\), P\(^{2-}\), respectively, the value of -5.8 kilocalories per mole is equal to \(\Delta H_{PP} + (\Delta H_1 - \Delta H_P)\)
HEAT OF ATP HYDROLYSIS

25°, ionic strength 0.6 and 1.0 m) Ging and Sturtevant (32) found -5.8 kilocalories per mole. For the hydrolysis of trimetaphosphate (PPP) to 3 orthophosphates (pH 6.95, temperature 33°), after the buffer correction, Meyerhof et al. (28) found -18.6 kilocalories per mole, or, per phosphoric anhydride linkage, -6.2 kilocalories per mole. Thus the cleavage of the P—O—P structure seems energetically similar in ATP, PP, and PPP, as might be expected on structural grounds.

ATP Hydrolysis in Vivo—As implied in the foregoing analysis, and as discussed in the accompanying paper by Bernhard (6), the heat production resulting from the dephosphorylation of ATP in vivo is governed by the tissue pH (provided the pH is below 8.0) and the nature of the tissue buffers. Without information on these subjects it is impossible to infer from the gross heat production of a tissue the amount of ATP dephosphorylated. It has been shown above that the factor of -12 kilocalories per mole of ATP hitherto assumed is quite uncertain.

We are pleased to acknowledge the intellectual and material assistance accorded to us by colleagues in the Division of Physical Biochemistry, especially Dr. Sidney Bernhard. Dr. Leon Levintow kindly counseled us in certain analyses, and he and Dr. Alton Meister offered comments of value in preparing this manuscript.

SUMMARY

With myosin as a catalyst, the enthalpy change accompanying the hydrolysis of ATP to ADP and P has been measured calorimetrically in several individual buffer systems. After correction for the heat of neutralization (calculated from direct calorimetric measurements of the heat of ionization and titrimetric measurements of the moles of H+ produced per mole of ATP hydrolyzed), ΔH(pH 8) was in each case found to be -4.7 kilocalories per mole. The uncertainty in this measurement does not exceed 0.7 kilocalorie per mole. These measurements were made at 20°, pH 8.00, in 0.6 m KCl, but it is shown that the change in ΔH(pH 8) effected by a 10-fold reduction in ionic strength is less than the experimental uncertainty. This result does not support the view that ATP is a "high energy" substance in the usual quantitative sense; on the other hand, it shows that,ΔH_F1/(1 + K_F*) where ΔH_PP is the enthalpy change in the reaction H2O + PP^- → 2P^-, ΔH_4 is the heat of ionization of PP^-, and K_F is its ionization constant.

13 At pH 7 the predominant species of PPP is PPP^3-; hence 3(6.2) kilocalories per mole is equal to ΔH_PP + 3ΔH_2/(1 + 1/K_3*), where ΔH_PP is the enthalpy change in the reaction PPP^3- + 3H2O → 3P-. At 33°, ΔH_3 is negative (6), so actually |ΔH_PP|/3 is less than 6.2 kilocalories per mole.
energetically, the hydrolysis of ATP is similar to that of simple polyphosphates, e.g. pyrophosphate and trimetaphosphate.

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