MgATP and EG ($6.2 \times 10^3\ M^{-1}$) is only about six times that for ATP and EG which indicates that although MgATP is the actual substrate rather than ATP, the role of the metal ion cannot be only to enhance the binding of ATP to the enzyme. This is contrary to the idea frequently brought forth that the metal ion acts as a strong structural cement between substrate and enzyme. Instead, a good deal of the binding seems to occur through the adenine and/or ribose portions of ATP which is consistent with the extended conformation of MgATP in solution proposed on the basis of nuclear magnetic resonance experiments.[6] The primary role of Mg^{++} is most likely to polarize the oxygen–phosphate bond being broken while anchoring the phosphate group of ATP to the enzyme. A more detailed discussion of the function of the metal ion will be presented elsewhere.[7]

If the equilibrium constant were known, enough data is available to calculate all of the rate constants which are still unknown. Robbins and Boyer[4] have determined the equilibrium constant under different conditions (pH 6, 30° and variable ionic strength) than those employed in our experiments, but at least an approximate value of the desired constant can be obtained from their data. A reasonable estimate is that the desired equilibrium constant (as defined by equation 4) is about 300. Using this value and the ϕ values in Table I, the rate constants can be calculated to within a factor of 2 or 3. Of course, k_1, k_{-1}, k_2, k_{-2}, k_6 and k_{-6} have all been determined independently and are known to about ± 30%.[1-3] A tabulation of all of the rate constants is

$^*k_1 = 1.2 \times 10^7\ M^{-1}$ sec.$^{-1}$ $\quad ^*k_{-1} = 1.2 \times 10^3$ sec.$^{-1}$
$k_2 = 3.7 \times 10^6\ M^{-1}$ sec.$^{-1}$ $\quad k_{-2} = 1.5 \times 10^3$ sec.$^{-1}$
$k_3 = 4 \times 10^6\ M^{-1}$ sec.$^{-1}$ $\quad k_{-3} = 6.5 \times 10^2$ sec.$^{-1}$
$k_4 = 3 \times 10^3$ sec.$^{-1}$ $\quad k_{-4} = 2 \times 10^6\ M^{-1}$ sec.$^{-1}$
$k_5 = 1 \times 10^3$ sec.$^{-1}$ $\quad k_{-5} = 1 \times 10^5\ M^{-1}$ sec.$^{-1}$
$^*k_6 = 2.5 \times 10^3$ sec.$^{-1}$ $\quad ^*k_{-6} = 3 \times 10^5\ M^{-1}$ sec.$^{-1}$

The constants marked with * were determined at 25° and an ionic strength of 0.1 M KNO_3.

The magnitudes of k_2 and k_3 are fairly large but not nearly as large as many other enzyme–substrate reactions which approach the theoretical upper limit for a bimolecular rate constant ($\sim 10^9\ M^{-1}$ sec.$^{-1}$).[8,9] An interesting point is that MgADP binds to both EG and EG6P ($K = 3.3 \times 10^2\ M^{-1}$ and $2 \times 10^3\ M^{-1}$, respectively); on the other hand no experimental evidence exists for the binding of MgATP and EG6P. This binding should be detectable if the binding constant is greater than about 50. This indicates that the phosphate group on G6P blocks the binding of MgATP very effectively.

Finally it should be pointed out that the procedure used for obtaining this rather detailed mechanism for the hexokinase system is applicable to phosphate transferring enzymes in general.

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Addendum.—Since this work was completed a kinetic study of the enzyme pyruvate kinase was carried out by Reynard, Hass, Jacobsen and Boyer.[10] The mechanism involved is apparently somewhat different than that proposed for hexokinase.

(6) G. G. Hammes, G. E. Maciel and J. S. Waugh, *J. Am. Chem. Soc.*, **83**, 2394 (1961).
(7) G. G. Hammes and D. Kochavi, *ibid.*, **84**, 2076 (1962).
(8) L. Peller and R. A. Alberty, *ibid.*, **81**, 5907 (1959).
(9) R. A. Alberty and G. G. Hammes, *J. Phys. Chem.*, **62**, 154 (1958).
(10) A. M. Reynard, L. F. Hass, D. D. Jacobsen and P. D. Boyer, *J. Biol. Chem.*, **236**, 2277 (1961).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS]

Studies of the Enzyme Hexokinase. III. The Role of the Metal Ion

BY GORDON G. HAMMES AND DANIEL KOCHAVI

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Steady state kinetic experiments were carried out with hexokinase using Ca^{++} as the metal ion activator. Although Ca^{++} is a much poorer activator than Mg^{++}, both metals utilize the same mechanism. Comparison of the two metal ions as activators indicates that the rates of complex formation are not rate determining. In fact, Ca^{++} in general builds complexes about 100 times faster than Mg^{++}. Furthermore, the equilibrium quotients involving the metal ions are approximately the same. However, the breakdown of the quaternary intermediate complex occurs over one hundred times faster for the Mg^{++} system indicating that the rate controlling step involves polarization of the media (probably breaking of a chemical bond) since Mg^{++} should be much more effective than Ca^{++} for this purpose. Comparison of the rate constants for the two systems suggests that at least two quaternary intermediates are necessary to explain the results in a satisfactory manner.

Introduction

Recent studies[1,2] of the enzyme hexokinase have indicated that the most probable mechanism for the transfer of a phosphoryl group from ATP to glucose is

$$\mathrm{Me} + \mathrm{ATP} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{MeATP} \qquad \mathrm{EG6P} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \mathrm{E} + \mathrm{G6P}$$

$$\mathrm{E} + \mathrm{G} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{EG} \qquad \mathrm{MeADP} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} \mathrm{Me} + \mathrm{ADP}$$

$$\mathrm{EG} + \mathrm{MeATP} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{X_1} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \mathrm{EG6P} + \mathrm{MeADP}$$

$$\mathrm{EG} + \mathrm{ATP} \rightleftharpoons \mathrm{EGATP(inactive)}$$

Here E respresents hexokinase, ATP is adenosine triphosphate, Me is the metal ion activator (Mg^{++} in previous studies), G is glucose, X_1 is a quaternary

(1) G. G. Hammes and D. Kochavi, *J. Am. Chem. Soc.*, **84**, 2069 (1962).
(2) *Ibid.*, 2073 (1962).

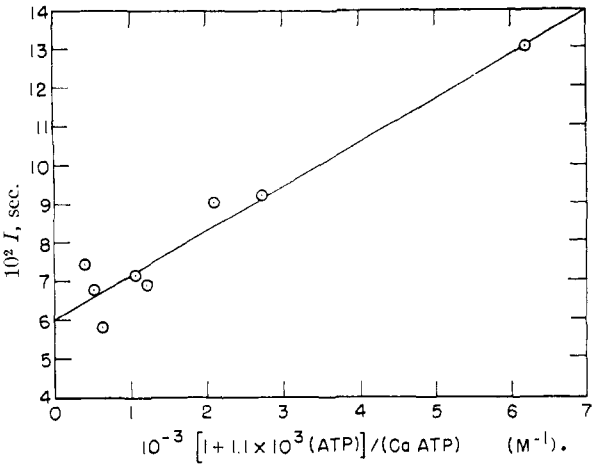

Fig. 1.—The intercepts of $(E_0)/v$ − 1/(G) plots *versus* [1 + 1.1 × 10^3(ATP)]/(Ca ATP).

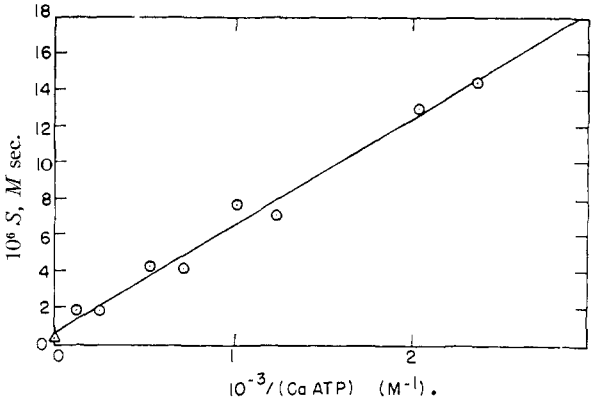

Fig. 2.—The slopes of $(E_0)/v$ − 1/(G) plots *versus* 1/(CaATP). The triangle represents the intercept found previously in the Mg^{++} system.

intermediate complex, G6P is glucose-6-phosphate and ADP is adenosine diphosphate. By coupling steady state kinetic studies[1,2] with equilibrium data[3] and kinetic data obtained with the temperature jump method,[4,5] all of the rate constants in the above mechanism were determined. From these results, the role of the metal ion, Mg^{++}, seemed to be primarily connected with a bond breaking step in the mechanism rather than with enhancement of the binding between the enzyme–glucose complex and ATP. This finding is contrary to many previous speculations. Since the role of metal ions in enzyme catalysis is still not very well understood, it seemed worthwhile to investigate further this aspect of the hexokinase mechanism. With this in mind, steady state kinetic studies have been carried out using Ca^{++} as the metal ion activator rather than Mg^{++}. The reasons for choosing Ca^{++} over other divalent metals will become apparent in the discussion section. The results show that Ca^{++} is a much poorer activator than Mg^{++} and that the main difference lies in their relative abilities to catalyze the step (probably bond breaking) characterized by the rate constant k_4. The results also suggest that at least one more intermediate X_2 should be postulated if the kinetic data is to be consistent with results obtained from studies of model systems.

Experimental

The experimental procedure employed in determining the equilibrium binding constant for CaATP and in making the kinetic measurements was exactly as previously described.[1] The only notable difference was that a much higher enzyme concentration had to be used, namely, 10^{-7} – 10^{-8} M.

Results

The equilibrium quotient for CaATP formation at 25.0° and in 0.3 M $(NH_3)_4NCl$ was found to be 3.7×10^3 M^{-1}. The kinetic data was treated exactly as previously.[1,2] The mechanism was assumed to be the same as that for the Mg^{++} activated system; therefore the expected rate law is

$$\frac{(E)_0}{v} = \phi_1 + \frac{\phi_2}{(G)} + \frac{\phi_3}{(CaATP)}[1 + K_i(ATP)] + \frac{\phi_3\phi_4}{(G)(CaATP)} \quad (1)$$

where $(E)_0$ is the total enzyme concentration, v is the initial steady state velocity, K_i is the equilibrium binding constant for EG and ATP, and the ϕ's are known functions of the rate constants

$$\phi_1 = 1/k_4 + 1/k_5 \qquad \phi_3 = (k_{-3} + k_4)/k_3k_4$$
$$\phi_2 = 1/k_2 \qquad \phi_4 = k_{-2}/k_2$$

As predicted, $(E_0)/v$ plotted *versus* 1/(G) at constant total concentrations of Ca^{++} and ATP gives straight lines with intercepts of $\phi_1 + \phi_3[1 + K_i(ATP)]/(CaATP)$ and slopes of $\phi_2 + \phi_3\phi_4/(CaATP)$. Figure 1 is a plot of the $(E_0)/v$ − 1/(G) intercepts obtained plotted against $[1 + K_i(ATP)]/(CaATP)$. The value of K_i is known to be 1.1×10^3 M from the previous work with Mg^{++} as an activator and the line was fitted by the method of least squares. Figure 2 is a plot of the slopes of the $(E_0)/v$ − 1/(G) plots *versus* 1/(CaATP). Again the line was drawn by using the method of least squares; the triangle is the intercept obtained in the Mg^{++} system. As is readily seen, the experimental results are in reasonably good agreement with equation 1. These data are not as abundant as those for Mg^{++} because 50–100 times as much enzyme had to be used for the Ca^{++} experiments. The relatively high enzyme concentration also caused a slight amount of buffering which made the kinetic measurements somewhat less precise. The relative error in the ϕ's is about ± 20% except for ϕ_2 which is $7.7 \pm 5 \times 10^{-5}$, the large error being due to the steepness of the slope and smallness of the intercept in Fig. 2. The values of the ϕ's found at 25.0° pH 8.0 in 0.3 M $(NH_3)_4NCl$ are

$\phi_1 = 6.0 \times 10^{-2}$ sec. $\qquad \phi_3 = 1.13 \times 10^{-5}$ M sec.
$\phi_2 = 7.7 \times 10^{-7}$ M sec. $\qquad \phi_4 = 5.2 \times 10^{-4}$ M

Discussion

The results for the Ca^{++} activated hexokinase system are consistent with the proposed mechanism,

(3) E. A. Robbins and P. D. Boyer, *J. Biol. Chem.*, **224**, 121 (1957).
(4) H. Diebler, M. Eigen and G. G. Hammes, *Z. Naturforsch.*, **15B**, 554 (1960).
(5) M. Eigen and G. G. Hammes, *J. Am. Chem. Soc.*, **82**, 5951 (1960); **83**, 2786 (1961).

In addition to following the correct rate law (eq. 1), ϕ_2 and ϕ_4 should also be the same as those measured for the Mg^{++} activated system since these constants are concerned only with the reaction of enzyme and glucose. Unfortunately ϕ_2 is not determined very precisely in the Ca^{++} system, but it is apparent from Fig 2, that the intercepts ($= \phi_2$) for Mg^{++} and Ca^{++} are the same within experimental error. The value $\phi_2 = 2.71 \times 10^{-7}$ obtained with Mg^{++} is much more accurate, and, therefore, will be considered as the correct value in all calculations of the rate constants. On the other hand, ϕ_4 is determined fairly well in both studies and is the same within experimental error: 4.1×10^{-4} M (Mg^{++}) and 5.2×10^{-4} M (Ca^{++}).

Although it is qualitatively apparent that Ca^{++} is a much poorer activator than Mg^{++}, a comparison between the individual rate constants is necessary if any specific information concerning the role in the metal ion in the mechanism is to be obtained. Since the rate constants k_2, k_{-2}, k_5 and k_{-5} are concerned with steps not involving the metal ions, they are identical for both metals. Using the value of k_5 determined in the Mg^{++} system, k_4 can be calculated directly from ϕ_1. In principle k_3, k_{-3} and k_{-4} should be obtainable from the data presented in the results section coupled with equilibrium data[3] and studies of the inhibition of the reaction by CaADP.[2] Inhibition studies were carried out and qualitatively gave the results expected; however, the relative magnitudes of the inhibition terms are too unfavorable to allow a quantitative evaluation of the rate constants. Nevertheless, the desired rate constants can be calculated by use of the results found for simpler systems involving Mg^{++} and Ca^{++}. In all reactions of Ca^{++} and Mg^{++} with a given ligand (*eg.* ATP and ADP), Ca^{++} always builds complexes about 100 times faster than Mg^{++}— this is a general kinetic characteristic of the two ions and is due to the fact that the rate controlling step in complex formation is the dissociation of a water molecule from the inner hydration shell of the metal ion.[6] Therefore, using the value of k_3 calculated for the Mg^{++} system,[1] k_3 in the present case can be estimated as $\sim 5 \times 10^8$ M^{-1} sec.$^{-1}$ (as shown previously,[1] k_3 for Ca^{++} must be greater than $1/\phi_3 \approx 10^5$ M^{-1} sec.$^{-1}$). Comparison of this rate constant with $1/\phi_3$ shows that $\phi_3 \approx k_{-3}/k_3k_4$ and thus k_{-3}/k_3 can be calculated. Using the estimated value of k_3, k_{-3} can now be found. Finally by estimating the equilibrium constant for the over-all reaction from available data[3] k_{-4} can be obtained.

$$K_{eq} = \frac{k_2k_3k_4k_5}{k_{-2}k_{-3}k_{-4}k_{-5}} = \frac{(CaADP)(G6P)}{(CaATP)(G)} \approx 400$$

A summary of the rate constants for both the Ca^{++} and Mg^{++} activated systems is given in Table I. One active site per molecule of molecular weight 96,600 has been assumed.

As previously indicated bimolecular rate constants in the forward direction are about 100 times larger for the Ca^{++} activated system, although the equilibrium constants involved are the same order of magnitude. On the other hand k_4 is much larger for Mg^{++}. Since Mg^{++} is smaller than Ca^{++}, reactions where polarization of the media are important should proceed faster with Mg^{++}. (*eg.* the dissociation of a proton from the inner hydration shell of the metal ion occurs over ten times faster for Mg^{++}.[7]) Therefore, it seems likely that the step characterized by k_4 involves polarization of the media—probably bond breaking. It should be noted that the binding constant for MeATP and EG is approximately the same for both metals; 6×10^3 M^{-1} and 5×10^3 M^{-1} for Mg^{++} and Ca^{++} respectively, while the same constant for ATP and EG is 1.1×10^3 M^{-1}. This is more evidence in favor of the previous suggestion[2] that the primary role of the metal ion is not to aid in binding the substrate to the enzyme but rather is to help catalyze the bond breaking step. The general role of Mg^{++} and Ca^{++} in enzymatic reactions will be discussed in more detail in another place.[7] However, it is worth mentioning that according to these considerations, Mn^{++} should be a very good activator for hexokinase.

TABLE I

RATE CONSTANTS FOR THE HEXOKINASE SYSTEM[a]

	Ca	Mg^{++} – (350 α)
$*k_1$ (M^{-1} sec.$^{-1}$)	$>10^9$	1.2×10^7
$*k_{-1}$ (sec.$^{-1}$)	$>2 \times 10^5$	1.2×10^3
k_2 (M^{-1} sec.$^{-1}$)	3.7×10^6	
k_{-2} (sec.$^{-1}$)	1.5×10^2	
k_3 (M^{-1} sec.$^{-1}$)	$\sim 5 \times 10^8$	4×10^6
k_{-3} (sec.$^{-1}$)	$\sim 10^5$	6.5×10^2
k_4 (sec.$^{-1}$)	17	3×10^3
k_{-4} (M^{-1} sec.$^{-1}$)	5×10^3	2×10^6
k_5 (sec.$^{-1}$)	1×10^3	
k_{-5} (M^{-1} sec.$^{-1}$)	1×10^5	
$*k_6$ (sec.$^{-1}$)	$>3.5 \times 10^4$	2.5×10^3
$*k_{-6}$ (M^{-1} sec.$^{-1}$)	$>2.5 \times 10^5$	3×10^6

[a] 25.0°, 0.3 M $(CH_3)_4NCl$, *p*H. 8.0 except for *. * 25°, 0.1 M KNO_3 *cf.* ref. 4 and 5.

Since Ca^{++} always builds complexes faster than Mg^{++}, k_{-4} should be greater in the case of Ca^{++}; instead it is greater for Mg^{++}. If this step is to fit in the large array of known data,[7] a more complex mechanism must be assumed in interpreting the data. The simplest modification of the mechanism originally proposed is to assume the existence of a second quaternary intermediate

$$EG + MeATP \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} X_1 \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} X_2 \underset{k_{-4}'}{\overset{k_4'}{\rightleftarrows}} EG6P + MgADP$$

The form of the rate equation for this mechanism is identical to the simpler case; however the ϕ's are more complex— that is they involve more rate constants. It is now necessary to demonstrate that the conclusions reached previously are consistent with the above mechanism. As might be expected, only the rate constants $k_{\pm 3}$ and $k_{\pm 4}$ are changed. In fact it is no longer possible to calculate these rate constants exactly. However, detailed consideration shows that these rate constants can be specified within certain limits. Two assumptions have been made in obtaining

(6) M. Eigen, *Z. Elektrochem.*, **64**, 115 (1960).

(7) M. Eigen and G. G. Hammes, in preparation.

these limits (1) the binding between MeATP and EG is at least as strong as that between free ATP and EG and (2) all reactions which can be classified as complex ion formation occur at least as fast for Ca^{++} as Mg^{++}. Both these assumptions are very reasonable and do not impose a severe limitation on the results which can be summarized as

For Mg^{++}: $6 \times 10^3\ M^{-1} \geq k_3/k_{-3} \geq 1.1 \times 10^3\ M^{-1}$; $k_4/k_{-4} \leq 5$; $k'_{-4}/k_4' \leq 5 \times 10^2\ M^{-1}$; $k_3 \geq 3.3 \times 10^6\ M^{-1}$ sec.$^{-1}$; $k_{-3} \geq 550$ sec.$^{-1}$; $k_4 \geq 3 \times 10^3$ sec.$^{-1}$; $k_{-4} \geq 6 \times 10^2$ sec.$^{-1}$; $k_4' \geq 3 \times 10^3$ sec.$^{-1}$; $k_{-4}' \geq 2 \times 10^5\ M^{-1}$ sec.$^{-1}$

For Ca^{++}: $5 \times 10^3\ M^{-1} \geq k_3/k_{-3} \geq 1.1 \times 10^3\ M^{-1}$; $k_4k'_4/k_{-4}k'_{-4} \geq 4 \times 10^{-3}\ M$; $k_{\pm 3}$ and $k'_{\pm 4}$ same as for Mg^{++}; $k_4 \approx 17$ sec.$^{-1}$

It is obvious that the more complex mechanism only serves to strengthen the conclusions reached earlier concerning the role of the metal ion in the hexokinase system. It should be mentioned that the inequalities presented above are probably equalities within a factor of 2 or 3 in the case of Mg^{++}. Work is now in progress to try and detect the proposed intermediates in a more direct manner.

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[CONTRIBUTION FROM THE CALIFORNIA RESEARCH CORPORATION, RICHMOND, CALIF.]

Inhibition of Cumene Oxidation by Tetralin Hydroperoxide

BY J. R. THOMAS AND C. A. TOLMAN

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Tetralin hydroperoxide is found to retard the rate of catalyzed oxidation of cumene at 57°. The retardation is explained in terms of tetralylperoxy radical formation by peroxidic hydrogen abstraction by cumylperoxy radicals and the more rapid termination reactions occurring between tetralylperoxy radicals with themselves and with cumylperoxy radicals than that between cumylperoxy radicals. The data yield a rate constant of 12 liters/mole sec. for the reaction $RO_2\cdot + TO_2H \rightarrow RO_2H + TO_2\cdot$. Possible reasons for the lack of a kinetic isotope effect, observed for this reaction, are discussed.

Russell[1] found that cumene solutions containing moderate concentrations of tetralin oxidize appreciably slower than either pure hydrocarbon alone. This behavior he showed to be due to the much faster termination reactions of tetralylperoxy radicals with themselves and with cumylperoxy radicals than that between cumylperoxy radicals alone.

If the hydrogen exchange reaction between cumylperoxy radicals and tetralin hydroperoxide proceeds with sufficient speed, it would be anticipated that tetralin hydroperoxide would retard the oxidation rate of cumene. We have observed that such an effect exists and find that as little as 10^{-3} M tetralin hydroperoxide significantly retards the oxidation rate of 4 M cumene solutions initiated with 4×10^{-3} M azo-bis-isobutyronitrile (ABN) at 57°. Figure 1 shows the oxidation rate plotted as a function of tetralin hydroperoxide concentration.

Utilizing the mechanism

$$ABN \xrightarrow{O_2} AO_2\cdot \qquad k_1$$

$$AO_2\cdot + RH \xrightarrow{O_2} AO_2H + RO_2\cdot \qquad k_2$$

$$AO_2\cdot + TO_2H \longrightarrow AO_2H + TO_2\cdot \qquad k_3$$

$$RO_2\cdot + RH \xrightarrow{O_2} RO_2H + RO_2\cdot \qquad k_4$$

$$RO_2\cdot + TO_2H \longrightarrow RO_2H + TO_2\cdot \qquad k_5$$

$$TO_2\cdot + RH \xrightarrow{O_2} TO_2H + RO_2\cdot \qquad k_6$$

$$TO_2\cdot + TO_2\cdot \longrightarrow \text{inactive products} \qquad k_7$$

$$TO_2\cdot + RO_2\cdot \longrightarrow \text{inactive products} \qquad k_8$$

$$RO_2\cdot + RO_2\cdot \longrightarrow \text{inactive products} \qquad k_9$$

where RH is cumene and TO_2H is tetralin hydroperoxide, the conventional steady state approximation yields the rate equation

$$\frac{-d[O_2]}{dt} = k_1[ABN] + k_2[RH]\left\{\frac{k_1[ABN]}{k_2[RH] + k_5[TO_2H]}\right\} + k_2[RH]\left[\frac{k_1[ABN]}{\frac{k_5^2k_7[TO_2H]^2}{k_2^2[RH]^2} + \frac{k_5k_8[TO_2H]}{k_2[RH]} + k_9}\right]^{1/2}\left[1 + \frac{k_5[TO_2H]}{k_2[RH]}\right] \quad (1)$$

It is assumed that $k_2 = k_4 = k_6$ and $k_3 = k_5$.

Using values of $k_7 = 2.3 \times 10^7$ liters/mole sec. and $k_9 = 3 \times 10^4$ liters/mole sec. as determined by Melville and Richards[2] and $k_8 = 10^7$ liters/mole sec. as determined by Russell,[1] the value of the rate constant for the exchange reaction k_5 is 12 liters/mole sec. The value of $k_1[ABN]$ was determined by use of diphenylnitric oxide as a radical trap (3.08×10^{-8} mole/liter sec. for 4×10^{-3} M ABN). This value is in good agreement with that determined by Hammond, *et al.*[3] The best literature value[2] of k_2 (0.45 liter/mole sec.) gives a calculated oxidation rate of cumene without tetralin hydroperoxide about 45% lower than observed. Consequently, k_2 was adjusted to 0.64 liter/mole sec. to bring experiment and expectation into agreement. Using these values the solid line in Fig. 1 is calculated in accordance with eq. 1.

Additional experiments were done with the following results: (1) Cumene hydroperoxide at concentrations up to 5×10^{-2} M had no influence upon the oxidation rate with or without added tetralin hydroperoxide. (2) The addition of tetralin up to 0.03 M had no influence upon the rate, demonstrating that abstraction of "normal" tetralin hydrogens is not involved. (3) There is no measurable kinetic isotope effect observable when TO_2D (65%) is used in place of TO_2H. These experiments were run at

(1) G. A. Russell, *J. Am. Chem. Soc.*, **77**, 4583 (1955).

(2) H. W. Melville and S. Richards, *J. Chem. Soc.*, 944 (1954).

(3) G. S. Hammond, J. N. Sen and C. E. Boozer, *J. Am. Chem. Soc.*, **77**, 3244 (1955).