

# FAST ELEMENTARY STEPS IN CHEMICAL REACTION MECHANISMS

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## INTRODUCTION

The essence of chemical rate studies is to learn about the detailed progress of a reaction, *i.e.* the elementary steps involved and their temporal combination in the over-all mechanism. For a long time there were essentially two time ranges, separated by about 10 orders of magnitude in time units, to which almost all our knowledge about molecular kinetics was restricted (*cf.* Figure 1). These were the ranges of "chemical kinetics", the lower limit of which is at about 1 sec (extending over, but hardly exceeding, 6 to 8 orders of magnitude), and the range of spectroscopy of which about 5 orders of magnitude, essentially the range between  $10^{-10}$  and  $10^{-16}$  sec, are of interest to the chemist. This latter range usually does not involve chemical transformations, but it gives information about the physical elementary processes (electron motion, vibrations, bond stretching *etc.*) underlying any chemical transformation. Elementary steps of actual chemical transformation are expected to lie just within the gap extending from 1 to about  $10^{-10}$  seconds.

Only recently has this time range been made accessible by modern techniques which allow the study of almost any type of reaction in solution with half-times from one second to fractions of a millimicrosecond. Figure 1

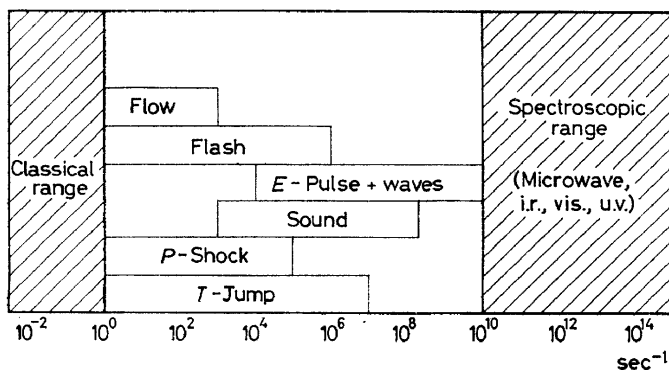


Figure 1. The time ranges of relaxation methods;  $E$  = electric field strength,  $P$  = pressure,  $T$  = temperature, each division of the abscissa represents one order of magnitude in reciprocal time (or frequency)

presents a survey of those techniques which allow a direct temporal recording of the progress of the reaction. These techniques are specifically adapted to



The periodic table in *Figure 2* (related to the usual electronic configurations of the free ions in aqueous solution) may serve as a guide for the following discussion. Bold type indicates where rate studies have been carried out already. The lanthanides and actinides are not included, since no systematic studies have been performed so far. Valence states of 4+ have also been omitted since the free ions in this state hydrolyze so rapidly that no studies of substitution rates are possible except under extreme conditions.

A great variety of different types of ligands were chosen, ranging from relatively weakly complexing ionic species, such as  $\text{NO}_3^-$ , to strongly chelating compounds, such as  $\text{EDTA}^{4-}$  etc. *Table 1* shows a survey of the ligands that were involved in the rate studies.

*Table 1.* The ligands\*

$\text{H}_2\text{O}$ ,  $\text{OH}^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$   
 $\text{SO}_4^{2-}$ ,  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{CrO}_4^{2-}$   
 $\text{ADP}^{3-}$ ,  $\text{HATP}^{3-}$ ,  $\text{ATP}^{4-}$ ,  $\text{TP}^{5-}$   
 $\text{CH}_3\text{COO}^-$ ,  $\text{H}_2\text{NCH}_2\text{COO}^-$   
 $\text{IDA}^{2-}$ ,  $\text{NTA}^{3-}$ ,  $\text{EDTA}^{4-}$ ,  $\text{PhTC}^{6-}$   
 Imidazole, Pyridine

### Alkali ions

For some time, two major difficulties prevented a systematic study of this group. First, the complexes are usually so weak that studies have to be carried out at relatively high concentrations where effects of the ionic atmosphere or unspecific ion pairing interfere. Second, the substitution rates, especially at high concentrations, are so high ( $\tau < 10^{-7}$  or even  $10^{-8}$  sec) that only techniques with a correspondingly high time resolution were applicable.

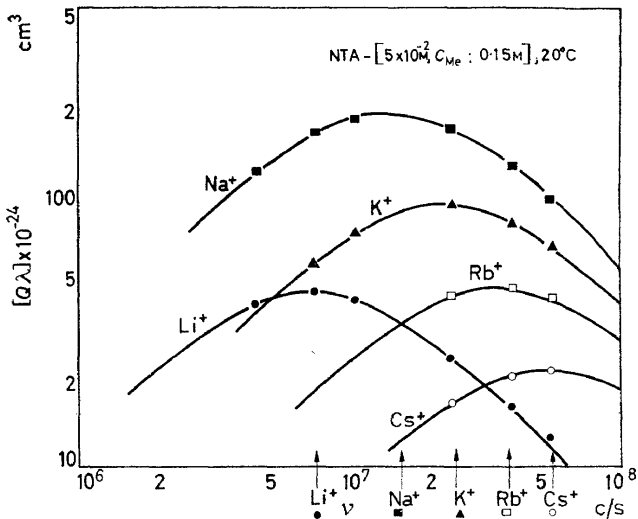
It was only recently that we were able to measure systematically the inner sphere substitution of ligands in this group by sound absorption techniques<sup>3</sup>. Specific effects could be found only for multidentate chelates, such as aminopolycarboxylic acids and for some polyphosphates, such as ATP and inorganic triphosphate.

As an example, *Figure 3* shows some sound absorption data for the different alkali complexes with nitrilotriacetate. A maximum in the absorption per wavelength (or the molecular absorption volume<sup>4</sup> as a function of frequency as shown in *Figure 3*) indicates the presence of a relaxation process, which from the shape of the curves, the concentration dependence, etc. could be identified as a specific chemical relaxation process representing the transformation between an ion pair and a (probably tetradentate) inner sphere complex. The rate-limiting step is the substitution of several water molecules by the ligands. The frequencies of maximal absorption show, as was to be expected, that the process is slowest for  $\text{Li}^+$  and fastest for  $\text{Cs}^+$ . The sequence parallels the ionic radii (*i.e.*  $\text{Li}^+ < \text{Na}^+ < \text{K}^+ < \text{Rb}^+ < \text{Cs}^+$ ) although the differences are not very large. For all ions definite stoichiometric complexes are present. For  $\text{Cs}^+$  and  $\text{Rb}^+$  these complexes, however, are quite weak. This is expressed by the absolute value of maximal absorption. (For evaluation of sound absorption data *cf.* ref. 4.) The highest values are to be found at about 50 per cent dissociation. Under the concentration

\*For explanation of symbols *cf.* legend to *Table 4*.

conditions in *Figure 3*,  $\text{Na}^+$  is closest to this condition, whereas  $\text{Li}^+$  is more and  $\text{K}^+$ ,  $\text{Rb}^+$  and  $\text{Cs}^+$  less strongly associated. This is in agreement with the known stability constants (for  $\text{Li}^+$ ,  $\text{Na}^+$  and  $\text{K}^+$ )<sup>5</sup>, for  $\text{Rb}^+$  and  $\text{Cs}^+$  no values are given in the literature).

The influence of ionic strength was investigated by measuring in excess of tetramethylammonium chloride. (No absorption is present for NTA with



*Figure 3.* Sound absorption (molecular absorption cross-section  $\times$  wavelength) as a function of frequency for alkali metal complexes of nitrilotriacetate ( $\text{pH} > 11$ ) (the relaxation times  $\tau$  are given by  $\frac{1}{2}\pi\nu_{\text{max}}$ )

tetramethylammonium as counter ion, showing the absence of any specific complexing with this ion.) The inner sphere substitution is considerably less influenced by the ionic strength than is ion-pair formation.

Such studies have been performed with EDTA, IDA (iminodiacetate) and some polyphosphates (*cf. Table 2*). The rates for a given metal ion depend

*Table 2.* Substitution rate constants for alkali metal ions  
\* $I = 0.5\text{M}$  ( $\text{NMe}_4\text{Cl}$ );  $\text{pH} > 11$ ;  $20^\circ\text{C}$

	*EDTA <sup>4-</sup>	NTA <sup>3-</sup>	IDA <sup>3-</sup>	TP <sup>6-</sup>
$\text{Li}^+$	$4.8 \times 10^7$	$4.7 \times 10^7$	$2.5 \times 10^8$	$9 \times 10^8$
$\text{Na}^+$	$4.7 \times 10^7$	$8.8 \times 10^7$	$2.5 \times 10^8$	$> 2 \times 10^9$
$\text{K}^+$	$7.5 \times 10^7$	$1.5 \times 10^8$		$> 5 \times 10^9$
$\text{Rb}^+$	$1.4 \times 10^8$	$2.3 \times 10^8$		$> 5 \times 10^9$
$\text{Cs}^+$	$2.1 \times 10^8$	$3.5 \times 10^8$		$> 5 \times 10^9$
	$1/\tau$ ( $\text{sec}^{-1}$ )	$1/\tau$ ( $\text{sec}^{-1}$ )	$1/\tau$ ( $\text{sec}^{-1}$ )	$k$ ( $\text{M}^{-1} \text{sec}^{-1}$ )

somewhat on the nature of the chelating compound, which shows that the chelate formation and not the substitution of the first  $\text{H}_2\text{O}$ -molecule is rate determining. Correspondingly, the rates are highest (and only with  $\text{Li}^+$  and  $\text{Na}^+$  still measurable) for the phosphates and become gradually lower if more ligand groups are involved in chelation. The rate constants of complex dissociation show much larger differences for the different alkali ions than do the rate constants of complex formation. Again, they are lowest for  $\text{Li}^+$  and, since the stability constants are involved, may even differ by orders of magnitude from those of  $\text{Na}^+$ ,  $\text{K}^+$  etc. If, for instance, the anion of uramil diacetic acid is considered, the rate of dissociation may be as low as  $10^3 \text{ sec}^{-1}$  for  $\text{Li}^+$  (since<sup>6</sup> the  $pK$  of the  $\text{Li}^+$ -complex is above 5, whereas for  $\text{Na}^+$  it is around 3 and for  $\text{K}^+$  below 2).

These large differences might be of some importance in the active transport of these ions across biological membranes. Table 3 exemplifies how the chemical rate processes can interfere with the transport mechanism of the metal ion carrier (which might be a strongly chelating compound). The first two rows give rate constants of complex formation and dissociation which may be characteristic of  $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Li}^+$ . The third row shows how far a carrier having a diffusion coefficient of  $\sim 10^{-6} \text{ cm}^2/\text{sec}$  can travel before dissociation occurs. It is seen that only for  $\text{K}^+$  is this distance within the thickness of a biological membrane ( $\sim 100 \text{ \AA}$ ) and only then is the transport mechanism rate limiting. For  $\text{Na}^+$  and especially for  $\text{Li}^+$ , the liberation of the ion from the carrier will be rate limiting. The carrier effect is optimal for  $\text{Na}^+$  in this example, since a relatively high stability of the carrier complex (as compared to  $\text{K}^+$ ) is combined with a sufficiently high rate of liberation (as compared to  $\text{Li}^+$ ).

Table 3. Examples of specific rate constants for alkali metal complex formation ( $k_R$ ) and dissociation ( $k_D$ ) as compared to diffusion lengths  $d = \sqrt{2D\tau}$  (where  $\tau = 1/k_D$  and  $D = 10^{-6} \text{ cm}^2/\text{sec}$ )

$k_R$	$2 \times 10^9$	$10^9$	$5 \times 10^8$	$(\text{M}^{-1} \text{ sec}^{-1})$
$k_D$	$2 \times 10^7$	$10^6$	$5 \times 10^3$	$(\text{sec}^{-1})$
$d$	33	140	2,000	$(\text{\AA})$

This, of course, is not intended as a proposal of a specific model for the active transport of these ions across membranes. However, any model which will explain such processes by carrier action has to take into consideration the rate phenomena discussed above, which for any particular carrier can be determined.

### Alkaline earth ions

A more detailed discussion of this group has been given already in a previous paper<sup>7</sup>. Therefore, we will concentrate here on some new results.

Measurements were extended to  $\text{Sr}^{2+}$ - and  $\text{Ba}^{2+}$ -complexes. As is to be expected, the rates for  $\text{H}_2\text{O}$  substitution by various ligands are slightly higher

than for  $\text{Ca}^{2+}$ . This difference could not be very large, since the rate of  $\text{H}_2\text{O}$  substitution for  $\text{Ca}^{2+}$  approaches that of a "diffusion-controlled reaction". Actually, the substitution rate for  $\text{Ca}^{2+}$  turned out to be even higher than previously reported<sup>7</sup>. Since it reaches almost the rate for substitution in the outer coordination sphere the observed relaxation spectrum, which consists of two time constants, shows the coupling of the two processes to such an extent that both time constants contain contributions from inner and outer sphere substitution. A more exact evaluation leads to rate constants of about  $10^8 \text{ sec}^{-1}$  for  $\text{H}_2\text{O}$  substitution in the inner sphere. Correspondingly high rate constants were found for the bimolecular over-all process of complex formation in the case of chelating compounds<sup>2, 8</sup>.

The values for  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$  (see *Table 4*) are thus very similar to those of the whole series of alkali ions. This finding is very plausible, since the electrostatic potentials are of the same order of magnitude.

The difference in the rate of water substitution  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  is very striking; it amounts to about 3 orders of magnitude. Apparently there is some limiting value of the size of the ion for a given valency below which relatively small differences cause quite appreciable effects in the substitution rates. The rates of  $\text{H}_2\text{O}$  substitution are quite independent of the type of ligand entering the inner coordination sphere, indicating that the splitting of the metal water bond is the rate limiting step in complex formation.

As has already been pointed out<sup>8, 9</sup>, the striking differences in rates for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are of significance for the explanation of the antagonistic behaviour of these ions as activators for enzymatic transformations.

The difference between  $\text{Mg}^{2+}$  and  $\text{Be}^{2+}$  is not as large as one would expect from the difference between  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . Here another effect comes into play, as has been discussed<sup>10</sup> already in connection with  $\text{Fe}^{3+}$  (*cf.* also ref. 7). In the case of very small ions of high valency the rates of hydrolysis often exceed the rates of substitution by orders of magnitude. In such a case the formation of a hydroxo complex which is more labile with respect to substitution (charge compensation) "catalyses" the exchange of a water molecule. If the hydrolysis becomes rate limiting the basicity of the incoming ligand will be of importance. As has been observed in these cases, the rate will not be independent of the ligand any more<sup>7</sup>.

Further divalent metal ions with an  $s^0p^0$ -configuration are represented by the group  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$ . However, they differ from the alkaline earth group by having a closed  $d$ -shell ( $d^{10}$ ), causing some differences in their affinity to various ligands (halide complexes) and their coordination structure (preference of coordination number 2, increasing from  $\text{Zn}^{2+}$  to  $\text{Hg}^{2+}$ ). Studies with various ligands<sup>9, 11</sup> show that the rates of  $\text{H}_2\text{O}$  substitution are practically unaffected by these differences.  $\text{Zn}^{2+}$ , due to its smaller ionic radius ( $r = 0.83 \text{ \AA}$ ), reacts somewhat slower than  $\text{Ca}^{2+}$  ( $r = 0.99 \text{ \AA}$ ) but definitely faster than  $\text{Mn}^{2+}$  ( $r = 0.91 \text{ \AA}$ ). This is probably due to some differences in coordination structure.  $\text{Cd}^{2+}$ , which has about the same size as  $\text{Ca}^{2+}$ , shows also a comparable rate, whereas the larger  $\text{Hg}^{2+}$  is faster by almost one order of magnitude (comparable to  $\text{Ba}^{2+}$  and also to  $\text{Pb}^{2+}$ ). Again, there are no appreciable differences for the various ligands (*cf.* *Table 4*). The rates of complex dissociation, of course, show the specificity as imposed by the complex stability constants.

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 Table 4. Dissociation  $pK$ 's of complexes of divalent metal ions and characteristic rate constants for  $H_2O$  substitution ( $k_{H_2O}$ )

	$pK_D$					$k_{H_2O}$ ( $sec^{-1}$ )
	Gly [0]	Ox [0]	ATP [0·1]	PhtC [0·1]	NTA [0·1]	
$Be^{2+}$	—	~4	—	—	—	$10^2$
$Mg^{2+}$	2·1	3·4	4·0	8·9	5·4	$10^5$
$Ca^{2+}$	1·4	3·0	3·6	7·8	6·4	~ $10^8$
$Sr^{2+}$	0·9	2·5	1·4	—	5·0	~ $2 \times 10^8$
$Ba^{2+}$	0·8	2·3	—	6·2	4·8	~ $5 \times 10^8$

	$Cl^-$		$Ac^-$		$SO_4^{2-}$	
	$pK_D$	$k_{H_2O}$ ( $sec^{-1}$ )	$pK_D$	$k_{H_2O}$ ( $sec^{-1}$ )	$pK_D$	$k_{H_2O}$ ( $sec^{-1}$ )
$Zn^{2+}$	-0·32	$2·5 \times 10^7$	~0·7	$3 \times 10^7$	2·31	$3 \times 10^7$
$Cd^{2+}$	1·54	$4 \times 10^8$	2	$2·5 \times 10^8$	2·31	$>10^8$
$Hg^{2+}$	6·74	$2 \times 10^9$	—	—	—	—

Gly = glycinate, Ox = oxalate, ATP = adenosine triphosphate, PhtC = phthaleine complexone, NTA = nitrilotriacetate, Ac = acetate

**Earth metal ions**

This group of trivalent ions has not yet been studied extensively and, therefore, some preliminary results<sup>12</sup> only will be given.

We may again distinguish the groups of  $d^0$  and  $d^{10}$  configurations, *i.e.*  $Al^{3+}$ ,  $Sc^{3+}$ ,  $Y^{3+}$ ,  $La^{3+}$ , and  $Ga^{3+}$ ,  $In^{3+}$ ,  $Tl^{3+}$ . Among the differences between these groups the decreasing tendency for hydrolysis from  $Al^{3+}$  to  $La^{3+}$  and the opposite behaviour in the second group, *i.e.* the increasing hydrolysis from  $Ga^{3+}$  to  $Tl^{3+}$  may be noted. The substitution kinetics, again, seem only to reflect the electrostatic behaviour of these ions just as in the case of other ions with inert gas-like electron configurations. Due to the triple charge the rates are relatively small: extending from less than  $1 sec^{-1}$  (for  $Al^{3+}$ ; hydrolysis influence) to about  $10^7 sec^{-1}$  for  $La^{3+}$ . The values for most members of this group (*cf. Figure 5*) are only tentative ones since the studies are still under way.

**Transition metal ions**

Systematic studies are still restricted to the divalent ions of the first series only and some of the results have already been discussed in detail<sup>7</sup>. More recent studies were extended to other kinds of ligands (including N-bases)<sup>12, 13</sup>, as well as to other members of the first series, such as  $V^{2+}$  and  $Cr^{2+}$ <sup>12</sup>. The relaxation techniques had to be specially adjusted for studies with these ions. Oxygen had to be completely excluded in order to prevent the occurrence of

small traces of the trivalent ions. For instance, small traces of  $V^{3+}$  perturb the spectrophotometric measurements seriously since the hydroxo compound which occurs around pH 2 shows a strong extinction (with an  $\epsilon$  about 100 times higher than for  $V^{2+}$ ) in the visible range obscuring the spectral changes which are brought about by  $V^{2+}$ -complexes<sup>14</sup>. Furthermore, fast electron transfer processes show up and complicate the interpretation of the relaxation spectra. These studies are not yet completed and the preliminary results reported here are to be considered as tentative. The data cannot be explained with a simple electrostatic model as was the case for ions with filled  $d$ -shells. Non-classical effects such as crystal field stabilization are known to be of importance for the stability of complexes in the transition series and it is therefore to be expected that these properties show up in the kinetic data, perhaps even in a more pronounced manner than for stabilities. Figure 4(a) and (b) show the radius and heat of hydration for the

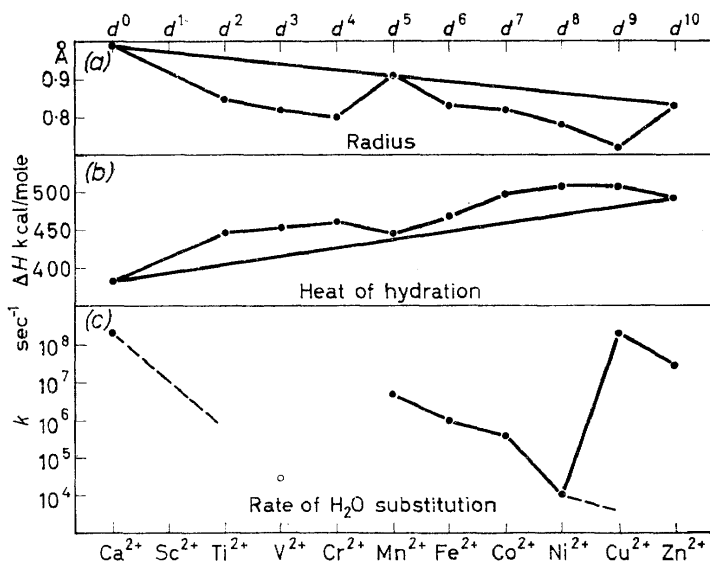


Figure 4. Radii, heats of hydration and characteristic rate constants for  $H_2O$  substitution of the divalent ions of the 1st transition series

divalent ions of the first transition series. (The data were taken from Basolo and Pearson<sup>15</sup> and Holmes and McClure<sup>16</sup>.) There is some periodicity with half filling of the  $d$ -levels as indicated by the straight line connecting  $Ca^{2+}$ ,  $Mn^{2+}$  and  $Zn^{2+}$ . It was shown by Holmes and McClure<sup>16</sup> (*cf.* also ref. 15) that crystal field stabilization can account for the deviation from this line found for the heats of hydration of  $Ti^{2+}$ ,  $V^{2+}$ ,  $Cr^{2+}$  and  $Fe^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ .

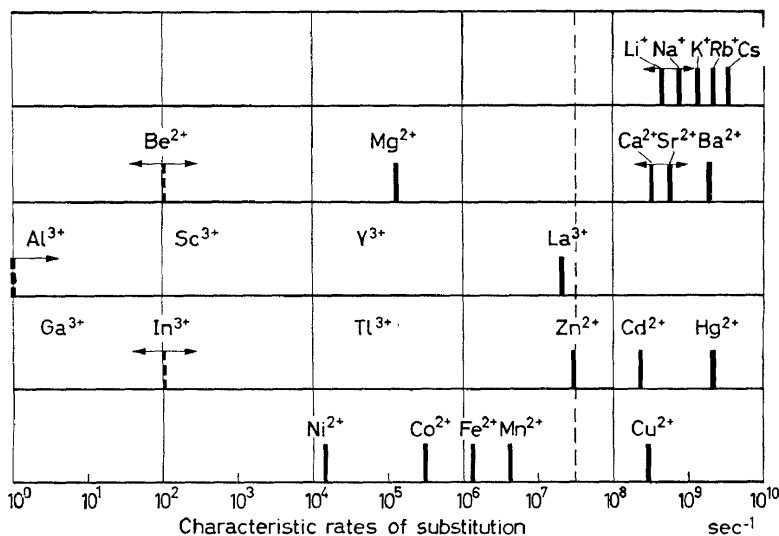
For the kinetic data, any stabilization in the transition states would be the decisive factor. Basolo and Pearson concluded in their review<sup>15</sup> (*cf.* also ref. 17, 18) that for the spin free systems only the  $d^3$  and  $d^8$  states should show an appreciable stabilization regardless of whether an  $S_N1$  or  $S_N2$  mechanism



is involved. Qualitatively, this conclusion is verified by the experimental results.  $\text{Ni}^{2+}$  with a (slightly) larger size than  $\text{Mg}^{2+}$  substitutes by a factor of 10 slower. Also,  $\text{V}^{2+}$  shows a "slow" substitution rate. Two rate effects have been observed so far with different ligands, one in the neighbourhood of  $\text{Ni}^{2+}$ , the other being appreciably slower. These studies are still under way, but there is no doubt that  $\text{V}^{2+}$  shows anomalous'y slow substitution rates.

On the other hand, there is a systematic trend to smaller values also for the  $d^6$ ,  $d^7$  and  $d^8$  configurations (and probably also for the  $d^1$ ,  $d^2$  and  $d^3$  configurations) resembling the above-mentioned periodicity with half filled  $d$ -shells. Actually, the deviations from a straight line are so large for the kinetic parameters (orders of magnitude) that they are beyond any error limits of their detection. Only  $\text{Cu}^{2+}$  and also  $\text{Cr}^{2+}$  which shows a similar value to  $\text{Cu}^{2+}$  do not seem to fit into this relationship. However, the reason for this is obvious. The change in the coordination structure from octahedral to square planar (as a consequence of the Jahn-Teller effect) results in a greater lability of the axial water molecules, which now can be substituted even more easily than in the case of  $\text{Zn}^{2+}$ . (The rates are comparable to those for  $\text{Ca}^{2+}$ ). The attack in the axial position ( $S_N2$ ) probably leads also to a rapid rearrangement of the plane upon incorporation of the incoming ligand since high rates have been found also for the formation of multidentate complexes. Furthermore, the characteristic rates represented in *Figure 4* agree well with Connick's<sup>19</sup> n.m.r. data for the exchange of labelled water molecules in the inner coordination spheres of paramagnetic ions.

These studies now have to be extended to other transition elements in order to complete our knowledge on substitution kinetics. Only a very small portion of such work necessary to establish a general and complete picture has been done so far.



*Figure 5.* Characteristic rate constants for  $\text{H}_2\text{O}$  substitution in the inner coordination sphere of metal ions (abscissa in  $\text{sec}^{-1}$ )

Figure 5 finally presents a survey on the characteristic rates of complex formation in aqueous media. Three groups may be recognized:

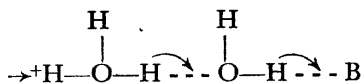
- (i) If the substitution rate constants are higher than  $10^7 \text{ sec}^{-1}$  interference with the diffusion controlled processes of ion pairing may occur. Furthermore, there may exist some specificity for the complexing partner if chelate formation rather than substitution of the first inner sphere water molecule is rate limiting. All alkali and more than half of the alkaline earth ions, but practically no trivalent earth metal ions belong to this category.
- (ii) For rate constants  $< 10^7 \text{ sec}^{-1}$ , independence of the particular ligand is observed if substitution is faster than hydrolysis. The rate limiting step then is  $\text{H}_2\text{O}$ -substitution and the rates are characteristic of the metal ion only. This category includes  $\text{Mg}^{2+}$ , most of the divalent transition metal ions in the 1st series (which only has been studied) and some of the earth metal ions.
- (iii) In the third category, usually showing quite slow substitution rates, the splitting of an  $\text{H}_2\text{O}$  molecule (hydrolysis) is faster than the substitution. Ligand specificity (basicity) may be observed in this group.  $\text{Be}^{2+}$ ,  $\text{Al}^{3+}$  and other strongly hydrolysing trivalent metal ions are members of this group.

### PROTOLYSIS AND ACID-BASE CATALYSIS

As another example of a fast elementary reaction we will consider in this section the "proton transfer". Many mechanisms, especially in organic chemistry, are decisively controlled by this type of reaction for which general relations can be deduced from rate studies. Although this type of reaction is the one which has been most extensively studied by relaxation methods, only a brief survey is given here, since many of the problems are beyond the scope of this conference.

#### Reactions involving $\text{H}^+$ or $\text{OH}^-$

The proton, which is present as the hydronium ion in aqueous media, has in common with the metal ions a strong hydration. Four water molecules form the "inner coordination sphere" yielding a very stable complex ion  $\text{H}_9\text{O}_4^+$ . Direct evidence for this ion has been accumulated by various methods<sup>20-23</sup>. As in the case of metal ions there is some evidence for further hydration (outer coordination). The kinetic properties of the proton, however, are strikingly different from those of the metal ions. The proton can recombine with a base molecule without "substituting" the water molecule which is hydrogen-bonded to the lone electron pair at the acceptor. In this process the proton "penetrates" any H-bonded complex by the same kind of jump mechanism, *e.g.*



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which is responsible for its anomalous high mobility in ice crystals<sup>24</sup>. For a hydrogen bond between equivalent centres, such as  $\begin{matrix} & \diagdown & & \diagup \\ & \text{O} & \text{---H}^+ & \text{---} & \text{O} \\ & \diagup & & \diagdown \end{matrix}$ , the

transfer rate has been shown to be as high as  $10^{13} \text{ sec}^{-1}$  (from measurements in ice crystals, *cf.* ref. 24). The relatively large isotope effect which was found for the deuterium<sup>25</sup> substantiates the non-classical nature of this mechanism. As a consequence, protolytic reactions between partners which are able to form H-bonds are very rapid, limited usually only by the frequency of (diffusive) encounters. The second order rate constants for reactions involving  $\text{H}^+$  or  $\text{OH}^-$  in aqueous media are, indeed, of the order of magnitude of  $10^{10}$  to  $10^{11} \text{ M}^{-1} \text{ sec}^{-1}$ . Table 5 presents a survey of acid and base systems together with their characteristic ranges of rate constants that have been investigated. The highest rates were found for the neutralization reaction<sup>26, 27</sup>. In this case the isotope effect is normal (*i.e.*  $\sqrt{2}$ ) as is to be expected from the mobilities in solution (which do not show the large isotope effect found in crystals, since the transfer rate is limited by the process of H-bond formation<sup>25</sup>). As is seen from Table 5, most organic acids and bases of the O, N

Table 5. Rate constants ( $\text{M}^{-1} \text{ sec}^{-1}$ ) of protolytic and hydrolytic reactions (characteristic ranges)

$\text{H}^+ + \text{OH}^-$ $\text{D}^+ + \text{OD}^-$	$1.3 \times 10^{11}$ $8.4 \times 10^{10}$		
$\text{H}^+ + \text{Inorganic acid anions}$	$10^{10}-10^{11}$	<u><math>\text{OH}^- + \text{Metal ions}</math></u>	$10^{10}-10^{11}$
Metal hydroxo complexes	$10^9-10^{10}$	Inorganic acids	$\sim 10^{10}$
Aliphatic carboxylates	$\sim 5 \times 10^{10}$	Amines (protonated)	$\sim 3 \times 10^{10}$
Aromatic carboxylates	$\sim 5 \times 10^{10}$	Amino-acids	$\sim 10^{10}$
Phenolates	$\sim 5 \times 10^{10}$	Protonated cyclic N-bases	$\sim 2 \times 10^{10}$
Enolates	$\sim 5 \times 10^{10}$	Pyrimidines, purines	$\sim 2 \times 10^{10}$
Amines	$\sim 10^{10}$	Aromatic and aliphatic hydroxo compounds	$\sim 10^{10}$
Cyclic N-bases	$\sim 10^{10}$	Inorganic and organic phosphates	$10^8-10^{10}$
Amino-acid anions	$\sim 5 \times 10^{10}$	Aminopolycarboxylic acids	$10^7-10^{10}$
Carbanions	$(<1)-10^{10}$	H-bond chelates	$10^8-10^9$
		C-acids	$(<1)-10^9$

or S-type, apart from a few exceptions, are characterized by very large rate constants, whereas C-acids show a very broad range of rate constants.

What are the factors determining the rate of a protolytic reaction? There are three specific influences which were found to be of only minor importance. These are size and shape of the acid or base molecule, steric and spatial restrictions, and electrostatic interaction. Size cannot be a very important factor since the reaction site for recombinations with  $\text{H}^+$  is always a lone electron pair at the acceptor molecule, and with  $\text{OH}^-$  the reaction site is a proton at the donor molecule. The accessibility of this site can be restricted by the shape of the molecule or by steric hindrances, but the factors determining these restrictions are usually within one order of magnitude unless



magnitude than the protonation at the  $O^-$  (enol formation). The differences in the deprotonation by  $OH^-$  are of a similar magnitude even though the enol form is stabilized by an internal H-bond and therefore reacts relatively slowly with  $OH^-$ . The difference between this rate and that of a diffusion-controlled reaction amounts to about 3 orders of magnitude. This difference corresponds to the  $pK$ -difference between acetylacetone and dimedone which is due to the existence of the internal H-bond in the former compound. (For a more detailed discussion *cf.* ref. 29.)

Similar behaviour can be found for many other keto-enol transformations or for certain hydrolysis and hydration reactions ( $CO_2$ ,  $SO_2$ , *etc.*). These systems are often called "pseudo"-acids or bases. It might be surprising that acid-base systems exhibiting symmetrical resonance structures (such as carboxylic acids, phenols, aniline *etc.*) show the large rate constants listed in Table 5. One might expect the change in resonance structure upon protonation or deprotonation to require some (free) energy of activation. However, one should bear in mind that the  $pK$ 's of  $H_3O^+$  and  $OH^-$  are such that the reaction itself can provide the necessary amount of (free) energy. This would not be the case if we consider a general acid-base reaction where the  $pK$ 's of the donor and acceptor are almost identical. Let us, therefore, consider this type of reaction in some detail.

### Proton transfer and acid-base catalysis

Figure 6 shows the rate behaviour to be expected for a proton transfer between "normal" acids and bases, *i.e.* if no change in electronic structure or molecular configuration is connected with the proton transfer. The curve in Figure 6(a) refers to a protonic charge transfer which is symmetrical with respect to the forward and reverse reaction, *e.g.*  $XH^+ + Y \rightleftharpoons X + YH^+$  or  $XH + Y^- \rightleftharpoons X^- + YH$ . The rate will be diffusion controlled ( $k \sim 10^9$  to  $10^{10} M^{-1} sec^{-1}$ ) if the acceptor binds the proton more firmly than the donor, *i.e.* if the acceptor is more basic than the donor. Then the rate constant  $\bar{k}$  (or  $\log \bar{k}$ ) for this "downhill" transfer is independent of the  $pK$  difference as long as  $pK_A \gg pK_D$ . Consequently,  $\log \bar{k}$  for the reverse process must be linearly related to the  $pK$ -difference of the donor and acceptor according to the equilibrium condition  $\log \bar{k} - \log k \equiv pK_A - pK_D$ . If we express the relation between  $\log k$  and the  $pK$ -difference by a coefficient  $\alpha$  (*i.e.*  $\partial(\log k)/\partial(\Delta pK) = \alpha$ ), this coefficient will be zero for the "downhill" and one for the "uphill" process. This behaviour is reversed if the  $pK$ -difference becomes negative, *i.e.* if the acceptor is a weaker base (or stronger acid) than the donor. The transition from  $\alpha = 0$  to  $\alpha = 1$  at  $pK_A \approx pK_D$  will be relatively sharp. For a symmetrical transfer (without activation) at  $pK_A = pK_D$  there should be a 50 per cent chance for the proton to go in either direction.

In the case where charge dissymmetry occurs, *i.e.* for processes like  $X^- + YH^+ \rightleftharpoons XH + Y$ , the limiting value for the diffusion-controlled process in the forward direction should be higher than that for the reverse direction thus producing a dissymmetry in the  $\log k - \Delta pK$  relation as shown in Figure 6(b). A similar dissymmetry is to be expected for any other stabilization of one of the species, *e.g.*, internal H-bonding (as discussed above), where the maximum rates of proton transfer in both directions are not the same.

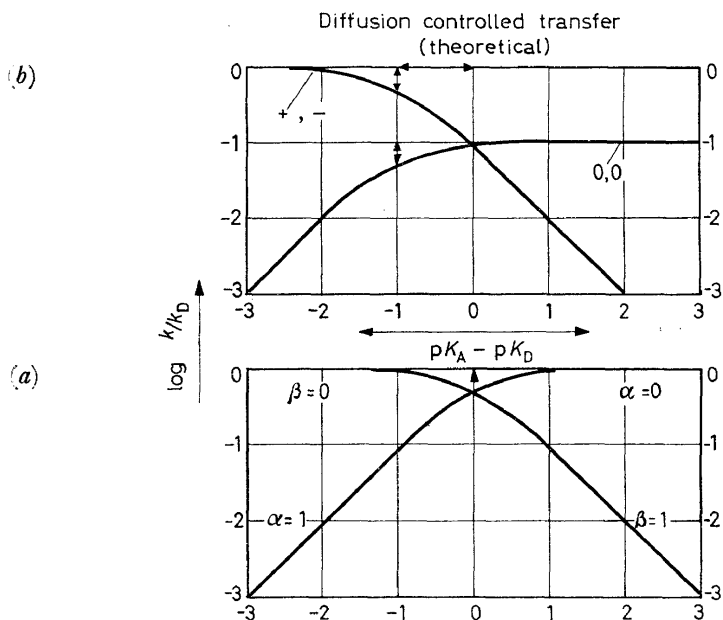


Figure 6. Log  $k$  for the rate of proton transfer as a function of the  $pK$  difference of acceptor and donor (theoretical curves); (a) for symmetrical charge type, (b) for charge neutralization (+, -  $\rightarrow$  0, 0); log  $k$  is normalized by log  $k_D$  where  $k_D$  represents the maximum value for a diffusion controlled reaction

Indeed, a behaviour according to Figure 6(a) and (b) has been found for some "normal" transfer processes involving hydrogen bonds  $\text{OH} \cdots \text{O}$ ,

Table 7. Systems in which proton transfer rates have been studied (each donor or acceptor respectively has been investigated with a number of acceptors or donors in the  $pK$  range listed)

Donor	Acceptor $pK$ -range	Acceptor	Donor $pK$ -range
Acetic acid	3-8	Aniline	3-7
Fumaric acid	3-7	Imidazole	4.5-12.5
Maleic acid	3-8	Hydrazine	5-10
ATPH	4-12	Ammonia	7-12.5
Veronal	5-11	Amines	7-12.5
Thioglycol	5-11	Piperidine	9-12
Phenol	7-12	Arginine	9-12.5
Glucose	7-12.5		

## FAST ELEMENTARY STEPS IN CHEMICAL REACTION MECHANISMS

$\text{OH} \cdots \text{N}$  and  $\text{NH}^+ \cdots \text{O}$  where donor or acceptor are not resonance-stabilized in either one of their forms. However, most of the organic acids and bases are not "normal" in this sense (*cf.* Table 7 which shows some systems that have been studied in collaboration with Kruse<sup>29</sup> and Maass<sup>30</sup>). For most of the systems where  $\text{p}K_{\text{A}} \approx \text{p}K_{\text{D}}$  (*cf.* Figure 7) a smoother transition from  $\alpha = 1$  to  $\alpha = 0$  is found, depending on the kind of H-bond (*cf.* nitrogen, such as in veronal, or SH-compounds, such as in thioglycol in Figure 7) and the degree of resonance-stabilization of one form with respect to its conjugate (*cf.* phenol and acetylacetone). For the keto form of acetylacetone (C-acid) the transition is so smooth that even the reaction with  $\text{H}_3\text{O}^+$  is not yet diffusion-controlled. However, the general shape of the curve is qualitatively the same as in the more normal cases, *i.e.* a transition from  $\alpha = 0$  to 1. Many other systems have been studied in this way (*cf.* Table 7), including "normal" types, H-bond and resonance-stabilized and also some of the so-called "pseudo" types of acids and bases. The general nature of the curves was the same in all cases, but the degree of smoothness varied.

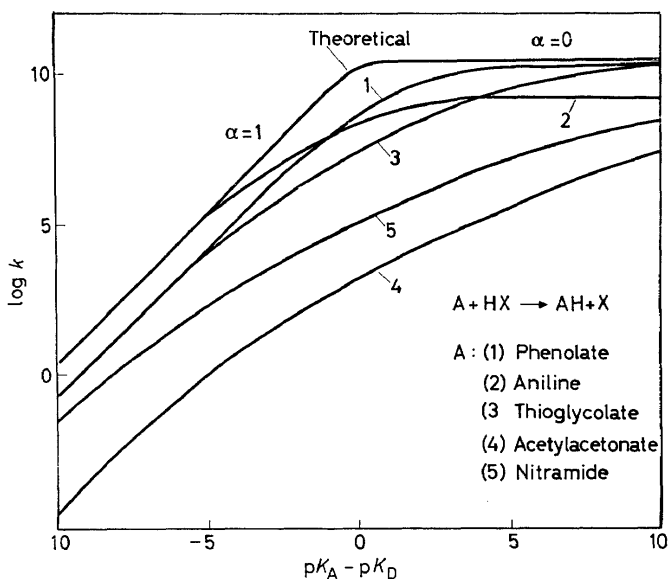


Figure 7. Log  $k$  for the rate of proton transfer as a function of the  $\text{p}K$  difference of acceptor and donor (experimental curves for some acceptor systems of different nature); for the irreversible reaction 5, the  $\text{p}K$  difference is arbitrary; note that the shape of curve 4 is similar to those of the other examples, although a different limiting value is approached

We may now compare this behaviour with the relations which were found for general acid–base catalysis (*cf.* Bell<sup>31</sup>), especially with Brønsted's catalysis law<sup>32</sup>. The above relationship between  $\log k$  and  $\Delta\text{p}K$  (or the catalyst  $\text{p}K$  if the substrate is given) governs almost all of the classical work on acid–base catalysis, but what is usually found is a constant value of  $\alpha$  which is between zero and one (if one does not consider the statistical corrections necessary in cases of polyfunctional acceptors and donors.) A relation of

this type, with an  $\alpha$  of 0.48, has also been found in the classical studies of acetylacetone<sup>33</sup>. However, the classical methods usually allow studies only in a relatively narrow time range where a smooth transition of  $\alpha$  still can be approximated by a constant. This is true for many keto-enol transformations (*cf.* also the variation of  $\alpha$  with the substrate  $pK$  for a series of ketones as quoted by Bell<sup>31</sup>) and other systems. Even the classical example of acid-base catalysis studied by Brönsted and Pedersen<sup>32</sup>, the nitramide decomposition, fits exactly into this scheme (*cf.* *Figure 7*, where the over-all rate is corrected for the tautomerization equilibrium of the ionized nitramide, showing that the actual proton transfer approaches the limit for a diffusion-controlled reaction at extreme  $pK$ -differences which in this case are arbitrary according to the irreversibility of the reaction). We may conclude that Brönsted's catalysis law represents the linear term in the Taylor or McLaurin expansion of a more general relationship with variable  $\alpha$  (varying between 0 and 1) which, however, in many cases holds for a quite appreciable  $pK$  range of catalysts. On the other hand, the general nature of the curves, as demonstrated by *Figure 7*, is not unexpected from a theoretical point of view. (Overlapping of potential curves<sup>31</sup> must lead to  $\alpha = 0$  or 1 for extreme differences in binding, but not necessarily to the limiting values for diffusion-controlled reactions). Also Brönsted and Pedersen in their first paper on general acid-base catalysis<sup>32</sup> anticipated such a behaviour, which in general, does not appear in the classical range of kinetics (a proton transfer slow enough to be measured by the classical techniques will be located in a very smooth transition range).

There seem to be also some examples with an unusual persistence of a constant  $\alpha$  (*e.g.* base catalysis of mutarotation of glucose<sup>34</sup>, dehydration of acetaldehyde hydrate<sup>35</sup> *etc.*). It can be shown that an analysis of such mechanisms in terms of pre-equilibria and rate-limiting proton transfer leads in some cases to the assumption of transfer rates larger than the measured elementary steps or even the limits for diffusion-controlled reactions. Then we may conclude that the separation into isolated steps was not justified and, instead, a concerted or coupled proton transfer mechanism is active. This seems to apply especially to mechanisms which include protonation and deprotonation at two different sites on the same molecule (as in the above quoted examples). The constancy of  $\alpha$  in a wide  $pK$  range results from the fact that the rate of a coupled three-centre transfer is far below the limit for diffusion-controlled processes.

### SOME VIEWS ON ENZYMATIC HYDROLYSIS

The scope of the present article is too restricted to allow any thorough discussion of this type of reaction. Only a few points of view resulting from the study of fast elementary reactions are presented in this concluding section (for more details *cf.* ref. 9).

One expects that an enzyme provides optimal conditions for a transformation to be catalyzed. It is, therefore, necessary to know the possible limits for all elementary steps which may be involved in the over-all mechanism. The results of the last section, which deal with the maximum speeds of protolytic and hydrolytic reactions are of special interest in this connection.



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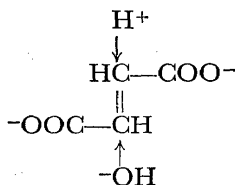
Enzymatic reactions, which include hydrolysis (water splitting) or other kinds of de- or trans-protonation, often reach, but generally do not exceed, "maximum velocities" of  $10^3$  to  $10^4$   $\text{sec}^{-1}$  (examples: fumarase, esterases *etc.*, *cf.* the reviewing articles in ref. 36). If some localized acid-base catalysis is involved we may expect that the active sites have to contain some of the proton donors or acceptors which have been recognized to be especially effective in transferring protons. It can be shown that (especially for transformations in the pH-range around 7) imidazole is one of the most

Table 8. Proton transfer reactions  
Acceptor: imidazole,  $\text{p}K_A$  6.95

Donor	$\text{p}K_D$	$k_{DA}$ ( $\text{M}^{-1} \text{sec}^{-1}$ )	$k_{AD}$ ( $\text{M}^{-1} \text{sec}^{-1}$ )
$\text{H}_3\text{O}^+$		$1.5 \times 10^{10}$	$1.7 \times 10^{3*}$
$\text{H}_2\text{O}$		$2.5 \times 10^{3*}$	$2.3 \times 10^{10}$
Acetic acid	4.76	$1.2 \times 10^9$	$6.3 \times 10^6$
$\text{HATP}^{3-}$	6.7	$2 \times 10^9$	$1 \times 10^9$
<i>p</i> -Nitrophenol	7.14	$4.5 \times 10^8$	$7.0 \times 10^8$
$\text{HP}_2\text{O}_3^{3-}$	8.45	$1.1 \times 10^8$	$3.6 \times 10^9$
Phenol	9.95	$8.5 \times 10^6$	$9.9 \times 10^9$
Bicarbonate	10.33	$1.9 \times 10^7$	$2 \times 10^{10}$
Glucose	12.3	$1.6 \times 10^5$	$2 \times 10^{10}$

\* Dimension( $\text{sec}^{-1}$ ); ionic strength  $\rightarrow 0$ , 25°C.

effective systems (*cf.* Table 8); its effectivity exceeds most of the known acids and bases occurring in the side chains of proteins. For a combined protolysis-hydrolysis mechanism in which the slowest step is rate limiting one can reach maximal rates of  $10^3$   $\text{sec}^{-1}$  (*cf.* the rate constants in Table 8, marked by\* or up to  $10^5$   $\text{sec}^{-1}$  if the transfer occurs between neighbouring groups. The fact that these limits are actually reached for substrates (such as fumaric acid), which are very poor proton or hydroxyl ion acceptors at the reacting site, *e.g.*:



stresses the point that the substrate has to be "activated" by a coupled action of both a proton donor and an acceptor which are kept in an exactly defined steric position. It is not possible here to give a complete explanation of this particular mechanism in terms of elementary steps as has been done already in other cases<sup>9</sup>. However it is evident that such an analysis using the results for isolated and concerted elementary mechanisms as given in the preceding section provides new information and will lead to a better

understanding of the mechanism of enzyme action. Apart from reaction steps which are directly involved in the hydrolytic process, a number of other elementary steps are required to bring the enzyme into optimal contact with the substrate. These are

(i) *The second order rate process of enzyme-substrate complex formation*—Studies with isolated systems, such as enzyme-inhibitor or antibody-hapten<sup>37</sup>, show that in most cases the rates are somewhat below the limiting value of about  $10^9 \text{ M}^{-1} \text{ sec}^{-1}$ . Values between  $10^6$  and  $10^8 \text{ M}^{-1} \text{ sec}^{-1}$ , including appreciable activation energies and entropics (which show that structural rearrangements are involved), seem to be typical for many of those processes. The rate constant of the reverse reaction (in  $\text{sec}^{-1}$ ), the complex dissociation, is often smaller by as much as 4 to 6 orders of magnitude than the formation rate constant (in  $\text{M}^{-1} \text{ sec}^{-1}$ ), indicating quite stable complex formation.

(ii) *Metal complex formation*—The combination of the ES-complex may be brought about by a metal ion and, for fast transformations, the rate of inner sphere substitution may be of importance for the stationary over-all mechanism. Examples of this kind of elementary processes have been quoted on pp. 98-106

(iii) *Structural conformation changes*—The exact "fit" between the active site of the enzyme and the substrate may require a structural conformation change of the protein. Studies of the rates of such processes are being carried out by relaxation techniques<sup>38</sup>. Preliminary studies have shown very large rates ( $\tau < 10^{-6} \text{ sec}$ ) for polypeptides such as polyglutamic acid and polylysine. This indicates that, if such processes are involved in the enzymatic mechanism, they are at least comparable with the high rates of chemical change mentioned above.

These remarks may show that our knowledge about the rates of elementary steps involved in enzymatic reactions is still quite incomplete, but that there are techniques available which are capable of elucidating this field.

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