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Immeasurably fast reactions*

Nobel Lecture, December 11, 1967

1. "*Prejudice and Pride*"

"The rate of true neutralization reactions has proved to be immeasurably fast". I found this quotation in Eucken's *Lehrbuch der Chemischen Physik*[1] while I was preparing for my doctor's examination. Although as a student of Eucken, this book was for me the "bible of physical chemistry", I was then at the age when one accepts practically nothing unquestioned, and so I started to reflect on just how fast an "immeasurably fast" reaction might be.

Clearly, the two reagents - the H^+ and OH^- ions in the case of neutralization - must come together before they can combine to form the reaction product: the H_2O molecule. The maximum rate will be determined by the frequency of such encounters or collisions, and this in turn will depend only on the diffusion rate and the mean separation of the reaction partners. This problem had already been analysed by Langevin[2], von Smoluchowski[3], Einstein[4], Onsager[5], and Debye[6], all of whom had derived expressions for the frequency of ionic collisions. It was fortunate that I was unable at that time to find in Göttingen Debye's paper in which this problem is solved in general form, for this meant that I had to sit down and derive again for myself the expression for the rate constant of diffusion-controlled reactions. The result was a theory which also applies to the mechanism of reactions in which not every encounter is successful. In such reactions the "lifetime of the collision complex" *i.* e. the time after which the partners diffuse apart once more if the encounter has not resulted in reaction, plays an important part. This time worked out as

$$\tau = \frac{R^2}{3(D_+ + D_-)} \frac{1 - e^{-\varphi}}{\varphi} \qquad (1)$$

* The investigation of fast reactions particularly the more recent applications in biological chemistry, has been dealt with in more detail in a number of recently published reviews[55,56,66,72,75]. The present lecture is not intended to duplicate these papers, but represents an attempt to record the development of a scientific idea from a personal point of view.

where R is the separation at closest approach, D_+ and D_- are the diffusion coefficients of the reagents, and φ is the energy of electrostatic interaction divided by kT (ref. 15).

If in this expression we expand the exponential term for the electrostatic interaction of the reaction partners, which is generally relatively small, and use Einstein's correlation between the diffusion coefficient and the "mean square displacement", we find that the encounter time is simply the time required by the reaction partners to diffuse a distance corresponding to their separation at closest approach. Using the known values for D $(10^{-4}–10^{-5}\,cm^2/sec)$, this time works out at

$$\tau = 10^{-10}–10^{-11}\ sec$$

If the actual "chemical" change takes place more quickly than this, the reaction rate is determined entirely by the collision frequency, and if it takes place more slowly, the reaction rate is independent of the rate of diffusion of the reaction partners (and hence of the viscosity of the solvent). It is then simply given by the rate of the chemical change (determined by a frequency factor and an exponential term containing the activation energy) multiplied by the statistical probability of the formation of the reaction complex.

At that time a reaction time of the order of magnitude of 10^{-10} set did in fact seem "immeasurable".

We generally describe as "fast" anything that takes place quickly compared to the rate of resolution of our sense perceptions. However, since our perceptions are in turn based on chemical processes - including the charge neutralization reactions that have just been mentioned - these processes must necessarily be "fast" - indeed "extremely fast".

But what does "immeasurable" mean? If we wish to measure the rate of a chemical reaction, we must do two things: (1) induce the reaction; and (2) follow the course of the reaction in time.

Chemists generally induce a reaction by mixing the reagents with each other. This must be done very quickly, and here the first difficulty appeared: in many cases the mixing takes longer than the entire reaction under investigation. Attempts were therefore made to reduce the mixing time, and Hartridge and Roughton[7] succeeded by using a flow method in which the partners flow together at high velocity and are mixed together within about one-thousandth of a second. At the same time, Hartridge and Roughton also solved the second problem, that of the rapid observation of the course

of the reaction. In the observation tube the time sequence of the reaction appears as a sequence in space, since the mixture flows at a constant velocity. The distance from the mixing chamber (in which the reaction is induced) is a measure of the age of the reaction mixture. This method was undoubtedly a great step forward, and it was subsequently refined and developed into an extremely effective instrument, especially for the enzyme chemist, by Britton Chance and his school in particular, but the reaction times were restricted to the range of milliseconds. Neutralization reactions thus still proved to be "immeasurably fast". If, as has just been shown, we had to assume that the shortest reaction time that might have to be observed was of the order of 10^{-10}-10^{-11} sec, there was a gap here of some 7 or 8 orders of magnitude. On a logarithmic scale, this is the same span as between a millisecond and a day or between a second and the duration of the studies for a doctor's degree, or again the span between the duration of the shortest "measurable" reaction at that time and the time spent in the laboratory to carry out the experiment. This was the situation around 1950, and in this sense neutralization reactions would still have to be regarded as "immeasurably fast" today.

However, there are always two possible courses when one is faced by an obstacle. One can either attempt to overcome the obstacle, as was done by Hartridge and Roughton, or to get around it.

In this case, the second course achieved our objective more quickly: we tried to get around the obstacle. This can be done in the following way: We know that chemical reactions are never entirely completed, and that an equilibrium between the various reagents is always established. Such chemical equilibrium is not static - it does not imply that the individual changes comes to a halt. The situation is, rather, that an opposing reaction sets in and ensures that eventually the number of products formed in unit time becomes equal to the number breaking down in that time. The number of units of the various partners will then on average remain constant. In the case of neutralization, "equilibrium" this means that H_2O molecules are continually breaking down into H+ and OH- ions, but that these ions very rapidly combine again to form water molecules, so that on average there are very few H+ and OH-ions "in equilibrium" with very many H_2O molecules. In pure water the ratio of the number of H_2O molecules to be number of H+ ions is almost 10^9. Unfortunately, it is not generally possible to see the rapid "to and fro" occurrence of these reactions in equilibrium, even if one attempts to "observe" the processes with "rapid resolution" methods. The individual reaction signals average out

precisely*. It would be necessary to seek to "align" or "synchronize" these mutually compensating individual signals. This can be done by rapidly disturbing the equilibrium, thus producing an "excess reaction" in one particular direction for a brief period (this reaction naturally vanishes when equilibrium is re-established). The only further requirement is that we must look quickly enough to see the decay of the excess reaction. We are therefore once again faced with the problem of inducing the reaction and observing it, but it is no longer necessary for us to mix the reaction partners. We need only transmit the inducing signal into the reaction system and then receive the reaction signal from the system. Since the signal must act through a large number of molecules, it must always travel a certain distance, and since there is a limit to the velocity of the signal it is not possible to measure "infinitely" short times. Where then does the limit lie?

The inducing signal can be either mechanical or electrical. A mechanical signal, for instance a pressure wave such as is observed in an explosion, propagates at about the velocity of sound, i.e. at about 10^5 cm/sec in condensed phases. An electric signal, for example a travelling electric wave, is about one hundred thousand times as fast, and its rate of propagation (in condensed phases) is only slightly less than the velocity of light in vacuum. If such signals are made to travel distances of 1-10 mm (the diameter of a measurement cell), we can attain "resolution times" of 1 μsec in the case of mechanical signals and of less then 10^{-10} set with electrical signals. It is naturally also necessary to use inertia-free, *i.e.* optical or electrical, signals to observe the reaction. We shall see that the main difficulty will lie in recording such signals. In the case of chemical equilibrium reactions, the signals emitted are so weak that they are only slightly above the "thermal noise". However, we can see that in principle we have already solved our problem, namely that of following chemical reactions down to within the time range of 10^{-10} sec. "Immeasurably" fast reactions should therefore be measurable after all.

However, I must now make two reservations: (1) When a problem has been solved in principle it is still far from having been solved specifically. (2) New discoveries are not generally made in this deductive manner, even if it appears so in retrospect.

First, chance had to come to our aid.

* We shall for the moment omit from consideration a fairly new class of methods in which the line width of resonance signals emitted by the reaction partners provides a certain amount of information about these individual processes.

2. A Trip to the Seaside

At the Third Physics Institute of the University of Göttingen, my colleagues Konrad Tamm and Günther Kurtze had investigated the absorption of sound by aqueous electrolyte solutions. Behind these investigations there was a technical problem, that of measuring distances in sea water by means of acoustic probes. When an acoustic signal is emitted in the sea, it does not travel far; it is absorbed. Now, it was found that in certain frequency ranges sea water absorbs sound even more strongly than distilled water.

What was the reason for this phenomenon?

Tamm and his colleagues - and also some groups of workers in the United States[9,10] - had found that it is the magnesium sulphate present in the sea water which is essentially responsible for the absorption. However, it was not clear to what interaction the energy loss of the sound waves was due. Was it the hydrodynamic properties of the ions, was it their interaction with the water, *i. e*. hydration of the ions, or was it some interaction between the ions themselves? Since I had considered such interactions in some detail in my dissertation, Tamm and Kurtze turned to me, and we started a joint programme of measurements. It was very quickly found that the absorption could not be caused solely by the interaction between the Mg^{2+} and SO_4^{2-} ions and the water, for neither magnesium chloride nor sodium sulphate dissolved on their own produced comparable effects. On the other hand, neither could a simple inter-ionic interaction be the explanation, either in terms of the Debye-Hückel ion clouds, for which we would expect a broad continuum of absorption at high frequencies[11], or in terms of ionic association as described by Nernst[12a] or Bjerrum[12b], which should give a single absorption maximum. Two separate maxima were in fact found in the ultrasonic region, one at about 10^5 Hz and the other at about 10^8 Hz, which had to be in some way related to each other because of the way in which they were found to depend on concentration[13]. In short, it appeared that there was an interaction between magnesium ions, sulphate ions, and water molecules in the form of a sequence of linked reactions. Here I should anticipate what will be said in a later section (Section 8a, p. 193) by stating that this was in fact the correct explanation: the interaction involves a stepwise substitution of the water molecules bound in the coordination shells of the magnesium-aquo complex by the sulphate ion, with the absorption continuum of the ion-cloud interactions appearing only at higher frequencies (which could not be reached at that time).

3. *Back to Physics*

At this point I feel I should interrupt my account of the historical course of events, and show how chemical interactions can cause the absorption of sound in the first place.

A sound wave represents an adiabatic pressure change progressing periodically in space and time. In water, as a result of the density maximum at 4°C, the simultaneously appearing temperature wave is very small in magnitude compared to the pressure wave. Thus, in the present case essentially we have to consider only the effect of the pressure change on the chemical equilibrium. A chemical equilibrium is always pressure-dependent whenever the reaction partners (in equilibrium with each other) differ in volume. When this is the case, a pressure change will induce a chemical excess reaction which takes place at a finite rate and leads to adaptation to the particular equilibrium state concerned. If the periodic pressure change takes place very rapidly in relation to the chemical reaction, the system will practically not "notice" these changes: the rapid positive and negative disturbances average out before the onset of any appreciable reaction (*cf.* Fig. 1a).

On the other hand, if the pressure change takes place very slowly compared to the chemical reaction, the system follows these changes with practically no lag. The sound then merely propagates at a slightly lower velocity, for the compressibility of the medium contains a contribution from the state of the chemical equilibrium (*cf.* Fig. 1b). Now, the interesting case is that in which the rate of re-establishment of equilibrium is comparable to the rate of the pressure change (i. e. when the time constant for the establishment of chemical equilibrium is of the same order of magnitude as the period of the acoustic wave). In this case the system tries to adapt continuously to the pressure change but does not quite succeed, so that it lags behind the pressure change by a finite phase difference. The chemical state is characterized by the concentrations of the reaction partners or the reaction variable. Because of the finite volume difference between the reaction partners in equilibrium, a volume increment characteristic of the chemical change follows the pressure change with a certain phase lag. In all fields of physics where there is this kind of phase difference between "conjugate" variables there is a transfer of energy (in this case a reduction in the amplitude of the sound waves). For a finite phase difference, the integral $\int P\mathrm{d}V$ is different for the compression and dilatation periods.

The physical situation just described can also be given mathematical expression. The essential steps in the calculation are shown in Tables 1A and 1B.

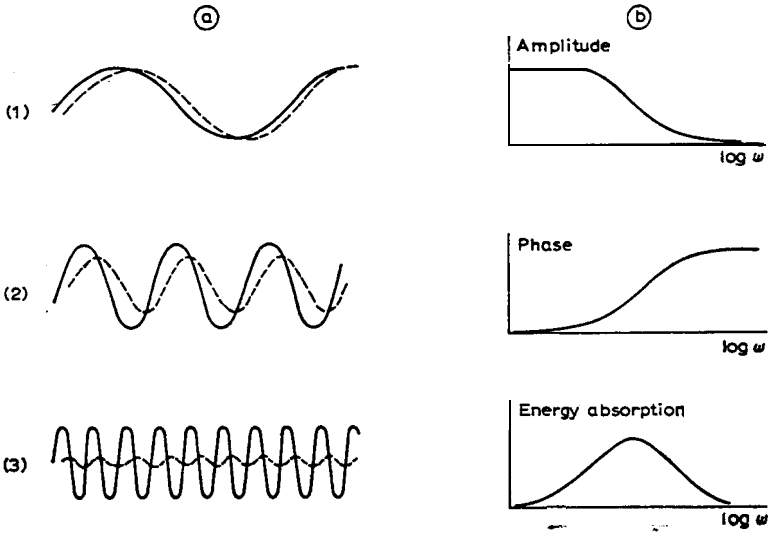

Fig. 1a. Periodic disturbances of a chemical equilibrium. Continuous line, disturbing parameter (e.g. sound pressure, affinity). Broken line, instantaneous value of an "internal" system parameter (e.g. concentration, reaction variable, volume increment of the chemical change). (i) The disturbance occurs slowly in relation to the chemical change. (2) The period of the disturbance and the reaction time are of the same order of magnitude. (3) The disturbance is rapid in relation to the reaction.

Fig. 1b. Amplitude and phase of the equilibrium disturbance and the energy absorption during one period, all as functions of the angular frequency ($\omega = 2\pi\nu$) in the relaxation range.

The starting point is the reaction rate of a particular system. For small disturbances the relevant differential equation can always be reduced to linear form. The concentration variable is then δc, i.e. the deviation of the concentration at any instant from a given reference value (e.g. the mean in the case of periodic disturbances) ; $\delta\bar{c}$ is then the deviation of the hypothetical-equilibrium value (relative to the instantaneous value of the pressure) from the same reference value. The deviation of the instantaneous value of the concentration from its equilibrium value is then $(\delta c - \delta\bar{c})$. This difference is proportional to the rate of the chemical change. In the case of periodic disturbances, the differential equation has a complex solution. The imaginary part ultimately re-appears in the absorption coefficient (*cf.* also Table 1B and Fig. 2). The decreases in amplitude, phase difference, and energy absorption per wavelength ($2\alpha\lambda$) are shown as functions of frequency in Fig. 1b. The chemical contribution to

Table 1

Dispersion and absorption of sound as a result of chemical relaxation
(A comprehensive theoretical treatment with bibliography is given in ref. 32)

(A)

Relaxation equation $\frac{d(\delta c)}{dt} = -\frac{\delta c - \delta \bar{c}}{\tau}$

Periodic disturbance $\delta c = A e^{i\omega t}$

$$\delta c = \frac{\delta \bar{c}}{1 + i\omega\tau}$$

Compressibility $-\frac{1}{V}\frac{\delta V}{\delta P} = -\frac{1}{V}\left(\frac{\delta V}{\delta P}\right)_{\delta c=0} - \underbrace{\frac{1}{V}\left(\frac{\delta V}{\delta c}\right)_P \frac{\delta c}{\delta P}}_{\text{complex}}$

Wave equation $\frac{\delta^2 P}{\delta t^2} = U^2 \frac{\delta^2 P}{\delta x^2}$; $\frac{1}{U^2} = -\frac{S}{V}\left(\frac{\delta V}{\delta P}\right)_S$

Reciprocal of the velocity of sound $\frac{1}{U} = \left(\frac{1}{U}\right)_{\text{real}} + \left(\frac{1}{U}\right)_{\text{imag}}$

Reciprocal of the phase velocity $-\frac{i\alpha}{\omega}$

(B)

$$\frac{\delta c}{\delta P} = \frac{\delta \bar{c}/\delta P}{1 + i\omega\tau} = \frac{\delta \bar{c}}{\delta P}\left[\frac{1}{1 + \omega^2\tau^2} - \frac{i\omega\tau}{1 + \omega^2\tau^2}\right]$$

$\alpha\lambda \sim \frac{\omega\tau}{1 + \omega^2\tau^2}$ Absorption within one wavelength

compressibility has been assumed to be small compared to the contribution of the solvent.

The calculations shown in Table 1 formed the starting point for our investigations on fast chemical reactions[14,15]. It can be seen that the relaxation time of the chemical equilibrium state can be read off directly from the variation of the absorption with frequency e.g. as the reciprocal of the frequency at the maximum absorption per wavelength. This relaxation time is simply related to the kinetic constants of the reaction system, as shown by the example in Fig. 3. This gives us a direct approach to the kinetics of fast reactions - even reactions that were hitherto "immeasurably" fast.

Of course this simple theory of single-stage relaxation processes cannot be applied directly to magnesium sulphate, but a sufficiently simple system was

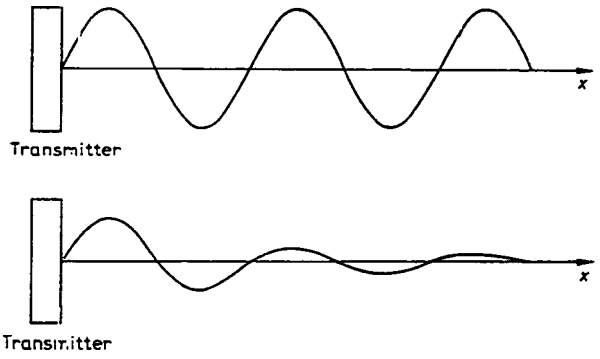

Fig. 2. Schematic representation of a plane propagating sound wave. Top, undamped (U real); below, damped (U complex).

$$P = P_0\, e^{i\omega(t-x/U)}$$

$$\frac{1}{U} = \frac{1}{U_{Ph.}} - \frac{i\alpha}{\omega}$$

$$P = P_0\, e^{i\omega(t-x/U_{Ph.})}\, e^{-\alpha x}$$

$$A + B \underset{k_D}{\overset{k_R}{\rightleftharpoons}} AB$$

$$1/\tau = k_R\,(\bar{C}_A + \bar{C}_B) + k_D$$

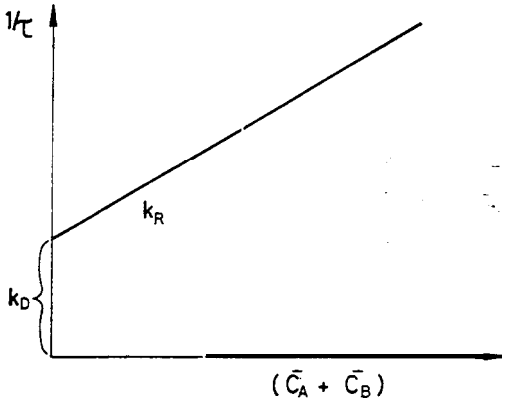

Fig. 3. Reciprocal of the relaxation time of a dissociation equilibrium as a function of the equilibrium concentration of the reaction partners. The gradient and the intercept on the ordinate give the rate constants for recombination and dissociation.

soon found: the hydrolysis equilibrium of ammonia in aqueous solution. A predictive calculation of the absorption and relaxation time (assuming diffusion-controlled recombination of NH_4^+ and OH^-) gave full agreement with the experimental data[15,16] (Fig. 4.).

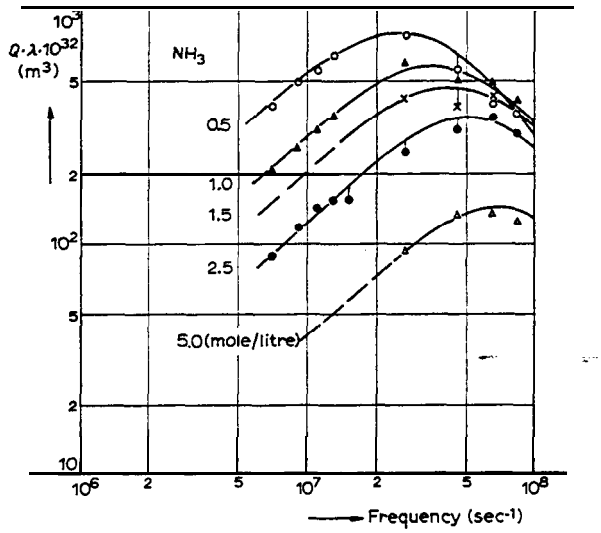

Fig. 4. Absorption of sound as a result of chemical relaxation in a system consisting of NH_3 in aqueous solution. Q. λ (absorption cross-section x wavelength) as a function of frequency (Q corresponds to the energy absorption coefficient 2α related to the total number of NH_3 molecules per unit volume). Parameter, molar concentration of NH_3.

At the same time it was also possible to solve a problem which had interested chemists for a long time. It was shown that in alkaline solution the reaction does not proceed *via* dissociation of the cationic acid (*cf.* the reaction scheme given in Fig. 5). In the reverse reaction, the dissociation of H_2O is accelerated by about ten orders of magnitude by the vicinity of NH_3:

$$NH_3 \cdot H_2O \rightarrow NH_4^+ + OH^- \quad k = 6 \cdot 10^5\ sec^{-1} \qquad (ref.\ 15)$$

$$H_2O \cdot H_2O \rightarrow H_3O^+ + OH^- \quad k = 2.5 \cdot 10^{-5}\ sec^{-1} \qquad (ref.\ 17)$$

The situation was much more complicated in the case of magnesium sulphate. Here it was first necessary to assume a scheme of stages for the stepwise formation of an aquo complex between the magnesium and sulphate ions (see Section 8a, p. 193). For the reaction rates of the different stages we get a system of linked differential equations from which, after reduction to linear form, the

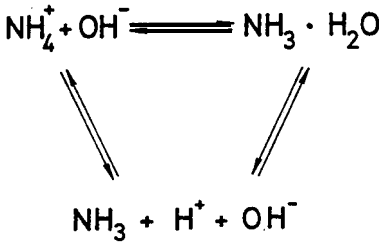

Fig. 5. Equilibrium state in the ammonia-water system. The direction of the reaction depends on the pH of the solution. There are three coupled reactions: (1) neutralization of the cationic acid NH_4^+ and splitting of water in the complex $NH_3 \cdot H_2O$; (2) deprotonation of the cationic acid and protonation of the anhydro base NH_3; (3) dissociation of the solvent water and recombination of the H^+ and OH^- ions originating from the solvent.

relaxation times are found as (negative, reciprocal) eigenvalues[18,19]. They cannot in general be attributed to individual stages of the reaction, as with the normal frequencies of a system of coupled oscillators, but the rate constants ofindividual stages of the reaction can be determined from the relaxation time spectrum with the aid of suitable transformations.

4. Historical Review

The principle of the method described above is quite well-known to physicists. Albert Einstein[20] had already shown in 1916 that relaxation effects appear in a dissociating gas subjected to the periodic temperature variations of a sound wave, and that these effects result in a dispersion of the velocity of sound. At about the same time, Walter Nernst and his associates attempted to detect this effect experimentally in the $2\,NO_2 \rightleftharpoons N_2O_4$ system[21]. However, these measurements were unsuccessful because the techniques of sound transmission were still insufficiently developed. Later, *i.e.* at about the beginning of the thirties, and following investigations by K.F. Herzfeld[22] and H.O.Kneser (ref.23), the interest of physicists and physical chemists turned to the relaxation effects which result from a lag in the establishment of equilibrium in "internal" temperature, *i.e.* to lags in energy transfers between the different degrees of freedom (translation, rotation, and vibration). It was only after these investigations that the "chemical" relaxation effects were detected and analysed. At about the same time, John Iamb and his school were studying the absorption of sound by rotation isomers[24,25]. The detection of chemical

relaxation effects in gases by means of sound absorption measurements was eventually achieved for the classic example of the dissociation of N_2O_4, but only after a delay of about 40 years[26]. In the meantime, the theory of the dispersion of sound based on the thermodynamics of irreversible processes[27,28] had also been developed in very general form, especially by J. Meixner[29,30].

Today we know the relaxation spectra of many chemical reactions, but very few of them derive from sound absorption measurements. The sound absorption method is generally too insensitive for studying chemical relaxation effects. One of the reasons for this is the high background absorption of the solvent. Since this increases with the square of the frequency, very different methods are required to cover a wide frequency range. Thus, at 100 kHz the amplitude of sound in pure water falls by about 37% (1/e) over a distance of 4 km, while at 100 MHz the same percentage reduction takes place over a distance of only 4 mm.

At low frequencies (< 100 kHz) resonance or reverberation techniques are generally used, and at very high frequencies (> 10 MHz) mainly pulse-echo methods are preferred. Several methods are available in the intermediate frequency range: interferometric methods and direct measurement of the damping from observations on the amplitude of the sound from the refraction of light waves in the acoustic field (after Debye and Sears). A review of the various techniques of measurement can be found in refs. 31 and 32. Because of the long wavelengths at low frequencies, large volumes of liquid and hence relatively large amounts ofsubstances are required at such frequencies. At high frequencies it is necessary to work with very high concentrations because of the strong background absorption of the solvent. Even if it has by now by and large proved to be possible to adapt various sound absorption methods to the requirements of chemical relaxation studies[33] - *e.g.* by considerably reducing the liquid volume while at the same time increasing the sensitivity by using difference methods - the application of this method, particularly to the more interesting multi-stage reaction mechanisms of biochemistry, remains very limited.

5. New Paths

Our first thoughts were along the lines of developing new relaxation methods in which the pressure of the sound waves was replaced by another variable, possibly electric field strength. It is of course impossible to carry out such

measurements in travelling electromagnetic waves, for the wavelengths are larger than those of sound waves of comparable frequency by a factor of 10^5. On the other hand, it should be relatively easy to determine dielectric dispersion and absorption directly by means of capacitance measurements using tuned circuits. On examining the literature, we found that although there was here a highly developed measurement technique and a great wealth of experimental data, it related exclusively to the relaxation of dipole orientation and not to chemical effects. The reason for this can be seen immediately from Fig. 6: the equilibrium constant for a chemical change of polar molecules is a quadratic function of the electric field. Small amplitudes are generally used in determining the dielectric constant (ε) or the energy loss ($\tan \delta$), so that the chemical increment does not appear in the linear field terms measured. It was but a short step from recognizing this to going on to develop an appropriate non-linear method[34], even if this left a whole series of technical problems to be solved. In a resonant circuit of high efficiency (with sharp resonance line), a strong steady field is superimposed on a low-amplitude alternating field. The associated chemical reaction leads to a broadening of, and shift in, the resonance line, which can be measured as a function of the field strength $(\sim E^2)$, concentration (corresponding to a second-order reaction) and fre-

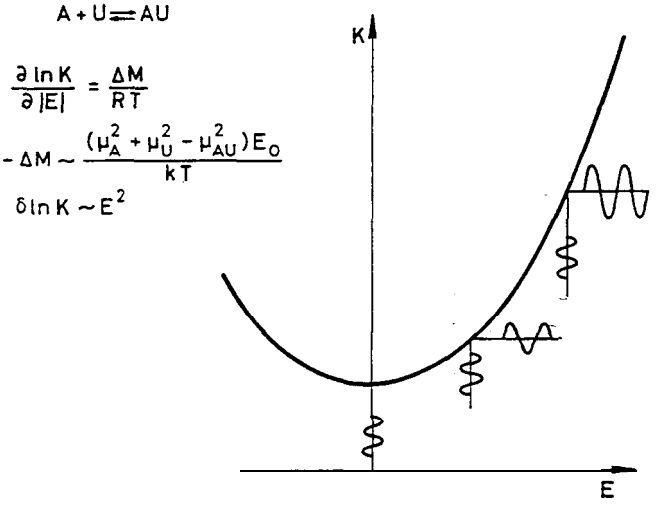

Fig. 6. Dependence of the equilibrium constant K of a chemical change on the electric field. (Example : the base pairing reaction adenine [A] + uracil [U].) In dilute solution the "reaction moment" ΔM varies as shown with the dipole moments of the individual reaction partners. E, electric field strength; kT, Boltzmann factor.

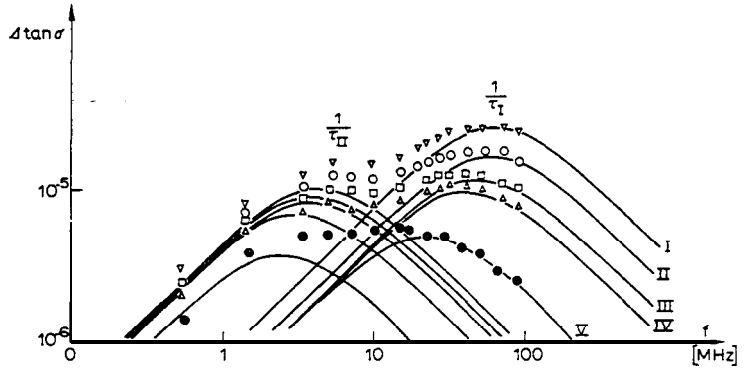

Fig. 7. Dielectric absorption in strong fields as a result of chemical relaxation. (Increment in the loss angle $\Delta \tan\delta$ as a function of frequency.) The two maxima are the result of a relaxation of the coupled chemical reactions indicated. A, ε-caprolactam; B, 2-aminopyrimidine, in cyclohexane, 22°C. 200 kV/cm.

$$A_2 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} A + A + B \underset{k_{31}}{\overset{k_{13}}{\rightleftharpoons}} AB + A$$

$1/\tau_I$ $1/\tau_{II}$

	c_{OA}	c_{OB}
I	0.18	0.01512
II	0.151	0.008
III	0.123	0.008
IV	0.079	0.008
V	0.0226	0.0167

quency $(\omega\tau/1+\omega^2\tau^2)$. In contradistinction to the simple orientation relaxation of dipoles (which generally occurs in the microwave range), the relaxation time of the chemical change is concentration-dependent in accordance with the order of the reaction. This enables the chemical effects to be distinguished from other relaxation effects relatively easily - once they have been made accessible to measurement in the first place. The main factor responsible for the technical difficulties of such measurements is that the effect is very small. Field strengths of up to 3.10^5 V/cm (the breakdown limit) were required, and tan δ (the loss angle) had to be accurate to fractions of 10^{-6}. My colleagues Klaus Bergmann[35] and Leo De Maeyer[36] developed a method of this accuracy. Fig. 7 shows some absorption curves ($\tan\delta$ as a function of the frequency of the alternating field) obtained by Julian Suarez[37]. The curves are for two coupled association reactions of hydrogen-bridge-forming substances in non-polar solvents. Unfortunately, the method cannot be applied directly to polar solvents (because their conductivity is too high), but pulse methods are available in this case, as will be shown later in Section 6 (p.184).

The dielectric method has attained great importance because it enables direct investigation of the kinetics of hydrogen bonding. This method thus enables the kinetics of the individual stages of base pairing in nucleic acids to be followed directly[38]. With long-chain macromolecules it can happen that orientation requires a longer time than the chemical reaction, and in this case the chemical relaxation appears in the linear terms as well. Gerhard Schwarz used a method like this (which does not require a strong steady field to be superimposed) to investigate the kinetics of structural changes in polypeptides[39].

6. Things «Get Even Simpler»

Periodic relaxation methods have added greatly to our knowledge of the kinetics of chemical reactions, particularly in the time range from microseconds to nanoseconds, but their use has always been confined to specific individual cases. In many cases the contribution of a chemical change to the thermodynamic parameters of the solution as a whole *(e.g.* its compressibility or dielectric permittivity) is so small that precise measurement is impossible. This is particularly so when we are concerned with multi-stage reactions, in which the relaxation spectrum could give detailed information on the intermediate stages and hence on the mechanism of the reaction. Here the approach to adopt was to follow the chemical change directly by means of specific properties of the reaction partners. This principle has now become familiar to us: in its simplest form, it can be formulated as follows:

The equilibrium constant must be rapidly changed by a constant (small) amount and the establishment of the new equilibrium followed immediately.

We then obtain a system of homogeneous linear differential equations for the reaction rates. The solutions are exponential functions with real, negative arguments.

However, it is not always simple to carry out in practice what appears to be simple in mathematical terms or in its physical principle.

Although we have now learned how to produce such step-like disturbances (with steep rising and descending branches), we initially attempted to use sinusoidal single pulses. As the disturbing parameter for investigating electrolytic dissociation equilibria we used the electric field strength. M. Wien[40] had already shown at the beginning of the thirties that binary electrolytes exhibit increased dissociation in strong fields. Lars Onsager[41] gave a complete theo-

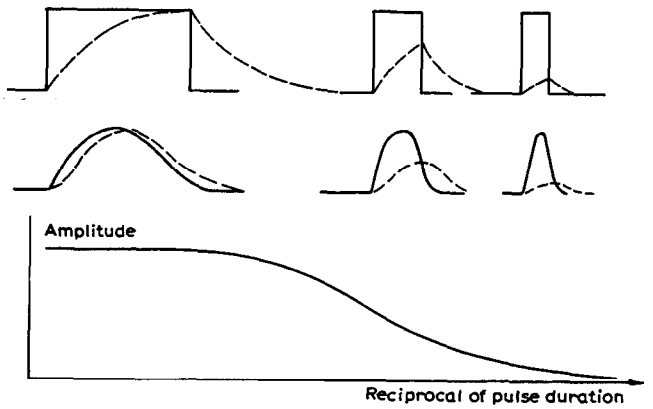

Fig. 8. Relaxation behaviour following pulse-like disturbance of equilibrium.

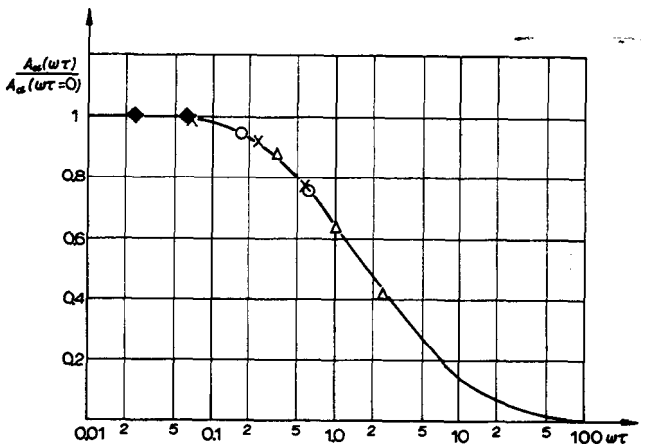

Fig. 9. Dispersion of the dissociation field effect in the systems ammonia andacetic acid in aqueous solution. The (normalized) change in the amplitude of the shift at equilibrium (*cf.* Fig.8) is plotted as a function of the reciprocal of the pulse duration (relative to the relaxation time). (ω is the angular frequency of the critically damped sinusoidal pulse; *cf.* ref. 42.) *t*, 20°C.

		C(moles/litre)	*(MHz)*
NH_3	●	$7 \cdot 10^{-3}$	0.44, 1.45
	×	$7 \cdot 10^{-4}$	0.44, 1.45, 3.60
	○	$2 \cdot 10^{-4}$	0.44, 1.45
	Δ	$8 \cdot 10^{-5}$	0.44, 1.45, 3.60
CH_3COOH	×	$3 \cdot 10^{-4}$	0.44, 1.45, 3.60
	○	$1 \cdot 10^{-4}$	0.44, 1.45

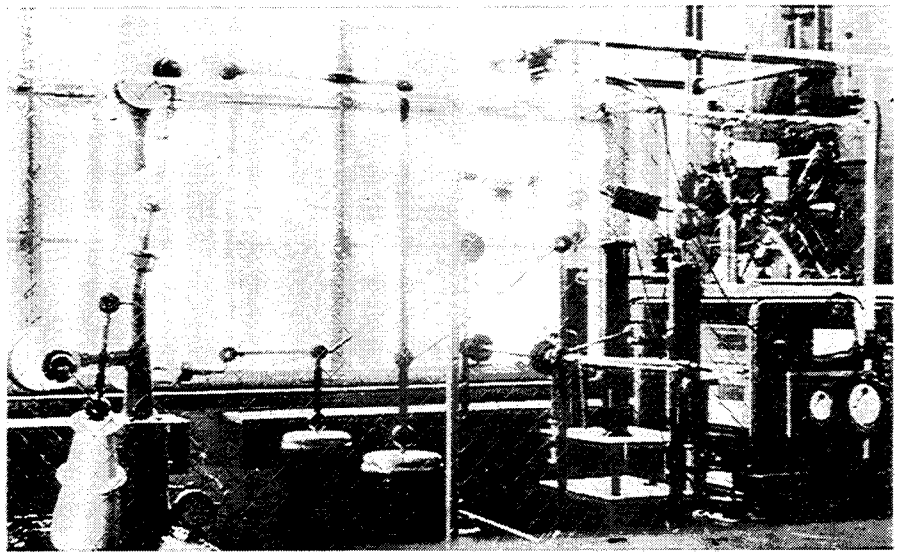

Fig.10. 200 kV high-voltage apparatus, pulse circuit, and impedance bridge with which the first chemical relaxation measurements using the dissociation field effect were carried out in 1953/1954 (*cf.* Fig.9).

retical interpretation of this "dissociation field effect". Fig. 8 shows how the dispersion of this effect can be measured with the aid of short-duration field pulses, and hence used to determine the relaxation time for the chemical equilibrium state. The amplitude of the shiftin equilibrium - measured as a change in conductivity - was determined directly with the help of a null method specially developed for this purpose in collaboration with Josef Schoen[42]. Fig. 9 shows the dispersion of the amplitude of the field effect as a function of pulse duration (or the frequency of a strongly damped harmonic vibration). The high-voltage apparatus used at that time is shown in Fig. 10.

A fairly large number of individual measurements with different pulse lengths were required to obtain a dispersion curve, but in contrast the relaxation time could be measured in a single experiment using a rectangular pulse. This advantage appeared to us decisive in determining the rate of a neutralization reaction. In this case it is possible to obtain a measurable disturbance in the equilibrium only by starting with "very pure" water, in which 10^{-7} moles of H^+ and OH^- ions are in equilibrium with about 55 moles of H_2O. A change in the equilibrium concentration of the ions can easily be followed by means of the electrical conductivity provided no contaminating ions get into the

solution. But this is precisely what happens when the highly purified water is subjected to too many high-voltage pulses. A *single* rectangular pulse - produced by a double spark circuit[17] - made it possible for the first time to measure the chemical relaxation of the dissociation of H_2O and hence the neutralization kinetics. At the same time, a stationary field method made possible the direct measurement of dissociation rates (*e.g.* in ice crystals)[43], thus enabling the kinetic parameters of the transport and neutralization of proton charges in hydrogen-bridge systems to be finally established.

7. On the Way to «Technical Perfection»

Today we use step-type disturbances, which are the most suitable for studying complex, multi-stage reaction mechanisms (see Fig. 11), almost exclusively. This procedure enables the relaxation spectrum to be followed directly by means of discrete steps (with logarithmic time scale). Fig. 12 shows the relaxation spectrum of a biochemical reaction. With a closely packed sequence of steps (a relaxation continuum) we obtain clearly defined mean values, either from the integral or from the initial gradient of the relaxation curve (mean values oft and $1/\tau$ respectively[44]).

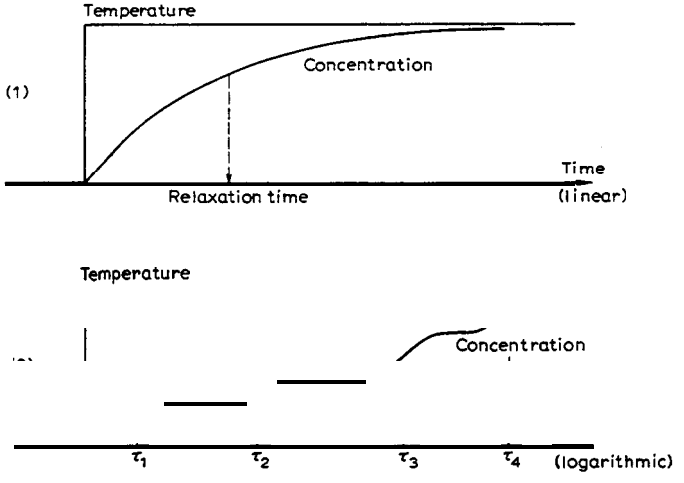

Fig. II. Relaxation resulting from the disturbance of an equilibrium by a temperature jump. (1) A single relaxation process on a linear time scale. (2) Relaxation spectrum on a logarithmic time scale.

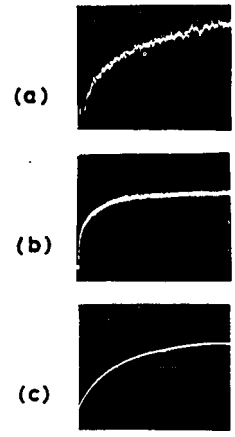

Fig. 12. Oscillogram of a relaxation spectrum with 3 time constants (the system glyceraldehyde-3-phosphate dehydrogenase+β-NAD). The spectrum describes the reaction mechanism of an allosteric enzyme. The relaxation measurements yielded important information on the nature of allosteric control (*cf.* refs. 66-68, see also Figs. 22 and 23). pH, 8.5; 40°C; $D_0 = 6.10^{-4}$(M). (a) 0.2 msec/cm, $1/\tau_1 = 7000^{sec-1}$. (b) 1.0msec/cm, $1/\tau_2 = 690 sec^{-1}$. (c) 500 msec/cm, $1/\tau_3 = 0.2\ sec^{-1}$.

The time resolution depends on the steepness of the step and also on the signal-to-noise ratio that can be attained. Fig. 13 shows as example the field effect of oxyhaemoglobin, measured by Georg Ilgenfritz[45] with a time resolution of about 50 nsec. In this case, the rectangular field pulse was obtained in the form of a travelling wave by discharging a high-tension cable across two spark gaps (Fig. 14)[46].

Pressure waves with very steep ascending and descending branches can also be produced in a shock tube on a similar principle. The shock tube is now a standard instrument for studying fast reactions in gas kinetics[47]. Fig. 15 shows

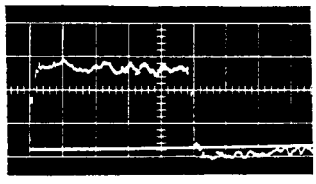

Fig. 13. Oscillogram of the dissociation field effect in oxyhaemoglobin (time scale 10^{-6} sec/cm, relaxation time ~ 50 nsec). The measurement was made by the method described in Fig. 14. System: oxyhaemoglobin (sheep), conc. 10^{-4} *M*, pH 8.9, $\lambda_{obs.}$ 577 mμ, field strength 75 kV/cm, time constant $\leqslant 10^{-7}$ sec.

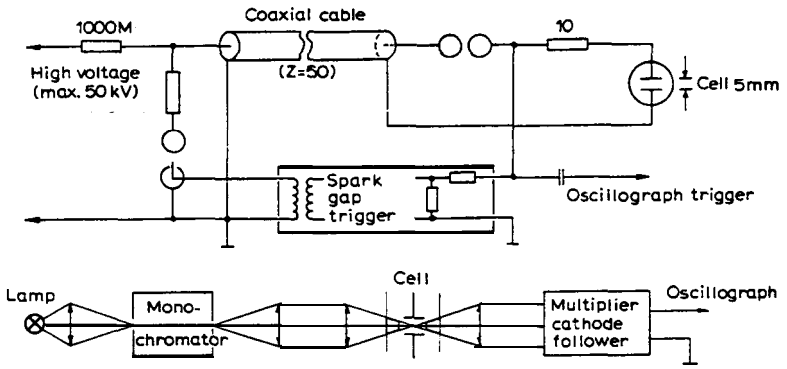

Fig. 14. Schematic representation of the field jump method. Travelling electric wave method for studying chemical relaxation. The (rectangular) electric pulse is produced by discharging a coaxial high-tension cable (time of travel about 3 μsec). The cable is connected through one spark gap (on the left in the figure) with a resistance matched to the impedance of the cable and through the other spark gap (above, right) to the (high-resistance) measuring cell. Depending on which of the two spark gaps is triggered first, it is possible to produce pulse durations that are fractions or multiples of the time of travel (in the former case the energy is dissipated in the matched resistance, and in the latter multiple reflection occurs at the measuring cell). The field effect is recorded spectrophotometrically. (The apparatus was constructed by Georg Ilgenfritz, Dissertation, Göttingen, 1966.)

the principle of a shock tube for liquids developed by Alexander Jost for studying fast reactions in solutions[48]. Simple pressure jump methods have been given by Hans Strehlow[48], among others.

However, particular mention should be made here of temperature jump methods. Very simple in principle, these methods are especially wide-ranging in their applicability to the study of fast reactions.

There are two ways of producing temperature jumps: firstly by adiabatic compression or dilatation, and secondly by heating by means of electrical impulses in electrolyte systems. The first method is not suitable for aqueous solutions, because of the maximum in the density of water at 4°C. Electrical heating can be achieved in two ways: (1) simply with a current impulse in solutions having a finite electrolytic conductivity; and (2) with microwave impulses in the X-band range (dispersion of H_2O orientation) for any conductivity. Strong fields (~ 100 kV/cm) are required in both cases. In the case of microwave heating, this means radar impulses with powers of the order of megawatts. The chemical change is best followed by optical methods (spectrophotometry, fluorimetry, polarimetry). After initial difficulties (inho-

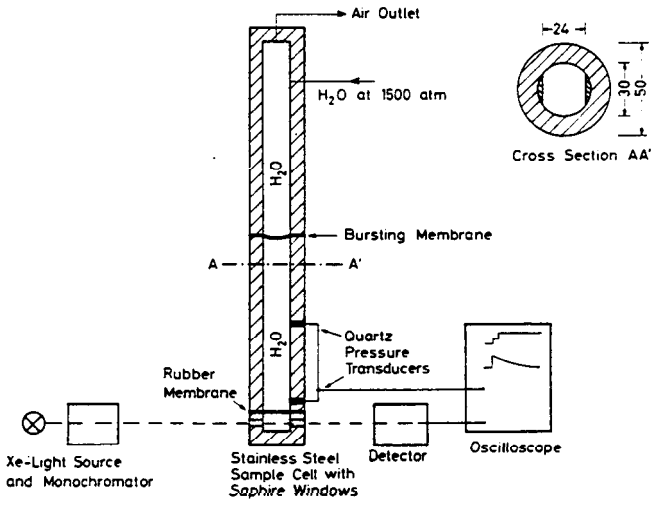

Fig. 15. Mechanical pressure wave method for studying chemical relaxation effects in liquids. The method is a mechanical analogue of the electrical impulse method shown in Fig. 14 (although in this case the time of travel is limited by the velocity of sound which is about 5 orders of magnitude slower). The thickwaled shock tube is completely filled with liquid. The shock wave originates when the metal membrane bursts following the production of a pressure of about 1000-1500 atm in the upper part of the tube. The measurement is made using the reflected shock wave (time of travel from the measurement chamber to the top end of the tube and back: about 10^{-2} sec; steepness of ascending and descending branches $< 10^{-6}$ sec). The solution in the measurement chamber (at the bottom end of the tube) is separated from the liquid filling the tube by a plastic membrane "transparent" to the pressure wave. The chemical reaction is followed spectrophotometrically. The apparatus was constructed by Alexander Jost, Dissertation, Göttingen, 1966, ref. 48.

mogeneous heating in "electric lenses", cavitation resulting from pressure waves, "cross-talk" of the high-voltage impulse on the electrical measurement equipment, unfavourable signal- to-noise ratio in the short time range) had been overcome, the temperature jump method was eventually developed into a standard procedure, which now has an extremely wide range of applications extending from inorganic to biological chemistry. This is particularly due to the development work of Leo de Maeyer[50], Georg Czerlinski[51], Hartmut Diebler[52], Gordon Hammes[53], Roland Rabl[54], and others. Fig. 16 shows a T-jump apparatus developed by Leo de Maeyer and now available commercially. Fig. 17 is a schematic representation of the circuit of a microwave T-jump apparatus developed by Roland Rabl and Leo de Maeyer[54].

Two important improvements considerably extend the possible range of applications : *(1)* A flow arrangement is used instead of a static measurement cell, so that it is also possible to carry out relaxation measurements on systems which are not in equilibrium (but have reached a stationary state). This improvement is of decisive importance in investigating many biochemical reaction mechanisms. *(2)* The stationary-state reaction mixture flowing through the observation capillary can be heated periodically with the aid of repeated microwave impulses. A "ime sampling" procedure can be used to obtain an average over many individual measurements, thus considerably improving the signal-to-noise ratio. The desicive limitation on previous methods in their application to chemical reaction systems concerned the sensitivity of recording rather than the time range involved. The greater the precision and sensitivity of our measurements, the more reaction stages become accessible to direct analysis (*cf.* also ref. 55).

Present-day methods of measurement[32] cover without a gap the time range between fractions of a nanosecond and several seconds, and thus bridge

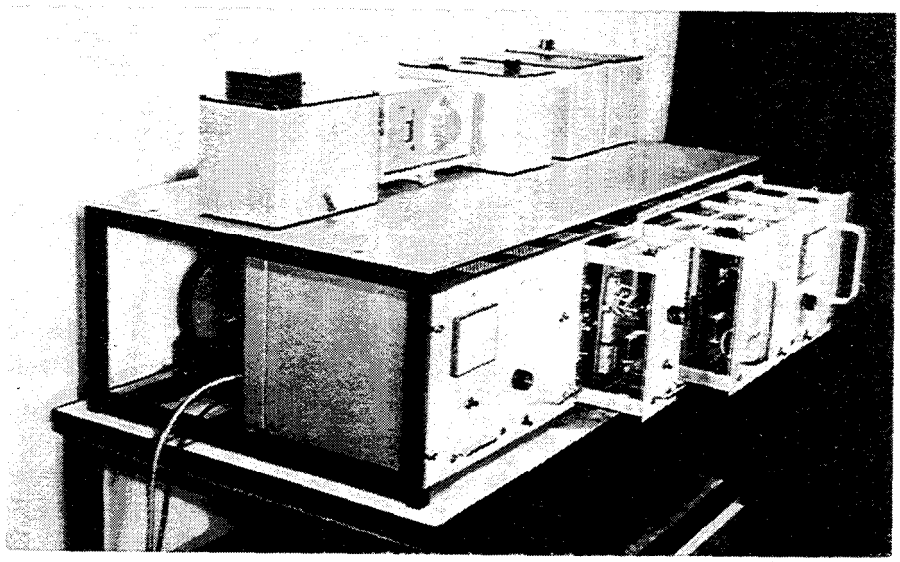

Fig. 16. Photograph of a standard present-day temperature jump instrument, developed by Leo de Maeyer and built by Messanlagen Studiengesellschaft, Göttingen. The lower part contains the high-tension generator, impulse circuit, lamps and multiplier power source and amplifier. The upper part consists of a spectrophotometer with the T-jump cell set up in its beam. This part contains interchangeable units which can also be combined to make a fluorimeter or polarimeter.

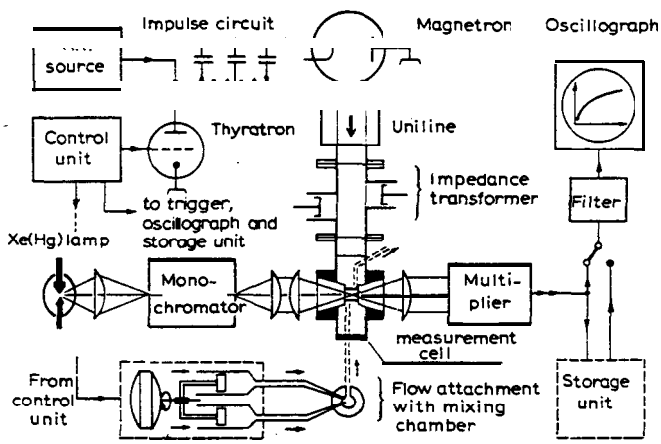

Fig. 17. Schematic circuit diagram of a microwave temperature jump apparatus. The temperature jumps are produced by microwave impulses from a magnetron (frequency 9.35 GHz, power 0.6 MW, pulse duration 0.5-3.0 μsec, temperature jump 3-15°C). The optical measurement cell, which is matched to the impedance of the hollow conductor, is either a static microcell (volume 30 μl) for simple T-jump studies or a capillary for relaxation studies on flowing reaction mixtures in a stationary state. In the latter case, the temperature jump can be repeated with a frequency < 300 Hz. Measurements by spectrophotometry. (Developed by Carl-Roland Rabl, Dissertation, in preparation.)

the gap between the time ranges of classical kinetics and molecular spectroscopy (*cf.* Fig. 18). It has therefore been possible to elucidate the individual steps in many of the reactions previously considered to be "immeasurably fast". Some examples of this will be discussed in the following sections.

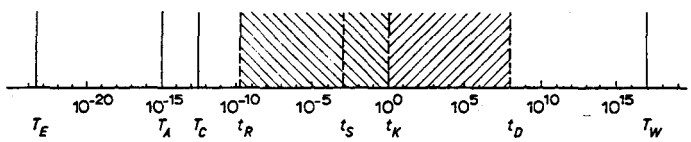

Fig. 18. The time scale of the chemist (see also ref. 56). Hatched part from left to right: proton transfer, electron transfer, formation and rupture of H bridges, isomerization, substitution in coordination compounds, secondary structure changes in proteins, enzymatic changes, elementary processes in biology. T_E, elementary time (dimension of the nucleus/velocity of light) ; T_A, time constant for processes in the atomic shell (excitation); T_C, time limit for chemical changes (~ h/kT); t_R, present lower time limit for direct measurements of reaction rate; t_S, time limit for flow methods; t_K, classical time limit for reaction rate measurements; t_D, time to obtain a doctor's degree; T_W, age of the world.

8. Applications

(a) For the inorganic chemist: a «periodic system» of reaction rates

We have already learned some of the applications of relaxation spectrometry from individual examples. Charge neutralization in the reaction $H^+ + OH^- \rightleftharpoons H_2O$ does in fact take place almost "instantaneously". Every encounter between the solvated ions results in combination ($k = 1.4 \cdot 10^{11}$ mole^{-1} sec^{-1} at 25°C). In this process the proton "tunnels" through a whole chain of hydrogen bonds, as has been shown directly by measurements on ice cyrstals[43]. Are all reactions involving a neutralization of charges then diffusion-controlled?

Let us first have a look at inorganic chemistry. Here we find many reactions in which a positively charged metal ion combines with a negatively charged ligand to form a neutral complex. It is precisely this kind of reaction that is frequently described in the literature as "immeasurably fast". The formation of an aquo complex between Mg^{2+} and SO_4^{2-}, mentioned at the beginning of this lecture, is a typical example of this class of reaction.

A metal ion in aqueous solution is surrounded by one or more shells of coordinated water molecules. If it is to combine with another ion of opposite charge, the latter must penetrate the hydration shells, substituting successive water molecules in the different shells. Since the water molecules in the inner coordination shell are bound most strongly, their substitution will be the slowest step of the process. Relaxation studies on very widely differing metal ions have confirmed this assumption. The mechanism of the stepwise substitution reveals itself in a relaxation spectrum with several time constants[57]. The chemist is primarily interested in substitution in the inner coordination shell. The specific properties of the metal ion, such as charge, radius, coordination number, and electronic structure should be expressed directly at this stage.

Fig. 19 summarizes the measured values of the rate constants for substitution in the inner coordination shell. With a few exceptions, these values are typical of the metal ion alone, *i. e.* they are practically independent of the nature of the substituting ligand. (A more detailed compilation of measurements can be found in ref. 58 and a discussion of the mechanisms involved in ref. 59). Metal ions with an electronic configuration similar to that of the noble gases exhibit the expected dependence on charge and radius. The smaller the radius and the higher the charge the more strongly are the H_2O molecules to be substituted bound, and hence the more slowly does the substitution take place. An in-

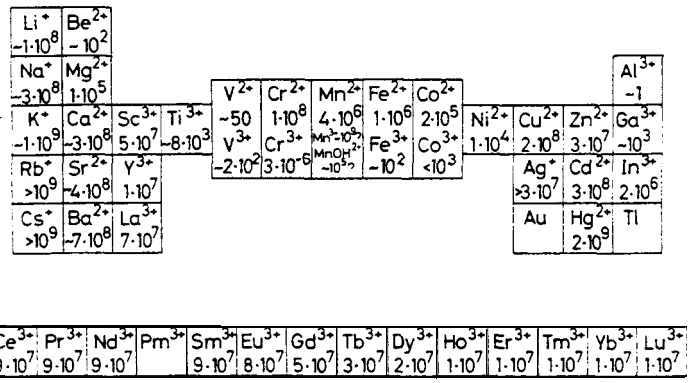

Fig. 19. Characteristic rate constants (in sec^{-1}) for H_2O substitution in the formation of metal complexes. Most of the values are specific to the metal ion and relatively independent of the nature of the ligand (*cf.* refs. 57-59), which also discuss in particular detail the exceptions to this rule). The rate constants for the alkali metal ions and alkaline earth metal ions are essentially determined by radius and charge Among the alkaline earth metal ions - and especially among the rare earths - the coordination number has an additional specific effect. The ions of the transition metals also reflect the specific properties of their electronic structure (ligand field stabilization and the Jahn-Teller effect.)

crease in coordination number makes the coordination shell more labile and therefore accelerates the reaction (*cf.* the lanthamides). Specific effects of electronic structure are revealed only in the case of the transition metals (filling of the d shell). V^{2+} and Ni^{2+} ions have strikingly slow rates of substitution, as a result of particularly strong ligand field stabilization in the transition state[59], whereas Cr^{2+} and Cu^{2+} ions are extremely labile to substitution because of distortion of the octahedral structure in consequence of the Jahn-Teller effect[59]. Fig. 19 shows that most of the rate constants for substitution lie in the range from 10^3 to 10^9 sec^{-1}. The mechanism of many inorganic reactions was thus inaccessible to experimental elucidation until the introduction of relaxation methods.

(*b*) *For the organic chemist: unused possibilities*

The method proved even more successful in measuring the rates of protolytic reactions. The kinetics of proton transfer have been studied exhaustively with reference to a large number of organic acids and bases[60]. These studies have resulted in the elucidation of the reaction mechanism of acid-base catalysis and enabled the Bronsted relations to be extended and generalized[61].

We shall now consider one example: the keto-enol transformation in heterocyclic compounds, e.g. barbituric acid[62].

The anion of this acid (enolate, E-) can acquire a proton either at the $O^{(-)}$ atom or at the $C^{(-)}$ atom. The first reaction results in the enol (E H) and the second in the ketone (KH). T he reaction scheme is as shown in Fig. 21. Both reactions take place so quickly that they cannot be followed by classical methods. When barbituric acid ($pK \approx 4.0$) is dissolved in water, the ionization equilibrium (conductivity) is established "at once". Relaxation measure-

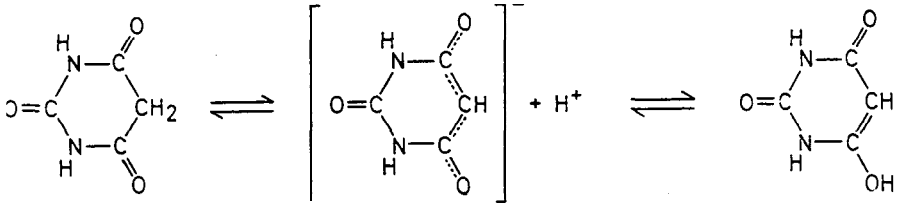

Fig. 20. The keto–enol transformation in heterocyclic compounds.

ments using the electric field method showed that enol formation, like most protolytic recombination processes, is entirely a diffusion- controlled reaction ($k \approx 10^{10}$ mole$^{-1}\cdot$sec^{-1}). Direct determination of the rate of this step is difficult when there is only a small amount of enol in the presence of a large excess of ketone. If we measure the relaxation time in such a system, then at low concentrations of H^+ and E^- we find a reaction of second order, namely the formation of KH from E^- and H^+. The second step, $E^- + H^- \rightleftharpoons E H$, is negligible in this case. However, at high concentrations of E^- and H^- we eventually reach a state in which the enol concentration becomes very much higher than the enolate concentration. What we then find is practically only the conversion of KH into EH (with $E^- + H^+$ as a stationary intermediate state), i.e. a first-order reaction (which can, however, be base-catalysed by E^-). The transition from a second-order type of reaction ($1/\tau$ increases linearly with $[E^- + H^+]$) to a first-order type of reaction ($1/\tau$ = constant) is shown in Fig. 21. Relaxation measurements by the temperature jump confirmed that this shape of curve applied to barbituric acid[62].

It was found that in the case of rapid keto-enol transformations this method enables small amounts of enol to be determined quantitatively in the presence of a large excess of ketone. The gradient of the linear part of the curve in

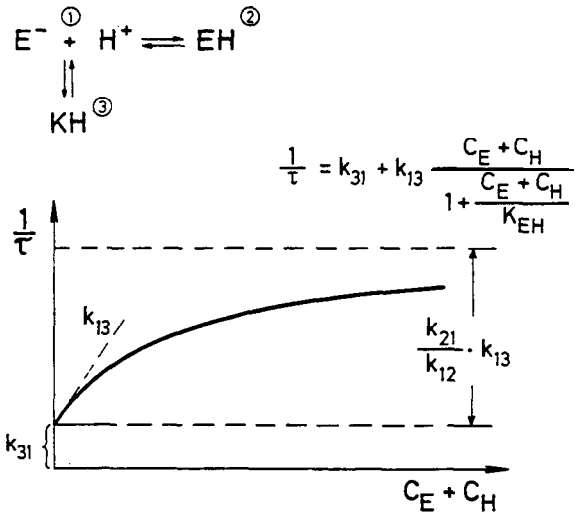

Fig. 21. Reciprocal of the relaxation time of a keto–enol equilibrium. The reaction scheme gives a relaxation spectrum with two time constants. The establishment of the enol equilibrium is very rapid compared to the establishment of the keto equilibrium. The shorter relaxation time is therefore of the form shown in Fig. 3 : $1/\tau = k_{12}(\bar{c}_E + \bar{c}_H) + k_{21}$. The longer relaxation time is also of this form (see Fig. 3) as long as $\bar{c}_E$ and $\bar{c}_H$ are small compared to K_{EH} (there is then so little EH present that the keto equilibrium may establish itself independently). However, at high concentrations of E- and H^+ the quickly established enol equilibrium is always coupled with the keto reaction. $1/\tau$ ultimately tends to a constant limiting value-behaviour characteristic of a first-order reaction (namely EH $\rightleftharpoons$ KH). (In the case of base catalysis by E-, the limiting value can be found by extrapolation.) This method permits the determination of all the kinetic constants and also a very precise determination of both equilibrium constants (and hence also of the keto/enol ratio, even when one form is present in large excess and *both* reactions are taking place quickly).

Fig. 21 and its intercept on the ordinate give the kinetic constants of the keto reaction. The plateau value contains the equilibrium constant of the enol. Other methods generally fail when the dissociation constant of the enol becomes very much larger than that of the ketone and the equilibrium between ketone and enol is rapidly established.

(c) *For the biochemist: «intelligent molecules»*

The main field of application of relaxation spectrometry is now in biology. Many methods of measurement have been developed solely for the purpose of studying complex sequences of biological rections or single specific elemen-

tary steps in such reactions. The slight disturbance to which the system is subjected enables us to "listen in" on the natural course of processes without going beyond the relatively narrow limits imposed by the "conditions of life".

Naturally enough, it was the enzymes, the key substances in the whole mass and energy balance of the cell, that first attracted our interest. Where previously only the gross, overall rates of enzymatic catalysis had been accessible to the biochemist, it was now possible to come to grips with the "fine structure" on the reaction mechanisms.

This opens up an entirely new world: a world of well-"planned", economically-functioning molecular "machines". The molecules of the inorganic chemist can say only "yes" or "no" -by reacting or not reacting. They may also occasionally exchange a "perhaps" by temporarily entering into a "non-specific" interaction, but this achieves little, for they "forget" everything as soon as they are parted. The molecules of the biochemist are quite different; they can "read", "program", "control", "correlate" different functions - and even "learn". Here is an example.

For a long time biochemists were uncertain how to interpret the fact that certain enzymes do not bind their substrates in accordance with the law of mass action (as any proper molecule should), but exhibit a cooperative behaviour, even though the individual binding groups are so far apart from each other that there is no possibility of a direct interaction between them.

In this case, it is evident that the affinity (or "attraction") of the enzyme for its substrate is initially only moderate, *i.e.* the first molecules of the substrate are bound with only a relatively low affinity but they succeed in arousing the "attraction" of the enzyme. This increases with the supply of substrate molecules, so that finally - close to saturation point - all substrate molecules are bound with high affinity. The interaction of haemoglobin with oxygen is such a case. In the lung, when there is a large supply of O_2, there is a complete (cooperative) saturation, but if the partial pressure of O_2 drops below a certain threshold value the haemoglobin suddenly becomes disinterested and once more gives up all its oxygen. If the saturation of the enzyme with substrate is measured as a function of substrate concentration, a sigmoid curve is obtained instead of the hyperbola expected from simple application of the law of mass action[63].

Monod, Wyman and Changeux[64] have proposed a model to explain this cooperative interaction. Their starting point is that an enzyme consisting of several subunits can occur in two different spatial conformations - one with affinity for the substrate or activator (or of high catalytic efficiency) and one

with low affinity (or low catalytic efficiency). In Fig.22, the two different conformations are indicated schematically by squares and circles; their frequency in the population at the various stages is indicated by the boldness of type used for the lines. In a given conformation all the subunits bind with the same affinity; the sites of binding are so widely separated that they have no direct influence on each other. The arrangement of the subunits is symmetrical. It is assumed that all the subunits are present in the same form, and that this can change only according to an "all-or-none" law. Hybrids are excluded, for the transformation of a single subunit would have a marked effect on the pattern of the interactions between the subunits. Such a model can be described by three parameters and can be made to fit the measured sigmoid binding curves perfectly. These three parameters describe the binding of the substrate by both forms and their isomerization. The Monod model makes a number of assumptions whose justification can be tested experimentally. D.E.Koshland *et al.*[65] put forward an alternative model, which also enabled the binding curves to be reproduced exactly with the aid of 3 parameters. One such alternative is the "induced-fit" mechanism, shown in the diagonal of Fig. 22, in which the change in the structure of a subunit is always associated with the uptake of substrate or activator by that subunit. A decision between the different models can be made with the aid of relaxation measurements. The individual stages of the reaction can be analysed in detail in the relaxation time spectrum, and inappropriate models can be eliminated[66].

My colleague Kasper Kirschner was able to show that the mechanism postulated by Monod, Wyman and Changeux applies for the enzyme glyceraldehyde-phosphate dehydrogenase, a key enzyme in glycolysis[67]. It was possible to give all the values for the rate and equilibrium constants of the individual stages[68]. The relaxation spectrum obtained has already been shown in Fig. 12. This mechanism dies not apply so simply in the case of haemoglobin, and there are deviations in the direction of the alternative mechanism proposed by Koshland *et al.*[69]. It has been found that the two models represent the two possible limiting cases of a single, more general reaction scheme (Fig. 22), and that it is possible to state the conditions under which the system will approximate to one case or the other[66].

These investigations have proved to be very important, for, as Fig.23 shows, such a mechanism can explain properties which we meet nowhere else on the plane of molecules and which we have only come to know through the man-made circuit and control elements of electronics and transistor technology. It has been known for a long time that such properties occur in biology,

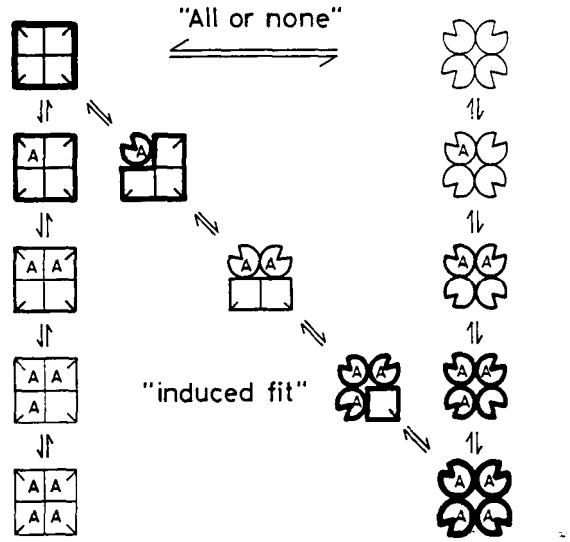

Fig. 22. Reaction scheme of allosteric control in enzymes. The enzyme consists of four identical subunits. The squares and circles indicate two different conformations, only one of which (circle) is capable of transforming the substrate, *i.e.* exhibits catalytic activity; this is indicated by "opening" a site of binding which is "closed" in the square form. The change in conformation is "regulated" by the binding of an activator A. Two alternative mechanisms are shown here: (1) The change in conformation of both forms. which have different affinities for A, always takes place cooperatively according to an "all-or-none" law, but with the square form being preferred in the absence of A and the circular form preferred when A is present at saturation concentration (Monod *et al.* [64]). (2) The change in conformation of the subunits takes place independently of each other, but must first be "induced" in the subunit concerned by the binding of A. A can thus be bound only in one conformation. The induced change in conformation affects the behaviour of adjacent units and thus also brings about cooperative binding (Koshland *et al.* [65]). The two alternatives are naturally only idealized limiting cases of a more general reaction scheme[66].

but it is a new discovery, confirmed by quantitative analysis of molecular mechanisms, that such properties are possessed by single - indeed programmed - molecules, and are not the consequence of a complex of mutually coupled reactions.

Many enzymes have been analysed in this way in various laboratories in recent years[70-72]. Here I should like in particular to mention the work of Britton Chance[73] and his school and that of H. T. Witt and his school[74] on the mechanism of photosynthesis[72].

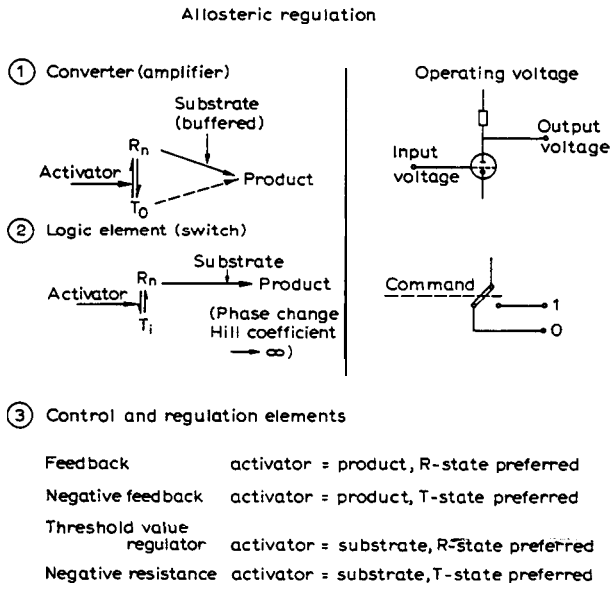

Fig. 23, Comparison of an allosteric enzyme (after Fig. 22) with an electronic circuit element. The activator has the role of a control lattice. Conversion of the substrate into a reaction product corresponds to the current to be regulated. T and R indicate the different conformations of the enzyme (e.g. T = squares, R = circles in Fig. 22). Enzyme activity is restricted to the R-form, *i.e.* there is no conversion of the substrate in the T-form (see also ref. 66).

Other classes of biological macromolecules have also been investigated in detail, for example nucleic acids and lipids. The dynamics of code translation in nucleic acids[66,75,76] are of particular interest. Here again we encounter extremely fast reactions. The lifetime of a base pair is measured in fractions of a microsecond. The reading of a code sequence makes use of these reaction steps. Replication, with many correlated individual steps per code unit (such as reading, linking, transporting), takes place in fractions of a millisecond.

9. Where Now

We are just beginning to understand how molecular reaction systems have found a way to <<organize themselves>>. We know that processes of this nature ultimately led to the life cycle, and that (for the time being?) Man with his central nervous system, *i.e.* his memory, his mind, and his soul, stands at the

end of this development and feels compelled to understand this development. For this purpose he must penetrate into the smallest units of time and space, which also requires new ideas to make these familiar concepts from physics of service in understanding what has, right into our century, appeared to be beyond the confines of space and time.

10. Epilogue

This description has perhaps failed to do justice to some things that were essential to the development of the main idea. I remember with gratitude those who taught and encouraged me: Arnold Eucken, Ewald Wicke, Karl Friedrich Bonhoeffer and Carl Wagner, who showed me the way and selflessly encouraged and helped my work. Karl Friedrich Bonhoeffer who turned my path from physical chemistry to biology. Much that I have described is based on the fundamental work of Lars Onsager, Josef Meixner, and many others I was unable to mention. Much was achieved by named and unnamed colleagues and associates, to two of whom I should like to give special mention as representing the others: Konrad Tamm and Leo de Maeyer.

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