

KINETICS FOR THE LIFE SCIENCES

Receptors, transmitters and catalysts

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1

The time scales of nature: a historical survey

1.1 The history of the application of physical principles

Input into biophysics from physics and biology

It is of interest to consider the historical development of ideas on the formal description and interpretation of chemical kinetics and the contributions made to this field by those with an interest in biological problems. In recent years we have witnessed a return to a multi-disciplinary approach in which more physical scientists are becoming involved in the study of biology. However, it is worth noting that this has been a recurrent feature of this field throughout history. In the eighteenth and nineteenth centuries it was easier to be a polymath than it is today. An outstanding example was Hermann von Helmholtz (1821–1894) who started his professional life as an army medical officer and held in succession chairs of anatomy, physiology and physics. In 1871 he was offered, but declined, the world's most coveted post in physics, the Cavendish Professorship in Cambridge. He developed a most ingenious method for measuring the rates of muscle contraction and the conduction of signals in nerve fibres (Helmholtz, 1850). The design and detailed description of these experiments are well worthy of study 150 years later. At that time he was also involved in establishing that the laws of conservation of energy applied to metabolic processes and physiological functions. His further important contributions were in the physics of vision (Helmholtz, 1856) and of hearing (republished Helmholtz, 1954). Last, but not least, he was associated with the theoretical physicist J. Willard Gibbs (1839–1903) in formulating some of the most important laws in chemical thermodynamics and electrochemistry. These laws are used in the interpretation of the mechanism of the transmission of signals in nerve fibres and of other physiological processes. Thomas Young (1773–1829) and Sir George Stokes (1819–1903) contributed to our knowledge of colour

vision and respiratory pigments, respectively, through the development of spectroscopic techniques. Many of the principal characters involved in the birth of molecular biology were inspired by Schrödinger's (1944) influential little book *What is Life?*. They were trained as physicists (Delbruck, Crick and many others) and wartime (1939–1945) experiences with the development of radar benefited the research on nerve conduction (see Hodgkin, 1992).

Chemical kinetics and biology

In the context of the theme of this volume it is specially pertinent to refer to those who have been among the first to recognize the importance of chemical kinetics to the study of reactions of biological interest. This is not intended to be a comprehensive treatment of the history of this subject, but just a reminder of some of the seminal contributions. Arrhenius (1859–1927), whose work features in sections 7.1 and 7.2, as well as van't Hoff (1884–1911) and Hood (1885) have shown how systematic studies of the temperature dependence of rates and equilibria of chemical reactions can be represented by a logarithmic relation. The empirical plot of $\log(\text{rate constant})$ against the reciprocal of the absolute temperature is still widely used to characterize chemical and biological processes. The theoretical significance and limitations of this treatment of experimental data are discussed in section 7.2. Arrhenius's contributions to the theory of electrolyte solutions (Nobel Prize, 1903) as well as to the theory of the influence of ions on the rates of reactions were of considerable importance for the quantitative study of physiological processes. He summarized his ideas in this field in a volume entitled *Quantitative Laws in Biological Chemistry* (Arrhenius, 1915). It is worth noting that textbooks of physiology often have more detailed discussions of the physical chemistry of electrolytes than all but specialized books on physical chemistry. Arrhenius was also interested in the origin of life on earth and was an early proponent of panspermia: the drift of microorganisms to earth from space.

Two major figures in the field of chemical kinetics, Hinshelwood (1897–1967) and Eyring (1901–1987), turned to the study of biological reactions during the later stages of their careers. The extensive investigations on the kinetics of bacterial growth and adaptation carried out by Hinshelwood and his colleagues are summarized in a monograph (Hinshelwood, 1946). These courageous studies preceded the discovery of the double helix, the definitive recognition of DNA as the transmitter of genetic information and the consequent development of molecular genetics. The reputation of

Hinshelwood's studies thus suffered from being overtaken by events. Eyring applied his ideas on the formulation of a theory of absolute reaction rates, through defined transition states, to a wide range of complex biological problems (Johnson, Eyring & Stover, 1974). Like a lot of controversial ideas, those of Eyring have stimulated many interesting developments. However, one does not have to be a vitalist to assume that a theory developed for simple reactions in the gaseous state will require much modification before it can be applied to complex molecules in aqueous solutions. Some of these modifications are discussed in sections 7.2 and 7.3 in connection with the effects of temperature and viscosity on reaction velocities. The theory of Kramers (1940), describing reactions in terms of a viscosity dependent diffusion over the energy barrier, has come into its own in the interpretation of the dynamics of protein molecules and protein–ligand interaction (see section 7.3).

Atkins (1982, p. 921) makes an interesting distinction between chemical kinetics and the field of chemical dynamics of individual steps. The former is descriptive in terms of rate equations defining the number of intermediates and their rates of interconversion. The latter is in the realm of chemical physics, but it is becoming of interest as modern techniques help to describe the dynamic behaviour of macromolecules. A similar distinction was made above between the descriptive and the mechanistic role of kinetic studies on systems at a more complex level. This emphasizes the important point that general questions about the mechanism of a reaction or a functional process are open ended. It is essential that specific questions are asked and methods are used which are appropriate to their resolution.

1.2 Kinetics and biological problems

Why measure the rates of biological processes?

The title of this chapter is a translation of one used by Manfred Eigen for a lecture given to the Max Planck Society (Eigen, 1966): 'Der Zeitmasstab der Natur'. It covers one of the aims of this volume, namely to provide information about the rates at which various biological processes proceed and how they form a temporal structure. This leads to the second aim, which is to show what kinetics can tell one about the mechanisms of complex functions. A third aim is the exploration of systems at different levels of organization, from the single step of a chemical reaction or the binding of a defined compound to a purified protein, to complex physiological events such as muscle contraction, or the electrical response of

photoreceptor cells to the absorption of a photon by the visual pigment. Ogden (1988), when reviewing a new technique for the initiation of reactions *in situ* (see section 8.4), wrote 'many processes in cell physiology will be understood only when the rates and equilibria of the reaction steps involved are known'. In some cases the aim is principally the elucidation of the detailed chemical mechanism of action of a natural effector at its primary site of interaction; this can be used for the design of drugs. In other, more complex systems the resolution of the sequence of events will help to characterize individual steps, demonstrate their contribution to the overall rate and indicate which change in condition affects specific steps.

Kinetics as a theoretical discipline plays a similar, though some might say less exalted, heuristic role in biology to that of formal genetics. It is, however, of more specialized application and although such analogies must not be pushed too far, some examples will illustrate the point. Genetics is both a theoretical discipline, proposing models and analysing data and also an experimental science concerned with the testing of such models as well as with the discovery of new phenomena. It is applied to systems of widely different levels of organization from large populations of higher animals to chemically well-defined events in prokaryotes, viruses and even to replication in systems containing nucleic acids and enzymes in free solution (Mills, Peterson & Spiegelman, 1967; Spiegelman, 1971). Model building and testing is an important part of genetics and new discoveries about the existence of functions under the control of a single or of multiple genes are made by such studies. Genetic techniques applied to the study of physiological functions can provide information about the sequence of the involvement of different proteins (gene \rightarrow products). For instance Hall (1982) presented an extensive review of the application of genetics to the analysis of the number of functional proteins and their role in phototransduction, chemosensory behaviour and membrane excitability.

A warning is necessary concerning the use of mathematical models in biology. These must be closely linked to the design of experiments which can test them. Examples will come up in the text which demonstrate the danger of models taking over from reality. It is also important to realize that a biological system does not necessarily work at optimum efficiency. Just as in private enterprise, to succeed one only has to be the most efficient in one's immediate environment.

Dynamics of populations

The application of kinetic equations to biological problems on the largest scale is found in modelling of the behaviour of populations. The use of

kinetic as well as genetic methods in this field is expanding with the wide availability of computers. Kinetic models of the time dependence of population sizes were formulated more than a century before the application of population genetics. The derivation of exponential growth curves for populations also preceded the use of such equations for the description of the exponential time course of chemical reactions discussed in section 2.1. The similarity of the approach is illustrated by the fact that one often talks about the population of some molecular species or of a state. Hutchinson (1978) provides a very elegant introduction to the development of equations describing the growth of populations. Other classic surveys of the history of this field are given by d'Arcy Thompson (1948) and Lotka (1956). Exponential growth results in population explosions. We can use a microbial example, discussed in more detail in section 3.3. If one starts off with one cell at time $t=0$, in 24 hours one would obtain (under ideal conditions) 2^{72} cells (i.e. 5×10^{21} descendants) weighing 10^6 kg. On Alexander Pope's advice 'the proper study of mankind is man' and the earliest applications were to human populations.

Demography is an application of quantitative population dynamics of importance for the planning of adequate resources for public services and for the correct assessment of insurance premiums. At the beginning of the nineteenth century Malthus drew attention to the dangers of unrestricted population growth and the resulting competition for resources. Equations for growth rates and survival rates were derived respectively by Verhulst (1804–1849) and Gompertz (1779–1865). Statistics about births and deaths had already been tabulated by Graunt (1620–1674), who discovered the slight excess of male over female births – a continuing trend in human populations. Gompertz's (1825) paper 'On the nature of the function expressive of the law of human mortality and on a new mode of determining the value of life contingencies' is often referred to as the origin of the Gompertz curve. His aim was to deduce life contingencies, or as he put it 'the power to oppose destruction', from tabulated data. There are no plots or curves in his publication, only algebraic expressions derived to fit the data (see also comments on Arrhenius plots in section 7.2).

The Gompertz equation is often written in its modern form:

$$N(t) = N(0) \exp\{-\exp(a + kt)\} \quad (1.2.1)$$

(with constants a and k) for N survivors at time t . Some algebraic properties of equation (1.2.1), which is obtained on integration of $dN/dt = kN(a - \ln N)$, are discussed by Winsor (1932). An equation

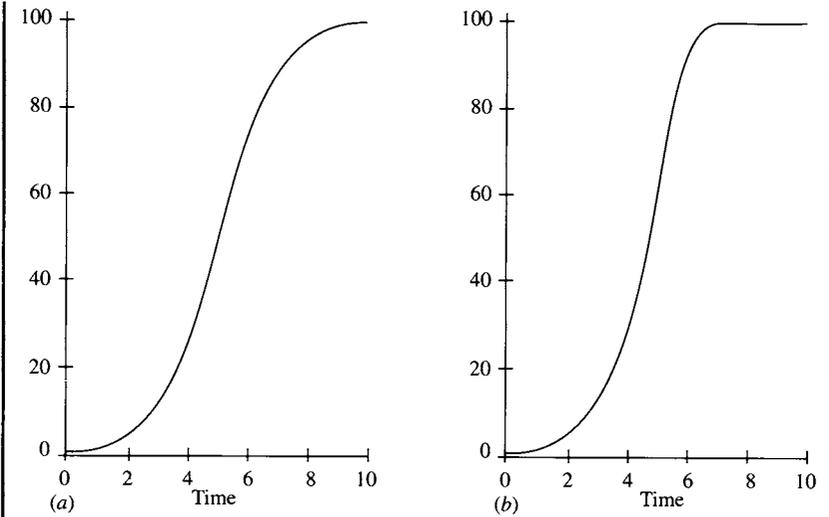


Figure 1.1 Simulation of (a) logistic (symmetrical) and (b) Gompertz (asymmetrical) growth curves. See equations (1.2.5) and (1.2.6), respectively.

for population growth proposed by Verhulst (1804–1849), which is, for unknown reasons, called the logistic equation, has the following mathematical foundation. If one starts from the premise that the rate of growth of a population is a function of its size (see the law of mass action, section 3.1), namely the number of its members N , then we can write

$$dN/dt = f(N)$$

This can be expanded by Taylor's theorem to

$$dN/dt = a + bN + cN^2 + dN^3 + \dots \quad (1.2.2)$$

(with additional constants b , c and d). The simplest rate equation for growth is

$$dN/dt = bN \quad (1.2.3)$$

If we add the next term

$$dN/dt = bN + cN^2$$

on substituting for $c = -a/K$ we obtain (K is a constant)

$$dN/dt = aN(K - N)/K \quad (1.2.4)$$

which gives, on integration, one of the forms of the logistic equation

$$N(t) = K / \{1 + \exp(-bt)\} \quad (1.2.5)$$

For this growth curve the term $(K - N)/K$ in equation (1.2.4) is added as a feedback term to the equation for exponential growth $dN/dt = bN$. This provides an inverse linear dependence of growth on population density.

The logistic curve, like the Gompertz curve, is sigmoidal (see figure 1.1) but the former is symmetrical while the latter is asymmetrical. Both equations can be used for growth or decay by change of a sign in an exponent and they have been used to model many quite varied systems. Easton (1978), for instance, used the Gompertz equation to describe the sodium and potassium conductance changes in giant squid axons in the following form (using Easton's nomenclature):

$$Y = a \exp\{-b \exp(-kt)\} \quad (1.2.6)$$

where the conductance Y is related to the asymptotic conductance a and

$$b = \frac{\text{asymptotic conductance}}{\text{initial conductance}}$$

The logistic equation (1.2.4) can be used to analyse autocatalytic reactions like the proteolytic activation of proteases and the activation of protein phosphokinases by auto-phosphorylation. Many other differential equations developed for the description of population growth, predator-prey interactions and the spread of epidemics have similarities to equations applied to the analysis of the progress of chemical reactions and of control phenomena. References will be made to common applications in relevant sections and simple examples of population problems are found in Pielou (1969) and Maynard Smith (1974). The more general treatment of the problems mentioned is a topic for much active mathematical research on the solution of non-linear differential equations. Incidentally, models of population genetics, of population dynamics and the kinetics of reactions in organized systems all involve diffusion phenomena (see section 7.4). Population growth as well as biochemical systems can, under certain feedback conditions, go into oscillations. Examples from the biochemical literature are given by Segel (1984).

Kinetics and physiology

Helmholtz's classic experiments of kinetic measurements on nerve conduction and muscle contraction have already been referred to. Further examples of the application of kinetics, which are used to demonstrate various points of physiological interest in detail in different sections, come from the kinetic analysis of visual perception and their molecular components. Important information about its mechanism can be obtained from studies at several levels of organization. Attempts can be made to correlate the kinetics of psychophysical observations with electrophysiological measurements on the retina or its component cells. These, in turn, can be compared with the rates of bleaching of rhodopsin and of the consequent cascade of enzyme reactions. The last of these reactions can be studied in solutions of reconstituted systems of purified proteins (see section 4.2). Fuortes & Hodgkin (1964) followed the time course of the first electrical response in the retina after bleaching the visual pigment rhodopsin. The equations which they developed to describe their results were used to postulate the number of distinct events during this process. Further electrophysiological studies and the recent elucidation of the biochemistry of a sequence of reactions subsequent to photon capture have confirmed the complexity of the mechanism of transmission of the message (see Stryer, 1988; and section 4.2).

In general there is a set of criteria which can be used to demonstrate whether an observed step is, or can be, on the direct pathway of a complex process. The rate of the step and its dependence on changing conditions has to be compatible with the overall rate. It is of course easier to exclude a step from the direct pathway, because it is too slow, than it is to ascertain its inclusion. This applies in the exploration of enzyme reactions, as will be discussed in detail in section 5.1, as well as to physiological responses. An example of the latter, which will be used to illustrate points in different sections, is the relation between steps in the hydrolysis of ATP by myosin and structural and mechanical changes during muscle contraction. Similarly the time course of calcium release and removal has to be correlated with the stimulation and relaxation of contraction and other phenomena.

It will be apparent to the reader that the study of rate processes in biological systems has several related objectives. First it can be used in a purely descriptive manner. The physiologist wants to know how fast different muscle types can contract, how fast a nerve can conduct a signal or how fast a cell can respond to a chemical messenger. Each of these is quite a complex process, which can be resolved into elementary steps. A second

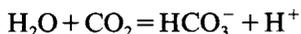
objective would be, for instance, to discover which step or steps are responsible for the difference in the rate of contraction of fast and slow muscle. The use of a range of methods for kinetic investigations increases the resolution by separating complex phenomena into a sequence of well-defined ones. Different methods of perturbation, that is initiations of reactions, and different monitors to follow them provide not only rate data but also characterize the nature of the events. The effects of a change in conditions (temperature, composition of medium, etc.) help to provide information on controlling factors and optimum performance (see section 5.2 for examples).

In the present volume information obtained about the temporal resolution of complex processes will be used as examples to define the problems which can be solved by the application of kinetic reasoning and techniques. There are systems of biological importance for which the rates at which they function are relatively unimportant. For example, the actual rates of oxygen uptake and release by the respiratory proteins haemoglobin and myoglobin are of marginal significance compared to the physiological importance of their equilibrium oxygen binding properties. Yet, without doubt, more kinetic investigations have been carried out on the reactions of haemoglobin than on any other protein. These experiments with a wide range of techniques were designed to explore the interesting mechanism involved in the cooperativity of the interaction with oxygen and other ligands. While equilibrium binding studies provide essential physiological and thermodynamic information about the reactions of haem proteins with oxygen and other ligands (they define the problem), only the results of kinetic investigations can describe the mechanism in terms of the number of reaction intermediates and their rates of interconversion. It is also often only possible to obtain equilibrium constants from kinetic data (see chapter 6).

Kinetic investigations tend to expose open ended questions since one can continue to divide steps into ever increasing resolution. This will be found to be a repetitive theme in the present volume. The exploration of rates of enzyme reactions can move from an interest in metabolic control by changing substrate and effector levels to the exploration of the detail of the mechanism of a single reaction, which would be principally of interest to a physical organic chemist. There are also many different levels of interest in most biological problems. For this reason the design of each investigation should start by asking specific questions. The word 'mechanism' does not mean the same to a physiologist and a chemist. An individual step in muscle contraction, as defined from the point of view of a physiologist, is a complex

series of events for the chemist. This kind of demarcation will be highlighted again in chapter 7 when physical factors controlling rates are discussed. The methods described in other chapters will be illustrated with examples which correspond to varying levels of molecular detail.

In some cases kinetic considerations provided the initiative for the search for a specific addition to the sequence of events during a process. For instance, the role of CO_2 in respiratory function requires a more rapid formation of bicarbonate than that achieved through the uncatalysed hydration



with a half life of 20 seconds. This led to the search for and discovery of the enzyme carbonic anhydrase (for a review see Roughton, 1935). This reaction is sometimes quoted as evidence that all, even the simplest, chemical reactions in biological systems are enzyme catalysed. Similarly, for the role of Ca^{2+} in excitation contraction coupling in muscle, Hill's (1948) calculations showed that the rate of diffusion of the ion is not fast enough to be compatible with the overall speed of response. The discovery of the role of the sarcoplasmic reticulum in calcium secretion and removal helped to resolve this problem.

1.3 Time scales of nature and of measurement

From elementary chemical steps to physiological processes

We shall now turn to a discussion of the range of rates of reactions from elementary chemical steps to physiological functions. This will then be linked to a discussion of methods which cover different time scales and monitors which are suitable for recording different signals. Table 1.1 shows the time scales over which biological processes, and some of the chemical steps associated with them, occur. The photochemical initiation of the utilization of solar energy for biosynthesis and the earliest reactions of the photopigments involved in visual response or bacterial ion transport occur in picoseconds (or faster). Some rate limiting steps in enzyme reactions are 12 or more orders of magnitude slower. Response to control signals and the adaptation of functions, such as contractile processes, occur on three separate time scales. The effect of a messenger or effector binding can be noticed within 10^{-6} to 10^{-1} seconds, the stimulation of the synthesis of a new protein through gene activation will take 10^2 seconds while adaptation to a new environment through natural selection takes of the order of 10^8

Table 1.1a. *Physiological responses and time scales*

Response	Time scale (frequencies per second)
Vibration of fly wings	200
Hummingbird	75
Rattlesnake (on a hot day)	100
Fastest human muscle	50
Human eye	50
Blow fly detecting flicker	250
Protein synthesis	10^{-3}
Range of enzyme turnover	1 to 10^9
Rate of signal conduction in myelinated nerve fibres	
outer diameter 2 μM	10 m s^{-1}
outer diameter 20 μM	100 m s^{-1}

Table 1.1b. *Time scales of kinetic methods*

Method	Time constants (range in seconds)
Fluorescence decay	10^{-6} – 10^{-10}
Ultrasonic absorption	10^{-4} – 10^{-10}
Electron spin resonance	10^{-4} – 10^{-9}
Nuclear magnetic resonance	1 – 10^{-5}
Temperature jump	1 – 10^{-6}
Electric field jump	1 – 10^{-7}
Pressure jump	down to 5×10^{-5}
Stopped flow	down to 10^{-3}
Phosphorescence	10 – 10^{-6}

seconds. The major interest in the present volume is focused on the time window from about 10^{-6} to 100 seconds, which covers the range of ligand binding to proteins and the subsequent conformational rearrangements and chemical transformations. According to molecular dynamics calculations on protein conformation changes the major observed events, with time constants ranging from 10^{-4} to 1 second, are in fact the results of picosecond structural fluctuations on a pathway searching for the new conformation. When considering the suitability of a kinetic technique for the study of individual steps of an enzyme reaction one is confronted with a wide range of turnover numbers which represent the slowest step. The fastest reported turnover rates (see section 7.4) are for superoxide dismu-

tase and carbonic anhydrase. They are of the order of 10^8 and 10^6 s^{-1} respectively, while some digestive enzymes and ribulose biphosphate carboxylase-oxygenase (probably the most abundant enzyme in nature) have turnover rates of seconds. The interesting developments in the application of kinetics to the study of enzymes and other functional proteins are in the design of new experimental methods and in the formulation of models suitable for algebraic description and, above all, for testing proposed models. The actual solution of the necessary equations by analytical or numerical methods does not involve any problems which are not, at least in principle, common to other fields using applied mathematics. The special cases do, however, prove to be of interest to mathematicians.

The approach to a correct model through kinetic investigations is an iterative process: preliminary experiments result in a proposed model. Simulation of this model allows predictions to be tested with further experiments. These experiments result in alterations or refinements of the model and so on (hopefully not *ad infinitum*). Methods for simulations of models and the least square analysis of experimental and simulated data, are discussed in chapter 2. Jencks (1989) gives the warning that models can take on a life of their own. He points out that mathematical models which explain all the data can be physically unrealistic. A *bon mot* attributed to Francis Crick is 'if a theory explains all the facts it is likely to be wrong because some of the facts will be wrong'.

There is an interesting relation between methods which have been developed for the study of rate processes and what actually occurs in biological systems. All techniques depend in some way on disturbing a system and observing the time course of the response to the disturbance as a new equilibrium or steady state is approached. Fluctuations about an equilibrium, which can be studied in 'small' systems containing relatively few molecules, can also be interpreted in terms of rates of transitions between different states. This approach to kinetic analysis is widely used in studies of ion channels of excitable membranes. Some very specialized methods are involved (Sakmann & Neher, 1983) and only their potentialities can be discussed in this volume. Similarly, perturbations due to changes in concentrations or in the physical environment occur in nature in response to stimuli, these in turn initiate reactions. The time course of these responses provides information about the temporal structure of the system and its function. This is analogous to studies of physical and structural properties of materials. These are usually carried out by perturbing the material to get information about elasticity, viscosity, stiffness, etc. from the way it responds to such perturbations. In research on the molecular

mechanism of muscular contraction mechanical responses of fibres are correlated with the kinetics of molecular events (see for instance Hibberd & Trentham, 1986).

Technical developments and time scales

In this discussion of time scales some reference must be made to examples of the major technical achievements, which make it possible to obtain kinetic data over a wide time scale and from responses to different perturbations. These methods, which have been selected on account of their special features and application to biological problems, will be referred to in discussions of the interpretation of the kinetic behaviour of different systems. The explanations are schematic rather than in technical detail. Many features relating to the monitoring of changes in reactant concentrations via changes in some physical parameter are common to all methods. A discussion of monitors would require chapters on electronics and optics. Factors which determine the time resolution are discussed below.

There are two criteria for the time resolution of a technique for following the rate of a process. The first is the precision with which zero time is determined and the second is the overall response time of the sensor (transducer of any physical change to an electrical one) and the system used for amplification and recording the signal. Some aspects of frequency response, time constants and signal to noise ratio are closely related to kinetic theory. The classic physiological experiments of Helmholtz, referred to above, were among the first in a long history of the use of galvanometers and smoke drums for the sensitive recording of electrical signals with a millisecond response. Interest in the theory of the frequency response of galvanometers has been maintained until fairly recently (Hill, 1965).

Whenever possible the basic molecular events of physiological processes are studied in detail in solutions either in parallel with, or as a guide to, experiments on the organized system. For example, the study of the reactions of myosin and the associated proteins of the contractile cycle, by the methods used for the investigation of mechanisms of soluble enzymes (see section 5.1), has helped in the planning of experiments and in the interpretation of the events observed in muscle fibres. The most useful methods for kinetic studies on physiological functions are those which can be applied to systems at different levels of organization. We shall return to them at the end of this chapter.

The time scale of methods for the study of reactions in solutions was

greatly extended by the rapid flow techniques initiated by Hartridge & Roughton (1923a, b). The stimulus for this development was their interest in the reactions of haemoglobin with oxygen and other ligands. Up to that time there was no general procedure available for the study of reactions in solution, which allowed a time resolution much better than, say, 30 seconds. The methods of Hartridge & Roughton relied on the rapid linear flow (about 10 m s^{-1}) of the reaction mixture along a capillary tube. The progress of the reaction was monitored, with a spectroscope or a thermocouple, at intervals along the tube. The initiation of the reaction, at the top of the capillary, was carried out either by injecting two reactant solutions through a mixing chamber, or by photoactivation by a constant strong light source. The use of the length of a capillary tube as a time axis was similar to that employed by Rutherford (1897) for the study of the recombination of ions. In his experiments, gas flowing down the tube was subjected to ionizing radiation at the top of the tube and the extent of recombination was monitored with electrodes at points downstream. The procedure involving mixing chambers, which made it possible to define the start of the reaction with an accuracy somewhat better than one millisecond, had many further applications. Initially the method could only be used when large volumes of reactant solutions were available since it relied on many individual readings during constant flow of the reaction mixture. The obvious interest in the application of flow techniques to the study of intermediates in the reactions of enzymes and respiratory proteins, which were being purified in small quantities, encouraged Roughton, Millikan, Chance and Gibson (see Roughton & Chance, 1963) to design equipment which was more economical in materials. Chance (1943) was inspired by Keilin & Mann's (1937) spectroscopic observations on different compounds of the enzyme peroxidase to carry out time resolved studies. He used an accelerated flow device to observe the kinetics of enzyme-substrate reactions. Keilin's studies of cytochrome spectra (for review see Keilin, 1966) also provided Chance (1952) with the background for his investigations of the kinetics of the reactions during cellular respiration. Rapid flow techniques were applied to studies on suspensions of mitochondria and whole cells. It thus became possible to study chemical reactions under conditions approaching those appertaining *in vivo*.

The modern commercially available stopped-flow apparatus has become a standard piece of laboratory equipment. A short burst of flow through the mixing chamber fills an observation tube with reaction mixture less than 1 ms old. When flow is stopped the signal from the photomultiplier or other transducer is fed into standard amplifiers and digital recording equipment