Meeting Report

OSCILLATORY PHENOMENA IN BIOLOGICAL SYSTEMS

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Recently, under the auspices of the European Molecular Biology Organization (EMBO) and supported by the Max-Planck-Gesellschaft and the Deutsche Forschungsgemeinschaft, a workshop on oscillatory phenomena was held, 3–6 October 1976, covering the thermodynamic and kinetic requirements for the generation of periodic phenomena, mathematical methods as well as specific chemical, biochemical and biological systems.

1. General aspects

In a fresh look at the fundamentals of oscillatory phenomena, P. H. Richter (Göttingen) discussed general aspects of stability from a fluctuation theory point of view. It was shown how the concept of time reversal could be useful in the study of such phenomena. Using fluctuation theory the onset of periodic behaviour related to symmetry breaking and limit cycle invariance was discussed from the point of view of breaking of time translation invariance. U. F. Franck (Aachen), in a comprehensive survey of experimentally observed oscillations, showed how all of the examples illustrated could be understood on the basis of simultaneous feedback mechanisms. Positive feedback could result in propagation phenomena while negative feedback could initiate recovery and overshoot reactions.

J. J. Tyson (Innsbruck) discussed the end-product feedback control in certain biochemical pathways as a general system of n-species. The model system of reaction equations, all of which are linear except that one in which the feedback directly acts, can be represented by an nth-order differential equation of the form

$$\left(\frac{d}{dt}+k_1\right)\left(\frac{d}{dt}+k_2\right)\ldots\left(\frac{d}{dt}+k_n\right)X(t)=f(x)$$

where the k_i, $i = 1, \ldots, n$ are related to the rate constants and $f(x)$ is the non-linear feedback term. Useful general results were found and proved for this system with particular emphasis on the existence of periodic solutions. Also presented were some general results on the character and stability (both linear and global) of the steady-states: a sound knowledge of these is a necessary prerequisite to establish the presence of periodic solutions. In a methods paper B. L. Clarke (Alberta) presented a method for the study of chemical networks, the basis of which is geometrical in character. It provides useful steady-state stability results, which are obtained by a simple computer algorithm. Results, which can be deduced from the application of this approach for feedback cycles of a general character, were illustrated.

Simple pedagogic models were described by J. D. Murray (Oxford) for kinematic (diffusionless) waves which have been observed with the Belousov-Zhabotinskii reaction. Some of the pitfalls were noted that can exist in making mathematical and chemical deductions from computed travelling wave solutions to reaction–diffusion systems. A two-species (ecological) model system was shown which exhibited stable spatial non-homogeneous patterns in a finite domain with zero flux boundary conditions. A model system based on the Field-Körös-Noyes mechanism for the Belousov-Zhabotinskii reaction was then described and travelling concentration wave profiles obtained for the Br^- and $HBrO_2$. The results for wave speed and thickness were in fair agreement with experiment. Finally a two-species (bi-molecular) model again based on the same mechanism was discussed which possesses single traveling solitary wave (or pulse) solutions.

R. Lefever (Bruxelles) presented a new aspect of the two-species tri-molecular model mechanism, often referred to as the Brusselator. In a finite domain with fixed boundary values for the species it was shown that spatial–temporal patterns appear as solutions of the pair of coupled equations for the model. Numerical results were given under conditions applicable to the phosphofructokinase reaction.

An interesting class of oscillatory phenomena which are non-periodic and are generically described as 'chaotic' was discussed by O. E. Rössler (Tübingen), in a brief and fascinating survey together with some new results obtained with analytical and numerical methods. A model 3-variable abstract reaction system, which has such non-periodic oscillatory behaviour was described and a numerical simulation of the model was shown. Another model reaction system, again of three species, was discussed in which there was only one non-linear term in the reaction kinetics. This model exhibited a chaotic property which was based on two distinct unstable foci. It was shown how such 'almost periodic' behaviour can be predicted in possible real biochemical systems, using information found from a structural study of the systems discussed in detail.

2. Chemical systems

R. M. Noyes (Eugene) stated that now three chemical oscillators are reasonably well understood: The Belousov-Zhabotinskii reaction (1958), the Bray reaction (1919) and the Morgan reaction (1916). All three systems have one feature in common, some intermediates are formed by processes of zero- and first-order in their concentrations and destroyed by processes of first- and second-order. E. Körös (Budapest) followed with an experimental study on the Belousov-Zhabotinskii system using polarographic and calorimetric measurements. The complex overall reactions can be separated into three phases (pre-oscillatory, oscillatory, and post-oscillatory), where reactions with identical stoichiometry proceed. During the pre-oscillatory phase bromomalonic acid is accumulated to a critical concentration required for the onset of the oscillation. The critical concentration depends both on the chemical composition of the system and on the temperature. In addition the oxidative bromination of malonic acid with bromate in the absence of a catalyst was described. A new facet of the Belousov-Zhabotinskii reaction was shown by H.-G. Busse (Kiel). If a collodion membrane-disc containing ferroin catalyst is put on the surface of a solution containing the rest of the system's reagents, periodic movements of the collodion-disc on the surface can be observed. The mechanism of this direct transformation of chemical into energy, resulting in translational diffusion, remains open.

3. Biochemical systems

This part of the meeting was initiated by B. Chance (Philadelphia) with a discussion of the general problems of data acquisition in the two- and three-dimensional reactor space of biological material, mainly with respect to his newer techniques of recording surface fluorescence of NADH in tissues, as well as of phosphorous-NMR-techniques allowing a continuous readout of phosphorylated metabolites in intact biological systems. Evidence of sustained metabolic oscillations in brain-tissue is obtained by the fluorometric observation of NADH-fluctuations accompanying spreading depression. This fluctuation is correlated with ion-movement and fluorescence-probe responses.

B. Hess (Dortmund) reported on experimental techniques to maintain dynamic-states in the analysis of oscillation, mainly in glycolysis, and to obtain information on the size and form of the dynamic domains as a function of the source-rate of substrate. Recently, with such methods, the type and range of entrainment of glycolytic oscillations by a periodic source of substrate in yeast extracts were determined. Domains of entrainment of the system by the fundamental frequency, one-half harmonic and one-third harmonic, of a sinusoidal source of substrate were obtained. In addition, the effect of stochastic input of the substrate on limit cycle behaviour on conditions for entrainment of the oscillating system were reported. Furthermore, it was stressed that the study of the entrainability of glycolytic oscillations presents analogies with findings on circadian rhythms and demonstrates a molecular mechanism of synchronization based on the phosphofructokinase-oscillator theory applicable to a large variety of periodic phenomena in biological systems.

A. Goldbeter (Bruxelles) complemented the experimental studies with a theoretical analysis on an allosteric model for the phosphofructokinase reaction. Theoretical predictions compare favourably with the experimental results in oscillating extracts of yeast and muscle and demonstrate that the dynamic behaviour of a complex biochemical system can be reduced to the molecular properties of a single-protein species operating at the master enzyme in a biochemical pathway, such as phosphofructokinase. Glycolysis offers the best known example of temporal organisation in a biochemical pathway beyond a non-equilibrium instability.

In an investigation of the interaction of a multiple enzyme system Th. Plesser (Dortmund) studied two models with phosphofructokinase, pyruvate kinase and a compensating enzyme function by computer simulation. For both flux directions – pyruvate kinase as sink or source enzyme, respectively – the oscillatory domain was computed and the large influence of the overall rate on the waveform and period was demonstrated. The experiments clearly showed the large variety of time-behaviour depending on the structure of the enzymic network. This view point was stressed by A. Boiteux (Dortmund) who presented experimental data on the stability and waveform control of soluble and membrane-shielded enzymic oscillators. Whereas the classical soluble oscillators produce all types of waveforms in a broad frequency range the shielded system exhibits only sinusoidal oscillations with narrow bandwidth, being rather insensitive to dilution and sensitive only to synchronizing signals. The mitochondrial oscillator operates in a concentration-range larger than 20:1 and is controlled by all parameters affecting the membrane potential.

H. Degn (Odense) discussed the only one-enzyme oscillator so far investigated: peroxidase. This enzyme, under conditions open to oxygen, exhibits autocatalysis, forward inhibition, oscillations and bi-stability in one single experiment, illustrating beautifully the multiplicity of dynamic-states that can be observed even in a one-enzyme reaction.

G. Siegel (Berlin) reported that a rhythmically varying energy supply from cell metabolism most probably is responsible for the spontaneous rhythmic fluctuation of the membrane potential, recorded in vascular smooth-muscle. That indeed the membrane potential affords a continuous and highly sensitive monitor of metabolic events in *Neurospora* was stressed by C. W. Slayman (New Haven). His experiments demonstrated that the coupling between membrane potential and metabolism is mediated by an electrogenic ion-transport system which, by feedback control, can give rise to oscillations. Another feedback loop was described by U.-P. Hansen (Kiel) in the pathway of the action of light on the membrane potential in *Nitella,* which leads to spontaneous oscillations with a period of about one hour.

P. Mueller (Philadelphia) reviewed the molecular aspects of membrane excitation. Several compounds of fungal or bacterial origin, incorporated into lipid bilayers, form molecular channels whose ionic-conductance can be gated by the membrane potential. Chemical and kinetic evidence indicate that a gating process involves a voltage-dependent insertion of the channel forming molecules into the hydrocarbon region and their subsequent aggregation by lateral diffusion into open 'barrel stave' channels. The mathematical description of this process accounts quantitatively for the observed conductance kinetics. This analysis was followed by a discussion of phenomena of hysteresis and oscillations occurring in systems, where enzymes were chemically bound to membranes (D. Thomas, Compiègne). He could show that the interaction between enzymic reactions and diffusion gives rise to spontaneous structurations in space and demonstrated experimental evidence for an unequal distribution of pH throughout the membrane.

The section on various dynamic properties of bioenergetic systems and its elements was closed with a theoretical discussion of the stability properties of general metabolic networks as a result of purely stoichiometric regulation by E. E. Sel'kov (Pushchino). It was interesting to see that the classical states of glycolysis and gluconeogenesis might simply alternate between two stable stationary-states by means of hysteretic transitions. It was suggested that energy metabolism could be a source of slow, even circadian oscillations setting a basis for the temporal organisation of cells.

4. Epigenetic and cellular systems

G. Gerisch (Basel) gave a summary on the phase of development of *Dictyostelium discoideum* which is controlled by oscillations of cyclic AMP. Prior to cell aggregation, *Dictyostelium* cells start to release periodic pulses of cyclic AMP, to which they respond by activation of adenylate cyclase. Cell membrane sites controlled by these pulses include cyclic AMP receptors, cyclic AMP phosphodiesterase as well as contact sites which are suggested to function in cell-to-cell adhesion. Further data on the signalling system controlling aggregation in *Dictyostelium discoideum* were presented by A. Robertson (Chicago), reporting that when aggregation is nearly complete the tip forms on top of the aggregate and releases a continuous cyclic AMP signal, as cells in the tip are continuously exposed to super-threshold cyclic AMP concentration and therefore fire as soon as they become sensitive. He showed that the tip can dominate any other signal source: explaining the capacity of tips from any multicellular stage of the developmental cycle to act as classical organizers in the sense of Speeman. He also reported that in the early chick embryo a propagated wave of cell movement was observed, seen as changes in refractive index in time-lapse film, with periods down to two minutes propagation and velocities at between 50 and 100 μm/min. It was inducable by an external cyclic AMP source, influencing even the direction of the developmental axis. The whole embryos were shown to release extracellular AMP and exhibit extracellular phosphodiesterase activity. It is suggested that early morphogenesis in the chick embryo is controlled by an extracellular signal release system employing the periodic propagation of pulses of cyclic AMP for organizing regions which release cyclic AMP continuously. This feature is common to vertebrates and possibly all regulative embryos.

An evaluation of the role of the membrane in the circadian rhythms of photosynthesis in *Acetabularia* was presented by T. Vanden Driessche (Bruxelles). This species displays several circadian rhythms, most of them associated with the chloroplast. In regenerating *Acetabularia*, B. Goodwin (Brighton) observed periodic propagating waves and formation of gradients. The polarity of the regenerating plant correlates with the direction of propagation of periodic electrical waves, whose polarity corresponds to a gradient of phosphate in the cortical layer. This gradient can be disturbed by agents such as the ionophore A 23187, which reversibly inhibits morphogenesis. This discussion was followed by the presentation of limit cycle models of the mitotic clock by S. Kauffman (Philadelphia) and an excellent review on the mechanism on circadian activity changes in enzymes by J. Hastings (Cambridge, USA) who reported that there is evidence that 'night'- and 'day'-luciferase molecules are different, the first direct evidence of biochemical phenomena related directly to a clock mechanism.

5. A summing up

There is a vast variety of periodic phenomena in living systems. Today only a few periodic processes can be reduced to the function of a single-enzyme or a few enzymes in a reaction sequence and enzymic network. Theoretical and experimental analysis indicate that important basic requirements for the generation of temporal and spatial organization in living systems are autocatalysis, feedback in the interaction network and the thermodynamic condition of being as far from equilibrium as an open system.