

## MEETING REPORT

## REPORT OF A SYMPOSIUM HELD IN HANGÖ, FINLAND, AUGUST 16–17, 1969

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A symposium on biochemical oscillations and membrane instabilities was held in Hangö/Finland on August 16–17, 1969, organized by Britton Chance and Ken Pye, University of Pennsylvania, Benno Hess, Max-Planck-Institute, Dortmund, and Nils-Erik Leo Saris, University of Helsinki. It was held under the auspices of Societas Biochemica, Biophysica et Microbiologica Fenniae, and supported mainly by the Sigrid Juselius Foundation with contributions from Orion OY, Helsinki. The participants numbered close to 50 from the Czechoslovakia, the German Federal Republic, the German Democratic Republic, Finland, Sweden and the United States while the Russian delegation was, unfortunately, not able to attend. This symposium was the second on biochemical oscillations following that held in Prague in 1968.

The contributions covered soluble oscillators, mainly the glycolytic ones, membrane-bound oscillators, such as mitochondrial, microbiological and electrogenic oscillators, and finally mathematical models to describe the experimental findings.

B.Chance (Philadelphia) described the two extreme conditions of mitochondrial volume change oscillations. In the expanded "charged" state mitochondria have accumulated ions and extruded protons, respiration is inhibited and respiratory carriers largely reduced; in the contracted "uncharged" state ions are lost, protons taken up, the respiratory rate is high and respiratory carriers more oxidized. Saris (Helsinki) gave a paper with A.J.Seppälä and M.K.F.Wikström on the effect of propranolol and local anesthetics as well as phenothiazines, such as chlorpromazine,

which inhibit the rate of contraction, thereby damping the oscillations in the expanded state. This seems to be an "energized" state, since contraction can be induced by classical uncoupling agents or by stopping the energy input.

R.Rybová and R.Metlicka (Prague) described their studies on the oscillation of the transmembrane potential in the alga *Hydrodictyon reticulatum* following changes in illumination. By using various inhibitors and changing the composition of the medium, chloride ion fluxes and changes in pH were found to be important. The membrane potential changes were inhibited by dichlorophenyl dimethyl urea indicating the involvement of the photosynthetic system II.

K.Janáček (Prague) read a paper on "Transient phenomena and their possible relation to the oscillations observed in frog skin". He suggested that the oscillations of the potential across frog skin, which occur in lithium and occasionally in sodium solutions, represent a special case of transient phenomena following sudden changes in potential difference. These effects might be explained by a feed-back mechanism: the higher the intracellular sodium, the higher the membrane resistance toward the entry of sodium ions.

A.Kotyk (Prague) read a paper entitled "Oscillatory transport of non-metabolized solutes by single cells". The uptake of monosaccharides by the yeast *Saccharomyces cerevisiae* shows rhythmic changes with a frequency of about  $0.8 \text{ min}^{-1}$ . The frequency is surprisingly enough insensitive to uranyl ions, metabolic inhibitors and temperature. Efflux of ammonium and potassium ions from the green alga *Scenedesmus quadricauda* shows similar oscillations with a frequency of  $0.75 \text{ min}^{-1}$ , which are again inhibitor- and temperature-insensitive. Pulsation of that part of the cell volume where the

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solutes are dissolved was suggested as a tentative mechanism.

Unsolved mechanistic problems of glycolytic oscillations were discussed by K. Pye (Philadelphia). Whereas it is commonly accepted that phosphofructokinase (PFK) in yeast is the primary source of the glycolytic oscillations, the control of the oscillatory dynamics in terms of activation and inhibition strength by various controlling ligands is poorly understood. Dr. Pye stressed the function of the relatively slow feed-in-step of glycolysis in the generation of the oscillation in yeast cell suspension. This is the permeation of glucose through the cellular membrane which is readily sensitive to a frequency controlling specific inhibitor of glucose transport, namely methylphenidate. B. Hess (Dortmund) presented a summary of the component structure of oscillating glycolysis in yeast extracts, where normalites of the various glycolytic enzymes in the range between  $5 \times 10^{-6}$  and  $10^{-4}$  M are found which are roughly equimolar to the levels of their respective substrates. NADH/NAD saturation figures for the dehydrogenases were computed and an action spectrum for the oscillatory "NADH"-compound (presumably a protein-bound species) with a maximum at "355" was presented. This component oscillates, depending on the input rate in phase or phase-delayed, with the pH oscillation (NADH max corresponding to alkaline max) indicating the activity state of PFK.

An analysis of the ionic membrane currents associated with step polarizations in the myelinated nerve fibre was presented by B. Frankenhaeuser (Stockholm). A negative step polarization was associated with a shortlasting capacity current and a leak current, while a positive step caused an initial transient current and a delayed current. The initial current was carried mainly by sodium ions moving passively under the electro-chemical driving force. The delayed current was a passive potassium current. Solutions of the simultaneous differential equations, fitted to the experimental data, quantitatively describe the excitability properties of the nerve and its regenerative impulse activity.

R. Stämpfli (Homburg-Saar) discussed "Oscillations in the membrane potential of Ranvier nodes" which occur spontaneously in low-Ca Ringer containing tetraethylammonium (TEA). Voltage clamp experiments show that Ca deprivation increases the

steady state Na permeability near the resting potential while TEA suppresses the K permeability. Under these circumstances the recovery of Na permeability after a spike leads to a slow depolarization to the threshold potential thus keeping the membrane in a state of repetitive activity.

W. Ulbricht (Kiel) described the same preparation under the influence of veratridine which induces a slowly changing Na permeability. In contrast to the fast, transient Na permeability which is responsible for the upstroke of the spike, the veratridine-induced permeability shows no appreciable inactivation. As a consequence, the steady-state current-voltage relation has a characteristic N-shape, if determined under voltage clamp conditions. This current-voltage curve contains a region of negative slope conductance, a prerogative of membrane instability. It is concluded that the veratridine-induced sodium permeability describes the features of degenerated Na channels whose normal behaviour is expressed by the fast, transient sodium permeability.

G. Adam (Konstanz) presented a theoretical model for the regulation of the electrical conductivity of biological membranes by cation-binding in the membrane. For the nonmyelinated axon membrane, the model, based on a protein subunit structure of the membrane permeable for electrolytes and maintaining two states of different electrical conductivity, is supplemented by the postulate of an ionic *psn*-layer (positive fixed charges – solution membrane – negative fixed charges) structure of the membrane. The model has the following quantitative properties: Electrical capacity and steady-state rectification, voltage clamp currents for small depolarizations and its dependence on previous hyperpolarization, equality of chemical sodium flux and charge flow in a succession of voltage clamp experiments, differential current flow effects by tetrodotoxin, negative quasi-steady-state resistance, heat cycle upon excitation and return to steady-state. The theoretical results agree well with published experimental results.

K. Heckmann, B. Lindemann and W. Vollmerhaus (Saarbrücken) discussed experiments on the relationship between the sodium conductance of a number of excitable membranes, the membrane potential and the calcium concentration, in which an equivalence of the increase of the potential to an increase in calcium concentration with respect to the sodium conductance

is found. Numerical value of this equivalent is about 7 mV for an  $e$ -fold increase of the sodium conductance. This finding can be explained by the assumption that sodium conducting pores can be blocked by calcium ions which are pulled into the pores by the electrical field, but are unable to completely cross the pores. The blocking affect of calcium on the sodium current must be amplified by additional sodium entering the pore behind calcium, decreasing the probability of calcium re-opening the pores.

N.F.Franck (Aachen) presented a general phenomenological theory of oscillatory behaviour of biochemical and physiological systems. The theory is based on the relationship between the conjugated forces and fluxes. The necessary conditions for the excitability, propagations and oscillations were given. One of these conditions is the existence of a non-monotonic relationship between one pair of force and flux. Such non-monotonic force flux characteristics are mainly found in systems in which strong field strengths of driving forces are effective, as in the case in membranes and all systems containing thin conducting interphases. Dr. Franck discussed three examples: Porous ion exchange membranes, passive ion wire and tunnel diode system.

T.Teorell (Uppsala) presented his theory for the behaviour of a membrane-electrolyte system subject to an electrical current flow. His system can be expressed by a set of non-linear differential equations. Depending on the form of non-linearity and the values of the parameters of the system, different kinds of oscillatory behaviour may be obtained, such as damping oscillations or limit cycle oscillations.

B.Neumcke (Komstanz) explained the observed non-linear current characteristics for the ion flux across lipid bilayer membranes using the following principles: Ion injection, image force correction of the membrane potential, Wien effect and non-linearities arising from membrane surface charges.

W.A.Knorre (Jena) presented a (simple) conservative non-harmonic oscillator.

G.Viniegra (San Francisco) described the stability properties of a sequence of enzyme reactions giving the conditions for the stability of the stationary state and the conditions for the existence of limit cycles.

The symposium was closed with an eloquent summary given by Dr. Teorell, in which he stressed the formally equal mathematical treatment of all oscillatory dynamics of the various systems discussed and their mechanistic analogies.