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989 — MECHANISMS OF MEMBRANE PROCESSES, ELECTRIC GENE TRANSFER AND CELL FUSION

Report on the workshop held at the "Zentrum für Interdisziplinäre Forschung" in Bielefeld on 3–4 April 1986

T. DORFMÜLLER, W. FÖRSTER and E. NEUMANN
Physikalische Chemie der Universität, P.O. Box 8640, D-4800 Bielefeld (F.R.G.)
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This workshop stems from the idea that compartmented partially ordered and partially fluid systems such as membranes could quite well provide a useful field of application and extension of the studies on complex liquids. The subject was also considered to be timely because of the important applications in biology. Electric field pulse techniques are indeed becoming increasingly important in cellular and molecular biology and biotechnology. It thus appeared appropriate to open the workshop with some historical remarks on electric field effects on cell membranes. Most of the contributions, however, deal with the different effects of high-field pulses on membranes which are observed experimentally and which, in part, were theoretically predicted.

The study of natural membranes as physical–chemical systems is meant to clarify those properties that provide the analytical framework for the study of cell processes. Thus, for example, information about the factors that control passive ion transport across membranes should, in general, help us to approach the unusual ion fluxes of excitable membranes. However, as we learn more about the physical properties of excitable membranes, especially the surface properties, we appear to understand many of the cellular properties better as well.

The first experimental observation of a non-bilayer lipid structure in the membrane of viable eukaryotic cells was reported. High electric fields induce reversible modification of the cell transmembrane potential and can lead to the formation of transiently permeable structures, this phenomenon being totally reversible. The pulsed cell membranes were found to return to the normal configuration after some time.

Electric impulses also cause transient structural changes in biological membranes and lipid bilayers, leading to apparently reversible pore formation (electroporation) with cross-membrane material flow and, if two membranes are in contact, to irreversible membrane fusion (electrofusion).

From the plant biological side, the effect of electric impulses is studied because there is an urgent need for a plant-derived expression system capable of transcribing and also translating cloned genes of interest such as the *Acetabularia* system.

The special case of alamethicin, an antibiotic peptide of 20 amino acid residues which is known to form electrically sensitive pores in phospholipid bilayers, was investigated. The circular dichroism spectrum of aqueous alamethicin is substantially changed once unilamellar vesicles are added, reflecting an *association* of the peptide with the lipid medium. This phenomenon was analysed quantitatively by means of a quite general approach.

It was shown that electric and electromagnetic field effects in biology exhibit new possibilities: electroincorporation of drugs, electrotransformation and electrofusion of cells by single high impulses, as well as electrostimulation of cell metabolism and bone healing by weak pulsating currents.

A quantitative theory of rupture and reversible, electrical breakdown which also suggests a microscopic mechanism for electroporation was advanced. The theory is based on the hypothesis that a bilayer contains a large number of transient aqueous pores. Such pores are viewed as fundamental entities in bilayer membranes, but are not fixed structures.

A mathematical formulation of the bilayer couple concept of red blood cell shape determination was presented based on the assumption of a minimum membrane bending energy. It was thus shown that this analysis indicates as a possible consequence also the formation of vesicles.

As an alternative to electric pulse methods, application of the glass capillary mediated injection technique was also discussed in two examples:

(1) the correlation between locomotory activity, shape and anchorage of animal tissue culture cells with a balanced equilibrium of cytoskeletal components; and

(2) the role of actin in RNA polymerase II dependent transcription.