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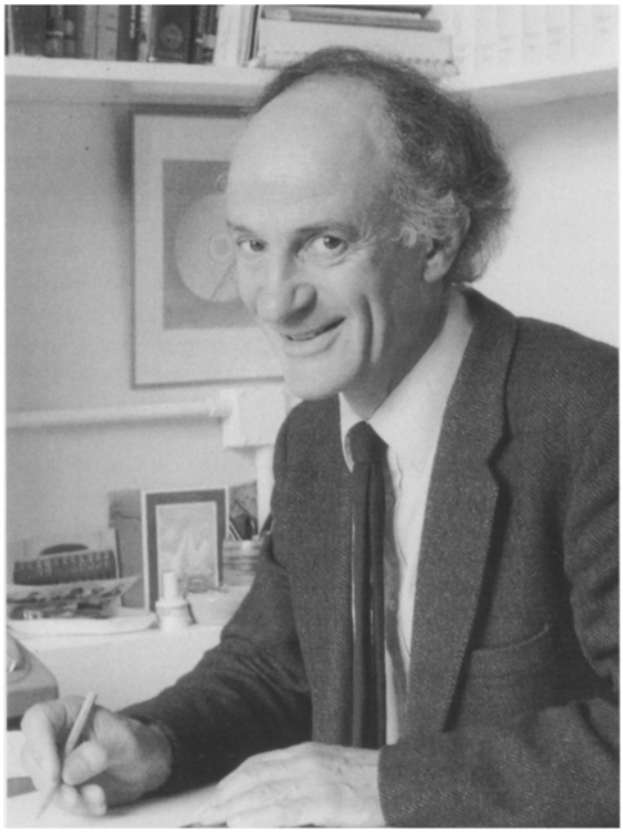
In Memory of
Denis Arthur Haydon



Denis Arthur Haydon
1930–1988

With the death of Denis Arthur Haydon on November 29, 1988, membrane biophysics has lost a prominent figure. A physical chemist by training, he devoted most of his early work to monolayers and electrically charged interfaces. From these studies in surface science arose his interest in lipid bilayers and biological membranes, and it was in this field where he made his most significant contributions to science.

Denis Arthur Haydon was born at Wilmington, Kent, in 1930, and educated at Dartford Grammar School at King's College, London, where he took his Ph.D. under the late Sir Eric Rideal in 1955. He continued to work at King's College on mass transfer at liquid/liquid interfaces and interfacial turbulence and spent two years at Imperial College, London, as an I.C.I. research fellow. In 1959 he joined the Department of Colloid Science, Cambridge, as the Assistant Director of Research; when the department was closed in 1970, he moved to the Physiological Laboratory in Cambridge. He was a Reader in Surface and Membrane Biophysics and became Professor of Membrane Biophysics in 1980. Haydon took an active part in the life of his college, Trinity Hall, where he was a director of studies from 1965 to 1978 and then Vice-Master for four years. He was elected a Fellow of the Royal Society in 1975 and received the Chemical Society Medal for Surface and Colloid Chemistry in 1976.

When Denis Haydon began his well-known work on lipid bilayers, he had already a name in surface science. In the years between 1956 and 1963 he published a number of substantial papers on thermodynamics of interfaces, electrical double-layer potentials, electrophoresis, and rheology of interfacial films. His interest in biological interfaces goes back to his thesis work in the laboratory of Eric Rideal who suggested to him to study surface charges of *Escherichia coli* cells by microelectrophoresis. But after these studies had been finished, Denis turned to better-defined systems, monolayers and liquid-liquid interfaces, more suited for the kind of rigorous physicochemical analysis which is so characteristic for his work.

Attempts to prepare artificial lipid bilayers in aqueous phase have been made as early as 1938 by Langmuir and Waugh. But it was not until 1962, when Paul Mueller and his collegues in Philadelphia succeeded in developing a practicable method for the formation of optically black lipid films. Two months after the paper of Mueller, Rudin, Tien and Wescott had appeared in Nature, Haydon and Taylor submitted a theoretical paper in which they discussed the structure and stability of bimolecular films in water. In 1964, Hanai, Haydon and Taylor published their study on the electrical characteristics of lecithin-in-hydrocarbon films, which was the first in a long series of experimental investigations by the Cambridge laboratory on the properties of lipid bilayers. These studies on the composition, electrical impedance, surface tension and water permeability of black lipid films contributed much in establishing lipid bilayers as a model system for quantitative physicochemical analysis of biological membrane phenomena. A characteristic example how Denis Haydon used to master seemingly difficult tasks by skillful experimentation is his work (with Janet Taylor and Jaime Requena) on the thermodynamics of black films. The change of free energy associated with the formation of a thin film from a thick layer of bulk solution is given by the difference of the surface tensions of bulk solution (γ) and film (γ_f). γ and γ_f are related by the contact angle ϑ between film and bulk solution with which the film is in equilibrium according to $\gamma_f = \gamma\cos\vartheta$. Haydon realized that ϑ can be measured by observing the interference fringes formed in monochromatic light reflected from minute lenses of bulk lipid solution trapped in the film. The free energy change per unit area of the film, $\Delta F = 2(\gamma_f - \gamma)$, which could be accurately determined in this way, was found to agree with the predictions of the Lifshitz theory for the interaction of two water phases across a layer of hydrocarbon. By the same ingenious method, Requena and Haydon demonstrated that a lipid bilayer obeys Lippmann's equation $-d\gamma_f/dU = UC/2$ which relates the dependence of γ_f on voltage U to the specific membrane capacitance C.

Undoubtedly, the most influential contribution of Denis Haydon to membrane biophysics is his joint work with Steven Hladky (and later with V.B.

Myers and B.W. Urban) on the gramicidin channel. From studies of B.C. Pressman, P. Mueller, D.C. Tosteson and others it was known that the hydrophobic pentadecapeptide gramicidin A is capable of increasing the permeability of biological and artificial membranes to small monovalent cations. In 1970 Hladky and Haydon reported in *Nature* that in the presence of minute amounts of gramicidin A, the conductance of a lipid bilayer fluctuates in a stepwise manner with unit conductance increments corresponding to the translocation of 10^7–10^8 ions per second. To measure currents in the picoampere range they mounted the bilayer set-up on a paving stone slung by nylon ropes from the ceiling of a basement room in the Cambridge Physiological Laboratory—an arrangement which may have been inspired by Denis Haydon's love of climbing. Discrete conductance fluctuations indicative of ion-channel formation had been observed before by P. Mueller and D.O. Rudin and by R.C. Bean and collegues with planar lipid bilayers in the presence of "EIM," a substance of bacterial origin of unknown nature. But it was Hladky and Haydon's experimental work on gramicidin A together with D.W. Urry's structural studies that made ionic channels a chemical reality. From now on, the properties of ionic channels could be investigated at the level of single molecules, a development that culminated in the work of Neher and Sakmann six years later. The structural model of the gramicidin channel proposed in 1971 by D.W. Urry, which was based on the findings of Hladky and Haydon, has stimulated extensive theoretical work on ion-channel interaction and translocation mechanisms. Chemical modification of the gramicidin molecule opened up the possibility of studying the relationship between channel structure and transport properties. In the years 1970–72, the Cambridge group analyzed the behavior of the gramicidin channel in considerable detail. They showed that the ratio g_A/g_B of single-channel conductances of two ion species A and B is different from the permeability ratio P_A/P_B obtained from biionic potentials, and that g_A/g_B and P_A/P_B depend on ionic concentration. This behavior has been explained later by Sandblom, Eisenman and Neher and by Urban, Hladky and Haydon on the basis of binding-site models of the channel.

The work on gramicidin was soon followed by studies of another model channel, the channel formed by the amphiphilic peptide alamethicin. From the experiments of Mueller and Rudin it was known that the alamethicin-induced membrane conductance is strongly voltage dependent, in a manner reminiscent of the behavior of channels in nerve membranes. In a short paper published 1972, L.G.M. Gordon and D.A. Haydon reported that alamethicin induces discrete conductance fluctuations which appear in groups ("bursts"). Within a group the conductance assumes several sublevels which can be arranged in an ordered sequence. Gordon and Haydon later showed that the current-voltage characteristic of the single conductance states is nearly ohmic and that the strongly nonlinear behavior of the average conductance results from the effect of voltage on the frequency of occurrence of bursts. Several structural models have been proposed to explain this peculiar behavior. Gordon and Haydon's original model consisted of an array of several parallel channels, but later work made the model of a single oligomeric channel more likely in which transitions between conductance sublevels occur by uptake or release of single alamethicin molecules.

In the years after 1977, Denis Haydon turned to a new subject, the action mechanism of anesthetics. He became interested in the curious observation that inert, lipophilic compounds, such as *n*-alkanes are able to block the conduction of nerve impulses. He noticed that the cut-off in anesthetic potency on ascending the homologous series is closely related to the decrease in adsorption of the *n*-alkane to a phospholipid/cholesterol bilayer. In addition, the anesthetic hydrocarbons were found to produce a concentration-dependent increase in bilayer thickness. On the basis of these two observations, and by analogy to the behavior of the gramicidin channel in artificial lipid bilayers, he proposed that a thickening of the bilayer regions of the nerve membrane by the alkane destabilizes the open state of the channels responsible for nerve excitation. To test these ideas, Denis Haydon started a long series of experiments with nerve axons, in collaboration with J. Requena, B.M. Hendry, S.R. Levinson, B.W. Urban, J.R. Elliot, J.E. Kimura, D. Needham, R.D. Murrell, A.A. McElwee and A.J.B. Simon. He went to the laboratory of Jaime Requena, his friend and former Ph.D. student, in Caracas, where he got acquainted with voltage-clamp experiments and learned to dissect squid giant axons. He later continued these experiments in the Marine Biological Laboratory in Plymouth, U.K. Haydon and his collegues indeed found that in the presence of blocking concentrations of alkanes, the membrane capacitance of squid giant axons decreased by 0.1–0.15 $\mu F/cm^2$, indicating an increase of membrane thickness by absorption of the hydrocarbon. Analysis of voltage-clamp experiments revealed that alkanes affect the sodium channel on the axon in at least three different ways, by a change of the voltage dependence of steady-state activation and inactivation (possibly resulting from an increase of bilayer thickness and a concomitant decrease of electric field strength), by a suppression of maximum conductance, and by a reduction of the time constants of

activation and inactivation. Haydon and his associates later extended the analysis to *n*-alkanols and other surface-active anesthetics. They concluded that these compounds are likely to insert into the membrane-solution interface rather than to adsorb into the bilayer, and that their blocking potency is not related to thickness changes of the membrane. These studies, carried out with a wide variety of blocking agents, led to the notion that multiple sites of interaction between anesthetic compound and excitable membrane exist.

Denis Haydon actively pursued his studies on the action mechanisms of anesthetics till his last days. For about two years, his close collaborators knew that his health was in serious danger. But he endured his illness with admirable courage, finding pleasure and strength in his scientific work, as always in his life. It was a life devoted to science.

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Konstanz, F.R.G.

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