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Phil. Trans. R. Soc. Lond. B 1975 **270**, 433-447
doi: 10.1098/rstb.1975.0021

Phil. Trans. R. Soc. Lond. B. **270**, 433–447 (1975) [433]
Printed in Great Britain

Potential-dependent conductances in lipid membranes containing alamethicin

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This article is concerned primarily with the mechanism of the potential-dependent conductance induced in artificial lipid membranes by the cyclic polypeptide antibiotic alamethicin. It has already been shown from studies of the fluctuations that can be detected in very small membrane currents, that alamethicin forms transient pores of some 0.6 nm in diameter and that, for small inorganic ions, these are poorly selective. The origin of these pores, their spatial distribution and interaction are discussed. It is demonstrated that the sensitivity of the membrane conductance to the applied potential arises only to a slight extent from the current–voltage relations for the individual pores, and that the main effect stems from the influence of the potential on the frequency of opening of the pores. From the properties of lipid membranes containing alamethicin in a wide variety of electrolytes, and from other evidence, it is concluded that the polypeptide reacts to the electric field more probably because it has a large dipole moment than because it binds ions. It is proposed that the conducting complex is capable of functioning in either of two orientations, and that it is these two possibilities that give rise to certain differences in the single channel characteristics for the two directions of the field.

Strongly potential-dependent conductances are now a well known phenomenon in artificial lipid membranes, as well as in biological membranes. In the artificial systems the potential dependence arises from the presence of certain macromolecules, the most notable of which are the so-called excitability inducing material (e.i.m.) (Mueller, Rudin, Tien & Westcott 1962; Bean, Shepherd, Chan & Eichner 1969; Ehrenstein, Lecar & Nossal 1970), alamethicin (Mueller & Rudin 1968; Gordon & Haydon 1972; Haydon, Hladky & Gordon 1972; Eisenberg, Hall & Mead 1973) and monazomycin (Mueller & Rudin 1969; Muller & Finkelstein 1972; Mauro, Nanavati & Heyer 1972). The e.i.m. has not been fully characterized and will not be discussed further. Both alamethicin and monazomycin are relatively well characterized and the prospects of understanding their mechanisms of action seem reasonably good. This paper will be concerned solely with alamethicin and, from the evidence at present available, an attempt will be made to explain at the molecular level how the potential-dependent conductances in this system arise. The studies which have led to the present conclusions have involved the use of black lipid films formed from a variety of different lipids. The alamethicin used in the early experiments was from Upjohn, and was known to be a mixture of several species. For later experiments and much of the more quantitative work a single fraction (the '30' fraction supplied by the Microbiological Establishment, Porton) was used. This material had a free carboxyl group in the eighteen position and was readily soluble in aqueous electrolytes.

The current–voltage relations

A typical current–voltage curve is given in figure 1, and the corresponding integral conductance, plotted logarithmically, is shown in the inset. The slope of the $\log_{10}$ conductance against voltage plot is slightly variable from one experiment to another but, as earlier authors have found, the conductance increases roughly e-fold for voltage increments of 4 mV. It has been reported (Cherry, Chapman & Graham 1972) that at high applied potentials the conductance levels off (as presumably it eventually must) but, in the present work, the membranes always

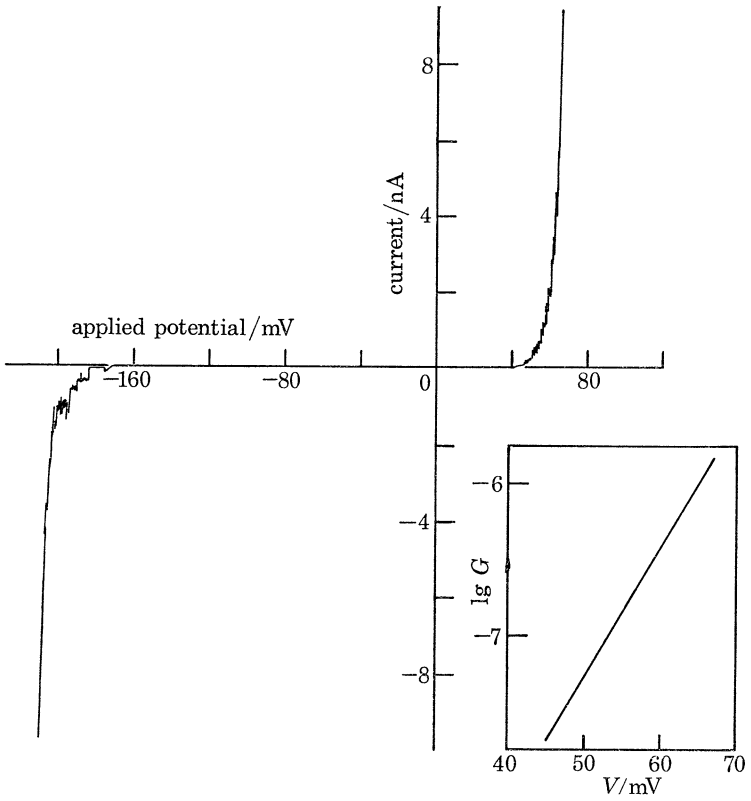

Figure 1. The current–voltage relation for a membrane formed from glyceryl mono-oleate (6×10^{-3} mol dm^{-3}) and cholesterol (1.2×10^{-2} mol dm^{-3}) in *n*-hexadecane, in aqueous KCl (2 mol dm^{-3}). Alamethicin (10^{-6} mol dm^{-3}) was added to one only of the two aqueous compartments. The sign of the potential is given for the alamethicin side of the membrane. The temperature in this and subsequent experiments, unless otherwise stated, was 20 ± 1 °C. The inset shows the logarithm of the integral conductance as a function of the applied potential.

broke before this point was reached. At a given applied potential, the conductance is extremely sensitive to the aqueous phase alamethicin concentration and quite sensitive to the electrolyte concentration. Thus, as either of the two concentrations are increased the log conductance–voltage curves are shifted towards smaller voltages. Detailed descriptions of these effects have been given by, among others, Mueller & Rudin (1968) and Eisenberg *et al.* (1973). In figure 1 the data is for a membrane to which the alamethicin was introduced on one side only. The asymmetry of the *I–V* curve is therefore not surprising, but it is notable that for phosphatidyl ethanolamine membranes no conductance results when the alamethicin side is negative

(Eisenberg *et al.* 1973; Gordon & Haydon, unpublished). The significance of this observation will be discussed again later.

If attention is confined to one branch of the *I–V* curve in figure 1, it is found that the membrane conductance, *G*, is given by the expression

$$G \propto c_{\mathrm{A}}^{9}\, c_{\mathrm{E}}^{4}\, \mathrm{e}^{\frac{1}{4}|V|}, \tag{1}$$

where c_{A} and c_{E} are respectively the alamethicin and electrolyte concentrations and *V* is in millivolts. Mueller & Rudin (1968) reported both the concentration terms to be raised to the sixth power but, in the present work, it has been found, in agreement with Eisenberg *et al.* (1973), that 4 and 9 (both with an uncertainty of *ca.* ± 1) describe the data rather better. Equation (1) suggests that even towards zero applied potential the membrane conductance may rise merely as a consequence of increases in alamethicin and/or electrolyte concentration. This effect is indeed observed and, for sufficiently high alamethicin concentrations, as Mueller & Rudin showed, a region of negative resistance may occur in the *I–V* curve.

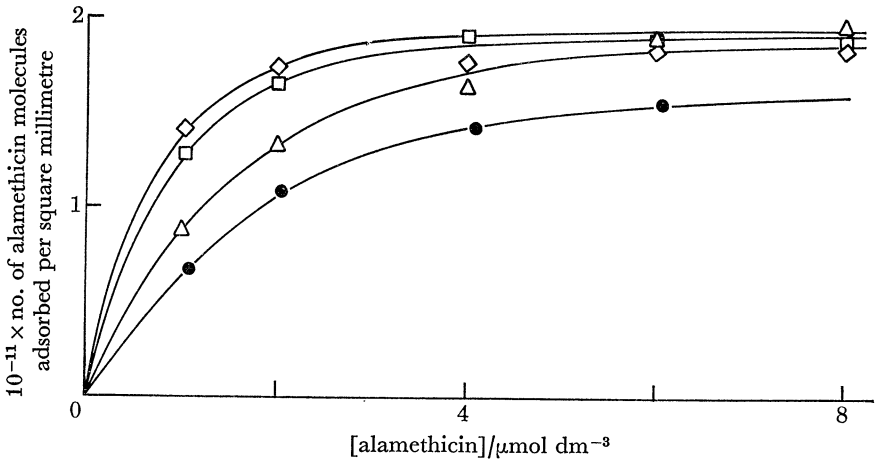

FIGURE 2. The adsorption of alamethicin at the interface between glyceryl mono-oleate (6×10^{-3} mol dm^{-3}) in *n*-decane and sodium chloride solutions. ●, 0.2; △, 0.5; □, 1.0 and ◇, 2.0 mol dm^{-3} NaCl. ($T = 20.0$ °C.)

THE DISPOSITION OF THE ALAMETHICIN

For glyceryl mono-oleate–hydrocarbon systems, alamethicin adsorbs reversibly at the bulk lipid solution/aqueous phase interface. As a consequence, it has been possible to measure the interfacial tensions of such interfaces as a function of alamethicin concentration and hence to use the Gibbs equation to estimate the adsorption of the polypeptide. The details of this study will be published elsewhere, but the results are shown in figure 2. It can be seen that the adsorption tends to level off (giving *ca.* 5.3 nm^2 per molecule) above about 4×10^{-6} mol dm^{-3} and that at lower alamethicin concentrations especially, it is a function of the NaCl concentration. Unless there is an appreciable interaction of the alamethicin across the black film, the adsorption at the film interfaces should be comparable to that at the bulk interface (Cook, Redwood, Taylor & Haydon 1968; Andrews, Manev & Haydon 1970; Fettiplace, Andrews & Haydon 1971). In fact, the amount of alamethicin actually conducting in a membrane is always very small and, moreover, no detectable effect of alamethicin on the specific capacitance of the membrane has so far been found (Takashima, Schwan & Mueller 1974; Fettiplace &

Haydon, unpublished). Indeed, there is no evidence for a strong interaction of the polypeptide across the membrane and it will be assumed that the adsorptions shown in figure 2 apply also to the membrane interfaces.

The only other assumption in the calculation of the adsorptions given in figure 2 is that the activity coefficient of the alamethicin in the aqueous phase is unity. At 10^{-7} to 10^{-6} mol dm^{-3} there is no direct evidence on this question (the ultracentrifuge studies of McMullen & Stirrup (1971) show strong aggregation in the 10^{-3} mol dm^{-3} range but do not extend to lower concentrations). However, if the activity coefficients were substantially less than unity the data would yield adsorptions larger than would be expected from the molecular area of the polypeptide. The assumption of ideality thus seems reasonable.

The interfacial pressure versus area per molecule data may be fitted quite closely by the Volmer equation (Aveyard & Haydon 1973) with a co-area term of 3.3 nm^2 per molecule. It may be inferred from this that the extent of any aggregation of the alamethicin in the membrane is quite small and that, in general, there is always a large excess of monomeric polypeptide over aggregates.

On the basis of the above discussion, the concentration of alamethicin monomers in the membrane is (at low concentrations) proportional to the aqueous phase concentration but the power of nine in equation (1) suggests that aggregates, though necessarily few in number, of approximately nine molecules, are the principal conducting units. The alamethicin (A) therefore appears to be involved in the following equilibria:

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \overset{K_3^+}{\nearrow\!\!\nearrow}\ A_9^+\text{ (membrane)} \\ A\text{ (aq)} \overset{K_1}{\rightleftharpoons} A\text{ (membrane)} \overset{K_2}{\rightleftharpoons} A_9\text{ (membrane)} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \underset{K_3^-}{\searrow\!\!\searrow}\ A_9^-\text{ (membrane)} \end{array} \qquad (2)$$

The species A_9^+ and A_9^- are intended to represent the conducting complexes when, for alamethicin on one side only of the membrane, the alamethicin side is made positive and negative respectively. Grounds for making this distinction will be given subsequently. The species A_9 represents a non-conducting ninefold complex, for which there is no direct evidence, but which must have some finite probability of existence and will be convenient for purposes of discussion.

Since the conducting aggregates are at very low concentration it will be assumed that they do not interact with each other, and that the specific conductance G of the membrane may be written

$$G(V, c_A, c_E) = G_a(V, c_E)\, N_a(V, c_A, c_E), \qquad (3)$$

where G_a is the mean aggregate conductance and N_a is the number of aggregates conducting at any given time in unit area of membrane. In accordance with the evidence of the single channel studies described below, G_a has been taken to be independent of the alamethicin concentration. In attempting to elucidate the origin of the potential dependence of G, G_a will be considered first.

The conductance and characteristics of a single complex

Information concerning G_a can be obtained from the fluctuations in the membrane current at very low levels. The technique of detecting and measuring these fluctuations has been described by Hladky & Haydon (1970, 1972) and results for alamethicin systems have been described by Gordon & Haydon (1972), Haydon *et al.* (1972) and by Eisenberg *et al.* (1973). A record showing the occurrence of current pulses as a function of time can be seen in figure 3.

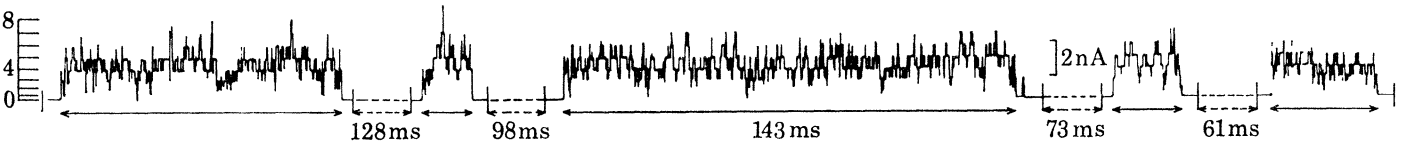

FIGURE 3. Current fluctuations for the membranes of figure 1 in KCl (2 mol dm^{-3}) with alamethicin (nominally 10^{-7} mol dm^{-3}) on the negative side. The applied potential was -220 mV.

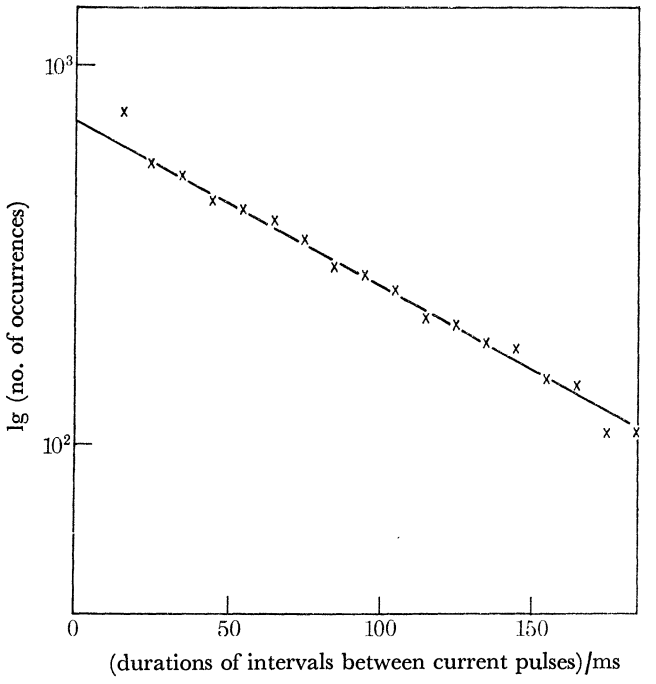

FIGURE 4. An illustration of the random occurrence of current pulses in a record similar to but more attenuated than that in figure 3. The membrane and aqueous phase were similar to those described for figure 1. The alamethicin concentration was nominally 5×10^{-8} mol dm^{-3} and the applied potential was $+150$ mV on the alamethicin side of the membrane.

From more attenuated but otherwise similar records it can be deduced that the pulses occur randomly (figure 4). The fact that, even at very high levels of membrane conductance the surface concentration of aggregates is very low ($\lesssim 10^4/mm^2$) leads to the expectation that the high conductances arise simply from the superposition in time of the individual current pulses. Eisenberg *et al.* (1973) have concluded that this is the case from an analysis of the fluctuations in moderately high levels of current.

A record of a single current pulse for alamethicin on the negative side of a glyceryl monooleate–cholesterol–hexadecane membrane is shown in figure 5. Transitions tend to occur only

to adjacent levels and it is notable that the spacing of the levels is not uniform, but increases with the height of the level. The conductance of the first level in 0.5 mol dm^{-3} NaCl is *ca.* $6 \times 10^{-10}\ \Omega^{-1}$.

The maximum number of levels observed is greatest when the alamethicin side of the membrane is negative and, although the infrequent occurrence of the higher levels leaves room for some uncertainty, as many as 9 (± 1) seem possible. While the correspondence of this number with the power of the alamethicin concentration in equation (1) suggests that the nine levels arise from an array of nine molecules, each of which constitutes a separate channel, it is necessary to consider also the obvious alternative that the levels arise from a single expanding channel. Selectivity data help to distinguish between the two possibilities.

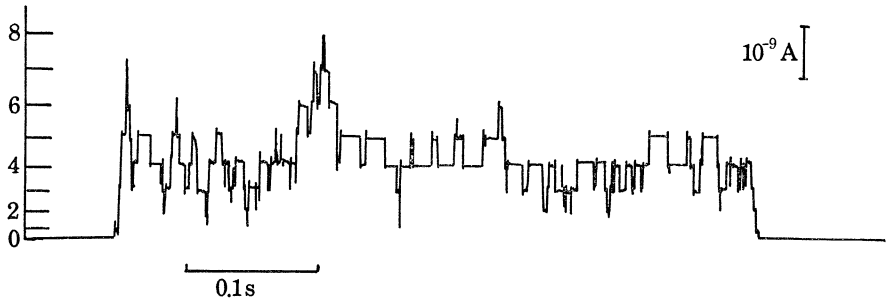

FIGURE 5. A record of a single current pulse. The membrane and aqueous phase were as described for figure 1 and alamethicin, nominally at 10^{-7} mol dm^{-3}, was present in one aqueous compartment. The applied potential was -260 mV on the alamethicin side of the membrane.

It has been shown (Haydon *et al.* 1972; Eisenberg *et al.* 1973) that the alamethicin channels in membranes with zero nett surface charge are effectively non-selective for the more common inorganic electrolytes. It has also been shown (Gordon 1974) that large ions such as citrate and tris appear not to permeate the channels. Examination of the unit channel conductances in solutions of the methyl-substituted ammonium chlorides suggests that there is an abrupt cut-off in the permeability to the cation at a certain ion radius. Figure 6 gives the experimental results. If the first level is disregarded, the ratio of the channel conductance increments to the bulk aqueous phase conductance for the various electrolytes remains constant until the dimethyl derivative is reached, beyond which it drops abruptly for each of the levels examined. For tetramethylammonium and tris the channel conductance increments are the same and correspond to conduction by chloride ions alone. (The rise in the curves in figure 6 for tris originates merely from the fact that the bulk conductance of tris hydrochloride is lower than that for tetramethylammonium chloride.) It appears therefore that although the conductance of the aggregate increases many-fold as successive levels appear, each conductance increment occurs at constant selectivity. These observations are very difficult to reconcile with the idea of a single pore (that the alamethicin channels must be pores has been shown in earlier papers) which expands discontinuously. Thus, in order to account for the range of conductances represented by the various levels, a single pore consisting of a cylinder of bulk solution, would have to increase its radius by a factor of at least two, and probably more, without altering its discrimination between dimethylammonium and tetramethylammonium ions. The observations are not, however, inconsistent with the notion of the aggregate as an array of pores of similar size and selectivity. The first level is nevertheless anomalous. Apart from the evidence of figure 6

this fact also emerges from the current–voltage relations (figure 7). In figure 7*a*, *I–V* curves are shown for levels up to six. The nonlinearity of the curves decreases with the height of the level. If the current for the first level is substracted from those for the higher levels the plots of figure 7*b* are obtained, where it appears that all channels other than the first to open are ohmic. This result tends to reinforce the conclusion from the selectivity data that one channel differs from the others.

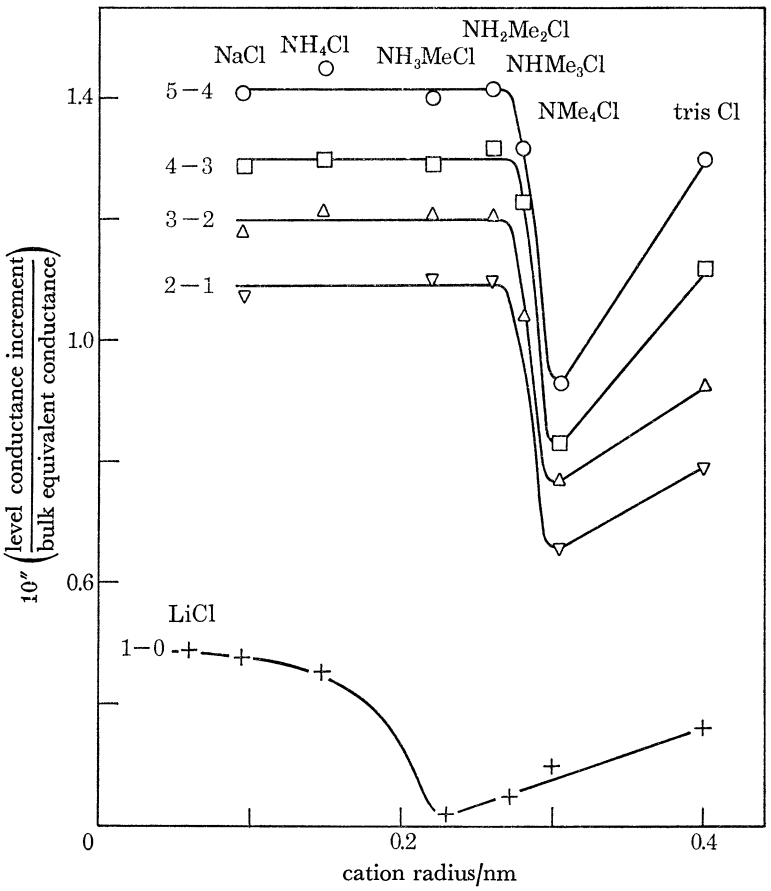

FIGURE 6. The selectivity of the conductance levels of the aggregate to cations of varying size. The level increments (indicated to the left of the curves) have been divided by the bulk phase equivalent conductance for the electrolyte in question. The cation radii are either the crystal radii or, when these were not available, a mean radius estimated from measurements on molecular models. All the electrolytes were at 1 mol dm^{-3}.

The conductance of an aggregate may be written

$$G_a\,(V, c_E) = \sum_{l=1}^{l=n} G_l\,(V, c_E)\,P_l, \qquad (4)$$

where G_l is the conductance and P_l the probability of occurrence of each level, and n is the maximum number of levels. G_l is, from figure 7, slightly potential-dependent. P_l is shown in figure 8 for a membrane with the alamethicin in turn on the positive and negative sides. Eisenberg *et al.* (1973) found for aggregates on the positive side of phosphatidyl ethanolamine membranes that P_l was similar to that in figure 8*a* and that, as for the present systems, no more than five levels were discernible. When the polypeptide is on the negative side, however, P_l is rather different (figure 8*b*) and, although as drawn the histogram does not show the point,

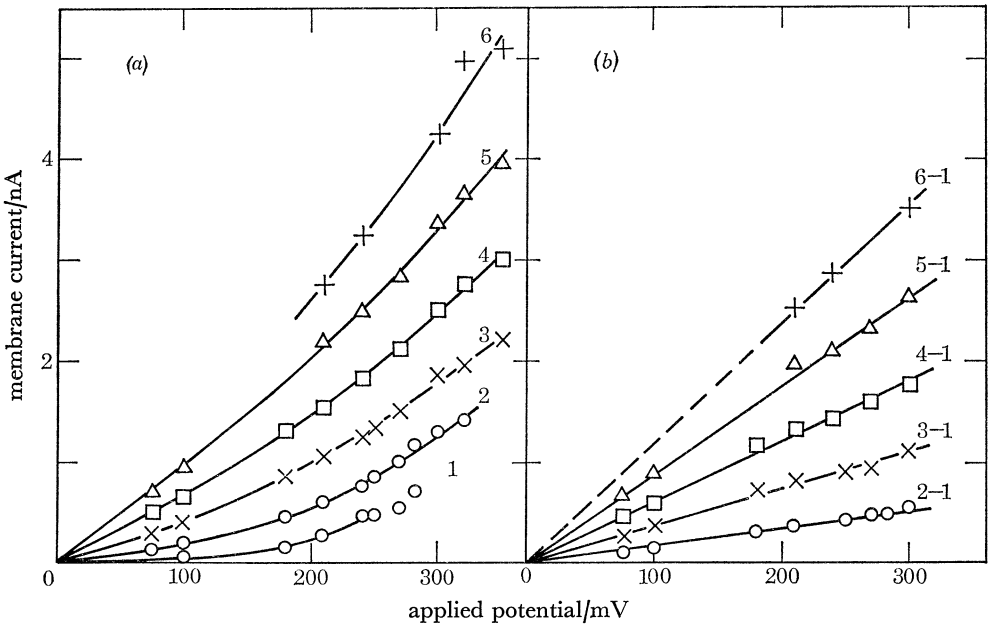

FIGURE 7. (*a*) Current–voltage curves for alamethicin aggregate levels. The aqueous phase was KCl at 2 mol dm^{-3}. (*b*) The curves of (*a*) with the current for the first level subtracted.

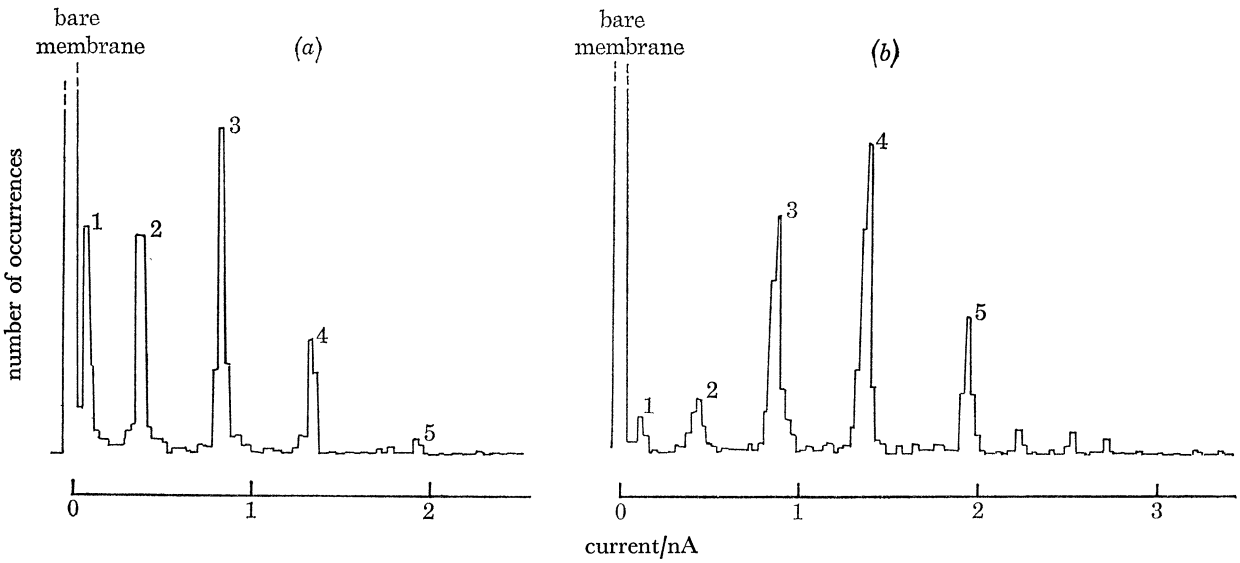

FIGURE 8. Current distributions for a glyceryl mono-oleate–cholesterol–hexadecane membrane in the presence of alamethicin. The aqueous solution was 2 mol dm^{-3} KCl and 3×10^{-8} mol dm^{-3} alamethicin was present on one side only of the membrane. The potential applied to the alamethicin side was in (*a*) 190 mV and in (*b*) −190 mV. The two records were obtained at the same absolute potential by waiting for different periods (the longer for (*b*)) after the addition of the polypeptide.

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there are apparently up to nine levels which do not originate from overlapping pulses. Eisenberg *et al.* state that their P_l for the 'positive' aggregates was not potential dependent. From the present investigation this seems to be roughly true, but it is difficult to rule out an upward shift of up to one level in the peak of P_l for a potential increase of *ca.* 100 mV.

Apart from its bearing on the origin of the potential-dependence of G_a, and hence of G, the results of figure 8 have interesting implications for the mechanism of channel formation. If it is accepted that each molecule in the multimolecular aggregate gives rise to one channel or pore, the question arises as to whether the individual molecules react to the applied field independently, or as one unit (to take the two extreme cases). If they reacted independently, increasing applied potential might be expected to result in an increase in the number of open channels, or probability of finding the aggregate in a high level of conductance and, in the limit of high potential, all the channels should be open all the time. The absence of any substantial change in the level distribution with applied potential suggests that the aggregate responds to the field as one unit or electrical particle.†

It is clear from the evidence of figures 7 and 8 that the contribution of G_a to the strong potential dependence of the membrane conductance, G, is very small. According to equation (2), therefore, the main part of the potential-dependence must originate in the variation of the number of aggregates conducting at any given time.

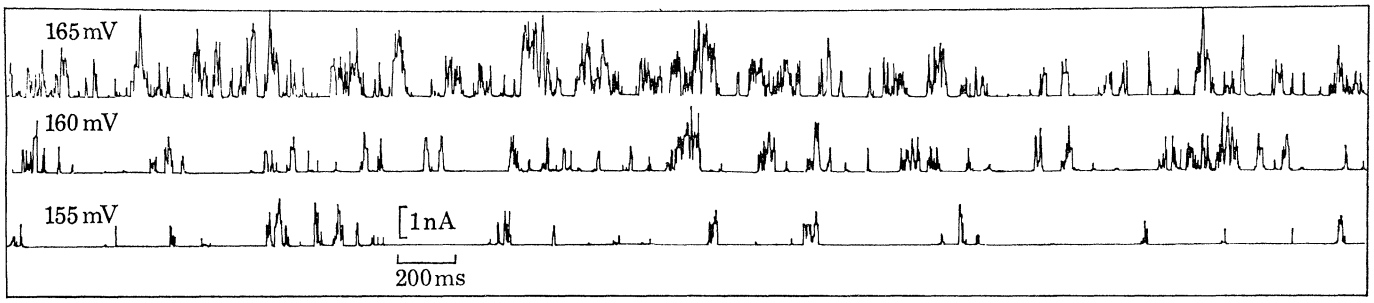

FIGURE 9. Current pulses for alamethicin (nominally 10^{-7} mol dm^{-3}) added to the positive side of a glyceryl mono-oleate–cholesterol–hexadecane membrane in 2 mol dm^{-3} KCl.

THE POTENTIAL DEPENDENCE OF N_a

In figure 9 are three records of current fluctuations for a membrane for which the potential has been changed in 5 mV steps. It is immediately obvious that the probability of finding at least one aggregate conducting at any given time increases considerably with the applied potential. It is less clear, however, to what extent this increase results, on the one hand, from a higher frequency of formation and, on the other, from a larger mean duration of the conducting aggregates. A statistical analysis shows that in the present membranes, at least, the mean duration lengthens by approximately 40 % for a 10 mV rise in potential (figure 10). It follows that the main potential dependence should lie in the frequency of formation, but a satisfactory proof of this is difficult to provide owing to the long term drifts in the mean membrane conductance which are common in this and other bilayer–polypeptide systems. However, rough

† The foregoing argument does not, of course, *prove* that the aggregate moves in the field as one unit but merely constitutes evidence in favour of it. A more detailed discussion of this question will be presented in a subsequent paper.

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estimates from the records of the probability of finding an aggregate functioning, yields a potential dependence for this probability which is quite close to that of the membrane conductance.

The effect of the field is thus to shift the equilibrium between the non-conducting and conducting complexes in favour of the latter. This shift will in turn affect the equilibria between the monomers and the non-conducting complexes, and between the aqueous phase alamethicin and the monomers. As pointed out earlier, however, the conducting complexes are, at most, so few (*ca.* 10^4 mm^{-2}) compared to the monomers (*ca.* 10^{11} mm^{-2}) that saturation effects, should be negligible even if diffusion times from the aqueous phase are very long.

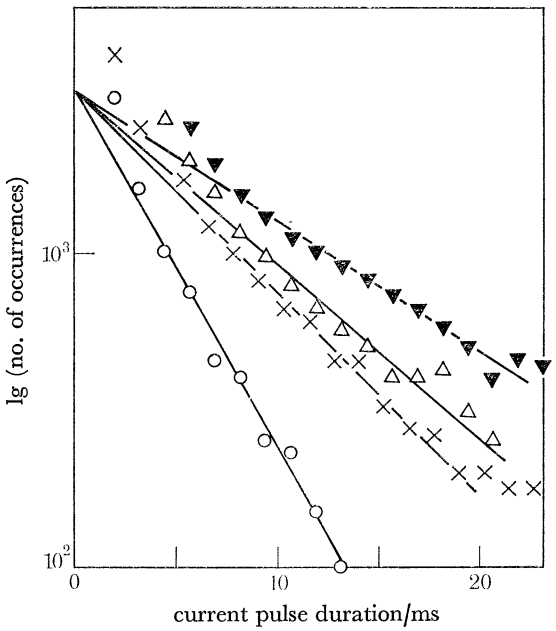

FIGURE 10. Current pulse durations as a function of applied potentials for a glyceryl mono-oleate–cholesterol–hexadecane membrane in 2 mol dm^{-3} KCl. The alamethicin (nominally at 5×10^{-8} mol dm^{-3}) was added to the positive side of the membrane. ○, 100 mV; ×, 120 mV; △, 150 mV; ▼, 170 mV.

THE INTERACTION OF THE COMPLEX WITH THE ELECTRIC FIELD

In one of its forms, alamethicin has an ionizable glutamyl carboxylic acid group (Payne, Jakes & Hartley 1970), and although, in its charged form, this group could contribute to the observed potential-dependent effects, the evidence does not support this possibility. Thus, even in solutions at pH $\approx$ 2, the membrane conductance is not obviously impaired and, moreover, an un-ionizable form of alamethicin in which the glutamic acid is replaced by glutamine continues to show potential-dependent conductance.

Several authors have proposed that alamethicin binds univalent cations and that the field acts on these charges. There are a number of observations to suggest that this might be so. First, Pressman (1968) found that alamethicin extracted alkali metal ions into non-polar media, and concluded that this resulted from a binding of the ions by the polypeptide. Secondly, as Mueller & Rudin (1968) noted originally, the membrane conductance is very strongly dependent (to the sixth power, according to the original report, and to the fourth power

according to Eisenberg *et al.* (1973) and to the present authors – cf. equation (1)) on the electrolyte concentration. One interpretation of this finding is that the conducting complex binds the inorganic ions. Thirdly, Eisenberg *et al.* (1973) have shown that if alamethicin is introduced to one side only of a phosphatidyl ethanolamine membrane, only positive and not negative potentials on the alamethicin side enhance the conductance – consistent with the binding of cations. It is nevertheless instructive to examine these various pieces of evidence more closely and in the light of other experimental data.

Pressman's experiments have not been described in detail and, although the results are reasonably convincing, it may be asked whether, since the alamethicin was probably mostly in the carboxylic acid form, it did not extract the inorganic cations into the non-polar phase simply as a consequence of salt formation or of the requirement for electroneutrality of the bulk phase. Mueller & Rudin's finding that the membrane conductance is strongly dependent on the electrolyte concentration is accounted for by the fact that the adsorption of alamethicin to the glyceryl mono-oleate–decane/NaCl solution interface (figure 2) is dependent on the square root (approximately) of the sodium chloride concentration (at least in the range 0.2–1.0 mol dm^{-3}). When this dependence is combined with the 9th power relation between the adsorbed alamethicin monomer and the conducting aggregate concentration, and the dependence of G_a on electrolyte concentration is also included, it can be deduced that a term in approximately c_E^4 should appear in equation (1). The evidence of Eisenberg *et al.* (1973) that conduction occurs only for one direction of the applied field seems to be restricted to phosphatidyl ethanolamine membranes. In all the other systems so far described, conduction occurs for both field directions. The latter result would be expected if, in these instances, the alamethicin diffused across the membrane such that conduction in the reversed field could arise from the polypeptide on the far side. There are, however, two reasons to suppose that diffusion across the membrane is not a satisfactory explanation. First, the character of the unit conducting aggregates (and certain features of the onset of conductance) is different for the two field directions. While the positions of the current levels are very similar under similar conditions, the probability of the higher levels is substantially greater for the alamethicin side of the membrane negative (figures 3, 5 and 8). This fact alone suggests that 'negative' complexes are not simply 'positive' ones acting on the far side of the membrane, but this possibility seems even less likely when the distribution of the alamethicin is considered. Thus, if the asymmetry of the *I–V* curve really does reflect the presence of polypeptide on the far side of the membrane equation (1) requires that its concentration on this side should be approximately one-twentieth of that on the side to which it was originally introduced. Now although there is no obvious effect of alamethicin concentration on level distribution, it would be at least curious to find a greater probability for the high levels at lower concentrations. Secondly, although no quantitative data is available regarding the ability of alamethicin to cross the membranes, it is quite clear from Eisenberg *et al.*'s results that the polypeptide does not appreciably permeate phosphatidyl ethanolamine membranes. It is arguable therefore that alamethicin may not permeate other types of lipid membrane, either, and that there is another explanation for the absence of a negative branch to the *I–V* curve for the phosphatidyl ethanolamine system. It is, of course, possible that whenever conducting pores are formed, some alamethicin crosses the membrane and remains on the far side. The quantities involved would, however, be extremely small and, owing to the reversibility of the adsorption, this material should pass on into the alamethicin-free aqueous phase.

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Probably the most convincing piece of evidence that alamethicin does not bind ions is that the membrane conductances show the same potential-dependence over a considerable range of electrolyte concentration and in a wide variety of electrolytes, including sodium chloride, acetate and citrate, and calcium,† tetramethylammonium and tris chlorides. Ion binding of the necessary strength which is as non-specific as this is, to the authors' knowledge, unknown.

If ion binding is to be discounted, the possibility that alamethicin or, at least, its aggregates, have a large dipole moment must be considered. By assuming that the whole of the membrane potential falls across a dipole of length 0.5 nm it can be calculated that in order to account for the current–voltage data the dipole moment of an aggregate would have to be approximately 5×10^{-28} C m. This should be regarded as a minimum value since if the potential fell across a distance larger than 0.5 nm, a larger dipole moment would be required. The aggregate value of 5×10^{-28} C m corresponds to a dipole moment per molecule of *ca.* 5.5×10^{-29} C m. The existence of such a dipole has not been directly demonstrated, although from surface potential studies of alamethicin monolayers at the air/water interface, it has been shown that the normal component of the surface dipole changes by *ca.* 2×10^{-29} C m for each polypeptide molecule adsorbed (Gordon & Haydon, unpublished data). Consideration of the structure of alamethicin suggests that dipole moments well in excess of 5.5×10^{-29} C m are feasible but, apart from the model of McMullen, Marlborough & Bayley (1971) (which seems unlikely to have a high enough dipole moment) there is little on which to base detailed computations.

If the alamethicin conducting aggregates may be regarded as dipolar rather than ionic, several aspects of the potential-dependence of the conductance are more readily accounted for. Thus, it is understandable that, even with the polypeptide on one side only of the membrane, conductance is stimulated by both directions of the field and also that, if one side of the aggregate interacts with the lipid more strongly than the other, the current–voltage curve would be asymmetric with respect to the origin. Indeed, if the interaction were sufficiently strong, one branch of the I–V curve could effectively disappear (cf. equation (10) below), as in phosphatidyl ethanolamine membranes. Also with two possible orientations for the conducting aggregate, the occurrence of different level probabilities for the two field directions (figure 8) is no longer surprising.

The aggregate structure and the conducting channels

The experimental results of the previous sections seem a sufficient basis for some tentative proposals as to the nature of the alamethicin aggregates and the means of formation of the conducting channels.

The only obvious possibility for arranging eight to ten molecules so as to form eight to ten channels, one of which differs from the others, is a radially disposed array, in which the peripheral molecules form the similar channels and the molecule or hole in the centre constitutes the odd channel. These structures form in significant numbers even at zero membrane potential provided the alamethicin concentration is high enough, and must be sufficiently deeply embedded in the membrane lipid to make possible the formation of pores, even if only transiently, connecting the two sides. If the notional non-conducting complex is thought of as being

† No effects of di- and polyvalent cations on the potential-dependence of the membrane conductance, such as have been reported by Cherry *et al.* (1972), were found in the present studies.

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similar to the conducting complex with the exception that it is situated on, rather than in, the membrane, the electric field, E, can be considered to influence the equilibrium constant for the two species through a term of the form $\exp(\mu E/kT)$, where μ is the dipole moment of the aggregate.

The above inferences may be combined within the formalism of equations (2)–(4) to yield a general expression for the specific conductance, G, of a membrane to which alamethicin has been added to one side only. First, equation (3) is rewritten so as to include the possibility of conduction in both directions of the membrane field, i.e.

$$G = G_a^+ N_a^+ + G_a^- N_a^-, \tag{5}$$

where, as previously, G_a and N_a are respectively the mean conductances and numbers per unit area in the membrane of the conducting aggregates and the plus and minus superscripts distinguish between the contributions of the aggregates for positive and negative potentials, respectively, on the alamethicin side. The equilibrium between the conducting and non-conducting aggregates may be expressed

$$N_a^+ = K_3^+ N_a^0 \quad \text{and} \quad N_a^- = K_3^- N_a^0, \tag{6}$$

where N_a^0 is the number per unit area of non-conducting complexes and $K_3^{+,-}$ are equilibrium constants which are field-dependent. Thus, if μ is the dipole moment of the aggregate,

$$K_3^+ = K_3^{0,+} e^{\mu E/kT} \quad \text{and} \quad K_3^- = K_3^{0,-} e^{-\mu E/kT}, \tag{7}$$

$K_3^{0+,-}$ being the equilibrium constants for zero field. But, if N is the number of monomeric alamethicin molecules per unit area of the membrane, according to equation (2)

$$N_a^0 = K_2 N^9, \tag{8}$$

and, for equilibrium with the aqueous phase,

$$N = K_1 c_A. \tag{9}$$

In writing equations (6), (8) and (9), saturation effects have been ignored. This should be permissible, for (6) and (8) because, as already pointed out, both $N_a^{+,-}$ and N_a^0 are much smaller than N and, for (9), provided $c_A \lesssim 10^{-7}$ mol dm^{-3} (see figure 2).

Combining equations (5)–(9), the membrane conductance can be written

$$G = K_2 (c_A K_1)^9 \{K_3^{0,+} G_a^+ e^{\mu E/kT} + K_3^{0,-} G_a^- e^{-\mu E/kT}\}. \tag{10}$$

$G_a^{+,-}$ will be given by equation (4) provided appropriate allowance is made for the differing values of P_l for the two directions of the field. G depends on the electrolyte concentration through $G_a^{+,-}$ and through K_1, at least. Empirical expressions for both $G_a^{+,-}(c_E)$ and $K_1(c_E)$ may be deduced from the experimental data and incorporated into equation (10) but the result is cumbersome and the exercise unnecessary for present purposes. The only aspect of equation (10) which perhaps warrants further comment is the role of $K_3^{0,+}$ and $K_3^{0,-}$ in determining the asymmetry of the G–V curve. These two constants represent the stability at zero field, relative to that of the non-conducting aggregate, of the conducting aggregate in its two orientations. Since, in general, larger negative than positive potentials are required to produce a given level of conduction, it must be concluded that the 'positive' orientation is preferred and that

$K_3^{0,+} > K_3^{0,-}$. This inequality apparently varies from one membrane to another and, in phosphatidyl ethanolamine membranes, is sufficient to effectively prohibit any conduction at accessible negative potentials. Thus, although the influence of the membrane field is to stabilize the conducting aggregates in one or other of their orientations, the 'chemical' interactions represented by $K_3^{0,+,-}$ may render one of these tendencies imperceptible.

To conclude this section it is appropriate to comment briefly on how the channels may form. It has been emphasized that although the formation of conducting aggregates is potential-dependent, the formation of the channels within the aggregate is at most only weakly so. Moreover, such statistical analyses as have so far been achieved on the current fluctuations for a single aggregate suggest that all the opening and closing processes involved are of approximately the first order. One possibility is, therefore, that the alamethicin aggregate resembles a sieve, one side of which is in contact with an aqueous phase (that via which the alamethicin was introduced). The other side (e.g. in the state of the non-conducting aggregate) would normally be covered with lipid but, in the conducting state, this lipid layer might become unstable (perhaps owing to its small thickness) and recede transiently uncovering between one and nine channels in the alamethicin aggregate. The most probable number of channels exposed would depend on whether the aggregate was 'positively' or 'negatively' oriented, so producing the results of figure 8. The divergence of the conductance levels (figure 5) must obviously be explicable in terms of the structure of the aggregate and its situation in the membrane. Several possibilities come to mind, but to express these quantitatively requires more information than is at present available.

The authors with to thank Dr S. B. Hladky for helpful discussions.

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