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# Electrical Noise from Lipid Bilayer Membranes in the presence of Hydrophobic Ions

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*Summary.* In the presence of the hydrophobic ion dipicrylamine, lipid bilayer membranes exhibit a characteristic type of noise spectrum which is different from other forms of noise described so far. The spectral density of current noise measured at zero voltage increases in proportion to the square of frequency at low frequencies and becomes constant at high frequencies. The observed form of the noise spectrum can be interpreted on the basis of a transport model for hydrophobic ions in which it is assumed that the ions are adsorbed in potential-energy minima at either membrane surface and are able to cross the central energy barrier by thermal activation. Accordingly, current-noise results from random fluctuations in the number of ions jumping over the barrier from right to left and from left to right. On the basis of this model the rate constant $k_i$ for the translocation of the hydrophobic ion across the barrier, as well as the mean surface concentration $N_t$ of adsorbed ions may be caluculated from the observed spectral intensity of current noise. The values of $k_i$ obtained in this way closely agree with the results of previous relaxation experiments. A similar, although less quantitative, agreement is also found for the surface concentration $N_t$.

In recent years numerous studies have been carried out in which electrical noise from biological membranes has been analyzed with the aim of obtaining information on the mechanisms by which ions traverse the membrane (Verveen & DeFelice, 1974; Conti & Wanke, 1975). A well-known difficulty in the analysis of noise spectra from biological membranes lies in the fact that usually several different sources contribute to the total noise signal. Besides this, the physical basis for the interpretation of noise spectra in terms of ion transport mechanisms is still poorly developed. For this reason, studies with simple and well-defined transport systems are very valuable for a better understanding of noise phenomena in membranes. So far, such noise studies have been performed with artificial lipid bilayer membranes in the presence of pore-forming substances like gramicidin, alamethicin or monazomycin (Zingsheim & Neher, 1974; Kolb, Läuger & Bamberg, 1975; Moore & Neher, 1976; Kolb & Bamberg, 1977; Kolb & Boheim, 1977).

Hydrophobic ions such as tetraphenylborate or dipicrylamine belong to the simplest charge transport systems so far studied in artificial lipid membranes (Mueller & Rudin, 1967; Liberman & Topaly, 1968; Le Blanc, 1969). These ions are able to penetrate the hydrocarbon core of the membrane without the aid of a carrier or a channel as the coulombic energy of the ion is compensated (in part) by the hydrophobic interaction with the membrane. The potential energy of a hydrophobic ion has a deep minimum in either membrane solution interface, the two minima being separated by a dielectric energy barrier (Ketterer, Neumcke & Läuger, 1971; McLaughlin, 1977). The transport of hydrophobic ions across the lipid membrane may therefore be described as a three-step process: (i) adsorption to the membrane-solution interface, (ii) migration over the central barrier, and (iii) desorption into the aqueous solution. Electrical relaxation studies have provided information on the rate constant for the migration over the barrier as well as on the partition coefficient of the ion between water and the interfacial energy minimum (Ketterer *et al.*, 1971; Andersen & Fuchs, 1975; Bruner, 1975; Benz, Läuger & Janko, 1976; Szabo, 1976; Benz & Läuger, 1977).

In this paper we report on noise studies with lipid bilayer membranes in the presence of dipicrylamine. The measurements were carried out with identical external solutions and with zero applied voltage. Under these conditions the average current through the membrane vanishes, but at any moment the instantaneous current differs from zero. This current, which fluctuates around zero, arises from statistical fluctuation in the number of ions jumping over the central barrier from left to right and from right to left. The current noise expected in this case is similar to the so-called shot-noise which has been postulated on the basis of theoretical arguments (Stevens, 1972; Verveen & DeFelice, 1974; Läuger, 1975), but which has not been described from membrane experiments so far. For a single potential barrier without adsorption, theory predicts "white" shot noise, i.e., noise with a frequency-independent spectral intensity (Läuger, 1975). In the case considered here, however, the existence of adsorption sites at the membrane surfaces introduces a capacitive element into the behavior of the system with a characteristic correlation time $\tau_i$ which is equal to the mean lifetime of a fluctuation in ion distribution between the two adsorption planes. This means that the spectral density should decrease with frequency $f$ below $f \approx 1/\tau_i$. In the following we show that lipid bilayer membranes in the presence of hydrophobic ions exhibit current noise of the expected spectral characteristic. Furthermore, we describe a theoretical model which accounts for the experimentally observed spectral intensity.

## Materials and Methods

The lipids used for membrane formation were D,L-1,2-diphytanoyl-3-phosphatidyl serine or L-1,2-diacyl-3-phosphatidyl cholines with mono-unsaturated fatty acid residues of different chain length: dipalmitoleoyl (16:1)-, dioleoyl (18:1)-, di-$\Delta^{11}$-eicosenoyl (20:1)- and dierucoylphosphatidyl choline (22:1). These lipids have been synthetized by K. Janko (Benz *et al.*, 1976; Janko & Benz, 1977). Optically black membranes were formed in the usual way (Läuger, Lesslauer, Marti & Richter, 1967) from a 1–2% (w/v) lipid solution in *n*-decane (Merck standard for gas chromatography). The membrane area usually ranged between 0.34 and 0.37 mm$^2$, except for the experiments with dierucoylphosphatidylcholine where the membrane area was between 0.68 and 0.7 mm$^2$. If not otherwise stated, the unbuffered aqueous solutions (pH$\simeq$6) contained 0.1 M NaCl and various concentrations of dipicrylamine (Fluka, purissimum). Prior to membrane formation the Teflon cell with inserted Ag/AgCl electrodes was incubated for about 30 min with the aqueous solution. The measurements were usually started 60 min after the membrane had turned completely black. It was checked that varying the membrane area $A$ had no effect other than changing the spectral density of current noise proportional to $A$ (*see below*). Furthermore, the noise spectrum remained unchanged when an external capacity (of about the same magnitude as the membrane capacity) was added in parallel to the membrane.

The current noise of the black film at zero voltage was measured in the following way. The membrane together with the preamplifier (Analog Devices Model 52 K) were contained in a completely closed metal box acting as an electric shield. In addition the membrane cell was shielded from mechanical vibrations by mounting the metal box on a large stone slab which was supported by an inflated automobile innertube. The feedback resistance which was adapted to the resistance of the membrane (*see below*) ranged between 60 and 500 MΩ. With an open-loop gain of the preamplifier of $\geqq 10^6$ the input resistance of the amplifier was always smaller than 500 Ω. A constant feedback capacitance of 1 pF was used in order to prevent oscillations in the amplifying system. The output of the preamplifier was fed into the main amplifier (Princeton Applied Research Model 113) which was used in the ac-coupled mode with the lower cut-off frequency at 0.03 Hz. The upper cut-off frequency of the PAR 113 was set well above the frequency limit determined by the low-pass frequency of the feedback circuit of the preamplifier.

The amplified current noise of the membrane was processed with a Honeywell-Saicor 52 B real-time spectrum analyzer. The spectral intensity $S_I(f)$ of the current noise obtained in this way was either plotted logarithmically on an *xy*-recorder (Philips PM 8/25) as a function of frequency $f$, or the frequency output of the instrument was fed into a logarithmic amplifier (Analog Devices Model 755 N) in order to obtain a $\log S_I$ *vs.* $\log f$ representation. The calibration of the spectrum analyzer was checked by recording the noise from metal-film resistors of known resistance $R$ and comparing the measured spectral intensity $S_I$ with the Nyquist relation

$$S_I(f)=\frac{4kT}{R} \tag{1}$$

($k$ is the Boltzmann constant and $T$ the absolute temperature). The recorded spectrum usually was an average over 256 or 512 summations.

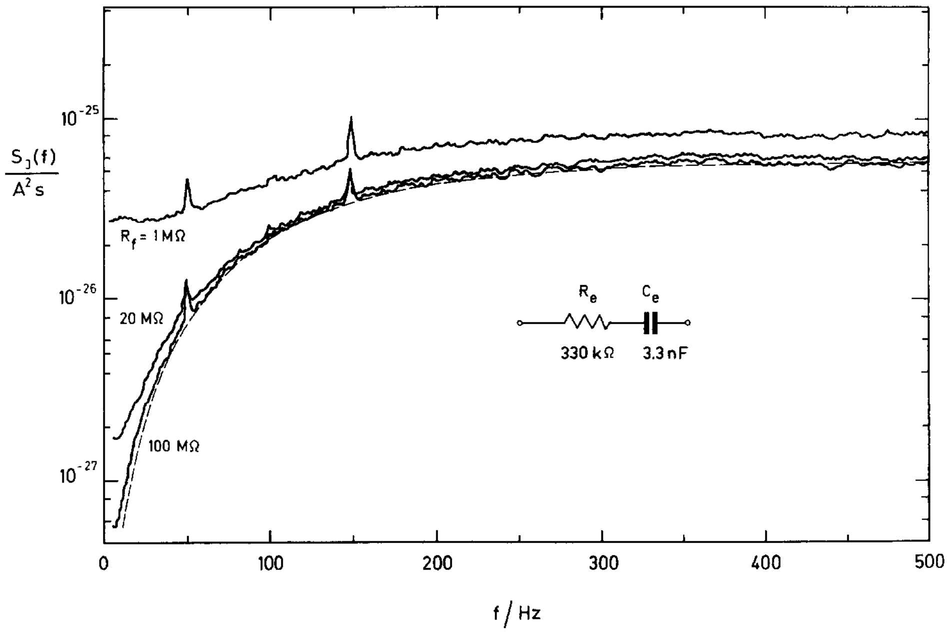

Fig. 1. Spectral intensity $S_I(f)$ of current noise from a dummy circuit consisting of a series combination of a resistance $R_e = 330\,\mathrm{k}\Omega$ and a capacitance $C_e = 3.3\,\mathrm{nF}$ as a function of frequency $f$. $S_I(f)$ has been measured at different values of the feedback resistance $R_f$ of the preamplifier. The dashed curve is the theoretical thermal (or Nyquist) spectrum of the dummy circuit calculated from Eq. (3). The spikes at 50 and 150 Hz are artifacts from stray signals derived from the ac line

The whole set-up was carefully checked with equivalent circuits. As will be shown later, the current noise from the lipid membrane in the presence of dipicrylamine yields a noise spectrum of the form ($\omega = 2\pi f$):

$$S_I(\omega) = S_I(\infty)\,\frac{\omega^2 \tau_i^2}{1+\omega^2 \tau_i^2} \tag{2}$$

where $\tau_i$ is a characteristic time-constant. A suitable equivalent circuit simulating the membrane therefore consists in a series combination of a resistance $R_e$ and a capacitance $C_e$ which yields a thermal current-noise spectrum of the form (*see* Appendix C):

$$\begin{aligned} S_I(\omega) &= \frac{4kT}{R_e} \cdot \frac{\omega^2 \tau_e^2}{1+\omega^2 \tau_e^2} \\ \tau_e &= R_e C_e. \end{aligned} \tag{3}$$

In order to record an undistorted noise spectrum from such a series combination of $R_e$ and $C_e$, the feedback resistor $R_f$ of the preamplifier has to be properly chosen. It was found that an agreement of the experimental power density with the calculated thermal spectrum [Eq. (2)] was obtained only for $R_f \gtrsim 100\,R_e$ (*see also*: Poussart, 1971; Fishman, Poussart & Moore, 1975). This is illustrated in Fig. 1 in which the noise spectra from a series combination of $R_e = 330\,\mathrm{k}\Omega$ and $C_e = 3.3\,\mathrm{nF}$ are given for different values of the feedback resistance $R_f$ of the preamplifier. It is seen that for $R_f = 100\,\mathrm{M}\Omega$ the measured spectral intensity is close to the theoretical spectrum (dashed line) which has been calculated from Eq. (3). On the other hand, at low values of $R_f$ serious distortions of $S_I(f)$ occur. These distortions originate from current noise in the feedback resistor of the preamplifier which is superimposed on the "signal" from the dummy circuit (*see* Appendix C). In the experiments with black films in the presence of dipicrylamine a large value of the feedback resistance $R_f$ was chosen so that a further increase of $R_f$ had no perceptible effect on the observed noise spectrum. Experiments with dummy circuits also showed that the geometrical membrane capacitance $C_m$ had no influence on $S_I$ in the experimental frequency range (an effect of $C_m$ on $S_I$ is seen, however, at frequencies above 1 kHz where the intrinsic voltage noise of the preamplifier which produces a frequency-dependent current noise through the capacitance $C_m$ leads to an overall increase of $S_I$).

## Results

An example of the current-noise spectrum $S_I(f)$ observed in the presence of dipicrylamine is given in Fig. 2, (curve *a*). $S_I(f)$ exhibits a characteristic shape with a frequency-independent part at high $f$ and a decline towards low $f$. This shape of $S_I(f)$ has been observed under all

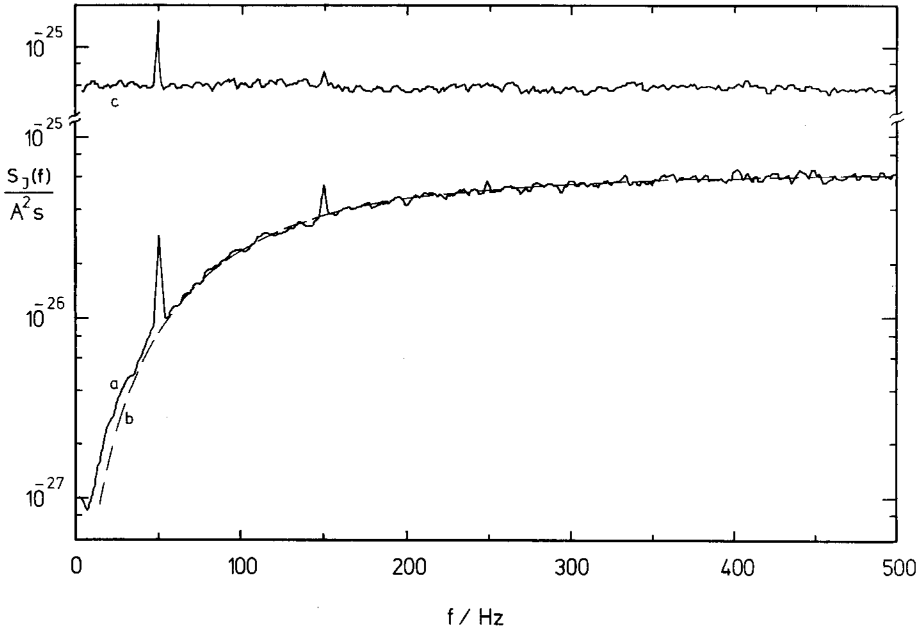

Fig. 2. Spectral intensity $S_I(f)$ of current noise from a dioleoyllecithin/*n*-decane membrane in the presence of 30 nM dipicrylamine and 0.1 M NaCl as a function of frequency $f$ (curve *a*). The membrane area was $A = 0.36\ \text{mm}^2$; $T = 25\ ^\circ\text{C}$. Curve *b* is the theoretical curve which has been calculated from Eq. (2) with $S_I(\infty) = 5.9 \times 10^{-26}\ \text{A}^2\text{sec}$ and $\tau_i = 1.15$ msec. These parameter values have been determined from a least-squares fit starting at the highest frequencies down to the frequency at which $S_{\text{exp}} - S_{\text{theor}}$ exceeded the mean scatter of the trace. Curve *c* represents the result of a control experiment without dipicrylamine, in which the dipicrylamine-induced (high frequency) conductance has been simulated by an external resistance of 278 kΩ in parallel to the membrane

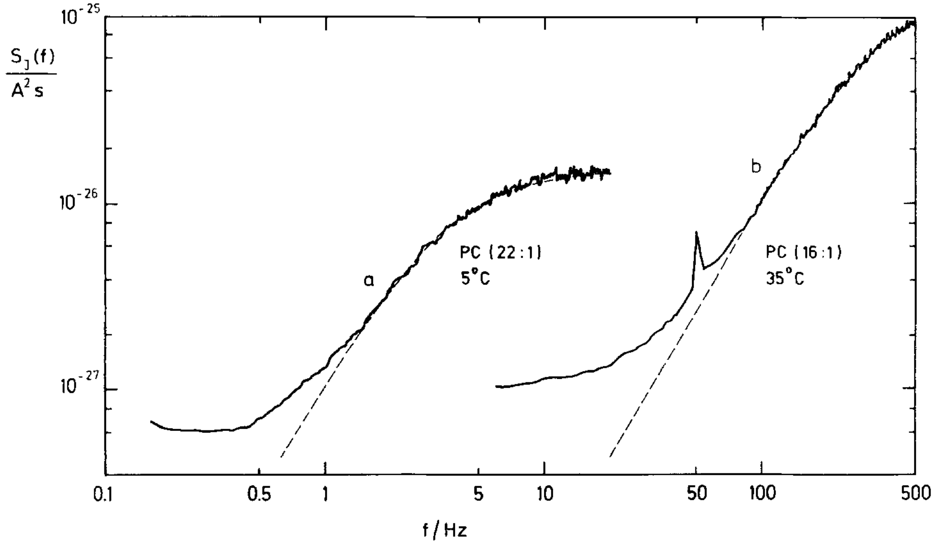

Fig. 3. Spectral intensity $S_I(f)$ of current noise in the presence of 30 nM dipicrylamine and 0.1 M NaCl. Curve *a*: dierucoyllecithin/*n*-decane membrane with $A = 0.36\ \mathrm{mm}^2$ at 5 °C; theoretical curve (dashed line) drawn according to Eq. (2) with $S_I(\infty) = 1.4 \times 10^{-26}\ \mathrm{A^2sec}$ and $\tau_i = 44$ msec. Curve *b*: dipalmitoleoyllecithin/*n*-decane membrane ($A = 0.35\ \mathrm{mm}^2$) at 35 °C; theoretical curve drawn with $S_I(\infty) = 9.7 \times 10^{-26}\ \mathrm{A^2sec}$ and $\tau_i = 0.44$ msec

experimental conditions (different dipicrylamine concentrations and temperatures, different lipids). The noise spectral intensity in the presence of dipicrylamine is thus strikingly different from the well-known Lorentzian spectrum which is frequency independent at low $f$ and declines in proportion to $1/f^2$ at high $f$.

It is found empirically that $S_I(f)$ may be represented (with the exception of the low-frequency end of the spectrum) by a function of the form of Eq. (2); a theoretical interpretation of this finding will be given in the next section. An example of a fit of Eq. (2) to an experimental curve is given in Fig. 2 (curve *b*). From such a fit two parameters are obtained, the high-frequency limit $S_I(\infty)$ and the time constant $\tau_i$; $1/2\pi\tau_i$ is the "corner frequency" at which $S_I(f)$ has declined to the value $S_I(\infty)/2$.

Depending on the temperature and on the nature of the lipid the values of $S_I(\infty)$ and $\tau_i$ vary in wide limits. Two extreme cases are represented in Fig. 3. It is seen that despite a variation of $\tau_i$ by a factor of about 100 the general shape of the spectrum remains the same.

With increasing temperature both the high-frequency limit $S_I(\infty)$ and the corner frequency $f_c = 1/2\pi\tau_i$ shift to higher values (Fig. 4). The results

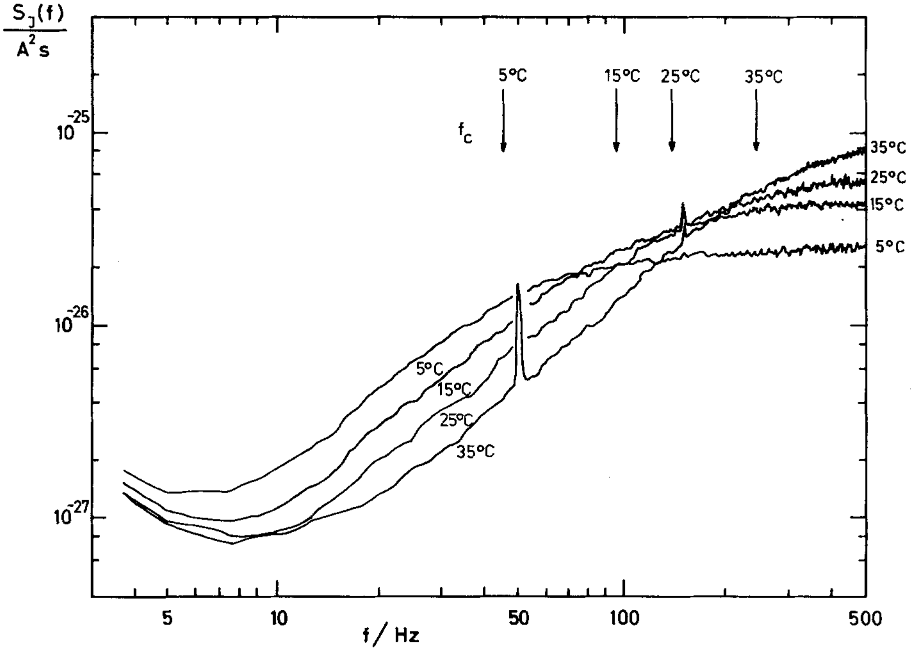

Fig. 4. Spectral intensity $S_I(f)$ of current noise at different temperatures. Dioleoyllecithin/*n*-decane membranes ($A = 0.35$ mm$^2$). The aqueous phase contained 30 nM dipicrylamine and 0.1 M NaCl. A different membrane was used for each temperature. The arrows mark the corner frequencies $f_c = 1/2\pi\tau_i$

obtained at different temperatures with lecithins of different chain-lengths are summarized in Table 1. It is seen that, despite a strong increase of $\tau_i$ with increasing chain length, $S_I(\infty)$ only slightly decreases.

Both $S_I(\infty)$ and $\tau_i$ depend on aqueous dipicrylamine concentration, as shown in Table 2. The significance of these results will be discussed later. In a further series of experiments the influence of surface potential on the noise spectrum was studied. For this purpose the current noise from neutral diphytanoylphosphatidylcholine membranes and from negatively charged diphytanoylphosphatidylserine membranes was compared at different electrolyte concentrations in the aqueous phase (Table 3). It is seen that for the neutral membrane $S_I(\infty)$ and $\tau_i$ are insensitive to changes in the aqueous concentrations of $Na^+$ and $Ca^{++}$ whereas strong effects of salt concentration are found for the negatively charged membrane. A strong influence of ionic strength on $\beta$ for membranes formed by diphytanoylphosphatidylserine was also found by Janko and Benz (1977).

Table 1. Analysis of current noise from lecithin membranes in the presence of 30 nM dipicrylamine and 0.1 M NaCl[a]

| Lecithin | $n$ | $T$ (°C) | $\tau_i$ (msec) | $E(k_i)$ (kJ/mole) | $k_i$ ($\text{sec}^{-1}$) | $S_I(\infty)$ ($10^{-26}$ $\text{A}^2$ sec) | $N_t$ ($\text{pmole/cm}^2$) | $\beta$ ($10^{-2}$ cm) | $\Delta H_{\text{ads}}$ (kJ/mole) |
|---|---|---|---|---|---|---|---|---|---|
| 16:1 | 4 | 5 | 1.61±0.10 | 32.8±2.9 | 311±19 | 5.2±0.5 | 1.55±0.16 | 2.6 | −15.2±5.8 |
| | 4 | 15 | 1.07±0.06 | | 467±26 | 6.3±0.8 | 1.25±0.12 | 2.1 | |
| | 5 | 25 | 0.61±0.04 | | 820±54 | 8.4±0.9 | 0.95±0.1 | 1.6 | |
| | 6 | 35 | 0.42±0.03 | | 1191±85 | 9.6±1.0 | 0.75±0.07 | 1.3 | |
| 18:1 | 5 | 5 | 3.45±0.29 | 37.8±2.9 | 145±12 | 2.9±0.4 | 1.9 ±0.3 | 3.1 | −19.4±4.3 |
| | 6 | 15 | 1.69±0.11 | | 296±21 | 4.7±0.6 | 1.5 ±0.2 | 2.4 | |
| | 11 | 25 | 1.17±0.06 | | 427±22 | 5.3±0.8 | 1.2 ±0.2 | 1.9 | |
| | 10 | 35 | 0.68±0.03 | | 735±32 | 7.3±1.0 | 0.92±0.1 | 1.5 | |
| 20:1 | 3 | 5 | 14.7 ±0.9 | 50.1±3.2 | 34±2 | 2.1±0.3 | 5.7 ±0.6 | 9.5 | −38.8±5.4 |
| | 5 | 15 | 6.76±0.4 | | 74±4 | 2.6±0.2 | 3.2 ±0.3 | 5.4 | |
| | 7 | 25 | 3.55±0.17 | | 141±7 | 2.9±0.3 | 1.9 ±0.2 | 3.2 | |
| | 5 | 35 | 1.78±0.08 | | 281±13 | 3.4±0.5 | 1.1 ±0.1 | 1.8 | |
| 22:1 | 5 | 5 | 42.5 ±3.1 | 64.9±4.5 | 12±1 | 1.6±0.3 | 12.6 ±1.9 | 20.9 | −49.2±7.5 |
| | 3 | 15 | 15.5 ±1.2 | | 32±3 | 2.1±0.3 | 6.0 ±0.7 | 10.0 | |
| | 5 | 25 | 6.7 ±0.4 | | 75±4 | 2.4±0.4 | 3.0 ±0.5 | 5.0 | |
| | 4 | 35 | 2.9 ±0.3 | | 172±18 | 2.9±0.3 | 1.6 ±0.2 | 2.6 | |

[a] $n$ is the number of membranes used for each set of experimental conditions. Mean values are given together with the standard deviations. The high-frequency limit $S_I(\infty)$ and the time constant $\tau_i$ have been obtained by fitting Eq. (2) to the experimental spectrum. $S_I(\infty)$ is referred to a membrane area of $A = 0.69\ \text{mm}^2$ for (22:1)-lecithin membranes and to $A = 0.35\ \text{mm}^2$ in the other cases. $k_i$, $N_t$ and $\beta$ have been evaluated from $S_I(\infty)$ and $\tau_i$ according to Eqs, (4) and (8)–(10). $E(k_i)$ is the activation energy for the translocation across the central barrier. $\Delta H_{\text{ads}}$ is the enthalpy change associated with the adsorption of dipicrylamine from the solution to the membrane.

Table 2. Analysis of current noise from dioleoyllecithin/*n*-decane membranes at 25 °C[a]

| DPA (nM) | $n$ | $\tau_i$ (msec) | $k_i$ ($\text{sec}^{-1}$) | $k_i^*$ ($\text{sec}^{-1}$) | $S_I(\infty)$ ($10^{-26}$ $\text{A}^2$ sec) | $N_t$ (pmole/ $\text{cm}^2$) | $N_t^*$ (pmole/ $\text{cm}^2$) | $\beta$ ($10^{-2}$ cm) |
|---|---|---|---|---|---|---|---|---|
| 3 | 7 | 1.05±0.05 | 476±25 | 460± 90 | 0.98±0.11 | 0.19±0.02 | 0.25±0.05 | 3.2 |
| 30 | 11 | 1.17±0.06 | 427±22 | 412±110 | 5.3 ±0.8 | 1.2 ±0.2 | 2.9 ±0.6 | 1.9 |
| 300 | 8 | 1.60±0.05 | 312±10 | 268± 44 | 10.5 ±1.2 | 3.1 ±0.3 | 8.8 ±1.0 | 0.52 |
| 1000 | 5 | 6.5 ±0.7 | 77± 8 | – | 5.3 ±0.6 | 6.4 ±0.8 | – | 0.32 |
| 6000 | 4 | 38.5 ±4.6 | 13± 2 | – | 1.0 ±0.4 | 7.1 ±0.9 | – | 0.059 |

[a] The aqueous phase contained 0.1 M NaCl and various concentrations of dipicrylamine (DPA). $k_i^*$ and $N_t^*$ are the values of $k_i$ and $N_t$ taken from Benz *et al.* (1976). $S_I(\omega)$ is referred to a membrane area of $A = 0.35\ \text{mm}^2$. *See* legend of Table 1 for further explanations.

Table 3. Analysis of current noise from diphytanoylphosphatidylcholine (diphtanoyllecithin) and diphytanolphosphatidylserine membranes in the presence of dipicrylamine (DPA) and various electrolyte concentrations in the aqueous phase ($T = 25\ ^\circ\mathrm{C}$)[a]

| DPA (nM) | $Na^+$ (M) | $Ca^{2+}$ (M) | $n$ | $\tau_i$ (msec) | $k_i$ ($sec^{-1}$) | $S_I(\infty)$ ($10^{-26}$ $A^2$ sec) | $N_t$ (pmole/ $cm^2$) | $\beta$ ($10^{-2}$ cm) |
|---|---|---|---|---|---|---|---|---|
| | | | | diphytanoylphosphatidylserine | | | | |
| 30 | 0.1 | – | 8 | 1.05±0.05 | 476±23 | 0.33±0.04 | 0.064±0.011 | 0.11 |
| 300 | 0.1 | – | 4 | 0.69±0.02 | 725±21 | 0.86±0.09 | 0.11 ±0.02 | 0.018 |
| 300 | 0.1 | 0.01 | 4 | 0.36±0.01 | 1389±39 | 1.35±0.18 | 0.09 ±0.02 | 0.015 |
| 300 | 1 | 0 | 10 | 0.68±0.03 | 735±32 | 8.8 ±0.6 | 1.1 ±0.1 | 0.18 |
| 300 | 1 | 0.05 | 3 | 0.40±0.02 | 1250±63 | 17.6 ±1.4 | 1.3 ±0.2 | 0.22 |
| | | | | diphytanoylphosphatidylcholine | | | | |
| 30 | 0.1 | 0 | 6 | 0.95±0.06 | 526±33 | 2.7 ±0.3 | 0.51 ±0.09 | 0.85 |
| 300 | 0.1 | 0 | 5 | 1.25±0.05 | 400±16 | 8.2 ±0.5 | 1.9 ±0.2 | 0.32 |
| 300 | 0.1 | 0.01 | 6 | 1.29±0.04 | 388±12 | 8.4 ±0.9 | 2.0 ±0.3 | 0.33 |
| 300 | 1 | 0 | 3 | 1.28±0.06 | 391±18 | 8.8 ±0.6 | 2.1 ±0.3 | 0.35 |
| 300 | 1 | 0.05 | 4 | 1.30±0.08 | 385±24 | 9.6 ±1.2 | 2.3 ±0.4 | 0.38 |

[a] $S_I(\infty)$ is referred to a membrane area of $A = 0.35\ \mathrm{mm}^2$. *See* legend of Table 1 for further explanations.

## Discussion

### *Theoretical Analysis*

In the following we show that the properties of the spectral intensity $S_I(f)$ which is observed in the presence of dipicrylamine can be interpreted on the basis of a simple model. For this purpose we consider the transport mechanism of hydrophobic ions in lipid bilayer membranes which has been previously proposed on the basis of kinetic experiments (Ketterer *et al.*, 1971; Andersen & Fuchs, 1975; Bruner, 1975; Benz *et al.*, 1976). According to this model, the hydrophobic ions are assumed to be located in deep potential-energy minima at either membrane surface (Fig. 5). If $c$ is the concentration of the hydrophobic ion in the two (identical) aqueous solutions and $N_t$ its total concentration referred to unit area of the membrane, then for sufficiently low values of $c$ the adsorption equilibrium may be described by

$$\frac{N_t}{2c} = \beta \tag{4}$$

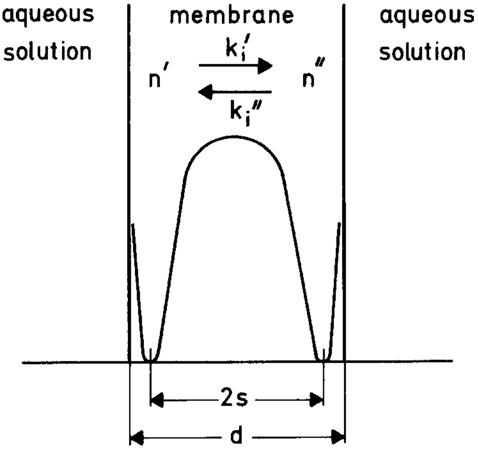

Fig. 5. Simplified potential energy profile of a hydrophobic ion in a lipid bilayer membrane. *d* is the membrane thickness

where $\beta$ is a partition coefficient ($\beta$ is the thickness of a solution layer containing the same amount of ions as the membrane surface). By thermal activation ions may cross the barrier at an average rate $k_i$ which is identical in both directions in the absence of an external voltage. $k_i$ depends on the shape of the barrier and on the mobility of the ion in the membrane (Benz *et al.*, 1976). In contrast to the average rates, the instantaneous transport rates from left to right and from right to left in general do not cancel each other so that there is a time-dependent net flow $\Phi(t)$ of ions:

$$\Phi(t) = \bar{\Phi} + \delta\Phi(t) \tag{5}$$

which fluctuates around zero ($\bar{\Phi} = 0$). If $z$ is the valency of the hydrophobic ion and $e_0$ the elementary charge, the corresponding fluctuating current $\delta I(t)$ is given by

$$\delta I(t) = \alpha z e_0 \delta\Phi(t) \tag{6}$$

where $\alpha$ is a dimensionless factor of the order of unity (*see below*). $\delta\Phi(t)$ in turn leads to fluctuations in the numbers $n'$ and $n''$ of ions in the left-hand and right-hand potential energy minimum (Fig. 5). A second process also contributes to the variation in the number of adsorbed ions, namely, statistical fluctuations in the partition equilibrium between aqueous solution and interface. This means that the time behavior of fluctuations in $n'$ and $n''$ will be governed by two processes, namely, (i) equilibration with the aqueous phases by adsorption/desorption and diffusion in water and (ii) equilibration across the central barrier. The situation becomes comparatively simple when the two processes have widely different time scales. This is the case for the experimental system studied here, because, as we will show, diffusion of dipicrylamine in water is much slower than exchange over the central barrier. This may be shown by the following argument. We consider the adsorption of molecules from an infinitely-extended convection-free solution to a planar surface and assume that the rates of adsorption and desorption are only limited by the diffusion rate in the solution. If the adsorption equilibrium between surface and solution is suddenly disturbed, the system relaxes back to equilibrium with a time constant $\tau_a$ which is given by (Appendix A):

$$\tau_a = \frac{\beta^2}{D}. \tag{7}$$

$D$ is the diffusion coefficient of the adsorbing molecule in the solution. According to the fluctuation-dissipation theorem (Onsager, 1931), $\tau_a$ is identical with the correlation time of fluctuations in the number of adsorbed molecules. For any real system in which the adsorption and desorption rates are not necessarily diffusion-controlled, $\tau_a$ represents a lower limit for the true correlation time. For dipicrylamine and a lecithin membrane, the partition coefficient $\beta$ is about $2 \times 10^{-2}$ cm (Ketterer *et al.*, 1971); with an estimated value of $D \simeq 5 \times 10^{-6}\,\mathrm{cm}^2\,\mathrm{sec}^{-1}$ one obtains $\tau_a \simeq 100$ sec. This time is much larger than the time constant $\tau_i = 1/2k_i \simeq 3$ msec for the redistribution of dipicrylamine ions across the central barrier (Ketterer *et al.*, 1971). Furthermore, $\tau_a \simeq 100$ sec corresponds to a frequency of 0.01 Hz which is outside the experimentally studied frequency range. Thus, changes in $n'$ and $n''$ originating from fluctuations in the adsorption equilibrium are much too slow to be detected in these experiments.

Under these circumstances the random motion of hydrophobic ions within the membrane may be described by assuming that (within the time scale of these experiments) the ions are unable to leave the membrane. This assumption is consistent with the transient behavior of the dipicrylamine-doped membrane after a voltage jump (Ketterer *et al.*, 1971). In the voltage-jump relaxation experiment the current decays from a high initial value (corresponding to the high transport rate over the central barrier) to a much lower steady-state current which is limited by diffusion in the aqueous phase. Thus, the slow diffusional exchange between adsorption plane and bulk aqueous solution acts in the same way as an energy barrier separating the adsorption plane from the aqueous phase. The fluctuations of the membrane current (at zero voltage) may therefore be analyzed on the basis of the simple potential profile depicted in Fig. 5 with the two energy minima separated from the aqueous solutions by barriers of virtually infinite height. Random transitions of ions between the energy minima induce current pulses in the low-impedance external circuit which is coupled to the membrane dielectric via the conducting aqueous phases. The analysis of this model given in Appendix B leads to the following expression for the spectral intensity $S_I(\omega)$ of the current fluctuations ($\omega = 2\pi f$):

$$S_I(\omega) = \frac{\omega^2 \tau_i^2}{1+\omega^2 \tau_i^2} S_I(\infty) \tag{8}$$

$$S_I(\infty) = 2 n k_i (\alpha z e_0)^2 \tag{9}$$

$$\tau_i = \frac{1}{2k_i}. \tag{10}$$

$n = A N_t$ is the average number of ions adsorbed to the membrane of area $A$ (both surfaces); $k_i$ is the translocation rate constant of the ion across the central barrier, and $\alpha \simeq 2s/d \simeq 1$ is a dimensionless factor depending on the location of the energy minima (Fig. 5).

The theoretical expression given in Eq. (8) predicts a spectral intensity which increases with the square of frequency at low $\omega$ and becomes independent of frequency in the limit $\omega \gg 1/\tau_i$. The experimentally observed current-noise spectra closely agree with this prediction. An example is given in Fig. 2 in which the theoretical curve has been drawn according to Eq. (8) with a suitable choice of the parameters $S_I(\infty)$ and $\tau_i$. A significant deviation between the observed and the calculated spectrum is only found at low frequencies where the observed values of $S_I$ are higher than the spectrum predicted from Eq. (8). This deviation is not unexpected, since other noise sources of the system (such as noise originating from the nonzero steady-state conductance of the membrane) which are not included in the model tend to keep the measured $S_I(\omega)$ at a finite level at low frequencies, whereas the model predicts $S_I(\omega) \rightarrow 0$ for $\omega \rightarrow 0$.

As mentioned previously, the spectrum represented by Eq. (8) is formally identical with the thermal equilibrium (or Nyquist) noise spectrum in an equivalent circuit consisting of a capacitance $C$ in series with a resistance $R$. For this equivalent circuit the Nyquist relation

(Appendix C) predicts a current-noise spectrum of the form

$$S_I(\omega)=\frac{4kT}{R}\cdot\frac{\omega^2\tau_e^2}{1+\omega^2\tau_e^2} \tag{11}$$

$$\tau_e=RC. \tag{12}$$

Eqs. (11) and (12) become identical with Eqs. (8)–(10) if the substitutions $R=2kT/nk_i(\alpha ze_0)^2$ and $C=n(\alpha ze_0)^2/4kT$ are made. This formal equivalence gives a clue for an intuitive interpretation of Eqs. (8)–(10). The high-frequency limit $S_I(\infty)$ of the spectral intensity [Eq. (9)] corresponds to the Nyquist noise of a resistance $R_0$ which is given by the reciprocal value of the initial ohmic conductance $\lambda_{00}$ of the membrane after a voltage jump (Ketterer *et al.*, 1971; Benz *et al.*, 1976):

$$S_I(\infty)=\frac{4kT}{R_0}=4kTA\lambda_{00}$$

$$\lambda_{00}=\frac{(\alpha ze_0)^2}{2kT}k_iN_t. \tag{14}$$

$A$ is the area of the membrane and $\lambda_{00}$ is referred to unit area. This Nyquist noise arising from transitions of ions over the central barrier is modified by a capacitive component, which originates from the presence of adsorption sites acting as storage elements. In this way fluctuations at low frequencies are strongly damped.

### *Evaluation of the Parameters of the Model*

From the experimentally determined values of $S_I(\infty)$ and $\tau_i$ the rate constant $k_i$ and the surface concentration $N_t=n/A$ of absorbed ions may be calculated according to Eqs. (9) and (10). The values of $k_i$ and $N_t$ obtained in this way are summarized in Tables 1–3. For some of the systems studied here the values of $k_i$ and $N_t$ as determined from noise analysis may be compared with the results of relaxation measurements carried out under comparable experimental conditions (Ketterer *et al.*, 1971; Benz *et al.*, 1976). Such a comparison is shown in Table 2. It is seen that the values of the translocation rate constant determined by the noise method ($k_i$) and by electrical relaxation experiments ($k_i^*$) agree within the limits of error. A similar agreement of $k_i$ and $k_i^*$ is found for the other lecithins (Fig. 6) at 25 °C where the relaxation measurements have been carried out (Benz *et al.*, 1976). The surface concentrations $N_t$ determined

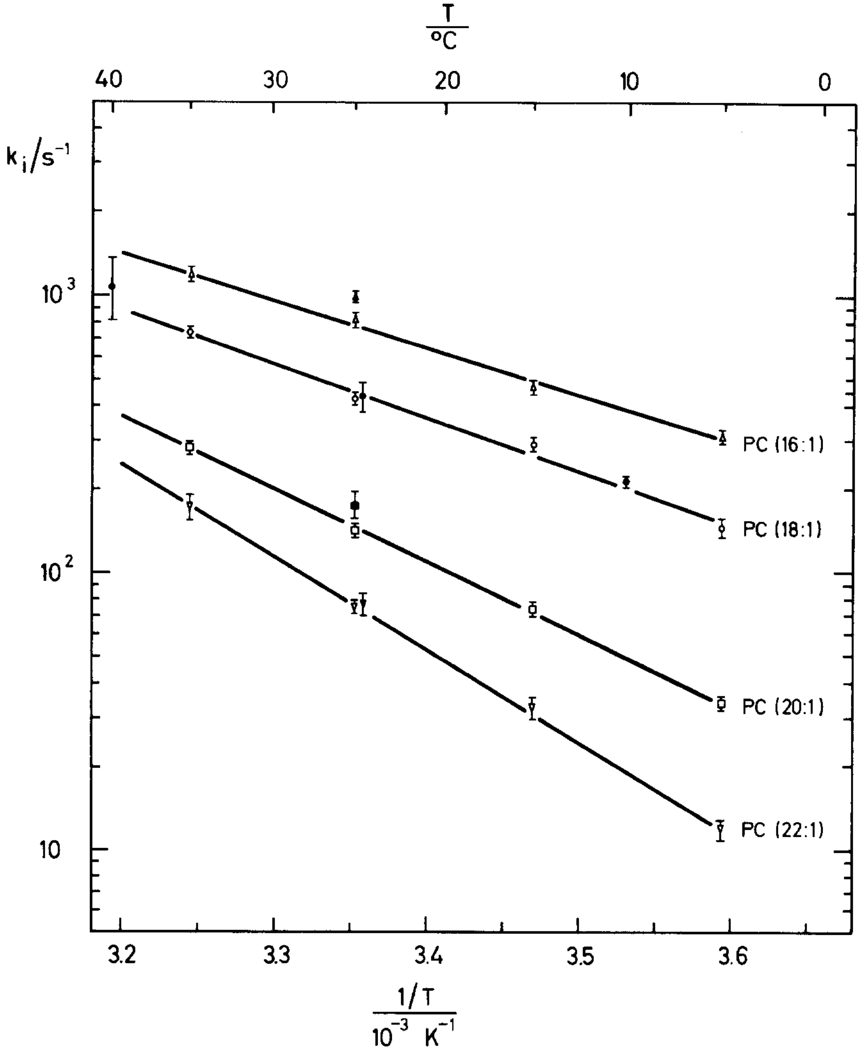

Fig. 6. Logarithmic plot of the translocation rate constant $k_i$ as a function of reciprocal temperature (values taken from Table 1). Open symbols: noise analysis. Filled symbols: charge-pulse relaxation studies (Benz *et al.*, 1976)

by noise analysis are of the same order as the values from relaxation studies ($N_t^*$) but being consistently larger than $N_t$ (Table 2). Whether this deviation results from a defect of the theoretical model or from a difference in the experimental conditions is not clear at the moment. A more detailed discussion of this question should be based on a direct experimental test of Eq. (13) which requires measurements of $\lambda_{00}$ and $S_I(\infty)$ at one and the same membrane.

At a given temperature the equilibrium concentration $N_t$ of adsorbed dipicrylamine increases with the chain length of the lipid (Table 1). An even stronger effect of chain length on the translocation rate constant $k_i$ is observed; at 25 °C $k_i$ decreases by a factor of eleven when the chain length is increased from $C_{16}$ to $C_{22}$. These findings are in agreement with previous results of Benz *et al.* (1976). For a given lipid, $N_t$ decreases and $k_i$ increases with temperature (Table 1). From a logarithmic plot of $k_i$ *vs.* $1/T$ (Fig. 6) the activation energy $E(k_i)$ of the translocation process may be determined (Table 1); it is seen that $E(k_i)$ considerably increases with chain length of the lipid, the value for the di-(22:1)-lecithin being about twice as large as for the di-(16:1)-lecithin. Similarly, from the temperature dependence of the partition coefficient $\beta$ the enthalpy change $\Delta H_{\text{ads}}$ associated with the adsorption of dipicrylamine from the solution to the membrane may be calculated using the relation $d\ln\beta/dT = \Delta H_{\text{ads}}/RT^2$ ($R$ is the gas constant). The values of $\Delta H_{\text{ads}}$ (Table 1) become increasingly negative with increasing chain length of the fatty acid residue (a negative value of $\Delta H_{\text{ads}}$ means that heat is liberated upon adsorption).

As seen from Table 2, the translocation rate constant $k_i$ decreases with dipicrylamine concentration above about 30 nM. This concentration dependence of $k_i$ has first been described by Bruner (1975); *see also* Wulf, Benz and Pohl (1977). Similar results have been obtained also for tetraphenylborate (Anderson & Fuchs, 1975; Benz *et al.*, 1976; Szabo, 1976). The partition coefficient $\beta$ also decreases with dipicrylamine concentration above 30 nM. As shown by McLaughlin (1977) this finding may be explained by the change in surface potential brought about by the adsorption of the negative ions to a plane located some distance away from the interface toward the interior of the membrane.

## Conclusion

In the presence of the hydrophobic ion dipicrylamine, lipid bilayer membranes exhibit a characteristic type of electrical noise which is different from the well-known Lorentzian noise and which is also distinct from the $1/f$ noise frequently observed with ion-permeable membranes (Dorset & Fishman, 1975). The current-noise spectrum at zero voltage increases in proportion to the square of frequency $f$ at low frequencies and becomes constant at high frequencies. This peculiar form of the noise spectrum can be explained by assuming that hydrophobic ions are adsorbed in potential-energy minima at either membrane surface and may jump by thermal activation over the central energy barrier (Ketterer *et al.*, 1971; Anderson & Fuchs, 1975). According to this model the observed current noise results from fluctuations in the number of ions crossing the barrier from right to left and from left to right. As a consequence of the slow diffusion rate in water, the ions behave as if they were trapped in the membrane. This introduces a capacitive element into the behavior of the system leading to a decline of the spectral intensity toward low frequencies.

On the basis of this model the translocation rate constant across the barrier, $k_i$, as well as the mean surface concentration of adsorbed ions, $N_t$, have been calculated from the observed current-noise spectrum. The values of $k_i$ obtained in this way closely agree with the results of previous relaxation experiments. Also, the $N_t$ values determined from noise analysis and from relaxation studies agree, but only to the order of magnitude. The origin of the difference between the $N_t$ values obtained by the two methods is not clear as yet. One possibility is that the theoretical analysis (Appendix B) is oversimplified in that the detailed shape of the energy barrier is not taken into account. A definitive answer to this question requires further experimental and theoretical studies. The type of noise which we have described here can be expected in all cases where a membrane contains bound charges able to move between two (or more) discrete equilibrium positions (potential-energy minima). There is strong evidence that the operation of gating mechanism of sodium channels in nerve involves the movement of membrane-bound charges (Bezanilla & Armstrong, 1975; Rojas & Keynes, 1975; Neumcke, Nonner & Stämpfli, 1976). It seems therefore possible that fluctuations in the gating mechanism manifest themselves in a characteristic noise with a decline of spectral intensity at low frequencies.

It is pertinent to mention that the corresponding voltage-noise spectral intensity exhibits a Lorentzian shape ([Eq. (B28)] of Appendix B). A similar spectral shape was found for the spectral intensities of voltage noise associated with active ion transport across frog skin. (Segal, 1972; *see also* Fishman & Dorset, 1973; Segal, 1973; Lindemann & Van Driessche, 1977). The underlying transport mechanism was theoretically described by Segal as a generation-recombination process of current carriers at one membrane interface. This approach leads to a current-noise spectrum which is also of Lorentzian type [Eq. (3) of Segal, 1972] and which is completely different from the spectrum found for the transport of hydrophobic ions. Therefore, by measurement of the current spectral intensity associated with active transport these two transport mechanisms may be discriminated. We want to point out that for the described transport process of hydrophobic ions the adsorption and desorption reactions at the membrane interfaces could be neglected. If the active transport is carrier-mediated these interfacial reactions in general should be taken into account.

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## Appendix A

### *Relaxation Time of an Adsorption Process* [*Derivation of Eq.* (7)]

We consider an infinitely-extended planar surface which is in contact with a convection-free infinitely-extended solution of molecules absorbing to the surface. We assume that at time $t<0$ the solution is uniform and is in adsorption equilibrium with the surface. If $c_e$ is the equilibrium concentration in the solution, the equilibrium concentration $N_e$ of adsorbed molecules per unit area is given by

$$N_e = \beta c_e \tag{A1}$$

where $\beta$ is the partition coefficient. At time $t=0$ the system is perturbed by suddenly changing the concentration $N$ of adsorbed molecules from $N_e$ to $N_0$. Thereafter an adsorption ($N_0 < N_e$) or desorption process ($N_0 > N_e$) sets in, in the course of which $N$ relaxes back to the equilibrium value $N_e$. We assume that this process is purely diffusion-limited so that there is always partition equilibrium at the surface:

$$N(t) = \beta c(0, t). \tag{A2}$$

$c(x,t)$ is the concentration in the solution and $x$ is the distance from the surface. If $D$ is the diffusion coefficient of the adsorbing molecules in the solution, the change of $N$ with time is given by

$$\frac{dN}{dt} = D\left(\frac{\partial c}{\partial x}\right)_{x=0}. \tag{A3}$$

The concentration $c(x, t)$ obeys Fick's second law:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}. \tag{A4}$$

The solution of Eqs. (A2–A4) which has to fulfill the boundary condition

$$c(x,0)=c_e \qquad (x>0) \tag{A5}$$

may be obtained using the method of Laplace transformations:

$$c(x,t)=c_e+\left(\frac{N_0}{\beta}-c_e\right)\exp\left(\frac{x}{\beta}+\frac{Dt}{\beta^2}\right) erfc\left(\frac{x}{2\sqrt{Dt}}+\frac{\sqrt{Dt}}{\beta}\right). \tag{A6}$$

The function $erfc(y)$ is defined by

$$erfc(y)=\int_y^\infty e^{-u^2}du \tag{A7}$$

and has the property

$$erfc(y)\approx 1-\frac{2y}{\sqrt{\pi}} \qquad (|y|\ll 1) \tag{A8}$$

$$erfc(y)\approx\frac{\exp(-y^2)}{y\sqrt{\pi}} \qquad (|y\gg 1). \tag{A9}$$

Eqs. (A2) and (A6) yield, together with Eq. (A1):

$$N(t)=N_e+(N_0-N_e)\exp(t/\tau_a)\, erfc(\sqrt{t/\tau_a}) \tag{A10}$$

$$\tau_a=\frac{\beta^2}{D}. \tag{A11}$$

This means that the relaxation process is nonexponential. From the tabulated values of the function $\exp(y^2)\cdot erfc(y)$ (Carlslaw & Jaeger, 1959) it is seen that $\tau_a$ is very nearly the time after which $N(t)-N_e$ has decayed to half the original value $N_0-N_e$. Thus, $\tau_a$ approximately gives the time scale for the re-establishment of an adsorption equilibrium after a sudden perturbation [Eq. (7)]. $\tau_a$ is equal to the diffusion time across a solution layer of thickness $\beta$ which contains as many dissolved molecules as there are adsorbed molecules at the surface.

## Appendix B

### *Spectral Intensity of Current-Noise in the Presence of Hydrophobic Ions [Derivation of Eqs.* (8)–(10)]

We consider the potential profile depicted in Fig. 5 with the two energy minima separated from the aqueous phases by barriers of virtually infinite height under equilibrium conditions. $n'$ and $n''$ are the numbers of hydrophobic ions presented in the left-hand and right-hand energy minimum, respectively. The total number $n$ remains constant, as the exchange with the aqueous solutions can be neglected within the time-scale of the experiment:

$$n' + n'' = n = \text{const.} \tag{B1}$$

$n'$ (and also $n''$) is a function of time $t$ and may be written as the sum of the average number $\bar{n}'$ and a randomly varying function $\delta n'(t)$:

$$n'(t) = \bar{n}' + \delta n'(t). \tag{B2}$$

In order to calculate the spectral density of $\delta n'(t)$ for the equilibrium state we use the method of Langevin (van der Ziel, 1970; van Kampen, 1976). In this method the rate of change of $n'$ is represented by the macroscopic differential equation with an added white-noise source $H(t)$:

$$\frac{dn'}{dt} = -k_i' n' + k_i'' n'' + H(t). \tag{B3}$$

$k_i'$ and $k_i''$ are the rate constants for "jumps" from left to right and from right to left, respectively, (Ketterer *et al.*, 1971). Although the passage of an ion over the barrier is a diffusion process rather than a single jump, a relation of the form of Eq. (B3) is justified as long as the barrier is high enough so that an ion spends most of the time in one of the energy minima (Andersen & Fuchs, 1975; Benz *et al.*, 1976).

As a generality, we do not require that $k_i'$ and $k_i''$ are equal; only in a completely symmetric system the relation $k_i' = k_i''$ holds. Unequality of $k_i'$ and $k_i''$ may occur for asymmetrical lipid composition of the membrane and for different electrolyte solutions on both sides of the membrane.

Implicit in Eq. (B3) is the assumption that $n'$ and $n''$ are sufficiently small so that interactions between ions may be neglected. Introduction of Eqs. (B1) and (B2) into Eq. (B3) leads, together with the equilibrium condition $k_i' \bar{n}' = k_i'' \bar{n}''$, to the relation

$$\frac{d(\delta n')}{dt} = -(k_i' + k_i'')\,\delta n' + H(t). \tag{B4}$$

We now make a Fourier analysis of the random functions $H(t)$ and $\delta n'(t)$ for the interval $0 \leqq t \leqq T$:

$$H(t) = \sum_{\nu=-\infty}^{\infty} \alpha_\nu \exp(j\omega_\nu t) \tag{B5}$$

$$\delta n'(t) = \sum_{\nu=-\infty}^{\infty} \beta_\nu \exp(j\omega_\nu t) \tag{B6}$$

where $\omega_\nu = 2\pi\nu/T$. The Fourier-series representation of $d(\delta n')/dt$ is then given by

$$\frac{d(\delta n')}{dt} = \sum_{\nu=-\infty}^{\infty} j\omega_\nu \beta_\nu \exp(j\omega_\nu t). \tag{B7}$$

From Eqs. (B4)–(B7) it is seen that the following relation holds:

$$\alpha_\nu = (k_i' + k_i'' + j\omega_\nu)\, \beta_\nu. \tag{B8}$$

The spectral intensity $S_n(\omega)$ of $\delta n'(t)$ is defined by (van der Ziel, 1970):

$$S_n(\omega) = \lim_{T\to\infty} 2T\overline{\beta_\nu \beta_\nu^*} \tag{B9}$$

where the asterisk denotes the complex conjugate of the quantity involved.

Similarly, the spectral intensity of the white noise source $H(t)$ is given by:

$$S_H(\omega) = S_H(0) = \lim_{T\to\infty} 2T\overline{\alpha_\nu \alpha_\nu^*}. \tag{B10}$$

From Eq. (B8) one finds

$$\overline{\alpha_\nu \alpha_\nu^*} = [(k_i' + k_i'')^2 + \omega_\nu^2]\, \overline{\beta_\nu \beta_\nu^*} \tag{B11}$$

so that

$$S_H(0) = [(k_i' + k_i'')^2 + \omega^2]\, S_n(\omega). \tag{B12}$$

In order to calculate $S_H(0)$ we use the relation between the mean square value of the function $\delta n'(t)$ and its spectral density:

$$\frac{1}{2\pi}\int_0^\infty S_n(\omega)\, d\omega = \overline{(\delta n')^2}. \tag{B13}$$

Introducing $S_n(\omega)$ from Eq. (B12) and integrating we find

$$S_H(0) = 4(k_i' + k_i'')\overline{(\delta n')^2}. \tag{B14}$$

For the calculation of $\overline{(\delta n')^2}$ we make use of the fact that $n'$ varies according to a binomial distribution, as $n' + n'' = n$ is a constant. If $p = \bar{n}'/n$ is the probability of finding a given ion in the left-hand energy minimum, the mean square value of $\delta n'$ is given by

$$\overline{(\delta n')^2} = \bar{n}'(1-p).$$

Using the equilibrium condition $k_i'\bar{n}' = k_i''\bar{n}'' = k_i''(n - \bar{n}')$ *one obtains*

$$\overline{(\delta n')^2} = n\frac{k_i' k_i''}{(k_i' + k_i'')^2}. \tag{B16}$$

This gives

$$S_H(0) = 4n \frac{k_i' k_i''}{k_i' + k_i''} \tag{B17}$$

and together with Eq. (B12):

$$S_n(\omega) = \frac{4n}{k_i' + k_i''} \cdot \frac{k_i' k_i''}{(k_i' + k_i'')^2 + \omega^2}. \tag{B18}$$

The same result is obtained if the master equation (van Kampen, 1976) is used for the derivation of the spectral intensity of $\delta n'(t)$ (E. Frehland, *personal communication*).

The calculation of the spectral intensity of the current $I$ may be based on the following model. We treat the passage of the ion over the barrier as a single jump, neglecting all unsuccessful attempts to climb the barrier. Again this is justified as long as the slopes of the barrier are sufficiently steep. We consider the membrane as a dielectric layer of thickness $d$ interposed between two conducting phases (the aqueous electrolyte solutions) which are connected by an external measuring circuit. If an ion of charge $ze_0$ is moved in the dielectric over a distance $2s$ (Fig. 5), a charge of magnitude $\pm ze_0(2s/d)$ is displaced in the external circuit. If $\delta\Phi$ ions are displaced per unit time, then the externally measurable current $\delta I$ is equal to $(ze_0 2s/d)\,\delta\Phi$.[1]

Implicit in this consideration is the assumption that the dielectric constant in the membrane is independent of position. If this is no longer true, a more general expression of $\delta I$ may be obtained by replacing $2s/d$ by the quantity $\alpha$ which depends both on the position of the minimum as well as on the dielectric constant $\varepsilon(x)$ in the membrane. If $x$ is the coordinate normal to the membrane surface with its origin in the center of the membrane, $C_m$ the specific membrane capacitance, and $\varepsilon_0$ the permittivity of free space, $\alpha$ is given by

$$\alpha = C_m \int_{-s}^{s} \frac{dx}{\varepsilon_0 \varepsilon(x)} = \frac{C_m}{C_i}. \tag{B19}$$

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[1] The introduction of an instantaneous flux $\delta\Phi(t)$ may seem problematic in view of the fact that the flow of ions over the barrier is composed of single, discrete events. Indeed, the definition of a particle flux always requires a certain time-interval over which the number of events is averaged. This difficulty, however, is only apparent because the single passages of ions over the barrier are recorded in the external circuit as current pulses which are broadened by the finite bandwidth $\Delta f$ of the measuring system. In this way the measuring circuit automatically introduces a characteristic time interval ($\sim 1/\Delta f$) over which the single events are averaged.

$C_i$ is the fractional membrane capacitance between $x=-s$ and $x=s$. We may therefore write

$$\delta I = \alpha z e_0 \delta \Phi. \tag{B20}$$

The derivation of Eqs. (B19) and (B20) is similar to that of Eqs. (A12) and (A17) of Benz *et al.* (1976). The spectral density of $\delta I$ may be expressed by the spectral intensity of $\delta \Phi$:

$$S_I(\omega) = (\alpha z e_0)^2 S_\Phi(\omega). \tag{B21}$$

$S_\Phi(\omega)$, in turn, may be obtained from Eq. B7 using the relation

$$\delta \Phi(t) = \frac{d(\delta n')}{dt}. \tag{B22}$$

This gives, together with Eq. (B9):

$$S_\Phi(\omega) = \lim_{T \to \infty} 2T\omega_v^2 \overline{\beta_v \beta_v^*} = \omega^2 S_n(\omega). \tag{B23}$$

The final result is then obtained by combining Eqs. (B18), (B21) and (B23):

$$S_I(\omega) = (\alpha z e_0)^2 \frac{4nk_i'k_i''}{k_i'+k_i''} \cdot \frac{\omega^2}{(k_i'+k_i'')^2+\omega^2}. \tag{B24}$$

For a completely symmetric system with $k_i' = k_i'' = k_i$ Eq. (B24) assumes the form ($\tau_i = 1/2k_i$):

$$S_I(\omega) = n(\alpha z e_0)^2 \frac{\omega^2 \tau_i}{1+\omega^2 \tau_i^2}. \tag{B25}$$

An alternative derivation of Eqs. (B24) and (B25) is possible on the basis of the generalized Nyquist theorem (Läuger, *to be published*).

The corresponding variance of current noise of this spectral intensity shows a formal divergence since the limitations in time of the underlying physical processes at high frequency are not taken into account. The same formal divergence occurs in the case of the variance of thermal white noise.

Under equilibrium conditions the corresponding voltage-noise spectrum at zero membrane current may be calculated as follows. Voltage fluctuations $\delta V$ are caused by fluctuations in the charge present in either energy minimum:

$$\delta V = \frac{\delta Q'}{C_i} = \frac{z e_0 \delta n'}{C_i} \tag{B26}$$

where in analogy to Eq. (B2) $\delta Q'$ denots the charge fluctuation of the left-hand energy minimum and $C_i$ the fractional membrane capacitance [Eq. (B19)]. The spectral intensity of $\delta V$ may then be expressed by the spectral intensity of $\delta n'$:

$$S_V(\omega)=\left(\frac{z e_0}{C_i}\right)^2 \cdot S_n(\omega). \tag{B27}$$

For a completely symmetrical membrane the final result is obtained by introducing Eqs. (B18) and (B19):

$$S_V(\omega)=\left(\frac{\alpha z e_0}{C_m}\right)^2 \cdot \frac{n\tau_i}{1+\omega^2\tau_i^2}. \tag{B28}$$

Together with Eq. (B25) one finds for the relation between the spectral intensities of voltage and current:

$$S_V(\omega)=\frac{1}{\omega^2 C_m^2} \cdot S_I(\omega). \tag{B29}$$

## Appendix C

### *Noise from the Preamplifier Stage*

We consider an experiment in which an operational amplifier is used to measure the thermal current-noise of a source of impedance $Z$. According to Nyquist's theorem the spectral intensity of this current noise is given by

$$S_I(\omega)=4kT\mathrm{Re}\left[\frac{1}{Z(\omega)}\right] \tag{C1}$$

where $\omega=2\pi f$ is the angular frequency and Re means "real part of". The intrinsic noise generated by the amplifier is described by introducing at the input of the amplifier a voltage-noise source $v_n$ with spectral intensity $S_v$ and a current-noise source $i_n$ with spectral density $S_i$; $v_n$ and $i_n$ may be assumed to be uncorrelated (Poussart, 1971). An additional noise source consists in the current noise of the feedback resistor $R_f$, which has the spectral intensity $4kT/R_f$. The spectral intensity $S_I^*$ of the overall noise from these three sources may be obtained from simple circuit analysis. In this way the "signal-to-noise-ratio" $S_I/S_I^*$ is obtained in the form

$$\frac{S_I}{S_I^*}=\frac{4kT\mathrm{Re}\left(\frac{1}{Z}\right)}{S_i+S_v\left\{\frac{1}{R_f^2}+\frac{1}{|Z|^2}\left[1+2\frac{\mathrm{Re}(Z)}{R_f}\right]\right\}+\frac{4kT}{R_f}}. \tag{C2}$$

A special form of Eq. (C2) describing the case of a parallel combination of a resistance and a capacitance has been given by Poussart (1971).

For a series combination of a resistance $R_e$ and a capacitance $C_e$ ($Z = R_e + 1/j\omega C_e$), Eq. (C2) assumes the form

$$\frac{S_I}{S_I^*} = \frac{\left(\frac{4kT}{R_e}\right) \cdot \left(\frac{\omega^2 \tau_e^2}{1+\omega^2 \tau_e^2}\right)}{S_i + S_v \left[\frac{1}{R_f^2} + \frac{1}{R_e^2}\left(1 + \frac{2R_e}{R_f}\right) \frac{\omega^2 \tau_e^2}{1+\omega^2 \tau_e^2}\right] + \frac{4kT}{R_f}} \tag{C3}$$

where $\tau_e = R_e C_e$. From the values of $S_i$ and $S_v$ given in the specifications of the Analog Devices 52K operational ampifier ($S_i \simeq 2 \times 10^{-29}\,\mathrm{A}^2\,\mathrm{sec}$, $S_v \simeq 4 \times 10^{-16}\,\mathrm{V}^2\,\mathrm{sec}$ at 100 Hz) it is seen that the term $4kT/R_f$ makes by far the largest contribution to the denominator of Eq. (C3). This means that the chief intrinsic noise-source of the amplifying system is the current noise of the feedback resistor.

## References

Andersen, O.S., Fuchs, M. 1975. Potential energy barriers to ion transport within lipid bilayers. *Biophys. J.* **15**:795

Benz, R., Läuger, P. 1977. Transport kinetics of dipicrylamine through lipid bilayer membranes: Effects of membrane structure. *Biochim. Biophys. Acta* **468**:245

Benz, R., Läuger, P., Janko, K. 1976. Transport kinetics of hydrophobic ions in lipid bilayer membranes: Charge-pulse relaxation studies. *Biochim. Biophys. Acta* **455**:701

Bezanilla, F., Armstrong, C.M. 1975. Kinetic properties and inactivation of the gating currents of sodium channels in squid axon. *Phil. Trans. R. Soc. London B* **270**:449

Bruner, L.J. 1975. The interaction of hydrophobic ions with lipid bilayer membranes. *J. Membrane Biol.* **22**:125

Carlslaw, H.S., Jaeger, J.C. 1959. Conduction of Heat in Solids, 2nd ed. Clarendon Press, Oxford

Conti, F., Wanke, E. 1975. Channel noise in nerve membranes and lipid bilayers. *Q. Rev. Biophys.* **8**:451

Dorset, D.L., Fishman, H.M. 1975. Excess electrical noise during current flow through porous membranes separating ionic solutions. *J. Membrane Biol.* **21**:291

Fishman, H.M., Dorset, D.L. 1973. Comments on electrical fluctuations associated with active transport. *Biophys. J.* **13**:1339

Fishman, H.M., Poussart, D.J.M., Moore, L.E. 1975. Noise measurements in squid axon membrane. *J. Membrane Biol.* **24**:281

Janko, K., Benz, R. 1977. Properties of lipid bilayer membranes made from lipids containing phytanic acid. *Biochim. Biophys. Acta* (in press)

Kampen, N.G. van. 1976. Fluctuations and noise in physical theory. *Physica* **83 B**:1

Ketterer, B., Neumcke, B., Läuger, P. 1971. Transport mechanism of hydrophobic ions through lipid bilayer membranes. *J. Membrane Biol.* **5**:225

Kolb, H.-A., Bamberg, E. 1977. Influence of membrane thickness and ion concentration on the properties of the gramicidin channel. Autocorrelation, spectral power density, relaxation and single-channel studies. *Biochim. Biophys. Acta* **464**:127

Kolb, H.-A., Boheim, G. 1977. Analysis of the multi-pore system of alamethicin in a lipid membrane. II. Autocorrelation analysis and power spectral density. *J. Membrane Biol.* (*in press*)

Kolb, H.-A., Läuger, P., Bamberg, E. 1975. Correlation analysis of electrical noise in lipid bilayer membranes: Kinetics of gramicidin A channels. *J. Membrane Biol.* **20**:133

Läuger, P. 1975. Shot noise in ion channels. *Biochim. Biophys. Acta* **413**:1

Läuger, P., Lesslauer, W., Marti, E., Richter, J. 1967. Electrical properties of bimolecular phospholipid membranes. *Biochim. Biophys. Acta* **135**:20

LeBlanc, O.H., Jr. 1969. Tetraphenylborate conductance through lipid bilayer membranes. *Biochim. Biophys. Acta* **193**:350

Liberman, E.A., Topaly, V.P. 1968. Selective transport of ions through bimolecular phospholipid membranes. *Biochim. Biophys. Acta* **163**:125

Lindemann, B., Van Driessche, W. 1977. Sodium specific membrane channels of frog skin are pores: Current fluctuation reveal high turnover. *Science* **195**:292

McLaughlin, S. 1977. Electrostatic potentials at membrane-solution interfaces. *In:* Current Topics in Membranes and Transport. F. Bronner and A. Kleinzeller, editors. Vol. 9, p. 71. Academic Press, New York (*in press*)

Moore, L.E., Neher, E. 1976 Fluctuation and relaxation analysis of monazomycin-induced conductance in black lipid membranes. *J. Membrane Biol.* **27**:347

Mueller, P., Rudin, D.O. 1967. Development of $K^+ - Na^+$ discrimination in experimental bimolecular lipid membranes by macrocyclic antibiotics. *Biochem. Biophys. Res. Commun.* **26**:398

Neumcke, B., Nonner, W., Stämpfli, R. 1976. Asymmetrical displacement current and its relation with the activation of sodium current in the membrane of frog myelinated nerve. *Pfluegers Arch.* **363**:193

Onsager, L. 1931. Reciprocal relations in irreversible processes. *Phys. Rev.* **38**:2265

Poussart, D.J.M. 1971. Membrane current noise in lobster axon under voltage clamp. *Biophys. J.* **11**:211

Rojas, E., Keynes, R.D. 1975. On the relation between displacement currents and activation of the sodium conductance in the squid axon. *Phil. Trans. R. Soc. London B* **270**:459

Segal, J.R. 1972. Electrical fluctuations accociated with active transport. *Biophys. J.* **12**:1371

Segal, J.R. 1973. *Reply to:* Comments on electrical fluctuations associated with active transport. *Biophys. J.* **14**:513

Stevens, C.F., 1972. Inferences about membrane properties from electrical noise measurements. *Biophys. J.* **12**:1028

Szabo, G. 1976. The influence of dipole potentials on the magnitude and kinetics of ion transport in lipid bilayer membranes. *In:* Extreme Environment; Mechanism of Microbial Adaptation. H.R. Heinrich, editor. Academic Press, New York (*in press*)

Van der Ziel, A. 1970. Noise. Sources, Characterisation, Measurement. Prentice-Hall, Englewood Cliffs, N.J.

Verveen, A.A., DeFelice, L.J. 1974. Membrane Noise. *Prog. Biophys. Molec. Biol.* **28**:189

Wulf, J., Benz, R., Pohl, W.G. 1977. Properties of bilayer membranes in the presence of dipicrylamine. A comparative study by optical absorption and electrical relaxation measurements. *Biochim. Biophys. Acta* **465**:429

Zingsheim, H.P., Neher, E. 1974. The equivalence of fluctuation analysis and chemical relaxation measurements: A kinetic study of ion pore formation in thin lipid membranes. *Biophys. Chem.* **2**:197