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Electron Flow at the Polarized Mercury-Water Interface in the Presence of Membrane Fragments Rich in Na^+-K^+-Activated ATPase

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Summary. Measurements of interfacial electron flow indicate that membrane fragments rich in Na^+-K^+-ATPase are capable of absorbing and releasing electrons in the form of random currents at an electrode surface. The electron transporting system, which functions in the presence or absence of substrate and activating ions, may be part of or in contact with the enzyme system, but it is not related to the ATPase activity. The observed electron transport at an electrode surface resembles physiological electron transport processes in being reversible, in extending over the same range of potential, and in being affected by some of the chemicals that interfere with electron transport and oxidative phosphorylation in mitochondria. Our experiments do not provide sufficient evidence to identify the substances that are responsible for the random currents, but the results suggest that the electro-active substances are similar to those which are involved in the reactions at the second phosphorylation site in mitochondria. Experiments with this technique provide a new approach to the study of the mechanism of biological electron transport processes and their possible relation to ATP synthesis and hydrolysis.

Many properties of the Na^+-K^+-ATPase parallel those of the ion transport system of the membrane, and it is quite probable that the enzyme is an essential part of the transport system in the membrane [11]. For this reason, it seemed desirable to determine if the enzyme could be studied at an interface, specifically with regard to its effect on the transport of charge across the interface. The mercury-water interface was chosen for this purpose, because it has many advantages: (1) the high interfacial energy leads to the adsorption and orientation of many substances; (2) the interface is almost ideally polarizable; and (3) there are well-developed physical techniques for the characterization of various interfacial phenomena with this system. This interface has been particularly useful for the study

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of ion transport through lipid monolayers [1, 8] by polarography, a technique in which one polarizes the interface and measures currents due to an electrode process, i.e., the transfer of electrons. The measured currents, which are due to redox reactions involving substances in solution, can be related to the concentrations and rates of arrival of these substances at the interface. (Although Na^+ and K^+, the ions which activate the ATPase, are not reduced under normal conditions, Tl^+, which substitutes for K^+ in activating the enzyme [2], can be studied by polarography.)

Measurements at the mercury-water interface can also give information about the involvement of the ATPase in the direct transport of electrons. This possibility is of special interest because ion transport, ATP synthesis and utilization, and electron transport are associated in a number of ways. During ion transport, when ATP is utilized, the Na^+-K^+-ATPase (E) is thought to interact with the substrate MgATP in the following way [11]:

$$E + MgATP \xrightarrow{Na^+} [E \cdot MgATP] \xrightarrow{K^+} E + MgADP + P.$$

This reaction is reversible, since ionic gradients can give rise to the synthesis of ATP [3]. *In vivo*, ATP is normally synthesized coupled to electron transport (with the aid of the mitochondrial ATPase) as indicated below:

$$MgATP \underset{+e}{\overset{-e}{\rightleftharpoons}} MgADP + P.$$

The mitochondrial ATPase system is also reversible since electron transport reactions normally result in ATP synthesis, but high concentrations of ATP can drive electrons in the reverse direction [5]. The actions of both ATPases depend on the ATP concentration, and both can cause a transport of charge as a result of ATP utilization. The two systems are also related through the effects of oligomycin which inhibits both the Na^+-K^+-ATPase activity [4] and the oxidative phosphorylation processes in mitochondria. These properties suggest that ion transport may be related to the electron transport and coupling mechanisms that exist in membrane systems, perhaps by the mechanism outlined by Mitchell and recently reviewed by Robertson [10].

The three aspects of the problem mentioned above will be treated in this paper. We shall consider (1) the properties of the interfacial currents in this system, (2) the relation between the currents and the activity of the Na^+-K^+-ATPase, and (3) the relation between the currents and electron transport or coupling mechanisms, such as they have been described for mitochondria.

Materials and Methods

The Na^+-K^+-ATPase was prepared from rabbit kidney by the methods described in a previous paper [2] except that in this investigation Tris-EDTA and mercaptoethanol were omitted from the enzyme solutions because they interfered with the physical measurements described below. The Na^+-K^+-ATPase activity and the protein content were also determined as described earlier.

Twice-distilled water was used, the second distillation being from alkaline permanganate in an all-glass apparatus. (This procedure is used to eliminate surface-active contamination.) The ethanol used as a solvent for some of the drugs was also distilled and free from surface-active impurities. The inorganic chemicals were reagent grade, and their aqueous solutions showed none of the effects to be described in this paper. The aqueous solutions were made up at regular intervals in relatively small volumes and stored in a refrigerator. The drugs used in these experiments were dicumarol, antimycin A, oligomycin, rotenone and ouabain from Sigma Chem. Co., pentachlorophenol and thenoyltrifluoroacetone from Aldrich Chem. Co., Inc., and 2,4-dinitrophenol from Fisher Scientific Co. In the course of the experiments, solutions of serum albumin (Sigma) and of hexadecyltrimethylammonium bromide (K+K Labs.) were introduced, and an aqueous dispersion of crude vegetable lecithin (Mann Research Labs., Inc.) was also studied.

The mecury-water interface was formed by having distilled mercury issue from a fine capillary into various aqueous phases. The capillary was part of a polarographic apparatus, the Metrohm Polarecord, equipped with a device for rapid polarography. The latter device helps to keep the polarographic currents small by knocking the mercury drop off the capillary tip at regular (one/sec) intervals before it has a chance to grow too large. The vessel that contains the aqueous phase was thermostated at 25.0 °C, and the dissolved oxygen was removed by bubbling purified nitrogen (water pumped grade from Ohio Chemical Co.) through the solution for about 20 min and continuing the flow of nitrogen above the solution during the measurements. The above is a brief description of a normal procedure for polarography; the only difference from the conventional apparatus is the device for rapid polarography. The mercury acts as one electrode (polarizable), and a Ag/AgCl electrode with a saturated KCl bridge is the indifferent electrode that is in contact with the solution. All polarizations of the mercury-water interface are with respect to the Ag/AgCl electrode.

Results and Discussion

Characteristics of the Interfacial Currents

The mercury-water interface is an almost ideal polarizable interface; that is, in the absence of substances that can be oxidized or reduced, there is no current across the interface. (The small residual current that does appear with each drop is due to the charging of the electrical double layer, and this is kept very small by the use of a charging current-compensating device on the polarograph.) For this reason, one would not expect to observe any currents across the interface due to Na^+, K^+ or $Tris^+$ ions in the polarization range studied, and indeed none are observed. However, in the presence of the (Na^+-K^+-ATPase)-rich membrane particles, there are interfacial currents that vary in frequency, intensity and direction with

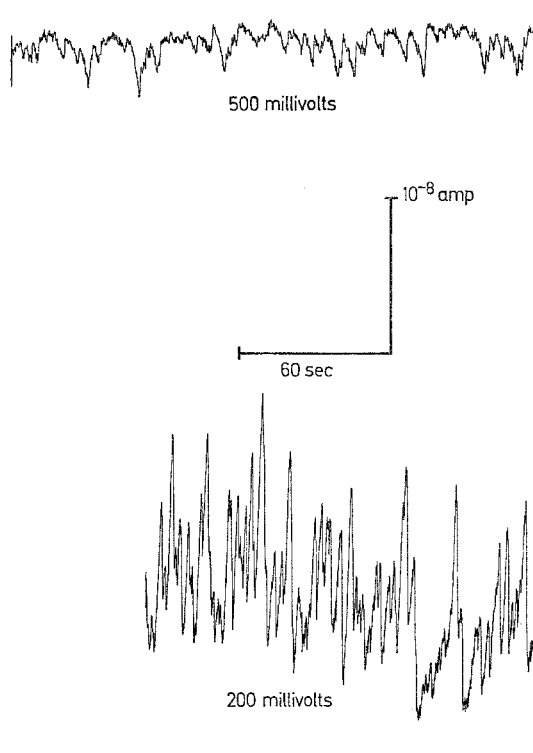

Fig. 1. The fluctuations in the base line with time for two different values of the polarization of the mercury-water interface relative to the Ag-AgCl electrode (saturated KCl), in the presence of membrane fragments of the ATPase preparation, dissolved in 0.1 M Tris NO_3, pH 7

potential (*see* Fig. 1). The currents, which occur in both the presence and absence of substrate and activating ions, look like noise superimposed on a base line, and suggest a random process as the basis for the observations.

It is apparent on inspection of Fig. 1 that a fluctuation in current may carry over several drops. This effect is not due to the recorder, since the response time of the pen is 0.02 sec/10^{-9} amp (at the sensitivity used) and experimentally the pen is able to make very large and rapid excursions between drops. It is possible that the events occurring during one drop influence the behavior of the following drop through the action of some material left behind as the drop separates. However, this explanation is also unlikely, since the proportion of surface carried over from drop to drop would seem to be no greater than 10 to 20%, whereas the carryover current appears closer to an order of magnitude greater. These considerations have led us to conclude that the variation in diffusion of particulate

matter to the surface of the drop is the major factor producing the observed fluctuations.

The magnitudes of the currents giving rise to the deflections seen in Fig. 1 are on the order of 10^{-9} amp, which corresponds to the movement of about 10^{-14} equiv/sec. If these currents are due to the diffusion of a substance in the aqueous phase at concentration C and of diffusion coefficient D, then the amount that diffuses to the drop in time t is equal to $C_0\sqrt{Dt}$ per unit area, or approximately 0.01 $C_0\sqrt{Dt}$ for a drop area of 0.01 cm^2. Therefore, the concentration required for the observed current is approximately $10^{-12}/\sqrt{D}$ equiv/cm^2. For small ions ($D \sim 10^{-5}$ cm^2/sec), $C_0 = 3 \times 10^{-6}$ equiv/liter, whereas for proteins and virus particles ($D \sim 10^{-7}$ to 10^{-8} cm^2/sec), $C_0 = 0.3 - 1 \times 10^{-5}$ M. Although the membrane particles are relatively large and D may be even smaller by an order of magnitude, each particle may contain several reactive molecules. Therefore, the concentration of reactive particles may still be on the order of 10^{-5} M. (It should be noted that this concentration of electro-active substances can supply a continuous current of 10^{-9} amp for over 5,000 hr.)

The current fluctuations seen with the ATPase preparations do not occur in solutions of any of the following: inorganic salts, a cationic surface-active agent, hexadecyltrimethylammonium bromide (CTAB) above or below its critical micelle concentration (CMC), and the proteins hemoglobin and serum albumin. Random currents were found to occur in dispersions of a vegetable lecithin that is a crude extract from soy beans. However, dispersions of pure *synthetic* lecithin (dipalmitoyl lecithin) do not show this kind of behavior [7], indicating that the effect is not due to the phospholipids *per se*, but requires other components of the cell that accompany the phospholipids when they are extracted. (It should be added that the crude vegetable lecithin dispersions have no detectable ATPase activity.)

Current fluctuations can also be induced in an inorganic solution upon the introduction of an electro-active substance that forms a suspension. CTAB solutions above the CMC (4×10^{-3} M) do not show random currents, but when ferricyanide ions are added to the solution at 10^{-3} M concentration, the solution becomes cloudy and random currents appear. The currents have a frequency and magnitude somewhat greater than in the case of the ATPase particles and, as expected, they are only reduction currents.

In the three cases studied where the random currents appear (the ATPase preparation, the crude lecithin suspension, and the CTA-ferricyanide suspension), the electro-active substances are present in particles

that are suspended in solution. A complete understanding of the random currents will require considerably more study, but the above results already indicate that one requires the presence of suspended particles containing electro-active substances. (The electro-active substances would cause normal polarographic waves if they were in free solution, and they give rise to the random currents only when the particles collide with the interface.) The particles in the enzyme preparation range in size from several tenths of a micron up to many microns, with the bulk of the particles less than several microns in diameter. The electro-active substance or substances in the particles have some unusual properties that we shall now discuss.

The random currents in the suspension of membrane fragments are markedly dependent on the polarization, and they disappear at the electrocapillary maximum (ECM), or point of zero charge at the interface. On opposite sides of the ECM, the current fluctuations have opposite directions, the mercury acting as an electron sink at polarizations more positive than the ECM, and as a source at polarizations more negative than the ECM. The magnitude and the average frequency of the electron currents rise and then fall to zero as one moves away from the ECM in either direction. From these observations it appears that the membrane fragments (and also the particles of the phospholipid dispersion) contain electro-active substances that are able to absorb and release electrons within a defined and measurable potential range at the interface.

To quantitate the observations, it was decided to count all rapid current jumps in excess of 10^{-9} amp and divide by the time to obtain an average frequency. The average frequency was then plotted as a function of the polarization of the interface (*see* Fig. 2). (Results essentially similar but with diminished amplitude were obtained if the count was limited to current fluctuations in excess of 2×10^{-9} amp.) The frequency-potential curve, which was obtained for all seven preparations of enzyme, as well as for the vegetable lecithin dispersion, showed a distribution of current fluctuations that was essentially symmetrical (except for sign) about the ECM. The fluctuations in either direction occurred over a range of about 600 mV, with a distribution that has a peak about midway in the range.

Using the value 10^{-9} amp as a threshold current jump, we can illustrate some of the quantitative properties of the random currents. For example, if the intervals between the current pulses are measured and their frequency plotted (as in Fig. 3), one obtains a characteristic Poisson distribution with the peak located (approximately) at the observed mean interval. If the number of pulses of a given magnitude is plotted vs. the magnitude (the current) of the pulse, there is an expected gradual decrease of the number

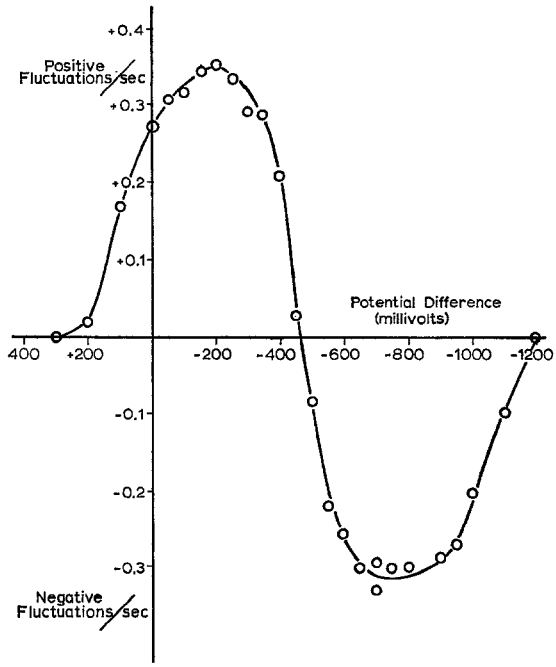

Fig. 2. The average frequency (fluctuations in excess of 10^{-9} amp/sec) as a function of the polarization of the mercury-water interface

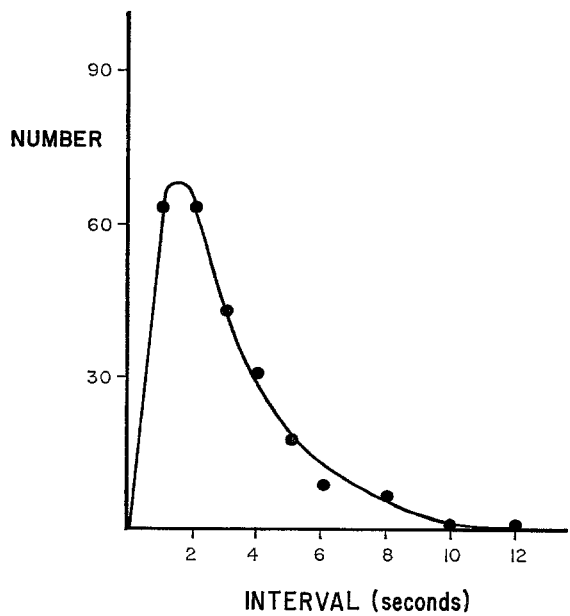

Fig. 3. The distribution of intervals between fluctuations over a 15-min period

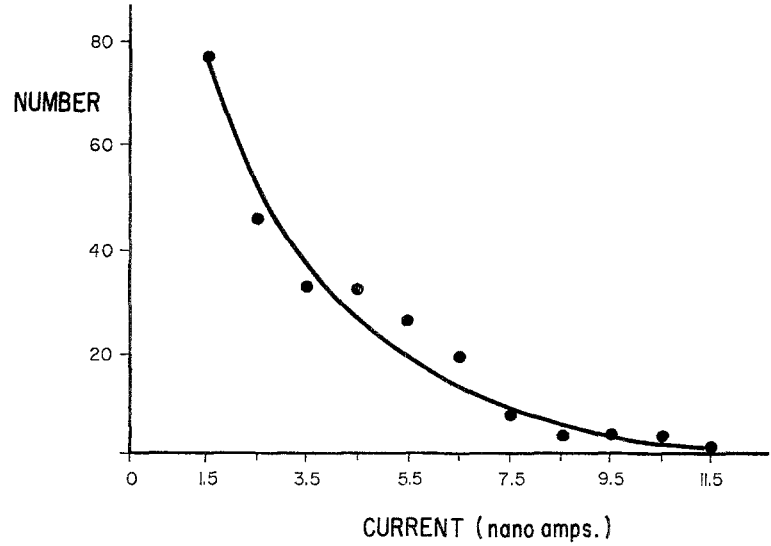

Fig. 4. The distribution of current magnitudes over a 15-min period

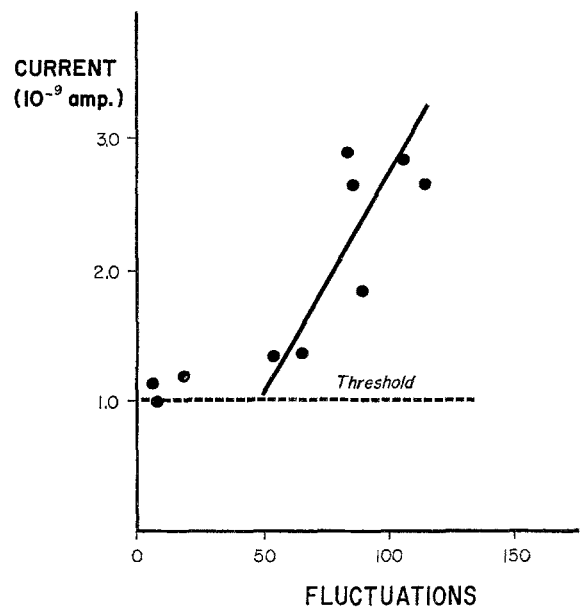

Fig. 5. A plot of the median of the distribution of currents vs. the number of fluctuations, in a 5-min period. The least squares line (for the points above 50 fluctuations) has a slope of 0.026 ± 0.008 and an intercept of 0.0 ± 0.8

as the magnitude increases (Fig. 4). The distributions of current magnitudes vary with the potential of an experiment, and they indicate a correlation between the average frequency of pulses (Fig. 2) and the magnitude of the

currents associated with them (Fig. 4). This correlation can be seen more clearly by plotting the magnitude of the median current pulse (Fig. 4) vs. the average frequency associated with those fluctuations. Fig. 5 shows that above the arbitrarily set threshold level in these experiments, the magnitude of the median current correlates with the average frequency. Measurement of the average frequency is much easier than measurement of the average amplitude, and so it is useful to know that the two quantities are directly related.

Relation Between the Currents and the Activity of the Na^+-K^+-ATPase

At low levels of protein concentration in an enzyme preparation, the amplitudes in the frequency-potential curve appear to increase with increasing concentration. The peak amplitude is a reasonable measure of the entire curve since it is roughly proportional to the area; it also contains information about the average currents. If one plots the magnitude of the peak in the frequency-potential curve as a function of the concentration of particles in the solution (*see* Fig. 6), one sees that the peak is proportional to the concentration only at very low values. At higher concentrations, there is almost no increase in the peak, suggesting saturation or no dependence on enzyme activity. In fact, the magnitude of the peak does not vary with the enzyme activity when comparing five different enzyme preparations ranging from zero to full activity, or the same enzyme preparation as the activity gradually decays with age. (There is also no correlation with activity in the presence of substrate.) In some experiments, it has been possible to dis-

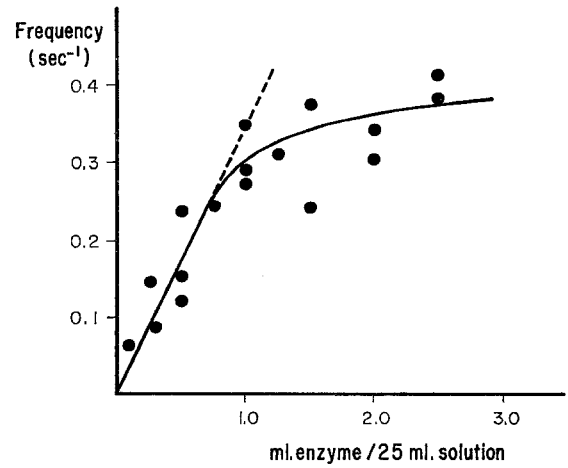

Fig. 6. The variation in the average frequency (fluctuations/sec) as a function of the concentration of the ATPase preparation (3.12 mg protein/ml in 0.1 M Tris NO_3, pH 7)

sociate the random currents completely from the enzyme activity by heat-denaturing the enzyme in a series of steps (with zero activity achieved at 60 °C for 2 min), and also by reversibly denaturing the enzyme with p-chloromercuribenzoate. In both cases, the random currents persisted in the absence of ATPase activity. In experiments with uncoupling agents, e.g., dinitrophenol, to be described later, it was possible to eliminate the random currents without affecting the ATPase activity. Therefore, it appears that the random currents are completely dissociable from the activity of the Na^+-K^+-ATPase. (This result was noted earlier in connection with the random currents from crude vegetable lecithin dispersions.)

The apparent inability to correlate any of the effects discussed here with the activity of the ATPase is a little disappointing, since this was the motivating force of the investigation. This negative result could mean that there is also no correlation between the electron currents and the ion pump mechanism. However, the mechanism of action of the ATPase is unknown, and it is possible that the electron currents are involved in the active ion transport mechanism but are not related to the properties of the ATPase. For example, this could occur if, according to the chemiosmotic theory [10], the energy available from the splitting of ATP by the pump enzyme leads to a pH gradient across a membrane through the action of an unknown (energy-absorbing) mechanism. If the latter mechanism can also operate by absorbing energy in the form of electrons directly at an electrode surface, then the ATPase activity would be completely unrelated. This may be the situation with regard to the ATPase, but we have no supporting evidence. The only relation that we have been able to show between the random currents and the ATPase is the effect of ouabain on both processes, and this effect will be discussed in the following section. As mentioned in the introduction, oligomycin also has an effect on both processes; however, the two compounds act differently since ouabain is more effective on the ATPase, and oligomycin on the random currents.

Relation Between the Currents and the "Electron Transport" and Coupling Mechanisms

Since the membrane fragments that are rich in Na^+-K^+-ATPase also contain other membrane-bound substances, the observed random currents may be due to the presence of elements of the electron transport chain. This is a distinct possibility, since the membrane fragments may contain mitochondrial components which are both oxidizable and reducible. The membrane fragments may also contain elements of an unknown mechanism coupling an oxidative pathway to the Na^+-K^+-ATPase in a manner similar

to the coupling of the electron transport chain and the oxidative phosphorylation mechanism in mitochondria. In either case, it would be interesting to determine the effect on the observed fluctuations of agents known to influence mitochondrial electron transport and coupling processes. From these and other observations, it may be possible to establish similarities between the mitochondrial system and the redox system which causes the observed fluctuations and, perhaps, to implicate the latter system in the biological ion transport mechanism.

There are several points which indicate similarities between the system studied and mitochondrial electron transport and coupling mechanisms. For example, the symmetry of the observed current frequencies (except for sign) about the ECM indicates the reversibility of electron transport at the interface and fits in with the known reversibility of the "electron transport" reactions in biological oxidations and of the coupling mechanism. Furthermore, the membrane particles can absorb or release electrons over a range that is about 600 mV on either side of the ECM, a voltage range that allows effective coupling to the approximately 1,100 mV involved in the electron transport chain [5, 6].

There is also a similarity with regard to the effects of known "uncoupling agents" (those that uncouple electron transport from oxidative phosphorylation) on the random currents. If one gradually increases the concentration of an uncoupler in the solution, there is a decrease in the average frequency and amplitude of the random currents in a given concentration range. The effect can be seen by plotting the peak average frequency of Fig. 2 vs. the concentration of a particular compound, to give a "dose-response" type of curve. Fig. 7 gives such a curve for dicumarol and pentachlorophenol, both of which eliminate the random currents completely at sufficiently high concentrations. The classic uncoupler, 2,4-dinitrophenol, is reduced at the mercury-water interface, so its effect can only be studied in a resticted potential range. However, it is evident that the random currents are reduced in about the same concentration range as in the case of pentachlorophenol, a concentration at which there is no effect on the Na^+-K^+-ATPase activity [4]. The concentrations at which the uncouplers reduce the average frequency of random currents to one-half the initial value are given in the Table. One can see that the effective concentrations in this system are approximately 10^{-5} M, and therefore in the range of the estimated concentration of electro-active substances given earlier on the basis of the observed current magnitudes.

The Table also lists the effective concentrations of several other compounds that can eliminate the random currents. These compounds may

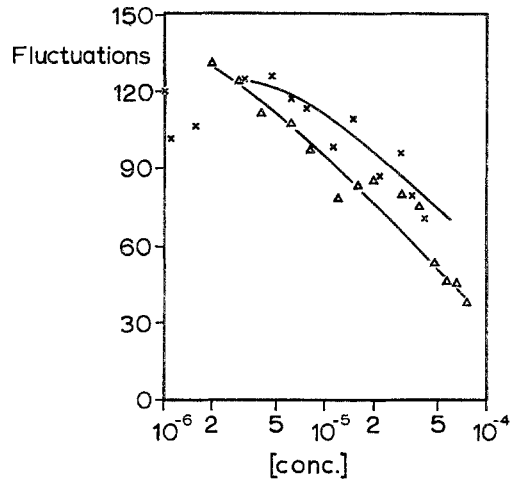

Fig. 7. The number of fluctuations in a 5-min period in a suspension of membrane fragments (0.5 ml, 3.12 mg protein/ml) in 24.5 ml 0.1 M Tris NO_3, pH 7, as a function of added dicumarol (▵) or pentachlorophenol (×)

Table. *Drug concentrations that reduce the average frequency of the currents to 50% of the initial value.* [The drugs are added to a suspension containing 0.5 ml enzyme preparation (3.12 mg protein/ml) and 24.5 ml 0.1 M Tris NO_3, pH 7.]

Substance	50%-Inhibition concentration (M)	Concentration of ethanol at 50% inhibition (M)
Antimycin (in alcohol)	3×10^{-5}	3×10^{-4}
Dicumarol	3×10^{-5}	—
Oligomycin (in alcohol)	5×10^{-5}	4.5×10^{-4}
Ouabain	5×10^{-5}	—
Pentachlorophenol	6×10^{-5}	—
2,4-Dinitrophenol	6×10^{-5}	—
Thenoyltrifluoroacetone[a] (in alcohol)	$2-3 \times 10^{-4}$	6×10^{-4}
Rotenone[a] (in alcohol)	$3-4 \times 10^{-4}$	1×10^{-3}
Ethanol	4×10^{-3}	4×10^{-3}

[a] The effects of these compounds are due in some measure to the alcohol used as a solvent.

interfere with coupling processes in mitochondria, but their primary effects are believed to be on other systems, such as the electron transport chain or the oxidative phosphorylation mechanism [5, 6]. Dose-response curves are given in Figs. 8 and 9 for antimycin A which blocks electron transport at the coupling point between cytochromes b and c, and for oligomycin which is believed to inhibit the phosphorylation of ADP. The effectiveness

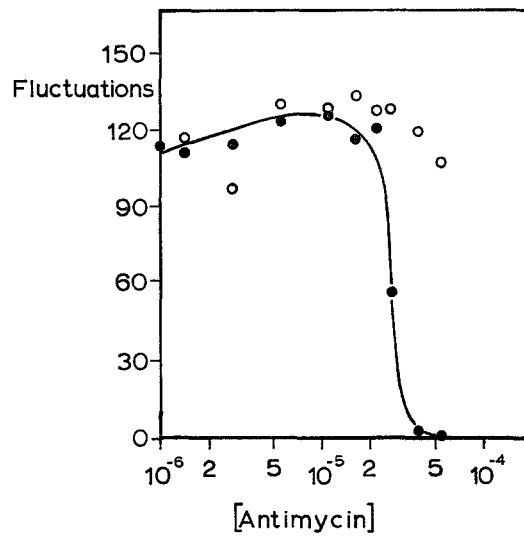

Fig. 8. The number of fluctuations in a 5-min period in a suspension of membrane fragments (0.5 ml, 3.12 mg protein/ml) in 24.5 ml 0.1 M Tris NO_3, pH 7, as a function of added antimycin dissolved in ethanol (•) or of the equivalent amount of ethanol (∘)

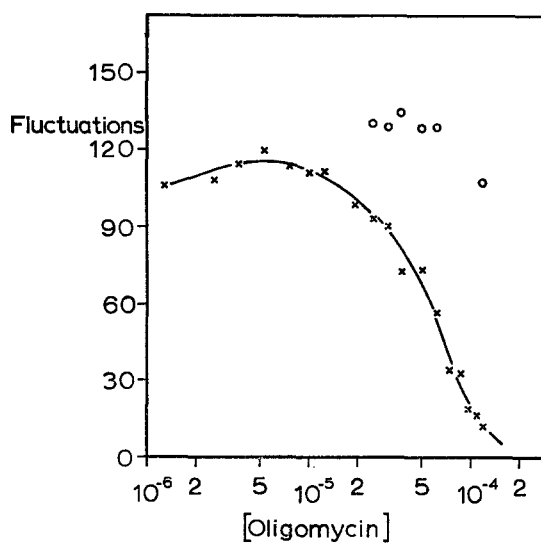

Fig. 9. The number of fluctuations in a 5-min period in a suspension of membrane fragments (0.5 ml, 3.12 mg protein/ml) in 24.5 ml 0.1 M Tris NO_3, pH 7, as a function of added oligomycin dissolved in ethanol (×) or of the equivalent amount of ethanol (∘)

of these two compounds in the elimination of the random currents is consistent with the idea of action at a coupling point or beyond. The effect of antimycin also suggests that there may be elements of the electron transport chain present in the membrane particles.

(The effectiveness of oligomycin on interfacial electron transport raises an interesting question about the mode of action of this compound. In mitochondria, oligomycin is believed to block a phosphorylation process involving an intermediate, but in the absence of ATP, ADP and P, it is difficult to justify the presence of phosphorylation reactions or of intermediates. One can speculate that phosphorylation is simultaneous with dehydration and that this kind of process is possible, or that the intermediate is a conformational state, but there is no evidence to justify these ideas from what we know regarding the nature of the electro-active substances or their redox reactions.)

By using inhibitors that function at different points in the chain, we attempted to determine if the mechanism producing the current fluctuations had properties similar to other elements of the electron transport chain. Since we found no effect of CN^- or azide which act in the region of the third phosphorylation site, we must conclude that, even if present, all elements of the chain are not functioning in our preparation. (The third phosphorylation site is normally inactivated by the sonic or mechanical fragmentation of mitochondria [9], processes that are similar to those used in extracting the ATPase from rabbit kidney, and it is possible that this element is destroyed in the preparation.) Two of the compounds listed in the Table, rotenone and thenoyltrifluoroacetone, normally act in the electron transport chain (and not at a coupling point or beyond) by blocking reactions before the site of action of ubiquinone. Their effects on the interfacial currents occur only at much higher concentrations (and may be due in part to the synergistic effect of the ethanol they are dissolved in), so they probably are not effective at the primary site of interfacial electron transport.

Four of the drugs listed in the Table were used in ethanol solutions, and, as can be seen in Figs. 8 and 9, ethanol itself has an effect on the random currents when present at higher concentrations. When ethanol is added alone, it causes a slight stimulation of the random currents at micromolar range, followed by a slow gradual inhibition, with half-maximal activity at about 4×10^{-3} M, or 0.06 vol%. The amphipathic nature of ethanol suggests that it (as well as the substances it can dissolve) may be effective in eliminating the random currents by action at a lipid-water interface (e.g., the disruption of a lipid membrane structure).

The only substance listed in the Table that has a rather specific effect on ion transport and the Na^+-K^+-ATPase is ouabain, which can apparently interfere with the interfacial electron transport mechanism in a manner that is quantitatively comparable to the actions of the compounds in Figs. 7, 8 and 9. This suggests that ouabain may act by blocking electron

transport processes when affecting the Na^+-K^+-ATPase and active transport processes. Although ouabain is far more effective in eliminating ATPase activity [2], by at least an order of magnitude in concentration, the fact that it acts at about the same concentration as the most effective inhibitors of interfacial electron transport means that the ouabain-sensitive site, which presumably is the Na^+-K^+-ATPase, is probably the site of interfacial electron transport. (One should bear in mind that much lower concentrations may be required to block electron currents within the membrane fragments than between the fragments and the interface.) Although interfacial electron transport is not related to ATPase activity, the strong possibility that the two processes occur at the same or adjacent sites lends support to the idea that the ATPase and an electron mechanism are both parts of the ion pump mechanism.

We can summarize the above discussion as follows: The electro-active substances in the enzyme preparation may be from mitochondrial contamination, but there is also the possibility that they are adjacent to the Na^+-K^+-ATPase and part of the ion pump mechanism. The reactions of the electro-active substances with the compounds listed in the Table suggest that they are similar to those that are involved in reactions at the second phosphorylation site in mitochondria [5, 6]. (The low concentration of antimycin required to eliminate the currents and the sharpness of the dose-response curve in Fig. 8 suggest that interfacial electron transport involves an antimycin-sensitive site, but there is relatively little difference between the effects of antimycin and of some of the other compounds in the Table.) The second phosphorylation site in mitochondria is also about halfway along the redox potential scale and therefore compatible with the ± 600 mV range of the random currents observed here. The ineffectiveness of p-chloromercuribenzoate on the random currents indicates the absence of SH groups at the electro-active sites, and the inability of heat (60 °C for 5 min) to eliminate the random currents suggests that the substances are probably not proteins. It is of some interest to note that, regardless of the identity of the electro-active substances, electron flow occurs when the particles containing them collide with the interface, i.e., via a mechanism that has been proposed as the basis of electron transport reactions in mitochondria [5].

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