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CHARACTERIZATION OF GASTRIC MUCOSAL MEMBRANES

VI. THE PRESENCE OF CHANNEL-FORMING SUBSTANCES

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SUMMARY

Highly purified gastric membranes were extracted by ionic or non-ionic detergents. Further fractionation was carried out on acrylamide gel electrophoresis. Some defined bands, incorporated into artificial bilayer membranes, produced discrete conductance changes characteristic of channel activity. A low molecular weight peak, purified through two cycles of electrophoresis was characterized as an anion-selective, positively voltage dependent channel, and a model to account for the action of the transport ATPase incorporating this channel concept is suggested.

INTRODUCTION

Conductance across epithelia may be described as the sum of the conductances of the cellular and paracellular route [1]. The relative magnitude of these conductances varies from tissue to tissue, and in the case of the gastric mucosa, the paracellular conductance is at most 20% of the cellular conductance [2]. It, therefore, follows that conductance measurements on the intact tissue, whether in vivo, or in vitro, determine the conductance of the cell membranes of the mucosa. The cell membrane presumably contains structures, be they channels or carriers, responsible for the conductance patterns observed in the intact tissue. Such molecules exist in abundance in unicellular organisms. Valinomycin [3], enniatins A, B, C, [4], the actins [5], nigericin [6] are just some examples of carriers produced by various bacteria or fungi. These cyclic compounds have as a common structural feature in hydrophobic environments, a central polar region and a peripheral hydrophobic shell. All of these compounds induce specific conductances in artificial bilayers up to $10^{-2}\,\Omega^{-1}\cdot cm^{-2}$.

Other molecules such as amphotericin [7] and Nystatin [8] are capable of inducing channels in bilayers. In this case it has been proposed that a relatively large (5 Å) pore is formed by cylindrical stacking of rod-like structures. There is a lipid

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specificity here in the form of a cholesterol requirement. Dehydration of solvated ions probably plays no role in selectivity with these compounds.

Linear peptides such as gramicidins [9–11] or *N*-formyl(Ala–Ala–Gly)$_4$-OMe [12,13] also form channels across bilayers characterized by quantal increments of conductance of the order 10^{-11}–10^{-10} Ω^{-1}. To account for these properties, a helical structure has been proposed such that there is a central polar and peripheral hydrophobic region, as for the cyclic compounds. This novel $\beta^6{}_{3,3}$ helix also shares a common mechanism for ion complexation with the cyclic carriers, consisting of competition between polar oxygen groups and the hydration shell for cation binding. Excitability inducing material [14], also of bacterial origin, forms voltage dependent channels of the same order of magnitude ($3 \cdot 10^{-10}$ Ω^{-1}) and appears to be a protein.

Information about ionophores derived from mammalian tissues is considerably more sparse. High ionic strength extracts of rat brain microsomes or frozen dried electroplax produced channel activity in bilayers. These were Na^+ selective or K^+ selective and voltage dependent [15]. Crude mitochondrial fraction from electroplax was separated on density gradients in a zonal rotor. Incorporation of the $(Na^+ + K^+)$-ATPase rich fraction in the presence of ATP was accompanied by an increase in zero current voltage which decayed in a stepwise fashion, suggesting a channel mechanism for voltage shunting [16]. In the same paper the authors showed that the low density fraction of the same gradient induced step changes in conductance. More recently the supernatant of trichloroacetic acid precipitated trypsin digest of electroplax membranes, when added to an oxidized cholesterol bilayers, lowered the conductance only in the presence of Na^+, and the conductance was cation selective [17]. Cholinesterase preparations have been shown to contain channels which require acetylcholine for activity [18].

In this work we showed that: (a) channel-active material can be extracted from highly purified gastric membranes by non-ionic detergents; (b) that there are various types of channel activity in the crude extract; (c) that these activities can be separated by polyacrylamide gel electrophoresis; and (d) that an anion channel has been obtained in a high degree of purity.

In relation to this, some of the conductance properties of gastric mucosa have been well defined. Importantly this tissue secretes HCl such that the secretion of H^+ is electrogenic, and we would, therefore, expect a fair quantity of anion selective channel material to be present in the cell membrane. Moreover, from experiments in the intact tissue, the $SO_4{}^{2-}$ conductance is considerably less than that of Cl^-, a property which we would predict to be shared by the anion selective channel shown to be present in the tissue extract.

METHODS

Bilayer technique

Electrical considerations. In order to be able to resolve individual channel events down to 10^{-12} Ω^{-1}, a bilayer with a background conductance of 10^{-9} $\Omega^{-1} \cdot cm^{-2}$ is required with a corresponding low noise level. Fig. 1 shows the technique we use to monitor the electrical characteristics. A voltage source or signal generator, and a current amplifier are connected through the bilayer, with the signal and am-

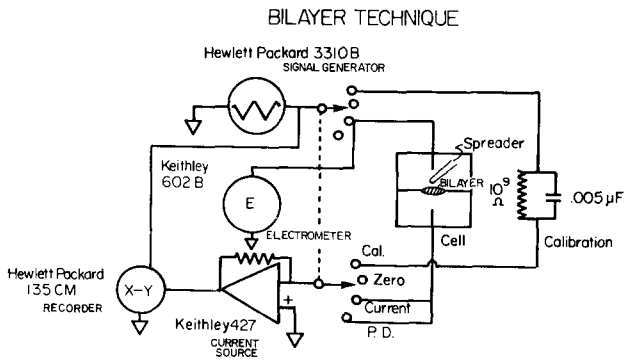


Fig. 1. Diagrammatic representation of the bilayer technique. A voltage generator (Hewlett-Packard 3310B) and a current amplifier (Keithley 427) are connected across a bilayer formed from soybean lecithin in decane and their output to an X–Y recorder. A switching mechanism is provided to connect an electrometer for measuring zero current voltage, and for capacitance and conductance calibration.

plifier output respectively to an X–Y recorder. The generator is used in two modes: a triangular sweep with $|dV/dt|$ constant, or as a piecewise constant voltage source (square wave). In the sweep mode, the curve obtained is shown in Fig. 2. The X-axis is the voltage and the Y-axis the current. The slope is the conductance, and the separation between the two sweep directions is due to the capacitance of the bilayer. This occurs because dV/dt changes sign at the extremes of voltage. Both channel and carrier incorporation can be detected. Channels are manifested as discrete conductance steps, and the size (conductance Δg) of the individual channel can be determined as the change in slope of the $I-V$ plots. If the studies are carried out in asymmetric

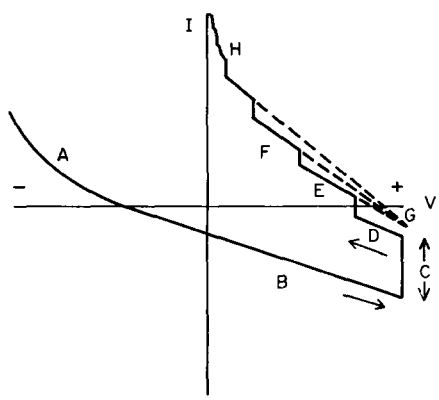


Fig. 2. Diagrammatic representation of type of result observed in sweep mode (triangular wave). A, effect of carrier incorporation showing smooth conductance changes even at very high sensitivity. B, background conductance. C, capacitance of bilayer, since dV/dt changes sign at extremes of plot, and is constant in between. D, E, F, successive single channel events in the bilayer, the change in slope giving Δg, the channel size or conductance. G, the point of intersection of the slope increments, giving the zero current voltage of the individual channels, i.e. their selectivity in asymmetric solutions. H, increase in conductance due to "unresolved" channels.

solutions (e.g. a $10 \times X^+Y^-$ gradient), the intersection of the slope gives the ion selectivity of the individual channel, i.e. V at $I=0$. Carrier effects show up as smooth conductance increments, and the asymmetry of the changes, if the solutions are asymmetric, shows whether anion or cation carriers are involved. In this set-up temperature effects can be studied since the chamber is provided with direct thermoelectric heating or cooling (Thermoelectrics SK-12).

Zero current voltage can be measured using the electrometer circuit and thus in asymmetric solutions gives the net channel or carrier selectivity. This circuit is used for studying active transport phenomena, i.e. voltage effects of ATP additions to ATPase preparations.

The square wave mode is used for measuring channel conductance and frequency distribution of conductance after some idea of selectivity and voltage dependence has been obtained on the sweep mode. A check on selectivity can be obtained in asymmetric solutions by determining the numbers of channels obtained with +ve or −ve current applied across the bilayer. One artifact must be considered in this context in that incorporation into the bilayer of a charged particle would also be voltage dependent, and this could not be distinguished from selectivity in this mode of operation.

Lipids. Various precautions have to be taken with the type of lipid used in order to reduce background conductance. The method we use as a routine is to dissolve soybean lecithin (Sigma, Type 11A) which contains some triglyceride, to 30 mg/ml in decane, and pass the solution over a neutral alumina (Bio Rad AG7) column immediately before use. All glassware is preheated to 400 °C to remove contaminating films.

Solutions. All solutions are made up in glass distilled water, and salts where necessary calcined to remove organic and bacterial contamination.

Cleaning procedures. The polyethylene inner chamber is disposable and is only reused after an extensive cycle of cleaning and testing of a sample batch. The spreader and electrode tips are not reused. The outer chamber is sonicated in detergent with prolonged water rinses.

Conclusion. The combination of these various procedures allows the production of a bilayer in which we can detect conductance changes as small as $2 \cdot 10^{-12}\ \Omega^{-1}$. In spite of all precautions, sometimes spontaneous events can be detected in the absence of any known additions. Usually they are quite large (approx. $10^{-9}\ \Omega^{-1}$) and hence can be distinguished from most of the natural channels we are studying. In our experience these spurious events are most often due to bacterial contamination.

Membrane preparation

The techniques we use have been previously described for gastric mucosa [19,20]. Briefly, the dog gastric mucosa is stripped from the underlying connective tissue, homogenized, and the post $20000 \times g$ supernatant spun at $100000 \times g$ for 60 min.

The precipitate is then distributed through a linear ficoll–sucrose gradient in a Beckman Ti XIV zonal rotor (47000 rev./min, 5–7 h) and two peaks of membranes are obtained, as well as a peak of mitochondrial activity. By both biochemical and morphological techniques the membrane fractions are not contaminated by other cell constituents. Membrane peak 2 is enriched in HCO_3^--ATPase, a marker for

the luminal cell surface of the oxyntic cell [21] and has been used for most of the work to be described.

Extraction of membranes

Since the membrane presumably contains proteins or peptides oriented so that the lipid portion of the membrane is associated with the hydrophobic portion of the protein, detergent extraction was the first method used. Since it was successful no other has as yet been investigated. Three types of detergents were used: sodium dodecylsulfate (SDS), Triton X-100, a non-ionic detergent containing a phenyl group, and Brij 36T, a non-ionic detergent that has no 280-nm absorption.

For sodium dodecylsulfate extraction, sodium dodecylsulfate was added at 1% final concentration, and the protein solubilized for 4 h at room temperature. Complete solubilization was achieved. For both Brij 36T and Triton X-100 extraction a 3:1 detergent/protein ratio was determined to be the most successful in solubilizing the HCO_3^--ATPase in active form. Solubilization was carried out by stirring at 0 °C for 1 h using conditions as previously described [22], i.e. approx. 1 mg/ml protein, 3 mg/ml detergent, 50 mM HEPES buffer (pH 7.4), 10 mM NaCl at 0 °C for 1 h. Following all three procedures the suspension was centrifuged at $100000 \times g$ for 60 min and the supernatant used for all further experimentation.

Characterization of extract

Although most of our recent experiments have been carried out with the Brij extract, the first active preparation was obtained with Triton X-100. All the detergent solubilized material was characterized on 7% analytical acrylamide gels. The positions of the peaks were determined on a Gilford gel scanner, following Coomassie Blue staining. Parallel gels were serially sectioned and fractions tested for activity in the bilayer.

Preparative gel fraction of the Brij extract was carried out on 7% gels using a 3.5% stacking gel. Reservoir buffer was 0.19 M glycine–Tris buffer (pH 8.6), stacking gel buffer was 100 mM Tris –HCl (pH 6.8), separating gel buffer was 0.4 M Tris–HCl buffer (pH 8.6) and flow buffer was as for the separating gel. All buffers usually contained 0.1% detergent. Similar conditions were used for analytical gel work.

Various fractions of the preparative run were tested in the bilayer, and subjected to analytical gel electrophoresis in 7 and 15% gels. The fastest moving active peak was then further fractionated on 15% preparative gel electrophoresis, the bands analyzed for activity and also for purity on 15% analytical gels.

Column chromatography

Separation of the detergent solubilized extracts was carried out on Sephadex columns of varying porosities. Elution was carried out using buffers containing 0.1% detergent to reduce precipitation of protein in the gels.

Bilayer experiments

Two types of solution were used in the bilayer chambers. When symmetric solutions were used, both inner and outer chambers contained 30 mM NaCl and

120 mM KCl at pH 7.4. For asymmetric solutions, the inner solution was the same as above, the external solution was 10-fold diluted.

The membrane extract was added in 10-μl quantities as a 400-fold dilution to the inner chamber, following a period during which the background had been shown to be satisfactory. The final protein concentration was usually about $1 \cdot 10^{-8}$ g/ml in the inner chamber. Usually both the sweep and constant voltage mode were used to scan the bilayer with the membrane extract incorporated.

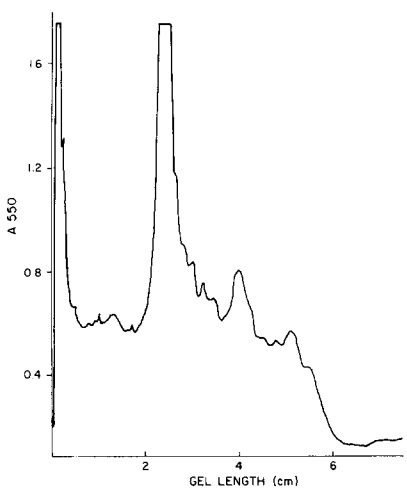
RESULTS

Acrylamide gel characterization

Sodium dodecylsulfate gels. The gel pattern was similar whether 1 or 4% sodium dodecylsulfate extraction was carried out. Fig. 3 shows the pattern obtained. There is clearly a major heterogeneous protein peak and several minor ones totalling about 25. Since sodium dodecylsulfate is charged, several artifacts were possible in our bilayer analyses, and relatively few experiments were carried out. More particularly, the negative charge on sodium dodecylsulfate would lead us to expect perhaps an artifactual cation selectivity on incorporation into the bilayer. Alternatively, better incorporation may be obtained with the negatively charged sodium dodecylsulfate being driven into the bilayer by the applied voltage.

The sodium dodecylsulfate was fractionated into two peaks on Sephadex G-50 columns. Two peaks were obtained and the included peak checked for activity in the bilayer.

Triton X-100 gels. Since membrane fragmentation by Brij 36T extraction seemed better than that of Triton X-100, coupled with the ease of determining the



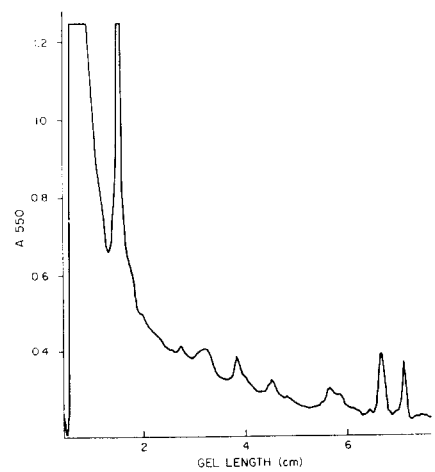


Fig. 3. Scan of protein bands present in 7% acrylamide gels following sodium dodecylsulfate solubilization of Peak 2 of gastric membrane fraction. The major peak which is heterogeneous has a mol. wt of 84000.

Fig. 4. Scan of protein bands present in 7% acrylamide gel following Triton X-100 solubilization. The highest R_F peak is a buffer front artifact. The second highest R_F peak has channel activity.

protein spectroscopically with Brij 36T, most of the work with Triton X-100 was confined to analysis of the total extract. The scan of a Triton X-100 gel is shown in Fig. 4. The fastest moving protein band has channel activity as will be discussed.

Sephadex G-100 chromatography using 8 mM HEPES buffer (pH 7.4) for

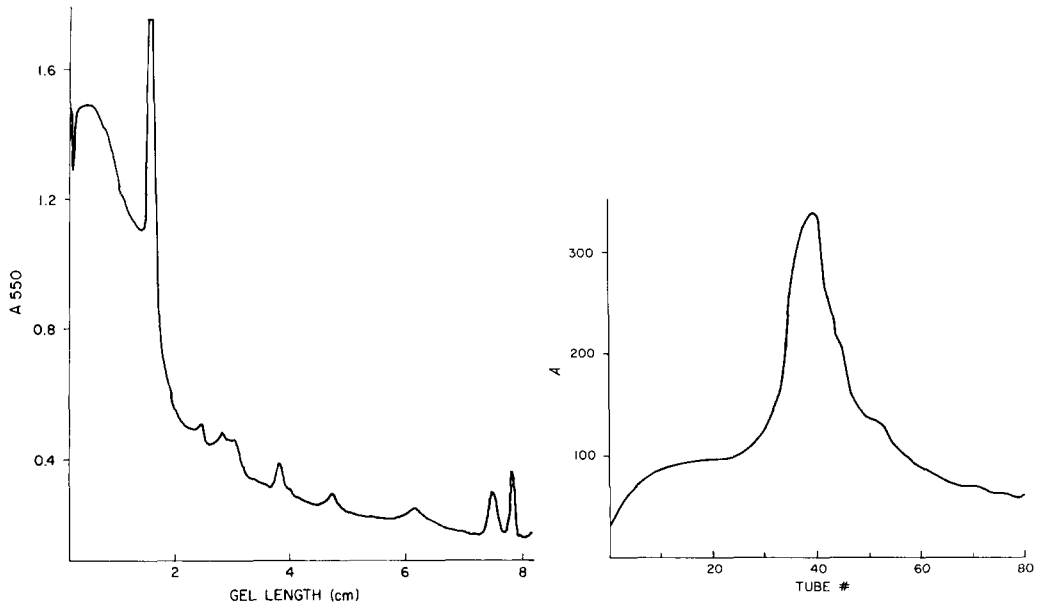


Fig. 5. Scan of protein bands present in 7% acrylamide gel following Brij 36T solubilization. The similarity of the pattern to Triton X-100 should be noted, especially the penultimate band.

Fig. 6. Profile of the low molecular weight band obtained from 7% preparative gel electrophoresis of a Brij 36T extract of gastric membranes. The initial major peak was studied further.

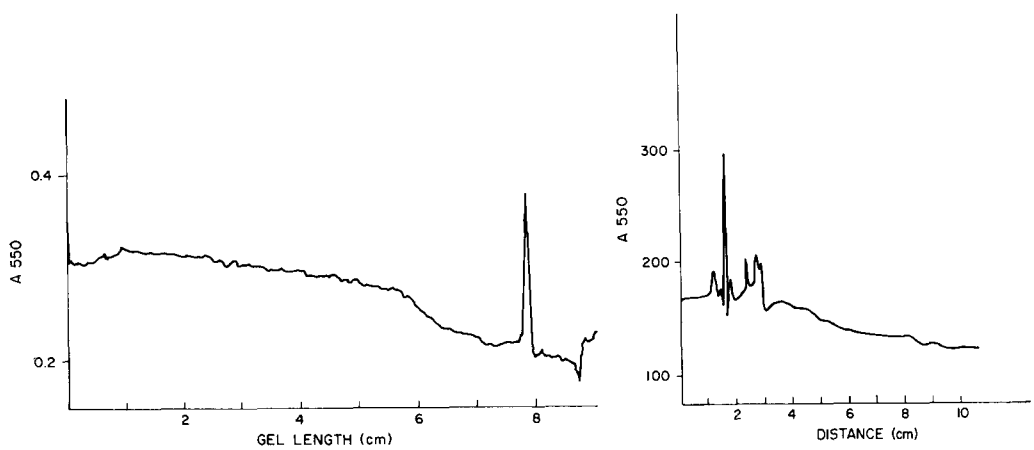


Fig. 7. 7% acrylamide gel pattern of peak obtained from preparative gel electrophoresis, showing the presence of a single protein band, corresponding to a mol. wt of about 8000.

Fig. 8. 15% analytical gel pattern of same preparative gel band as shown in Figs 6 and 7, showing the presence of six peaks at this resolution.

elution fractionated the Triton X-100 extract into two peaks. The included peak contained the majority of the channel activity.

Brij 36T gels. Fig. 5 shows a scan of a Brij 36T extract run in 7% acrylamide. The similarity of the faster moving bands in the Brij 36T and Triton X-100 runs should be noted. Fig. 6 shows the elution profile of a preparative gel run on the total Brij 36T extract. In many of the runs a fast moving peak was obtained which, although showing a peak absorption between 260 and 280 nm, did not contain protein. From Fig. 6 a heterogeneous low molecular weight band can be seen. Running this in the analytical gels showed that the preparative procedure had indeed resolved the mixture. Fig. 7 shows the analytical 7% gel pattern obtained from the early absorbance rise from the preparative gel showing the presence of the high R_F band. The presence of a single peak in 7% gel is misleading because the 15% gel pattern of the same peak shows the presence of six bands (Fig. 8). Combining several tubes, and running those on 15% preparative gels resulted in several fractions being obtained. The fastest running of these, subjected to 15% analytical gel electrophoresis, produced a double band as shown in Fig. 9. This, therefore, is considerably purified over our starting material.

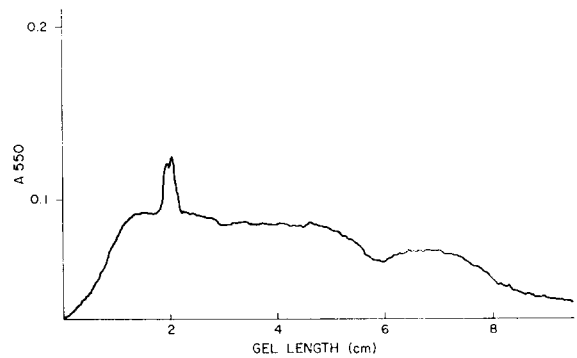


Fig. 9. 15% analytical gel pattern of initial eluate from 15% preparative gel electrophoresis of protein obtained in Fig. 6, showing the presence of only the doublet band of Fig. 8.

Bilayer results

Channel activity

Sodium dodecylsulfate extracts. From the analytical gel profile we decided to test the effect of the major protein on the bilayer. As a control an area free of protein was also checked. The control portion, when extracted with the inner chamber solution, produced no measurable effects.

The major peak (mol. wt 84000), however, produced an effect as shown in Fig. 10A, the representation of a square wave experiment. It can be seen that applying current so that the inner chamber was +ve with respect to ground (external solution) had no effect on conductance. Applying current in the other direction, however, produced a large number of channel events. Since in this experiment, asymmetric solutions were present, one may be tempted to conclude that anion selectivity had been demonstrated. However, sodium dodecylsulfate is negatively charged and it may be that the applied voltage is facilitating incorporation into the bilayer. Against

this argument, experiments which tested the Sephadex G-50 included peak of the sodium dodecylsulfate extract produced no evidence of selectivity in the square wave mode. Thus Fig. 10B shows that the sign of the applied voltage had no effect on channel appearance.

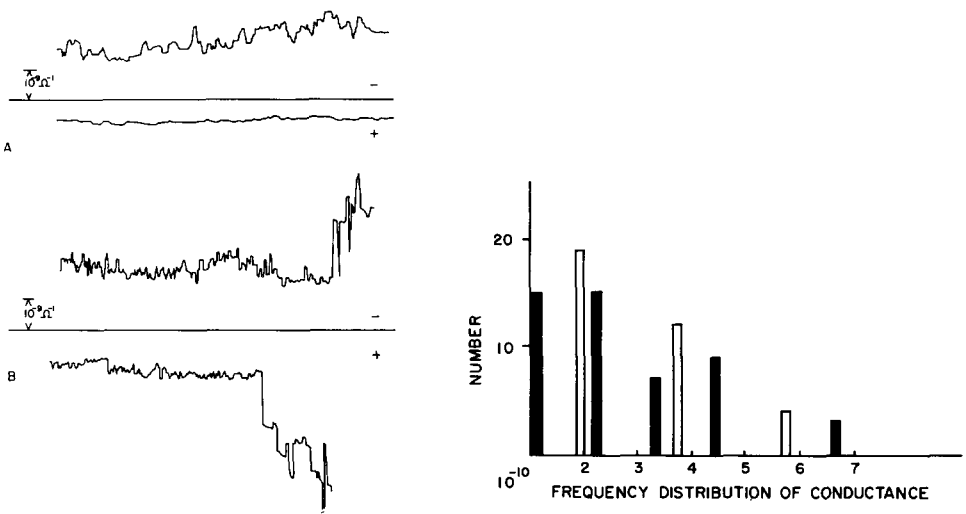


Fig. 10. (A) Square wave scan of bilayer following incorporation of high mol. wt (84000) band of sodium dodecylsulfate solubilized extract, showing channel activity essentially only when inner chamber is –ve, with a 10-fold concentration gradient (high salt in the inner chamber), suggesting the presence of anion channels. (B) Similar scan of bilayer following incorporation of material from included area of a Sephadex G-50 eluate showing channel activity that is presumably non-selective.

Fig. 11. Frequency distribution of channel events following incorporation of sodium dodecylsulfate of high mol. wt and low mol. wt material into the bilayer. The anion channel occurs as a multiple of $1.9 \cdot 10^{-10}\ \Omega^{-1}$ (clear bar), the non selective channel as a multiple of $1.1 \cdot 10^{-10}\ \Omega^{-1}$.

Fig. 11 shows the frequency distribituon of channel size (conductance) of both sodium dodecylsulfate studies. It can be seen that channel size varies quantitatively, as integral multiples of $1.9 \cdot 10^{-10}\ \Omega^{-1}$ for the anion selective channel, and of $1.1 \cdot 10^{-10}\ \Omega^{-1}$ for the included peak, non-selective channel.

Attempts to incorporate many of the other peaks into the bilayer, with demonstrable changes in conductance failed, coupled with the failure to observe changes with stain free areas. This suggests that we were not dealing with detergent artifacts, and also that since only certain bands were active, that this was not a non-specific effect.

Triton X-100 extracts. Applying the total Triton X-100 extract to the bilayer resulted in rapid increases in conductance. Starting at a sensitivity of 10^9, often changes of two or more orders of magnitude in conductance were observed. More often, however, as channels incorporated into the bilayer, it broke readily, hence most observations were carried out at a sensitivity of 10^9 only. Three types of channel activity were observed. Fig. 12 shows the sweep appearance following incorporation of a nonselective channel, which appeared to be the major activity. In addition anion and cation selective channels were observed. The fastest moving band on the Triton X-100

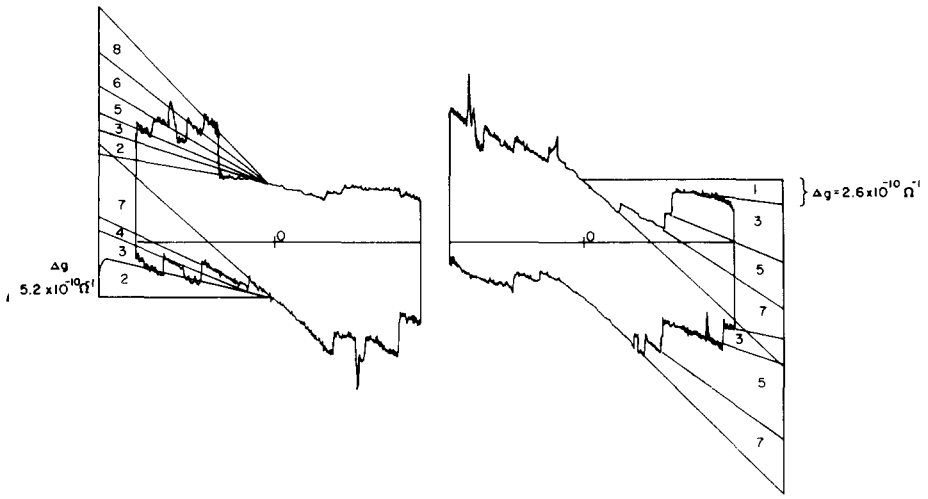


Fig. 12. Effect of total Triton X-100 extract on bilayer in sweep mode showing the incorporation of non-selective channel activity as single or multiple steps with unit conductance of $2.6 \cdot 10^{-10}\ \Omega^{-1}$.

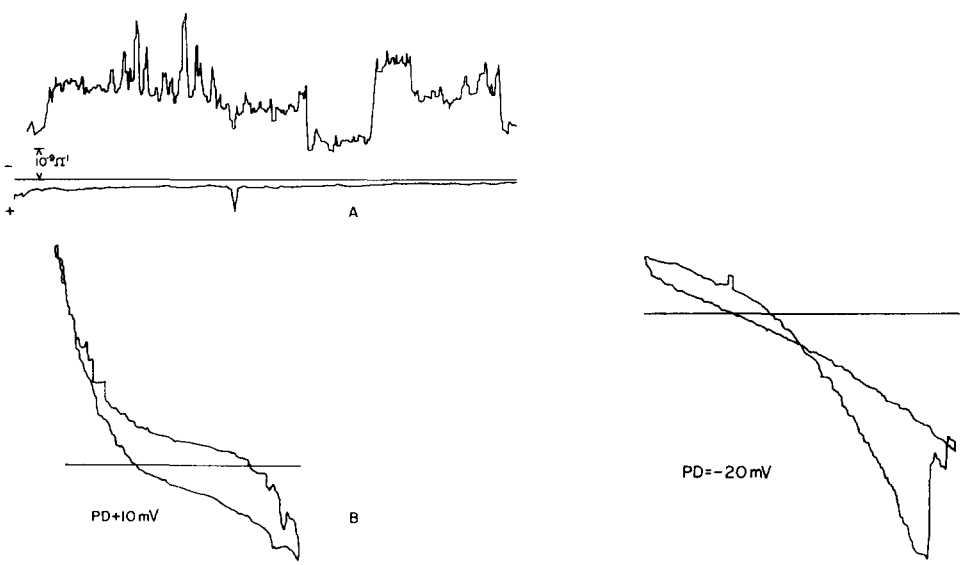


Fig. 13. Effect on bilayer of incorporation of purified low mol. wt material as shown in Fig. 9 in square wave A and sweep mode B. Zero current voltage was +10 mV, confirming weak anion selectivity of channel, as suggested by both sweep and square wave pattern. The +ve voltage dependence of activity should also be noted.

Fig. 14. Appearance of bilayer in sweep mode following incorporation of low R_F band of Fig. 8, showing the presence of "unresolved" cation selective channels generating a PD of −20 mV across the bilayer.

TABLE I

SUMMARY OF CHANNELS FOUND

Electrolyte concentration 120 mM NaCl.

Detergent	Sodium dodecylsulfate	Triton X-100	Brij 36T
	Low mol. wt < 50000 non-selective $1.1 \cdot 10^{-10}\ \Omega^{-1}$ High mol. wt approx. 84000 anion selective $1.9 \cdot 10^{-10}\ \Omega^{-1}$	Mixture of anion cation non-selective	Low mol. wt 7% gel Mixed activity 15% resolved anion selective $1.7 \cdot 10^{-10}\ \Omega^{-1}$ Cation selective mol. wt approx. 8000 $3.5 \cdot 10^{-10}\ \Omega^{-1}$

gel also had a mixture of activities. The discrimination of the selective types of channel was clear cut, but not absolute, since about 20 mV zero current voltage was developed in 10-fold concentration gradients.

Brij 36T extracts. The effect of the Brij 36T extracts on the bilayer was superficially quite like that of Triton X-100 extracts, but the bilayer appeared to be much more stable than with Triton X-100. Since both the sodium dodecylsulfate and Triton X-100 experiments had established the presence of channels, our Brij 36T experiments were carried out with the major purpose of separating and characterizing channel activity. Fig. 13 shows the data obtained when the material corresponding to the analytical gel of Fig. 9 is added to the bilayer. Based on the sweep, square wave and zero current voltage data (i.e. the potential developed across the whole membrane), this material is anion selective. Less than 0.1 μg of protein (based on absorbance at 280 nm) produces easily detectable activity. Based on molecular weight calibration of the gel, molecular weight of the channel is about 8000 or less [2]. The slowest moving peak of Fig. 8 when incorporated into the bilayer produced a sweep characteristic as in Fig. 14. Square wave, sweep and voltage analysis showed this to be a cation selective channel. Again, as with the Triton X-100, the selectivity ratio was usually less than 5, anion/cation for the "anion" channel and cation/anion for the "cation" channel. In the case of the frequency distribution for the anion channel, this showed that channel size is a multiple of $1.7 \cdot 10^{-10}\ \Omega^{-1}$, close to the sodium dodecylsulfate size.

DISCUSSION

The purpose of this work was to devise techniques wich could allow the study of natural ionophores occuring in animal cell membranes.

We selected gastric mucosa for this purpose since we know that conductance of this tissue is mainly cellular, hence the electrical properties of the epithelium in the Ussing chamber reflect the properties of the cell membrane. Hence structures responsible for anion conductance will show $P_{Cl^-} > P_{SO_4^{2-}}$, and if the electrogenic proton pump hypothesis is correct, will also show positive voltage dependence.

Another reason for the use of this tissue was that we had available a supply of highly purified membrane fractions from the zonal rotor, hence we could be fairly confident that the material extracted was membrane derived.

Examining the data presented we have the following facts: (a) The starting material is a highly purified plasma membrane suspension. (b) Direct addition of this material shows no activity in the bilayer, hence the active materials are not water soluble, or are tightly bound to the membrane. (c) Detergent extraction produces a highly active preparation which shows channel rather than carrier properties. Presumably, then, with adequate structural characterization, the mammalian analog to compounds such as digramicidin malonamide will be identified. (d) Several of the protein bands show no activity in the bilayer. Thus the property of channel induction is confined to only some of the proteins extractable from the membrane and this serves as a satisfactory control. (e) Ion selective channel activity is found in a relatively low molecular weight peptide band, corresponding to a molecular weight of about 8000 present in both Brij 36T and Triton X-100 extracts on 7% analytical gels. This band seems to have mixed anion and cation selectivities. (f) Preparative gel fractionation results in purification of this band which is further subfractionated on 15% gels (preparative and analytical) into six bands. The highest mobility band was anion selective. The cation selective compound appeared to be subject to aggregation and was of low mobility on 15% gels. The intermediate bands had no activity. (g) There was anion and cation discrimination of about 5:1. (h) The anion channel showed positive voltage dependence in that bilayer conductance increased with voltage. This could be due to alteration in channel incorporation or a voltage effect on the channel itself. (i) Several channel sizes were observed. The larger channels could be accounted for as multiples of a single channel event, suggesting associativity between channels. (j) Channel activity was heat labile, perhaps due to denaturation, or aggregation of the channels into an inactive form. (k) The conductance concentration curves for the channels were different for the anion and cation selective types. Although not conclusive, the simplest interpretation of the curves would be that the anion channel was charged, and the cation channel neutral. (l) In other regions of the gel, non-selective channels were found. The 84 000 mol. wt protein

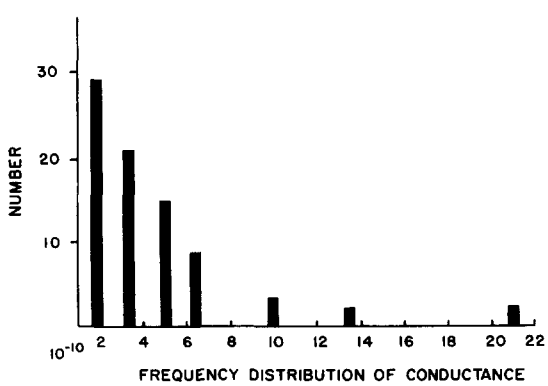


Fig. 15. Frequency distribution of channel events following incorporation of partially purified anion channel into the bilayer, showing that conductance increments occur as multiples of a unit conductance of $1.7 \cdot 10^{-10}\,\Omega^{-1}$.

found on sodium dodecylsulfate gels was anion selective, and the crude sodium dodecylsulfate fractions from Sephadex columns were non selective. (m) The anion channel we obtain shows considerable Cl^-/SO_4^{2-} discrimination, as we would predict from intact tissue studies. The positive voltage dependence was also an interesting property, since this could be one way of linking Cl^- flux to the activity of an electrogenic H^+ pump.

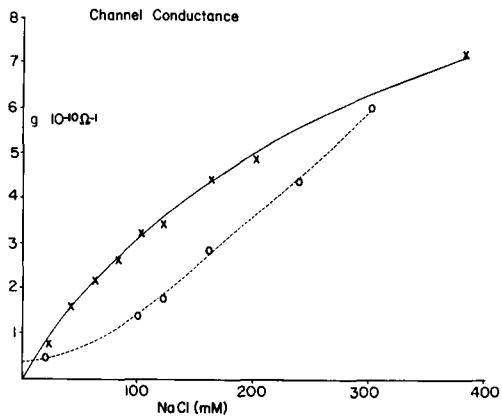


Fig. 16. Channel conductance as a function of NaCl concentration, showing, in the upper curve, the effect of ionic strength on the cation selective channel (×—×), and on anion selective channel (○---○).

This work thus provides the first evidence for channel activity in epithelial membranes. Their physiological significance has not as yet been defined, but it is encouraging that one of these channel activities is of low molecular weight, and relatively easily separated from the rest of the membrane material. It is hoped that controlled proteolysis will be able to reduce the molecular weight by about half without loss of activity so that studies can be initiated on its conformation.

With the application of any novel approach, one is cognisant of several possible artifacts. Many of these have been excluded by our work. Thus the effect of several detergents on bilayers has been well documented [22]. Control experiments showed that detergents at concentrations used had no effect on the bilayer (<3 μg/ml) and at much higher concentrations smooth conductance changes were observed which did not correspond to the changes we observed. Since the majority of the separable bands on acrylamide gel were also without detectable bilayer activity, it would appear that the property of inducing conductance changes is confined to only a few of the membrane constituents. This increases our confidence that the conductance changes are physiologically relevant.

The use of Brij 36T or Triton X-100 was initially based on their capacity to solubilize the ATPase activity of the membrane. The finding of channel activity raises the possibility that channels were an integral part of this enzyme, as has been suggested for the $(Na^+ + K^+)$-ATPase [17]. Clearly we can separate channel activity from ATPase activity which is located on top of the gel in the case of Triton X-100 extracts, and at about 1 cm for the Brij 36T preparation. We have not, however,

shown that purified ATPase is devoid of channel activity. As mentioned in the introduction, the PD obtained by the addition of ATP to ATPase preparations for $(Na^+ + K^+)$-ATPase decayed in stepwise fashion. Similar data were obtained in these preparations, and it was shown that this stepwise decay was due to the induction of channels by the bilayer voltage. This type of observation led us to postulate a function for the anion voltage dependent channel in the gastric mucosa. Thus the ATPase in Fig. 17 is visualized as an electrogenic pump, responsible for increased OH^- (HCO_3^-) translocation. The anion channel is the parallel limb of the circuit, and being voltage dependent would transport Cl^- in response to ATPase activity. The net result would be HCl secretion. With SO_4^{2-} instead of Cl^-, since the $P_{Cl^-}/P_{SO_4^{2-}} = 6$, the electrogenic nature of the proton pump would be unmasked, as is the case in SO_4^{2-} solutions [23].

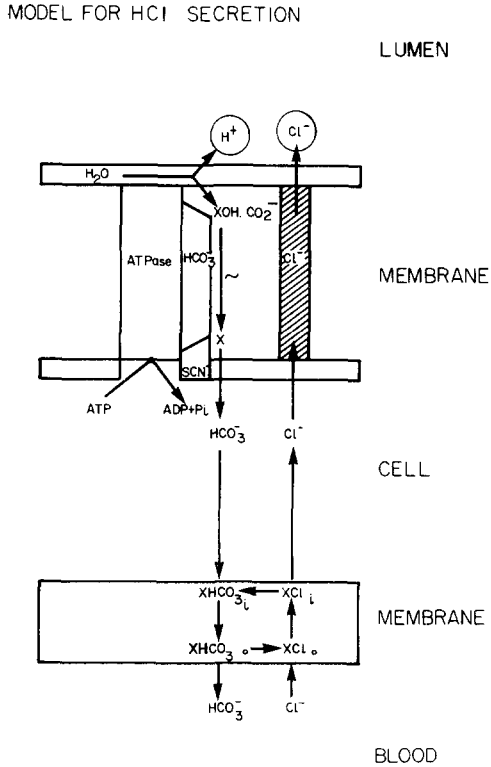


Fig. 17. Hypothetical model for acid secretion incorporating a voltage dependent Cl^- selective channel in parallel to an electrogenic ATPase pump of the Mitchell Type I, responsible for H^+ production. The net effect of these two systems is the secretion of HCl, and the intracellular production of HCO_3^-, which is dissipated by a Cl^-–HCO_3^- forced exchange mechanism in the basal membrane.

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