Nystatin as a Probe for Investigating the Electrical Properties of a Tight Epithelium

SIMON A. LEWIS, DOUGLAS C. EATON, CHRIS CLAUSEN, and JARED M. DIAMOND

From the Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550. The present address of Dr. Clausen and Dr. Diamond is the Department of Physiology, University of California at Los Angeles, Los Angeles, California 90024.

ABSTRACT We show how the antibiotic nystatin may be used in conjunction with microelectrodes to resolve transepithelial conductance $G_t$ into its components: $G_a$, apical membrane conductance; $G_{bl}$, basolateral membrane conductance; and $G_j$, junctional conductance. Mucosal addition of nystatin to rabbit urinary bladder in Na$^+$-containing solutions caused $G_t$ to increase severalfold to ca. 460 $\mu$mho/$\mu$F, and caused the transepithelial voltage $V_t$ to approach +50 mV regardless of its initial value. From measurements of $G_t$ and the voltage-divider ratio as a function of time after addition or removal of nystatin, values for $G_a$, $G_{bl}$, and $G_j$ of untreated bladder could be obtained. Nystatin proved to have no direct effect on $G_{bl}$ or $G_j$ but to increase $G_a$ by about two orders of magnitude, so that the basolateral membrane then provided almost all of the electrical resistance in the transcellular pathway. The nystatin channel in the apical membrane was more permeable to cations than to anions. The dose-response curve for nystatin had a slope of 4.6. Use of nystatin permitted assessment of whether microelectrode impalement introduced a significant shunt conductance into the untreated apical membrane, with the conclusion that such a shunt was negligible in the present experiments. Nystatin caused a hyperpolarization of the basolateral membrane potential in Na$^+$-containing solutions. This may indicate that the Na$^+$ pump in this membrane is electrogenic.

INTRODUCTION

Even the simplest epithelium consists of three types of membranes that generally differ in resistance and in relative permeability characteristics: apical and basolateral cell membranes and junctions. Several studies have sought to resolve the properties of these three membranes by cable analysis and measurement of voltage divider ratios (Frömter and Diamond, 1972; Reuss and Finn, 1974; Lewis et al., 1976), by analysis of transepithelial diffusion potentials (Koefoed-Johnsen and Ussing, 1958; Sachs et al., 1970; Barby et al., 1971), or by use of amiloride to vary apical membrane conductance alone (Reuss and Finn, 1974; Lewis et al., 1976). However, the validity of each of these three methods rests on assumptions. In particular, the first and third methods assume that apical membrane...
conductance is unaffected by impalement with a microelectrode, an assumption that has been questioned by Lindemann (1975).

The present paper describes a simple new method for resolving the three membrane conductances as well as for assessing Lindemann's objection. The method involves using the polyene antibiotic nystatin to decrease the apical membrane resistance to a very low value.

Our experiments have been carried out in rabbit urinary bladder, a tight epithelium whose properties were described by Lewis and Diamond (1976) and by Lewis et al. (1976). Briefly, this preparation has three cell layers, of which the one nearest the mucosal solution generates all the short-circuit current (I_{sc}) and is the site of virtually all the transepithelial resistance. Throughout this paper the terms "apical membrane" and "basolateral membrane" will be understood to refer only to the cell membranes of this transporting layer. I_{sc} arises entirely from active Na⁺ transport. Junctional resistance is much higher than transcellular resistance. Transepithelial conductance increases with I_{sc} because of a transport-related conductance channel in the apical membrane.

MATERIALS AND METHODS

Rabbit urinary bladders were dissected and mounted as described by Lewis and Diamond (1976). The chamber used by Lewis et al. (1976) for microelectrode studies differed from that used by Lewis and Diamond (1976) for gross electrode studies in ways that introduced some edge damage. Therefore, we used a new microelectrode chamber design (Fig. 1, from Lewis, 1977) that eliminates edge damage and harmful hydrostatic pressure differences across the membrane while facilitating solution changes and temperature regulation. Ag-AgCl electrodes on either side of the preparation were used for continuous

![Figure 1](image-url)
monitoring of transepithelial voltage ($V_t$), $I_{re}$, transepithelial conductance ($G_t$), and effective transepithelial capacitance ($C_t$; see Lewis and Diamond, 1976). The open top of the chambers allowed ready access of glass microelectrodes to the mucosal (apical) surface of the epithelium. A light source and Wild dissecting microscope placed at opposite ends of the chamber facilitated positioning the microelectrodes and monitoring cell viability.

Microelectrodes were of the fiber-filled type, pulled on an Industrial Associates horizontal pipet puller. They were filled with 3 M KCl and were rejected if their resistance was below 20 MΩ. Remote, fine positioning of microelectrodes within an accuracy of ±1 μm was achieved through a Stoelting hydraulic microdrive (Stoelting Co., Chicago, Ill.). The preparation rested on a steel plate, shock-mounted on tennis balls on a heavy table.

The voltage-divider ratio $\alpha$ (ratio of apical to basolateral membrane resistance, $R_{ap}/R_{bl}$) was measured by applying a transepithelial current pulse (Anapulse stimulator, W-P Instruments, Inc., New Haven, Conn.) and simultaneously recording $V_t$ and the voltage between the cell interior and the serosal solution (i.e. the basolateral membrane potential $V_{bl}$) on a Newport two-channel digital printer (Newport of North America, Inc., Villanova, Pa.) interfaced with two Weston digital voltmeters (Weston Instruments, Newark, N. J.). The printer was triggered by the stimulator to print $V_t$ and $V_{bl}$ during and after an applied current pulse. The lapsed time between measuring the voltage deflections and the base-line values was 600 ms. A 2-s transepithelial current pulse was applied at 6-s intervals during addition of nystatin to the mucosal solution, and at 30-s intervals during wash-off of nystatin. All voltages were measured to ±100 μV. This protocol permits accurate monitoring of spontaneous $V_t$, spontaneous $V_{bl}$, $G_t$, and $\alpha$ as a function of time.

For reasons discussed by Lewis and Diamond (1976), $G_t$ was normalized not to chamber area but to $C_t$ measured in the presence of amiloride, so that units of conductance are μmho/μF. Under these circumstances a $C_t$ value of 1 μF corresponds approximately to an apical surface area of 1 cm². $V_t$ is given as the potential of the serosal solution with respect to that of the mucosal solution, the apical and basolateral membrane potentials $V_a$ and $V_{bl}$ as the potential of the cell interior with respect to that of the mucosal and serosal solution, respectively.

The composition (mM) of the usual bathing solution ("Na⁺ solution") was: 111.2 NaCl; 25 NaHCO₃; 5.8 KCl; 2.0 CaCl₂; 1.2 MgSO₄; 1.2 KH₂PO₄; and 11.1 glucose, buffered at pH 7.4 and gassed with 95% O₂-5% CO₂. The serosal solution always had this composition. The mucosal solution was sometimes changed to "K⁺ solution" or "choline solution," which differed by equimolar replacement of NaCl-NaHCO₃ with these salts, all other solutes remaining at the same concentration. Under some circumstances, in an attempt to mimic the intracellular solution, a "K-sulfate solution" was used. Its composition (mM) was: 58.5 K₂SO₄; 25 KHC₂O₃; 10 Ca (methanesulfonate); 1.2 MgSO₄; 1.2 KH₂PO₄; 11.1 glucose; 80 sucrose. Nystatin (Sigma Chemical Co., St. Louis, Mo.) dissolved in methanol at a concentration of 5 mg/ml (88,000 U/ml) was added in 1-μl portions to the mucosal solution. The final nystatin activity was 120 U/ml except in experiments to determine the dose-response curve. Except in the experiments described in Results (Dose-Response Curve and Effect of Nystatin Added to the Serosal Solution), nystatin was added only to the mucosal solution. Bathing solution temperature was maintained at 37°C. Errors are given as standard errors of the mean.

**RESULTS**

**Nystatin Effect on $G_t$ and $V_t$**

Mucosal addition of nystatin to a final activity of 120 U/ml rapidly caused $G_t$ to increase in either Na⁺ or K⁺ mucosal solutions, $V_t$ to decrease in Na⁺ solutions,
and $V_t$ to increase in K$^+$ solutions. For example, after 160 s in the experiment of Fig. 2, nystatin in Na$^+$ solution caused $V_t$ to decrease from +81 to +49 mV (i.e. serosal solution positive) and $G_t$ increase from 250 to 470 $\mu$mho/\mu F, but in K$^+$ solution caused $V_t$ to increase from +13 to +38 mV and $G_t$ to increase from 49 to 400 $\mu$mho/\mu F. If bladders were exposed to mucosal nystatin in Na$^+$ solutions for over 200 s, $V_t$ continued to decrease, $G_t$ continued to increase, and microscopic observation revealed cell swelling and desquamation. With nystatin treatment in K$^+$ solutions, $V_t$ and $G_t$ reached plateau values, and cell swelling was not seen.

![Graph showing time course of spontaneous transepithelial potential ($V_t$) and transepithelial conductance ($G_t$) after addition of 120 U/ml nystatin (at arrow) to the mucosal solution of rabbit bladder in Na$^+$ solution or in K$^+$ solution. Note that with time $V_t$ and $G_t$ for both conditions converge. Rectangular steps in $V_t$ are caused by passing a brief transepithelial current pulse (9.29 $\mu$A and 3.93 $\mu$A for Na$^+$ and K$^+$ solutions, respectively), so that the height of the steps is proportional to transepithelial resistance = 1/$G_t$.]

**Figure 2.** Time course of spontaneous transepithelial potential ($V_t$) and transepithelial conductance ($G_t$) after addition of 120 U/ml nystatin (at arrow) to the mucosal solution of rabbit bladder in Na$^+$ solution or in K$^+$ solution. Note that with time $V_t$ and $G_t$ for both conditions converge. Rectangular steps in $V_t$ are caused by passing a brief transepithelial current pulse (9.29 $\mu$A and 3.93 $\mu$A for Na$^+$ and K$^+$ solutions, respectively), so that the height of the steps is proportional to transepithelial resistance = 1/$G_t$.

**Locus of the Nystatin Effect**

Is the rapid increase in $G_t$ upon mucosal addition of nystatin due solely to an increase in apical membrane conductance $G_a$, or does nystatin also increase basolateral membrane conductance $G_{bl}$ and junctional conductance $G_j$? To answer this question, we used an intracellular microelectrode to monitor the voltage-divider ratio $\alpha$ after addition of nystatin to the mucosal solution. Fig. 3 A depicts the change in log (1/$\alpha$) = log ($G_a$/$G_{bl}$) and in log $G_t$ as a function of time after addition of nystatin (Fig. 3 A *left*) and after removal of nystatin (Fig. 3 A *right*). It is apparent that not only $G_t$ but also (1/$\alpha$) were increased by nystatin. These effects were reversed by removal of nystatin, but reversal of the effect was about 60 times slower than onset of the effect (note difference in time scale of Fig. 3 A between left and right).
Since $\alpha$ measurements are independent of $G_1$, and since nystatin increases both $G_t$ and $1/\alpha$, the nystatin effect must at least partly involve an increase in $G_a$. If nothing more than an increase in $G_a$ were involved, then Fig. 3A would imply that nystatin increased $G_a$ 200-fold, since $G_a/G_{bl}$ goes from 0.05 to 10. However, more complex interpretations are also possible: nystatin might also alter $G_1$, an effect that would not be detectable by $\alpha$ measurements; and a change in $G_{bl}$ might be masked by a larger increase in $G_a$. For example, nystatin added to the mucosal solution could affect $G_{bl}$ in two ways: nystatin might enter the cell, reach

![Figure 3A](image_url)

**Figure 3A.** Above, logarithms of the inverse voltage-divider ratio $1/\alpha$ (○) and of $G_t$ (●) as a function of time after addition of 120 U/ml nystatin to the mucosal solution (left), or after removal of nystatin from the mucosal solution (right). Note different time scales on left and right. Fig. 3B below, plots of $G_t$ vs. $(1 + a)^{-1}$ from the data of Fig. 3A left and right. The straight lines were obtained from linear regression analysis and yield values of $G_1$ and $G_{bl}$ as discussed in the text.

the basolateral membrane, and increase $G_{bl}$ directly; or a change in $G_a$ might permit sufficient ion or water flow across the apical membrane to alter intracellular ion concentrations or cell volume, thereby indirectly increasing or decreasing $G_{bl}$. If these effects were significant, we might expect an initial rapid increase in $G_t$ and $G_a/G_{bl}$ as nystatin reached the apical membrane, followed by a slower increase in $G_t$ and change in $G_a/G_{bl}$ as nystatin began to affect $G_{bl}$. In fact, in nystatin-containing Na$^+$ solutions there is a decrease in $G_a/G_{bl}$ at long times (>5 min) that could be interpreted as nystatin entry into the cell, causing a delayed increase in $G_{bl}$. This observation need not, however, indicate nystatin entry into the cell, since the increase in intracellular Na$^+$ expected in nystatin-containing Na$^+$ solutions could cause cell swelling or directly affect $G_{bl}$ (Bezanilla and Armstrong, 1972). To assess the possibility of nystatin entry requires an experiment in which the ionic composition of the mucosal solution approximates the
intracellular composition, so that an indirect effect of nystatin on G_b because of changes in intracellular composition could be minimized. Treatment with nystatin in K⁺ solutions is such an experiment. We found that in nystatin-containing K⁺ solutions, G_b and V_b remained constant for at least 10 min, and G_a/G_b did not change further during this time after reaching a plateau in about 2 min. This implies that nystatin does not reach the basolateral membrane.

A more quantitative assessment of any nystatin effects on G_a and G_j is possible as follows. From a simplified equivalent circuit of an epithelium, where the junctional conductance G_j is in parallel with the series combination of G_a and G_b (Lewis et al., 1976; Lewis, 1977), G_t may be related to the three individual conductances as:

\[ G_t = G_j + G_a G_b/(G_a + G_b). \] (1)

Substituting \( G_a = G_b/\alpha \) into Eq. (1) yields:

\[ G_t = G_j + G_b [1/(1 + \alpha)]. \] (2)

If brief exposure to nystatin does not affect G_b (as suggested by the preceding paragraph), and if G_j is also unaffected, then Eq. (2) means that a graph of G_t vs. \( (1 + \alpha)^{-1} \) should yield a straight line with a slope G_b and ordinate intercept G_j. From Fig. 3 A, the sets of \( (G_t, 1/\alpha) \) values at different times have been replotted as graphs of G_t vs. \( (1 + \alpha)^{-1} \) in Fig. 3 B. For addition of nystatin (left) the slope G_b is 570 \( \mu \text{mho}/\mu\text{F} \) \( (R_b = 1,800 \Omega \cdot \mu\text{F}) \), and the intercept G_j is 12 \( \mu \text{mho}/\mu\text{F} \) \( (R_j = 83,000 \Omega \cdot \mu\text{F}) \), with a correlation coefficient of 1.00. For wash-off of nystatin (Fig. 3 B right) G_b is 550 \( \mu \text{mho}/\mu\text{F} \) \( (R_b = 1,800 \Omega \cdot \mu\text{F}) \) and G_j is 46 \( \mu \text{mho}/\mu\text{F} \) \( (22,000 \Omega \cdot \mu\text{F}) \), with a correlation coefficient of 0.99. Table I summarizes the results of seven such experiments. These excellent fits to straight lines (in which the data points displayed no consistent deviation from linearity), and the agreement between the two G_b values, mean that brief exposure to nystatin does not measurably affect G_b or G_j but only G_a. However, G_j estimated from wash-off of nystatin (Fig. 3 right) is greater than G_j estimated from addition of nystatin (Fig. 3 left). This discrepancy is probably caused by secondary delayed effects on G_j due to some cell swelling, lysis, or desquamation, rather than by incorporation of nystatin into junctional complexes.

Additional evidence that corroborates our hypotheses that (a). G_b does not change significantly after addition of nystatin; (b). G_a and G_b are not directly related; and (c) the mucosal solution composition does not directly affect the

1 Estimates of G_a in rabbit urinary bladder using AC analysis \( (670 \pm 100 \mu \text{mho}/\mu\text{F}, n = 9, \text{Clausen, Lewis and Diamond, personal communication}) \) and nystatin \( (790 \pm 70 \mu \text{mho}/\mu\text{F}, n = 7, \text{Table I}) \) are in good agreement \( (t\text{-test yields } P > 0.2) \). In another tissue, rabbit descending colon, the estimate for G_b using nystatin (Dr. N. Wills, personal communication) and amiloride (Schultz, Frizzell and Nellans, 1977; Dr. N. Wills, personal communication) are likewise in good agreement. These two lines of evidence support our contention that mucosal nystatin does not alter the basolateral membrane conductance. However, if one considers the difference of the mean G_a between the methods \( (18\%) \), one can predict the effect that this difference will have on the I-V relationship and relative selective permeability of the basolateral membrane. The I-V relationship, if linear (see Results), will have a slope change of 18%. The relative selective permeability will: (a) approach unity if nystatin increases conductance in a nonselective manner; (b) increase, decrease, or remain unchanged if nystatin increases the conductance of only one ion; or (c) remain unchanged if nystatin increases the conductance of all ions proportionately.
basolateral potential or conductance can be obtained from the following experiment. By examining Fig. 4 the relationship between $G_0$ and $(1 + \alpha)^{-1}$ for apical solutions containing high Na$^+$ ("Na$^+$ solution" in Materials and Methods) or high K$^+$ ("K-sulfate solution" in Materials and Methods), one finds that there is virtually no difference in $G_{bl}$ for the two solutions and in both cases the

| $I_m$  | $R_a$ | $R_b$ | $R_j$ | $r$  |
|-------|-------|-------|-------|-----|
| $\mu A/\mu F$ | $\Omega/\mu F$ | $\Omega/\mu F$ | $\Omega/\mu F$ |     |
| 0.6   | 20,000 | 1,100 | $\infty$ | 0.98 |
| 0.8   | 45,000 | 1,400 | $\infty$ | 0.998 |
| 1.3   | 20,000 | 1,800 | $\infty$ | 0.998 |
| 1.4   | 22,000 | 1,800 | 80,000   | 0.998 |
| 2.4   | 21,000 | 1,100 | 31,000   | 0.999 |
| 3.0   | 14,000 | 1,000 | $\infty$ | 0.996 |
| 6.5   | 9,400  | 1,100 | $\infty$ | 0.99 |

Membrane resistance values in rabbit bladders in the absence of nystatin, calculated by extrapolation of the time course of nystatin action (addition of nystatin) as in Fig. 3 B. $r$ is the correlation coefficient for straight-line plots as in Fig. 3 B. Each line is based on a different bladder, whose short-circuit current is given in the first column.

Intercepts suggest an infinitely small conductance for $G_j$. This demonstrates that the $\alpha$ values produced by nystatin are not dependent upon the apical solutions if they contain permeable cations and that, at least for low transport rates (<3 $\mu A/\mu F$), $G_{bl}$ does not depend upon the composition of the apical solution.

**Basolateral I-V Relation**

Before or after nystatin treatment, $R_j$ is greater than $(R_a + R_b)$, so that most applied current flows across the cells rather than across the junctions. However,
$R_{bl}$ after nystatin is much greater than $R_a$, so that the transepithelial I-V relation of nystatin-treated bladders mainly reflects properties of the basolateral membrane. We measured this I-V relation in two bladders and found it to be linear over the range $-155$ mV to $+40$ mV (potential of cell interior with respect to serosal solution).

**Selectivity of Nystatin-Doped Apical Membrane**

We assessed the ionic selectivity of the nystatin channel in the apical membrane by comparing nystatin-induced changes in $V_a$ and $V_{bl}$ in Na$^+$, K$^+$, and choline mucosal solutions (Fig. 5).

As discussed by Lewis et al. (1976), $V_a$ in Na$^+$ solutions in the absence of nystatin varies greatly with changes in $I_{sc}$ and with changes in the Na$^+$ conductance $G_{Na}$ of the apical membrane. Lewis et al. (1976) found a range of $V_a$ from a negative value of $-40$ mV in bladders with low transport rates to values near zero in bladders with high transport rates. Subsequently, we have found reversed $V_a$ values as high as $+50$ mV in bladders with very high $I_{sc}$. Nevertheless, Fig. 6 shows that, independent of the initial value and even initial sign of $V_a$ (and hence independent of $G_{Na}$), nystatin changed $V_a$ in Na$^+$ solutions to approximately the same value: on the average, $-6.7 \pm 2.5$ mV ($n = 5$, cell interior negative). Evidently, nystatin completely masks the permeability characteristics of untreated membrane, and $V_a$ reflects only the properties of the nystatin channel. This is as expected from the great increase in $G_a$ caused by nystatin.
Nystatin caused $V_a$ in $K^+$ solutions to depolarize to $-5.8 \pm 3.2$ mV ($n = 3$; Fig. 5), a value essentially the same as that in $Na^+$ solutions, suggesting that the nystatin channel has similar permeability to $Na^+$ and $K^+$. In choline solutions $V_a$ of untreated bladders was larger than in $Na^+$ or $K^+$ solutions, and $V_a$ hyperpolarized rather than depolarized with nystatin to $-75 \pm 9$ mV ($n = 3$). This suggests that the native apical membrane has low permeability to choline, and that the permeability of the nystatin channel is much lower to choline than to $K^+$, $Na^+$, and possibly $Cl^-$. The large $V_a$ of nystatin-treated bladder in choline solutions is presumably a $K^+$ and/or $Cl^-$ diffusion potential. Independent of mucosal solution composition, $G_a$ increased to approximately 7 mmho/$\mu$F in the first 2 min after exposure to nystatin. Thereafter, however, $G_a$ in choline solutions began to decrease, due possibly to a decrease in intracellular ($K^+$) and hence in extracellular ($K^+$) in the unstirred layer immediately adjacent to the apical membrane. After 10 min in nystatin-containing choline solutions, $V_a$ depolarized towards zero.

In contrast to the large effects of solution composition and nystatin on $V_a$, effects on $V_{bl}$ were much slighter (Fig. 5). $V_{bl}$ was initially $-53 \pm 3.1$ mV ($n = 5$) in $Na^+$ solutions, $-53 \pm 6$ mV ($n = 3$) in choline solutions, and $-45 \pm 5$ mV ($n = 3$) in $K^+$ solutions. Nystatin caused no immediate change in $V_{bl}$ in choline solutions (but a depolarization towards zero after 10 min), a slight depolarization of a few millivolts in $K^+$ solutions, and invariably a hyperpolarization of 10 mV in $Na^+$ solutions. The significance of these observations is considered in the Discussion.

**Dose-Response Curve**

All results discussed so far were obtained at a nystatin dose of 120 U/ml. Fig. 7 is a dose-response curve of log ($1/\alpha$) against log nystatin activity, in $Na^+$ solutions. $\alpha$ was measured 2 min after addition of nystatin, because this period produced the maximum reversible response for the maximum nystatin dose of 120 U/ml in $Na^+$ solutions. Since $G_{bl}$ is unaffected by brief exposure to nystatin (see above), the ordinate of Fig. 7 is actually proportional to log $G_a$. It is apparent that the
lowest dose tested, 40 U/ml, scarcely affected log $G_a$, but that for higher doses the curve rises steeply with a slope of 4.6.

**Effects of Nystatin Added to the Serosal Solution**

All results discussed so far were obtained with nystatin added only to the mucosal solution. The effects of nystatin added only to the serosal solution differed in four respects.

1. The nystatin-dependent increase in $G_t$ was much smaller. This is expected, since $G_t$ of untreated bladder is primarily determined by $R_a$ rather than by $R_{bl}$, and the apical membrane is not accessible to serosally added nystatin.

2. $\alpha (= G_{bl}/G_a)$ increased rather than decreased, because serosally and mucosally added nystatin increase $G_{bl}$ and $G_a$, respectively.

3. The time course of nystatin action was up to 60 times longer, because the connective tissue and two cell layers on the serosal surface constitute a significant unstirred layer and perhaps a nystatin sink, whereas the transporting layer is in direct contact with the mucosal solution.

4. Effects of serosally added nystatin were poorly reversible, whereas those of mucosally added nystatin were readily reversible. Unstirred layers and nystatin sinks on the serosal surface are again the probable explanation.

**DISCUSSION**

We consider four problems: comparison of nystatin effects on urinary bladder and on bilayers; reassessment of microelectrode impalement artifacts; estimates of $R_a$, $R_{bl}$, and $R_i$; and reassessment of basolateral membrane properties.

**Comparison with Bilayers**

The epithelium on which the polyene antibiotics, nystatin and amphotericin B, were first tested was toad urinary bladder (Lichenstein and Leaf, 1965; Sharp et
The effects of these antibiotics on artificial bilayers were subsequently studied in detail. The effects of nystatin on apical membrane of mammalian urinary bladder resemble those of nystatin and amphotericin B on bilayers.

i. Bilayers must contain sterols for a maximal antibiotic-induced conductance change (Andreoli, 1973; Finkelstein and Holz, 1973). This requirement is satisfied by mammalian bladder cell membranes, which have a molar cholesterol:phospholipid ratio of 0.6 (Ketterer, et al., 1973).

ii. Nystatin added to one side of a bilayer imparts cation selectivity, while nystatin added to both sides imparts anion selectivity (Marty and Finkelstein, 1975). In our experiments nystatin could be added only to the extracellular side of the apical membrane, and the resulting selectivity was probably cationic (estimated $P_{\text{Cl}}/P_{\text{K}} = 0.28$; see below).

iii. The pore size of amphotericin B in layers is ca. 7 Å, and that of nystatin is similar. Molecules with a Stokes-Einstein radius greater than 4 Å are virtually impermeant (Holz and Finkelstein, 1970). The low permeability of nystatin-treated rabbit bladder to the large cation choline fits this pattern.

iv. The dose-response curve of nystatin has as steep a slope in bilayers (Finkelstein and Holz, 1973) as in rabbit bladder and red cells (Cass and Dalmark, 1973). This slope may indicate that several nystatin molecules are required to form one channel.

v. The steady-state conductance produced in bilayers by a given nystatin dose is not reproducible among nystatin preparations (Marty and Finkelstein, 1975). We found that effects on rabbit bladder conductance were consistent within a batch of nystatin but not among batches.

vi. Nystatin-induced conductance in bilayers varies inversely with temperature (Cass et al., 1970). We did not investigate the question for two reasons: rabbit urinary bladder is very sensitive to any temperature shift from 37°C; and nystatin-induced apical conductance at 37°C is already so high (7 mmho/µF) that any increase in conductance at lower temperatures would be difficult to quantitate.

Estimates of Chloride Permeability Induced by Nystatin

Because of the lack of information concerning the intracellular ion concentrations, quantitating the anionic permeability of the nystatin channels in the apical membrane is extremely difficult. However, by evoking certain assumptions we can calculate an approximate value for $P_{\text{Cl}}/P_{\text{K}}$ as well as an estimate for intracellular [Cl\(^-\)]. The value for intracellular [K\(^+\)] of 150 mM was estimated from the zero potential intercept of a plot of the serosal potassium concentration vs. basolateral membrane potential if one assumes that the basolateral membrane potential is close to the potassium diffusion potential; (Lewis and Eaton, unpublished observations). The potential for nystatin-treated apical membrane was $-5.8 \pm 3.2$ mV and $-74.4 \pm 9.2$ mV (cell interior negative) when the mucosal solution was KCl-KHCO\(_3\) Ringer's and choline Cl-choline HCO\(_3\) Ringer's, respectively. Since $R_\text{f}$ has a value much greater than $R_a$ or $R_{bl}$, we have not corrected for the influence of $R_\text{f}$ on $V_a$ or $V_{bl}$. If one assumes $P_{\text{choline}}$ to be negligible compared to $P_{\text{K}}$, and intracellular [Na\(^+\)] to be much lower than
intracellular [K+] in choline or K+ solutions (which are Na+-free), the Goldman-Hodgkin-Katz equation for the apical membranes becomes

\[ V_a = \frac{R T}{2F} \ln \frac{[K_o] + (P_{Cl}/P_K)[Cl_i]}{[K_i] + (P_{Cl}/P_K)[Cl_o]} \]  

(3)

Substituting the measured values for \( V_a \) in K+ and choline solutions (−5.8 and −75 mV, respectively), the values for \([K_o]\) and \([Cl_o]\) in these solutions (see Materials and Methods), and the estimate \([K_i] = 150 \text{ mM}\) into Eq. (3) yields two equations in the two unknowns \(P_{Cl}/P_K\) and \([Cl_i]\). The result is \(P_{Cl}/P_K = 0.28\), \([Cl_i] = 15 \text{ mM}\) and \([K_i] = 150 \text{ mM}\).

This \(P_{Cl}/P_K\) value agrees well with the value of 0.15 obtained for nystatin-treated Aplysia cell bodies (Russell et al., 1976). The value \([Cl_i] = 15 \text{ mM}\) agrees well with the value of 16 mM calculated from the spontaneous Vbl value of −53 mV on the assumption that Cl− is in electrochemical equilibrium across the basolateral membrane.

**Microelectrode Impalement Artifacts**

Lindemann (1975) has stressed the risk of errors in microelectrode study of epithelial cells. If the cell is small, microelectrode impalement may introduce a significant shunt conductance in parallel with the native membrane conductance. Such a shunt conductance in one cell will scarcely alter the conductance of the whole epithelial sheet but may seriously underestimate the voltage and resistance of the impaled membrane, and hence may cause to be in error the voltage-divider ratios and resistance values derived from cable analysis. How significant is this effect in our experiments?

This question can be answered by examining the graphs of \(G_t\) vs. \((1 + \alpha)^{-1}\) in Fig. 3 B. In native membrane or just after addition of nystatin, \(R_a\) and \(\alpha\) are high. A significant shunt in the apical membrane caused by a microelectrode would cause the measured \(\alpha\) to be artifactually low and the measured \((1 + \alpha)^{-1}\) to be artifactually high. As \(R_a\) and \(\alpha\) rapidly decrease with time after addition of nystatin (\(R_a\) decreasing to ~1% of its initial value), \(G_a\) becomes completely dominated by the nystatin channel, and any contribution from a microelectrode shunt is negligible. Thus, a microelectrode shunt would cause the graphs of Fig. 3 B to deviate below linearity at low \((1 + \alpha)^{-1}\) values (because the plotted abscissa values would exceed the true values for low \((1 + \alpha)^{-1}\)). In fact, no such deviation from linearity is apparent in Fig. 3 B. Any microelectrode shunt conductance must be considerably less than the native membrane conductance. Naturally, this conclusion applies only to the present experiments and gives no assurance that microelectrode shunt conductance is negligible in other epithelia impaled with other microelectrodes.

**Values for \(R_a\), \(R_{bl}\), and \(R_j\)**

Table I summarizes values for \(R_a\), \(R_{bl}\), and \(R_j\) in seven untreated rabbit bladders, determined by extrapolation of \(G_t\) and \(\alpha\) values after addition of nystatin as in Fig. 3 B. \(R_j\) values are consistently very high, as found by Lewis et al. (1976). \(R_a\) values vary inversely with \(I_{me}\), also as found by Lewis et al. (1976). However, our \(R_{bl}\) values are consistently several times lower than those of these investigators.
just as our $\alpha$ values are consistently higher than theirs. We attribute this difference to our improved chamber design and microelectrode techniques, hence more accurate measurements of $\alpha$.

**Reassessment of Basolateral Membrane Function**

By reducing apical membrane resistance to a low value, nystatin may offer a powerful tool for studying the basolateral membrane. Fig. 5 illustrates such a use. Nystatin consistently causes $V_{\text{m}}$ to hyperpolarize by 10 mV in Na+ solutions but not in K+ or choline solutions. This hyperpolarization must somehow result from the increase in [Na]$_i$ expected after addition of nystatin in Na+ solutions (Cass and Dalmark, 1973; Russell et al., 1976). $V_{\text{m}}$ appears in large part to be a K+ diffusion potential. The hyperpolarization actually observed cannot be an Na+ diffusion potential since the Na+ gradient is in the wrong direction, but could arise from either the Na+ pump in the basolateral membrane being electrogenic or an increased K+ gradient across the basolateral membrane formed by an electrically silent Na+-K+ exchange pump. Further work employing nystatin is required to differentiate between these possibilities.

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