ANTIBIOTIC INTERACTION WITH MODEL MEMBRANES[1]

STEPHEN C. KINSKY
Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri

INTRODUCTION

This paper will review some of the more recent investigations concerned with the interaction of antibiotics and model membranes. These studies serve as mute testimony to the principle that acquisition of new data and concepts usually follows the introduction of new tools. They also indicate the usefulness of certain drugs in elucidating problems of membrane structure and function. In this sense, the study of membranes has finally caught up with investigations on nucleic acids, proteins, and bacterial cell walls. Undoubtedly, the availability of agents (primarily, antibiotics) that interfere with the biosynthesis and function of these macromolecules underlies much of the phenomenal progress in each of these areas.

There are numerous antibiotics, as well as other drugs, for which various membrane systems have been postulated to be the site of action. A forthcoming article by F. M. Harold provides comprehensive coverage of this subject (1). Accordingly, the scope of the present review was limited to avoid duplication. We shall primarily consider the interaction of two classes of antibiotics, the polyenes and macrocyclics, with three types of artificial, or reconstituted, membrane prototypes. Slightly more emphasis will be placed on the polyenes because the effects of the macrocyclic antibiotics on natural membranes (particularly, mitochondria) is so extensively discussed in (1) that we have found it unnecessary to review this subject in any detail.

The three types of model membranes that have been employed in these

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investigations are popularly designated monolayers, bilayers, and liposomes. "Monolayers" (lipid monomolecular layers) should require no futher comment and, for the purpose of this article, will refer to films of lipid oriented at the air/water interface. "Bilayer" membranes are thin films formed across a small orifice in a septum separating two aqueous phases, and are so called because their transverse dimensions approximate twice the thickness of monolayers. These films are too thin to reflect ordinary light and hence appear black when viewed against a black background. For this reason, they are also known as "secondary black lipid membranes" (abbreviated BLM). The properties of bilayers have been recently reviewed by Henn & Thompson (2). Numerous electron microscopic studies have shown that the predominant structure of phospholipid dispersions in water has a lamellar appearance. This pattern has generally been interpreted as an array of concentric bimolecular lipid layers separated from each other by an aqueous compartment. "Liposomes" are dispersions of lipids prepared under conditions which favor trapping of various marker compounds within these aqueous regions. They have also been referred to as lipid "structures", "spherules", "liquid crystals", "smectic mesophase" and "Bangasomes" (in deference to their discoverer). Lack of space prevents a discussion of the theoretical and experimental foundations upon which these models are based but, fortunately, a recent article by Bangham (3) covers these points in detail. Nevertheless, a brief comparison of the more important features of these different models would seem appropriate to emphasize that each has certain advantages and disadvantages.

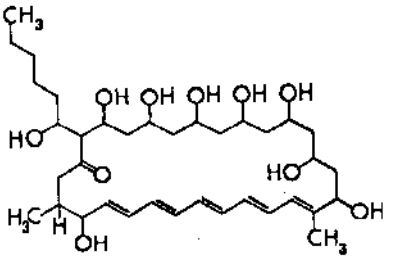
Composition.—The model membranes are generated from either phospholipids alone, or a mixture of phospholipids and other amphipathic compounds (e.g., cholesterol, dicetyl phosphate). During preparation of monolayers and liposomes, the organic solvents (e.g., benzene, chloroform) are removed completely. This is not the case with bilayers, and solvents such as n-decane or n-tetradecane are present in these films as hydrocarbon "fillers". The molar ratio of aliphatic hydrocarbon to phospholipid may be quite high [between 1:1 and 10:1 (2)] and, accordingly, bilayers are properly considered to be hydrocarbon films stabilized by phospholipid (3). Unfortunately, at the present time, it is not possible to predict precisely the chemical composition of a bilayer membrane from the composition of the lipid mixture used in its manufacture. This disadvantage is probably not shared by the monolayers and liposomal membranes with which this review is concerned.

Parameters.—Antibiotic interaction with monolayers is generally detected by a change in surface pressure or interfacial potential or both. Such changes, if observed, may indicate either penetration of the monolayer by the antibiotic or a change in the spatial orientation of the lipids. Monolayers may restrict gas exchange between aqueous substrate and air phase, and are perhaps appropriate models for the very few biological membranes that are found at air/water interfaces. However, to our knowledge, none of the anti-

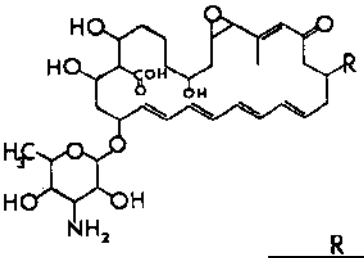
biotics have been tested for their effect on the permeability of lipid monolayers to gases.

Bilayers, and also liposomes (see below), are obviously more suitable models for the majority of membranes that separate two aqueous phases. A common method for determining the permeability characteristics of bilayers is to follow the migration of solutes (radioactive as well as nonradioactive) from one of the aqueous chambers to the other under the influence of a concentration gradient. This procedure requires that samples for analyses be removed under conditions that do not disturb the membrane. This is not necessary when determining the permeability of ions by measurements of electrical resistance and transmembrane potential. The fact that these parameters can be investigated with bilayers confers a unique advantage to this model membrane because many of the more interesting properties of natural membranes concern their electrical activity. The electrical resistance is, of course, the resistance to ion flow and is thus an inverse function of the membrane's permeability to both cations and anions. The individual contributions can be expressed as "transference numbers", which are defined as the ratio of current carried by a single ionic species to the total current; the sum of the transference numbers therefore equals one. Transference numbers, or related expressions, have been determined by several procedures: radioactive tracers, specific ion electrodes, and transmembrane potential measurements. The latter method depends on the fact that when permeability of the cation is different from the anion, diffusion processes will transfer more net charges to one side of the membrane and an equilibrium potential will result [see Andreoli et al. (68a) for a discussion of this technique]. However, transference numbers can be misleading. For instance, if an antibiotic increases membrane permeability equally to all ions, there will be no change in the transference numbers (cf. discussion of gramicidin), and situations can occur in which the transference number decreases as the absolute permeability increases dramatically (cf. discussion of nystatin and amphotericin B).

Of all the model membranes, liposomes are the easiest to generate and, in contrast to the others, measurement of their permeability properties involves equipment that should be present in any modern biological laboratory. This undoubtedly accounts for the rapid acceptance accorded liposomes following their introduction by Bangham and coworkers (4). These investigators originally employed leakage (exchange) of trapped radioactive ions as an index of permeability. Other substances, for which convenient analytical procedures are available (e.g., phosphate and chromate anions, glucose) may also be trapped by liposomes and the release of these markers has been used to study the influence of antibiotics on liposomal permeability. Unlike monolayers or bilayers, liposomes can be readily examined in the electron microscope with negative staining techniques. This is a distinct advantage because negative staining—in contrast to other "fixation" methods—may



FILIPIN



ETRUSCOMYCIN (LUCENSOMYCIN) R = $-(CH_2)_3CH_3$

PIMARICIN R = $-CH_3$

FIG. 1. Primary structures proposed for some polyene antibiotics; see text for appropriate references.

preserve the lipids as though they were in the presence of water (3). Alternative lipid configurations induced by some antibiotics (e.g., lamellar versus micellar) might thus be recognizable.

POLYENE ANTIBIOTICS

Chemistry.—Although numerous polyene antibiotics have been described in the literature [reviewed in (5)], investigations on their mode of action have mainly been conducted with five of these antibiotics. They are: amphotericin B, etruscomycin, filipin, nystatin, and pimaricin. Of the five, complete structures have been proposed for etruscomycin (6), filipin (7), and pimaricin (8), and partial structures have been suggested for nystatin (9) and amphotericin B (10). The formulas shown in Figure 1 serve to emphasize some of the features that all the polyene antibiotics share, as well as some of the more prominent differences. They are all characterized by a macrolide ring containing a number of conjugated double bonds; the latter accounts for the generic name "polyene". However, the number of conjugated double bonds may vary. Thus, etruscomycin (also known as lucensomycin), pimaricin, and nystatin are tetraenes; filipin is a pentaene; amphotericin B is a heptaene. The macrolide ring may also contain a number of different substituents. In particular, the existence of an amino sugar and carboxyl function in etruscomycin, pimaricin, amphotericin B, and nystatin confers amphoteric properties on these antibiotics. In contrast, filipin (which lacks these substituents) is a neutral polyene. The effect of these substituents, and the size of the macrolide ring, on the biological properties of the antibiotics is still not completely clear, although they may be responsible for quantitative differences in potency of the antibiotics. However, the available evidence (reviewed in the following sections) suggests little influence on the

basic mode of interaction with natural and artificial membranes. Molecular models of the polyenes for which structures are available clearly indicate a rigid hydrophobic region (the conjugated double bond chromophore) and a more flexible hydrophilic region in each of the antibiotics. This is consistent with the amphipathic behavior of the polyenes and is, perhaps, the essential feature underlying their mechanism of action.

Filipin deserves some special comments. This antibiotic has been extensively studied with model membranes because it is the most potent of the polyenes for which a complete structure has been suggested. Unfortunately, the formula illustrated in Figure 1 may possibly have to undergo some revision in the light of some recent observations by Bergy & Eble (11). They were able to show that crystalline filipin, which had been considered to be a single chemical entity, can be separated by thin layer and partition chromatography into at least eight components. Three of these components have been isolated and crystallized as apparently pure compounds. These three, designated filipins II, III, and IV, constitute approximately 25, 53, and 18 per cent, respectively, of the original filipin complex. Although filipin IV is more labile than the others, the components are closely related chemically as shown by elemental analysis, ultraviolet, infrared, and NMR spectra. Mass spectrometry of derivatives of filipins II, III, and IV indicates that they have an identical molecular weight (11, note added in proof). Therefore, the reason for the pronounced difference in chromatographic properties must still be established. Under certain conditions, these components can also produce different responses in natural and model membranes (particularly liposomes). Accordingly, in the succeeding sections we shall restrict use of the term, filipin, to the unfractionated antibiotic complex; the individual components will be referred to by Roman numerals as appropriate.

These observations naturally raise the possibility that recent advances in separation techniques might also reveal heterogeneity in the case of the other polyene antibiotics. It should become quite apparent from what follows that our knowledge of the chemistry of these antibiotics has not kept pace with studies of their effects on membranes. Certainly, the polyenes suffer by comparison to the macrocyclic antibiotics for which, as discussed later, there is far more information concerning their structure, spatial orientation of the constituent atoms, etc.

Biological effects.—Although a number of papers dealing with the effects of the polyene antibiotics on a variety of cells and tissues have been published within the past two years, they will not be considered in the present article. These papers convincingly confirm earlier studies that indicate that numerous changes in metabolic patterns can occur as a result of altered cellular permeability, but they provide relatively little information on the molecular basis of polyene action. The previous work (up to ca. 1966) on polyene mechanism has been adequately reviewed (12–15). From these studies, the following general picture of the biological effects of the polyenes has emerged. As a consequence of polyene interaction with the cell membranes

of sensitive organisms, the latter can no longer function as a selective restraining barrier. This is evidenced either by the entrance of material that is normally excluded (e.g., H^+ ions) into the cell, or by the loss of essential cytoplasmic constituents from the cell. The nature of the cytoplasmic material that appears in the medium apparently depends mainly on three factors: the particular antibiotic under investigation, its concentration, and the time of incubation. Usually, small molecular weight compounds (particularly, K^+ ions) appear within a few minutes of antibiotic addition. Over longer periods of time, higher molecular weight compounds (including some proteins and nucleic acids) may leak from the cell. This may also be accomplished by increasing the concentration of the antibiotic.

In the case of those cell types that lack a rigid external capsule or wall (e.g., fungal protoplasts, protozoa, mammalian erythrocytes), these permeability alterations also manifest themselves by the fact that the polyenes produce cell lysis. Studies (16, 17) on the kinetics of, and the critical threshold concentration required to induce, erythrocyte hemolysis indicate that the antibiotics cause increasing damage to the cell membrane in the following order: nystatin < pimaricin < amphotericin B < etruscomycin < filipin. Essentially the same order was established by the extensive investigations of Lampen and coworkers (12, 14) using a completely different criterion (i.e., the ability of various compounds to reverse polyene induced inhibition of yeast respiration and glycolysis). They have proposed an "inverse" correlation between the number of carbon atoms and the extent of membrane damage: larger polyenes with 46 or more carbon atoms (e.g. nystatin, amphotericin B) are generally "weaker" than the smaller antibiotics with 33 to 37 carbon atoms (e.g., filipin). It should be noted that this ranking and classification of the polyenes is based on the degree of membrane damage and not on the relative abilities of the antibiotics to inhibit growth of sensitive organisms.

It is now generally accepted that low concentrations of all the polyenes affect only those cells that contain sterols in their cell membrane, and that this is the basis for the selective toxicity of these antibiotics. It has, in fact, been possible to convert some normally insensitive organisms that lack sterols to polyene sensitivity by cultivating them in a medium supplemented with cholesterol (18–20). This is an extremely important consideration because any influence which the polyene antibiotics have on the properties of model membranes would a priori appear to have more relevance to their mechanism of action if the effect depends on incorporation of sterol into the membrane system. As discussed below, the effect of these antibiotics on natural membranes in many instances can be reproduced with the artificial membranes provided that sterol is present. In this connection, attention is directed to a novel phenomenon reported in a note by Woods & Ahmed (21). They have not only isolated various mutants of *Saccharomyces cerevisiae* that are resistant to nystatin, but also a mutant that apparently is dependent on the presence of nystatin in the medium for growth. The com-

plexity of cross-resistance patterns between nystatin, amphotericin B, and filipin suggests that resistance may have several different origins; for example: possible elaboration of an enzyme system which inactivates one or another of the antibiotics, reduction of the membrane sterol content to a level which is below the critical threshold required for polyene sensitivity (see below), or inaccessibility of membrane sterol to the antibiotics. On the other hand, the phenomenon of nystatin dependence cannot be readily explained on the basis of what is currently known about the polyenes and their interaction with membrane sterols.

Monolayers.—The ability of filipin and nystatin to interact with various lipid monolayers was studied by Demel et al. (22). They observed that both of these antibiotics increased the surface pressure of cholesterol and ergosterol monolayers when the latter were compressed to initial surface pressures above the collapse pressures of the antibiotics. Under identical conditions neither of the antibiotics showed any appreciable interaction with a variety of phospholipids or with a total lipid extract obtained from a polyene-insensitive bacterium. Filipin did, however, increase the surface pressure of monolayers prepared from a total lipid extract of beef erythrocytes (polyene-sensitive). This was apparently caused by the presence of cholesterol because, after separation of the extract into a neutral lipid (primarily the sterol) and phospholipid fraction, it was found that filipin could interact with monolayers prepared from the former, but not the latter, fraction.

These experiments were performed using low concentrations (ca. 10^{-8}M) of filipin and nystatin in the aqueous substrate, corresponding to a ratio of "moles antibiotic injected/moles spread lipid" between 0.09 and 0.14. In a subsequent study, prompted in part by some anomalous results obtained with liposomes (see below), Demel et al. (23) reinvestigated the apparent preferential interaction of the polyenes with sterol monolayers over a wider range of antibiotic concentrations. With cholesterol monolayers at initial surface pressures greater than 12 dynes/cm and at low molar ratios of antibiotic/lipid (ca. 0.11), they observed that filipin produced the largest increase in surface pressure with etruscomycin, amphotericin B, pimaricin, and nystatin following in this order. Because this corresponds to the order in which the antibiotics are able to cause membrane damage to mammalian erythrocytes or the yeast cell, it was suggested that differences in the potencies of the antibiotics may reflect varying affinities of the polyenes for sterols. This, in turn, may indicate an important role for the size of the macrolide ring in determining the affinity between the polyenes and sterols because, as noted by Lampen (12, 14), the antibiotics with more carbon atoms (and, therefore, presumably a larger ring) are generally less effective in causing membrane damage (see preceding sections). At low molar ratios of antibiotic/lipid, the presence of lecithin in mixed monolayers with cholesterol markedly reduced the magnitude of surface pressure increases produced by the polyenes. This "inhibitory" effect of phospholipid was consistent with earlier observations on mitochondria (24) which suggested that:

(*a*) the presence of sterols in natural membranes is a necessary prerequisite for polyene sensitivity, but not a sufficient condition, and (*b*) it is the phospholipid/sterol ratio that may be the significant factor in conferring antibiotic sensitivity to a membrane. The situation was quite different when higher concentrations of antibiotics were employed. At molar ratios of antibiotic/lipid in the range 2 to 20, filipin, pimaricin, and nystatin had an appreciable effect on the surface pressure of pure lecithin monolayers. For a variety of reasons, the authors minimize the physiological significance of polyene interaction with lipids other than sterols. It is clear, however, that the specificity of the polyene antibiotics for sterols is not as "absolute" as originally claimed (*22*), and that preferential interaction with the latter may not occur when high concentrations of the antibiotics are present.

The monolayer studies thus confirm the hypothesis that low concentrations of the polyenes are selectively toxic for organisms that contain sterol in their cell membrane but they do not provide much information on the mechanism by which polyene-sterol interaction produces membrane damage. In this regard, it should be emphasized that the physical basis for the observed surface pressure increases is not yet known. The surface pressure increases may be caused by actual penetration of the antibiotics into the monolayers, or may reflect accumulation of the antibiotics underneath the monolayers with a subsequent spatial reorientation of the sterol molecules, or both. At the present time, monolayers would appear to have their greatest applicability in the elucidation of the chemical and physical factors that favor polyene-sterol interaction (i.e., structure-activity relationships). Two examples may be cited: (*a*) Perhydro-, saponified, and irradiated filipins are far less potent hemolytic agents than the parent antibiotic (*25*) and these derivatives produce a much smaller increase in the surface pressure of cholesterol monolayers than filipin (*23*); (*b*) esterification of cholesterol, as well as introduction of 5 M urea into the aqueous substrate, reduces the magnitude of the surface pressure increase produced by filipin, suggesting a possible role for hydrogen bonds in stabilization of the polyene-sterol complex (*23*).

Liposomes.—The action of the polyene antibiotics on liposomes has been extensively studied by Weissmann & Sessa. In their initial investigation (*26*), they reported that filipin, etruscomycin, amphotericin B, and nystatin (at concentrations between 10^{-4} to 10^{-3}M) could promote the release of various markers (chromate and phosphate ions, glucose) from lecithin liposomes. In the case of amphotericin B and nystatin, the amount of marker released was approximately doubled by prior incorporation of cholesterol or ergosterol into the liposomes. However, inclusion of sterol had no effect on the ability of filipin and etruscomycin to produce loss of marker. Subsequent experiments (*27*) indicated that the absence of a sterol requirement for the action of filipin on liposomes was still manifest when positively charged liposomes (prepared with stearylamine instead of dicetyl phosphate) were employed.

These findings led to the suggestion that there were basic qualitative, and not only quantitative, differences in the mode of action of the several polyene antibiotics: filipin behaving like etruscomycin but different from either amphotericin B or nystatin. This division was in accord with the observations that the larger antibiotics (amphotericin B, nystatin) were less potent than the smaller polyenes (filipin, etruscomycin). Because incorporation of cholesterol was not required for maximal effects of filipin and etruscomycin on liposomes, it was proposed that the more rapid and extensive membrane damage by these antibiotics occurred as a consequence of interaction with membrane phospholipids. However, Weissmann & Sessa also observed that, under their experimental conditions, filipin and nystatin induced leakage of chromate from lecithin-cholesterol liposomes to the same extent; both antibiotics were more effective than amphotericin B, which in turn was more effective than etruscomycin (*26*). This order was at variance with the relative hemolytic potency of the antibiotics (see above). As emphasized by Lampen (*12, 14*), classification of the antibiotics on the basis of size is not clear-cut and the polyenes in fact exhibit a graded series of effects with filipin and nystatin as examples of the two extremes. Of even greater significance, the differential response of the liposomes to filipin and amphotericin B was not in agreement with investigations on the pleuropneumonia-like organism, *Mycoplasma laidlawii,* which had demonstrated that growth in a medium containing cholesterol was required to confer sensitivity to both of these antibiotics (*18, 19*).

These discrepancies necessitated a re-examination of the interaction between the polyenes and model membranes (monolayers and liposomes). The monolayer results (*23*), which have already been described in the preceding section, focused attention on the importance of antibiotic concentration (specifically, the antibiotic/lipid ratio). Using a more sensitive assay for following the response of liposomes, Kinsky et al. (*28*) were able to show that cholesterol incorporation into lecithin liposomes increased both the rate and extent of glucose release induced by low concentrations (10^{-6} to 10^{-5}M) of filipin; this should be contrasted with antibiotic concentrations in the range 10^{-4} to 10^{-3}M used initially by Weissman & Sessa. The subsequent experiments (*29*) by these investigators were extremely significant because they indicated that not only concentration, but also the individual filipin components, must be considered. Filipins II and III account for most of the antifungal (*11*) and hemolytic (*29*) activity of the antibiotic complex; these two components also caused the greatest release of chromate from liposomes (*29*). Filipin III, which is the major component of the antibiotic complex (see "Chemistry") released significantly more marker from lecithin liposomes prepared with cholesterol but, in contrast, incorporation of the sterol had no effect on the sensitivity of liposomes towards filipin II when tested under identical conditions. It was therefore concluded (*29, 30*) that the effects of filipin III ("cholesterol-reactive") predominate at low concentrations (such as those employed in studies with biological systems, monolay-

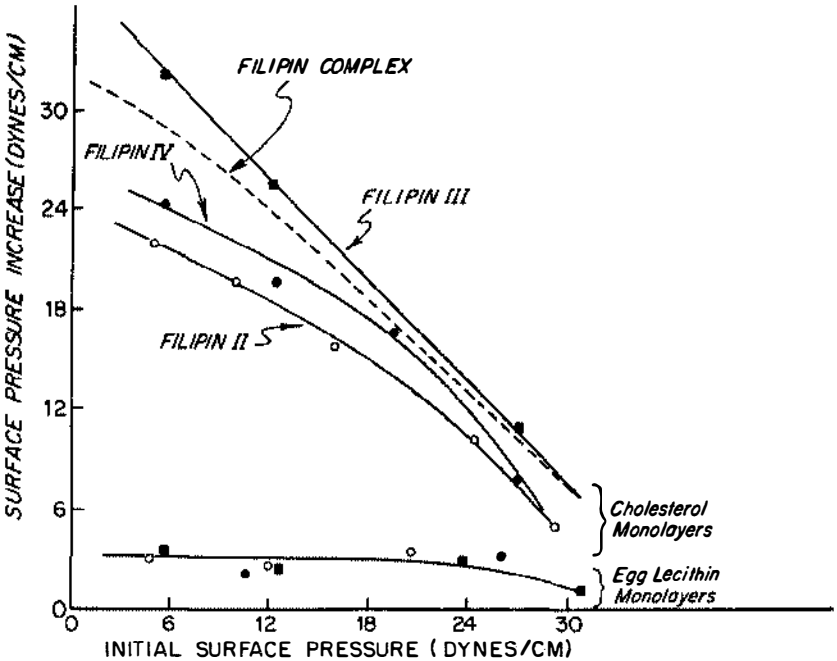


FIG. 2. Interaction of filipin complex and subfractions with lipid monolayers; experimental methods described in (23).

ers, and bilayers) and the effects of filipin II predominate at the higher concentrations that are required to elicit a response in liposomes. However, recent monolayer experiments indicate that filipin II also interacts preferentially with cholesterol at low antibiotic/lipid ratios (Fig. 2) and this component is no longer "the only clear exception to cholesterol preference among polyenes" (29).

Accordingly, it is not readily apparent why one or the other of the filipin components should have a greater influence depending on the amount added unless, as has been suggested (28), release of marker from liposomes may be caused by factors other than (or in addition to) direct interaction with lipids. The response of liposomes to the homologous antibiotics, pimaricin and etruscomycin (Fig. 1), bears on this point. Both antibiotics have been shown to interact with lipid monolayers (23) but, in the case of liposomes, etruscomycin (one of the most potent polyenes as far as lytic activity is concerned) induces only a slight release of marker, and pimaricin has no effect at levels of 10^{-3}M (26, 28). As noted by Weissmann & Sessa (26), pimaricin is the least soluble of the polyenes, which suggests that it might have the smallest effect (reduction) on the surface tension of water. By analogy, it is possible that filipin II has a greater tendency to form true solutions (as op-

posed to micellar solutions) than the other filipin components. The effectiveness of filipin II as a detergent could account for the observations of Balcavage et al. (31) who reported that it was the only component of the antibiotic complex that, at high concentrations (mM range), had an effect on the respiration of rat liver and yeast mitochondria (membranes with a high phospholipid/sterol ratio).

Bilayers.—The feasibility of using bilayers to study polyene mechanism was indicated by van Zutphen et al. (32) who reported that filipin reduced the survival time of lecithin-cholesterol (but not lecithin) films. The magnitude of this effect was dependent on the molar ratio of sterol:phospholipid in the lipid mixture used to generate the films. Nystatin also decreased the stability of lecithin-cholesterol bilayers but to a lesser extent than filipin, whereas perhydrofilipin had no influence.

Andreoli et al. (33, 34), and Finkelstein & Cass (35), have conducted extensive studies on bilayer permeability to ions and nonelectrolytes in the presence of the antibiotics. Nystatin and amphotericin B were examined in detail because, unlike filipin, they alter permeability under conditions which do not affect the stability of the film. There is essential agreement between the two groups not only as regards the effects of the antibiotics, but also concerning interpretation of the data. The antibiotics radically lower the DC resistance of bilayers prepared from either ox brain (35) or sheep erythrocyte (33) lipids; under optimal conditions, the initial resistance of ca. 1–5 $\times$ 10^8 ohms-cm^2 may be reduced as much as a hundred thousand- or millionfold. Three factors influence the extent of this reduction: temperature, the presence of sterol, and antibiotic concentration. A rise in temperature (over the range 31 to 38°) inhibited the ability of nystatin to increase the ionic conductance across bilayers (35). The antibiotics have no effect on the DC resistance of films that do not contain cholesterol above a critical threshold value (33). Of particular interest is the fact that the increase in ionic conductance varied as a high power between (4.5 to 10) of the antibiotic concentration (33, 35). This is in sharp contrast to the action of macrocyclic antibiotics which act as cation carriers and generally produce resistance decreases that are a direct function of antibiotic concentration. This distinction implies a fundamental difference in mechanism, and additional evidence (discussed below) supports this conclusion.

Both laboratories present data showing that amphotericin B and nystatin have a much greater influence on the permeability of certain small anions (e.g., Cl^-, acetate$^-$) than on K^+ or Na^+. This merely indicates that most of the current was carried across the antibiotic-modified membranes by the anions and not an absence of any effect on cation permeability (see "Introduction"). Indeed as emphasized by Andreoli & Monahan (33), the bilayer Na^+ conductance also increased between ten thousand- and a hundred thousandfold, and Finkelstein & Cass (35) mention that ion selectivity is lost in the presence of high antibiotic concentrations. It therefore remains to be determined whether the observed anion preference has any physiological significance.

In this connection, it is important to note that low concentrations of these antibiotics also facilitate passage of nonelectrolytes through bilayers. Lippe (36) has shown that amphotericin B increased the thiourea permeability of cholesterol-dodecane, but not dioleoyl lecithin-decane, films. In an abstract (37) and addendum to (35), Finkelstein & Cass report that nystatin increased the permeability of ox brain lipid bilayers to water and certain small hydrophilic solutes (e.g., urea, ethylene glycol, glycerol, propionamide, erythritol, but not glucose or sucrose). Andreoli et al. (34) have published a detailed paper along these lines and, using tracer flux and osmotic methods, they demonstrated similar effects of amphotericin B on sheep erythrocyte lipid films. As in the case of ionic conductance (see above), bilayer permeability to water and nonelectrolytes was not influenced by the antibiotic when cholesterol was absent from the membrane; also, the magnitude of the permeability increase was dependent on a high power of antibiotic concentration. Particularly significant is the finding that amphotericin B does not discriminately increase the permeability of all water-soluble solutes and, like nystatin (35), there is a graded effect on the basis of size (i.e., hydrodynamic radius). The available data therefore strongly suggest that these polyenes may form multimolecular complexes with cholesterol which, in a manner yet unknown, lead to the formation of pores of finite size through the membrane. Andreoli et al. (34) have determined the reflection coefficients for a variety of compounds (sucrose, raffinose, glucose, ribose, glycerol, acetamide, urea) and calculate that amphotericin B produces channels with an equivalent radius of ca. 7 to 10 Å. Similar measurements by Finkelstein & Cass (35, 37) suggest that the pores produced by nystatin may have a slightly smaller radius (ca. 4 to 7 Å). Additional studies by Dennis, Stead & Andreoli (34a) have provided some information on the structural requirements for pore formation and the factors that contribute to anion selectivity. These experiments are noteworthy for the extensive use made of a number of polyenes and sterols, appropriate synthetic derivatives of amphotericin B, and, particularly, bilayers prepared without phospholipid (i.e., containing cholesterol only). Their results emphasize the importance of stereospecific hydrogen bonding between the 3 β-OH function of sterols, with a rigid intact perhydrocyclopentanophenanthrene nucleus, and portions of the polyene molecule other than the amino or carboxyl groups. The concept of antibiotic-dependent pore formation is also consistent with the phenomena described in the following section and, undoubtedly, these bilayer investigations mark a singular advance towards understanding the molecular basis of polyene action.

Electron microscopy and osmotic stabilization.—It is of some interest to compare the preceding results with model membranes to the effect that the polyenes have on the morphology of erythrocyte membranes and lipid dispersions as viewed in the electron microscope. In this regard, reference should be made to earlier experiments which demonstrate that isotonic sucrose (but not isotonic NaCl or mannitol) markedly inhibited the lytic action of am-

photericin B and nystatin (but not filipin) on fungal protoplasts and mammalian erythrocytes (16, 38). The simplest interpretation of these observations is that any "holes" in the membrane produced by the weak polyenes were too small to allow the entrance of sucrose, whereas the damage initiated by filipin was sufficient to permit passage of this osmotic stabilizer into the cells. This hypothesis was supported by the finding of numerous "pits" in negatively stained (phosphotungstate) rat and human erythrocyte membranes obtained from cells which had been lysed with filipin (39). These pits were visualized as dark areas between 80 to 150 Å in diameter (where the negative stain had accumulated), surrounded by a light ring. The pits produced by filipin resembled those found in sheep and human erythrocyte membranes after lysis with appropriate antibodies in the presence of complement; they were, however, different in shape and distribution from pits caused by saponin which, like filipin, is also a sterol complexing agent. Pit production by the antibiotic requires the presence of cholesterol. Thus, filipin produces pits in lecithin-cholesterol, but not lecithin, dispersions (liposomes); pits are also found along the edges, and on the surface, of cholesterol crystals formed in the presence of the antibiotics. No pits were present in lecithin-cholesterol dispersions after incubation with filipin derivatives which have little (perhydrofilipin) or no (irradiated) hemolytic activity. These observations also suggest that the pits may be related to the terminal phase by which the antibiotic causes membrane damage.

In an extension of these studies (17) it was found that, in contrast to filipin, none of the other antibiotics produced these characteristic pits in erythrocyte membranes or lecithin-cholesterol dispersions. Nevertheless, electron micrographs of cholesterol crystals formed in the presence of etruscomycin, amphotericin B, pimaricin, and nystatin no longer displayed sharp edges and corners indicating interaction between the antibiotic and sterol. Large molecular weight compounds (including a variety of dextrans and hemoglobin) could not protect erythrocytes against hemolysis by low concentrations of filipin (ca. 1 μg/μl). However, under appropriate conditions, these substances could stabilize the cells against the lytic action of moderate concentrations of etruscomycin (ca. 40 μg/ml). Hemolysis by high concentrations (100 μg/μl) of amphotericin B, pimaricin, and nystatin was completely blocked by dextrans and hemoglobin as well as low concentrations of small molecular weight compounds in addition to sucrose (e.g., phosphate and sulfate anions). These results suggest that hemolysis by filipin does not occur by a colloid-osmotic mechanism and that the pits produced by this antibiotic may indeed represent large aqueous channels (80 to 150 Å) that permit flow of cellular constituents through the cell membrane. A colloid-osmotic mechanism is probably operative in the case of the other antibiotics, and the available data indicate that "holes" produced by amphotericin B, pimaricin, and nystatin are smaller than the effective diffusion radius of sucrose (ca. 5 Å). This proposal is consistent with the electron micrographs of erythrocyte membranes and lipid dispersions treated with the weaker poly-

enes, because pits of such small size would be difficult to visualize with the techniques employed. It is also in accord with the conclusions derived from the bilayer studies discussed in the preceding section.

Macrocyclic Antibiotics

Chemistry.—The term, macrocyclic antibiotic, is a descriptive designation that has been applied to several classes of antibiotics: valinomycin, enniatins A and B (depsipeptides); nonactin, monactin, and dinactin (macrotetrolides); gramicidin S and alamecithin (polypeptides).[2] Some noncyclic antibiotics are also included in this category for the purpose of this review because their effect on natural and model membranes resembles the action of the "true" macrocyclics, and the available evidence (see below) indicates that they can can cyclize noncovalently. Examples of such open chain antibiotics are: gramicidins A, B, and C; monensin, nigericin; X464 and X537A (Hoffman-La Roche). We shall also briefly consider several antibiotics whose structures have not yet been established: dianemycin and X206 (Hoffman-La Roche).

The earlier studies (up to ca. 1966) concerned with the chemical properties of the macrocyclics have been reviewed by Hunter & Schwartz (40–42), Shaw (43, 44), and Korzybski et al. (5). However, the past few years have witnessed so much work in this area that these articles are already somewhat out of date. In discussing the chemistry of these antibiotics, it is important to distinguish between their "primary" and "secondary" structure. Primary structures for some true (i.e., closed) and open-chain antibiotics are shown in Figures 3 and 4, respectively. Secondary structures refer to the spatial configuration of the constituent atoms when the antibiotics are either free or complexed with the appropriate cations for which these antibiotics act as "carriers" (see following sections). We have not attempted to depict the latter and, instead, recommend that the interested reader consult the papers cited below.

Secondary structure.—Valinomycin-cation binding has been measured by chemical equilibrium and conductivity determinations (45–47). Optical rotary dispersion (ORD), infra-red (IR), and nuclear magnetic resonance (NMR) spectra provide information on the secondary structure of the complex and free antibiotic (47–50). The potassium complex is a sixfold coordination clathrate and X-ray crystallographic studies further indicate that the valinomycin molecule completely surrounds a potassium ion (51).

Analogous investigations have been carried out with the enniatins (47, 52), and the secondary structure of the K^+-enniatin B complex has been proposed as a disc with coordination between the ion and six carbonyl oxygens (53).

Cation binding by the actin homologues has also been studied (54). X-

[2] It should be noted that polyene antibiotics also contain "large rings" and therefore, in a strict sense, can be considered macrocyclic antibiotics.

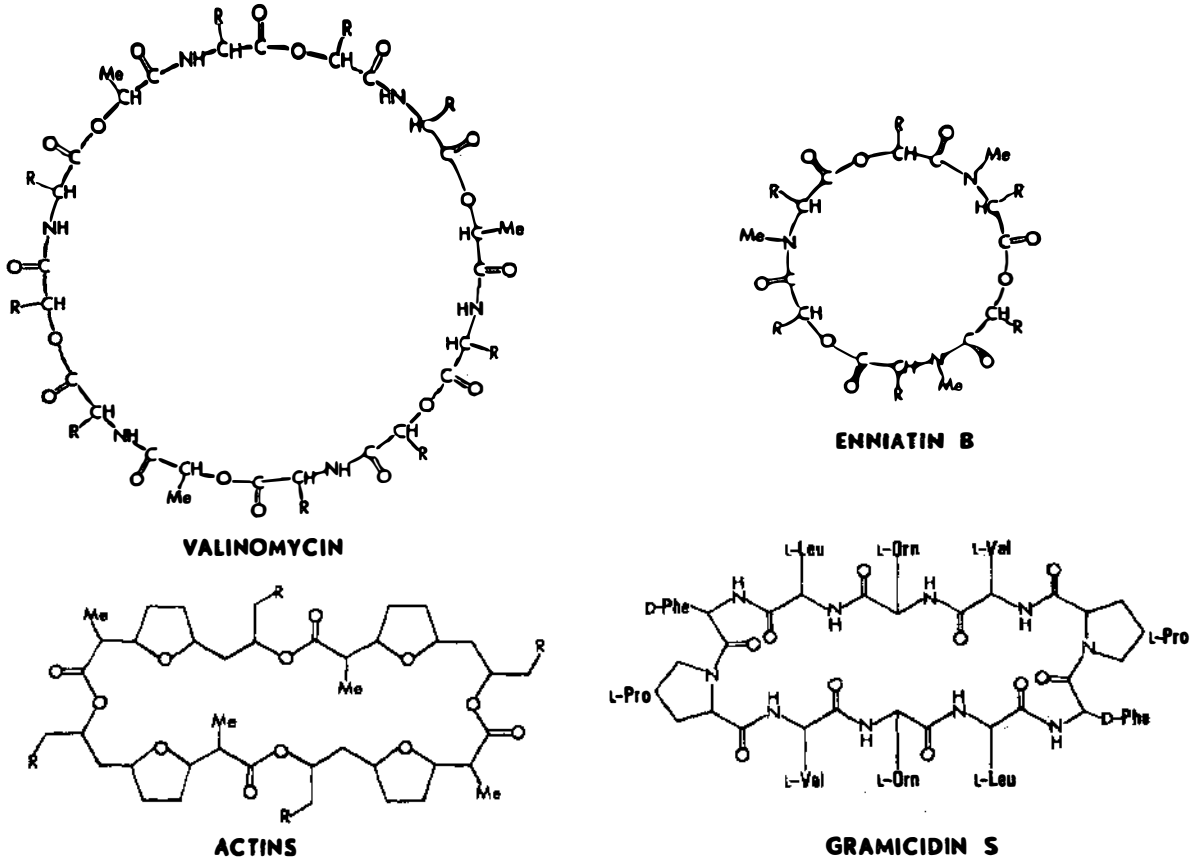
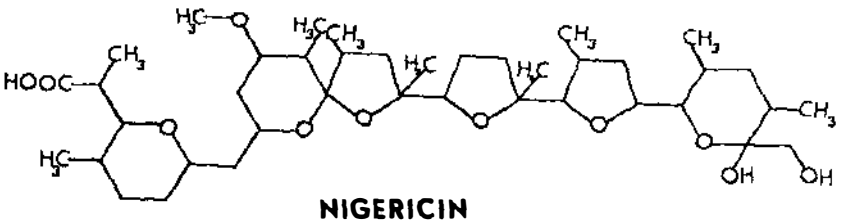


FIG. 3. Primary structures of some closed macrocyclic antibiotics. In valinomycin and enniatin B, R is an isopropyl residue; in enniatin A, some of these residues are replaced by secondary butyl groups. Nonactin. monactin, dinactin differ from each other depending on whether R is hydrogen or methyl. See text for appropriate references.



NIGERICIN

HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Try-D-Leu-
L-Try-D-Leu-L-Try-D-Leu-L-Try-NHCH$_2$CH$_2$OH

(Valine)-GRAMICIDIN A

FIG. 4. Primary structure of some open macrocyclic antibiotics. Monensin has a structure very similar to that shown for nigericin [see, e.g. (60)]. Gramicidins A, B, and C have different amino acid compositions but in each the *N* terminal and C terminal residues are "blocked" by formyl and ethanolamine substituents, respectively; they are therefore "nonamphoteric" compounds.

ray investigation of the K^+-nonactin complex places the cation in the center of a nonplanar molecule whose 32 membered ring "resembles the seam of a tennis ball" (55). The ion exists in eightfold coordination with the four carbonyl oxygens and the four oxygens of the furan rings.

As far as gramicidin S is concerned, the only available data (NMR) on the secondary structure of the antibiotic indicates that the ring is stabilized by four intramolecular hydrogen bonds (50, 56).

At the moment, we have not come across any information on the secondary or primary structure of alamecithin other than the suggestion that it may be a closed macrocyclic antibiotic containing amino acids in peptide linkage (57).

Sarges & Witkop (58) have proposed an antiparallel dimer configuration for gramicidin A and suggest, on the basis of IR spectra, that the dimer is cyclic.

The primary and secondary structures of monesin (59), nigericin (60), and X537A (61) have been determined by chemical analysis and X-ray crystallography. Lardy et al. (62) report that antibiotic X464 is identical with nigericin. All of these antibiotics have been shown to cyclize around a central cation; the secondary structure of nigericin is stabilized by bonds involving the carboxyl group (60, 61). In this regard, it should be noted that the two antibiotics (dianemycin and X206 (63)), whose structures have not yet been established, are also monocarboxylic acids (62).

Bilayers: valinomycin.—Five years after the introduction of this model membrane system by the laboratory of Mueller & Rudin (64, 65), they, and Lev & Buzhinsky, reported that several antibiotics markedly reduced bilayer

resistance (66, 67). Valinomycin, enniatin B, dinactin, and gramicidin A increased the permeability to some cations, such as rubidium and potassium, but had little effect on the permeability of other cations, such as sodium and lithium, or on polyvalent ions. These initial observations have since been confirmed and extended in numerous laboratories.

The antibiotic that has been studied most extensively is valinomycin (35, 66–71), and there exists virtually full agreement on the basic phenomenon. Using bilayers generated from various sources (e.g., beef heart, beef brain, and sheep erythrocyte lipid fractions; lecithin and sphingomyelin; oxidized cholesterol derivatives), it has been demonstrated that valinomycin preferentially increases cation permeability several thousandfold depending on their concentration in the medium. Two mechanisms have received serious consideration in an attempt to explain how valinomycin and the other macrocyclic antibiotics produce this effect. The first postulates that several molecules of the cyclic antibiotics aggregate to form a tunnel or physical channel that penetrates the membrane. According to this hypothesis, anions would be excluded by electrostatic repulsion because the interior of the channel contains many carbonyl and ether oxygens and thus represents a region of considerable electronegativity; cation ion selectivity occurs because the tunnel functions as a "sieve" and does not permit passage of the larger ions. The second alternative mechanism places primary emphasis on the ability of the macrycyclics to form stoichiometric complexes with some cations. These complexes then diffuse through the nonpolar interior of the bylayer, i.e., the antibiotics act as a carrier for the cation. Although Mueller & Rudin (66) initially felt that either mechanism could operate depending on antibiotic concentration, the second is presently favored by most investigators (35, 46, 69–71). The arguments in favor of the diffusion mechanism are impressive (and are valid for all the macrocyclics to the extent that they have been studied) and may be summarized as follows:

(*a*) The membrane conductivity varies with the first power of the valinomycin concentration (68, 69) which implies noncooperative behavior of the antibiotic.

(*b*) As discussed in the previous section, molecular clathrates of potassium and valinomycin have been demonstrated and the structural determination shows valinomycinin is "folded as it circles the potassium, describing what resembles three complete sine waves" (51). The cation is coordinated to six internal carbonyl oxygens of the antibiotic which replace the hydration shell and completely surround the ion. This complex is further stabilized by six intramolecular hydrogen bonds between the remaining carbonyl groups and the peptide nitrogens. It is not easy to see how such a structure could stack to form a fixed channel. Moreover, NMR studies of these clathrates do not indicate polymerization or aggregation at high concentrations (49).

(*c*) The ability of valinomycin to alter the distribution of cations between an aqueous and nonpolar solvent phase (in favor of the latter) follows the sequence of ionic permeabilities seen in bilayer membranes treated

with the antibiotic (46). Again, even though the antibiotic might aggregate in a nonpolar phase under some conditions, it is not clear why this should resemble pores.

(*d*) If valinomycin is added to a bulk lipid barrier separating two aqueous phases (i.e., a thick "membrane" of 1 to 10 mm) the lipid phase becomes permeable to potassium ions (51, 70, 72). An analysis of electrical resistance as a function of thickness has shown that the former has two components (69–70). One component is caused by the energy barrier at the surface and the other arises from the resistance of the interior; these components are added in series. Linear extrapolation to a 75Å bilayer membrane yields resistances typical of those measured in bilayer experiments. Thus it would appear that similar transport processes occur in thick membranes and bilayers. This finding is completely inconsistent with the notion of channels traversing a bilayer membrane.

There were some early reservations about the carrier transport hypothesis, based on the experiments indicating that the closed macrocylic antibiotics all imparted essentially identical membrane permeability sequences although their internal diameter could be markedly different. However, this objection was removed when more information about the secondary structures became available—especially, the X-ray crystallographic studies that showed that most of the rings were not planar (see "Chemistry"). Although the flexibility of the antibiotic molecules answers this one question, it raises another: what is it about the macrocyclics that determines their cationic preference? Many of the above references discuss the possibility that the number of oxygen atoms available for complex formation may be insufficient to complete the hydration shells of the larger ions; it has also been suggested that the electric field strength at the surface of the complex could be a contributing factor (70). A general review and theoretical discussion of ionic specificity, which may be relevant to this issue, has been presented by Diamond & Wright (73).

There is thus strong evidence in favor of the hypothesis that a single valinomycin molecule forms a clathrate with a suitable cation within the membrane. The exterior of this complex is relatively nonpolar and therefore soluble in hydrocarbons (indeed, valinomycin itself is quite insoluble in water). The valinomycin molecule is superbly adapted to function as a "shuttle bus". Ivanov et al. (48) have recently shown that the antibiotic undergoes a dramatic configurational transition during "reaction" with a potassium ion, allowing the cation easy access to the carrier, which then completely surrounds it. However, the valinomycin-ion complex has a net charge of +1 (the antibiotic per se is electrically neutral) and we must further inquire why it is admitted to the interior of a membrane whereas the free charged ion is excluded. A possible answer follows from the interpretation advanced by Finkelstein & Cass (35) to explain iodide transport through bilayers. They point out that the barrier to charges in a nonpolar medium is related to the surface charge density of the transported particle,

and that the repulsion energy decreases as the charge is spread over a larger surface. Moreover, the equilibrium constant ([K^+]membrane/[K^+]aqueous) is given by the Boltzman distribution where the repulsion energy term is present as an exponent, so that a relatively small change in the surface charge density will cause a much larger change in the equilibrium constant. Thus, spreading of the ionic charge over the valinomycin surface may facilitate transport of small cations through the membrane. In this regard, it should also be noted that the resistance to cation flow decreases even further in the presence of a lipid soluble counter-ion [see, e.g. (45)]. The obvious explanation is that the cation is transported as a neutral complex (valinomycin-ion–counter-ion).

A few open questions regarding valinomycin still remain. Perhaps the most important one concerns the position of hydrogen ions in the selectivity sequence. Some (67, 68), but not all (71, 74), investigators have found that this ion is rendered more permeable than the other cations by the antibiotic. An anomalous result has been reported by Andreoli et al. (68) who recorded membrane potentials as large as 80 mV when valinomycin was added to one side of a bilayer in the absence of any apparent salt gradient. Their observations can be completely explained by the Goldman equation (75, 76) if we assume potassium ion "contamination" of the valinomycin preparation.

Bilayers: other closed macrocyclic antibiotics (excluding alamecithin).— A similar story can be told, although less completely, about the actins [nonactin (69, 77–79), monactin (69, 70, 78–80), dinactin (66, 69, 70, 79), and enniatin B (66, 70, 81)]. No bilayer studies have yet been reported using enniatin A but it has been investigated with liposomes (see below) and has comparable properties to the other antibiotics of this class. The selectivity sequences and some physical properties of these antibiotics are presented in Table I.

The relatively planar (disc) shape of the K^+-enniatin B complex, and the fact that they stack when crystallized, has suggested to Dobler et al. (53) that the antibiotic may form a tunnel through the membrane. However, bilayer (66, 70) experiments indicate that it behaves similarly to other closed macrocyclics for which there is little evidence to support a pore hypothesis. Nevertheless, this secondary structure may explain why the enniatins must be present in higher concentrations than valinomycin (and the actin homologues) to obtain the same permeability increase, because incomplete shielding of the cation should result in higher repulsion energies. Lippe (81) has reported a threefold increase in the permeability of thiourea (an uncharged molecule) when enniatin B was present in sufficient concentration to cause a thousandfold increase in potassium permeability; the significance of this is not clear.

Tieffenberg & Tosteson have reported in abstract (82) that the potassium and hydrogen ion selectivity of a bilayer treated with a monactin-dinactin mixture can vary with the direction of current flow. These results may

TABLE I

SOME PROPERTIES OF OPEN AND CLOSED MACROCYCLIC ANTIBIOTICS

Antibiotic	No. of Ring Atoms	Charge at pH 7	Cation Selectivity Sequence	Cation Binding Sequence	K^+/Na^+ Transport Ratio
Closed					
Valinomycin	36	0	Rb>K>Cs>Na>Li (b, 1)	Rk, K>Cs>Na>Li	>100 (b)
Enniatin A	18	0	K, Rb, Cs, >Na>Li (b)	—	—
Enniatin B	18	0	K>Cs>Na (b)	—	—
Nonactin	32	0	K>Na (b)	—	>100 (b)
Monactin	32	0	K>Rb>Cs>Na>Li (b, 1)	—	—
Dinactin	32	0	Rb>K>Cs>Na>Li (b)	—	14–60 (b)
Gramicidin S	30	0	Inactive (b); K>Na (1)	—	—
Alamecithin	?	−1	little or none	K, Rb>Cs, Na	—
Open					
Gramicidin A	?	0	Rb>K>Cs>Na>Li (b)	—	4 (b)
Gramicidin B	?	0	little or none (b)	—	2.5 (b)
Nigericin	?	−1	K, Rb>Na>Cs>Li (1)	K>Rb>Na>Cs>Li	>10 (b)
Monensin	"18"	−1	Na>K, Li>Rb>Cs (1)	Na>K>Rb>Cs	—
Dianemycin	?	−1	little or none (1)	Na>K>Cs, Rb>Li	—
X 206	?	−1	K, Rb>Na>Cs>Li (1)	K>Rb>Na>Cs>Li	—
X 537A	?	−1	K, Rb>Na>Cs>Li (1)	Cs>Rb, K, >Na>Li	—

This table was kindly prepared for this review by Dr. Alfred L. Ochs. The cation selectivity sequence was determined from experiments using either bilayers (b) [see (66–68, 80, 84)] or liposomes (1) [see (74, 85). Reference 46 should be consulted for the data on cation binding sequence.

have some implications as regards the confusing position of hydrogen ions in the valinomycin induced permeability sequence (see above and the succeeding discussion of liposomes). An important theoretical study has been published by Eisenman, Ciani & Szabo (80). By means of a formal kinetic analysis, they derived expressions for membrane conductance, the transmembrane potential in the presence of an ionic gradient, and the partition equilibrium in a two-phase solvent system. To test the theory, extensive measurements were made with monactin and a synthetic analogue, the cycle crown ether, dicyclohexyl-18-crown-6 (83). Their experimental results fit the theory closely for monactin but not for the crown ether; preliminary calculations, using the data of other workers, indicate that valinomycin and the open chain gramicidins can be treated successfully with this theory.

Bilayers: open macrocyclic antibiotics.—The open chain antibiotics can be separated into two groups on the basis of their net charge at neutral pH (Table I). The experimental data available for these compounds is meager compared to that for closed macrocyclics.

The effects of the gramicidins on bilayers has been investigated in several laboratories (66, 69, 71). These compounds impart a stronger cation conductivity to bilayers than the closed macrocyclics at comparable concentrations. However, the cationic specificity is markedly less. For example, the K^+/Na^+ transport ratio is about 2 to 4 rather than > 100 with valinomycin

(69) although the selectivity sequence is comparable (66). There is evidence that gramicidin acts on bilayers as a dimer. Tosteson et al. (69) have shown that the membrane resistance follows second order dependence on antibiotic concentration. This is consistent with the chemical evidence for anti-parallel dimerization (see "Chemistry") and the initial confusion, by a factor of 2, in the assignment of molecular weight (42).

Most of the available information concerning the monocarboxylic antibiotics (monensin, nigericin (X464), dianemycin, X206, and X537A) has come from bulk fluid phase (45) and liposome experiments (see following section). Tosteson et al. (69) have reported a nigericin induced K^+/Na^+ permeability ratio of > 10; others could not detect any membrane potential produced by this antibiotic [see (45) for discussion]. In experiments with nigericin, the hydrogen ion concentration may have an important role, and this discrepancy could reflect differences in pH. The monocarboxylic antibiotics are negatively charged at neutral pH and should manifest somewhat different transport properties than the neutral antibiotics reviewed previously. This is supported by results obtained using liposomes (74), which have shown that these antibiotics, in contrast to valinomycin, the actins, and enniatin A, can exchange protons for monovalent cations.

Bilayers: alamecithin.—The detailed mechanism by which this closed macrocyclic antibiotic acts is not yet known, but its properties are remarkably different from any of the other antibiotics which have been reviewed. Mueller & Rudin (84) have shown that alamecithin can induce relatively unselective cation transport that is a function of both time and membrane current. In the presence of basic proteins such as protamine, histone, or spermine, the conductivity shows a sixth power dependence with respect to NaCl and antibiotic concentration. An ionic gradient will cause cationic potentials and a region of negative slope in the current voltage curve (i.e., a negative resistance which, being unstable, gives rise to a voltage jump when the potential reaches a critical value). In this regard, it is important to note that nerve and muscle fibers display a negative resistance during the rising phase of an action potential; the falling phase corresponds to the period when the negative resistance reverts to a positive resistance. Bilayer conditions can be adjusted so that the electrical activity resembles a nerve action potential. Furthermore, spontaneous voltage changes, which mimic repeating action potentials, can also be induced; in this case, the membrane permeability alternates from cation selectivity to anion selectivity. The full range of conditions under which these various electrical changes occur has not appeared in the literature but, in view of the significance of these preliminary observations, this information will undoubtedly be available in the near future.

Liposomes.—Although we have found it more convenient to discuss the action of the macrocyclic antibiotics on bilayers before considering liposomes, it should be emphasized that there is little "historical" justification for this approach. Indeed, the first indication that these antibiotics could af-

fect ion transport through model membranes was obtained with liposomes and reported by Chappel & Crofts in a 1965 symposium (85). They observed that lecithin-dicetyl phosphate liposomes became more permeable to sodium and potassium ions when treated with gramicidin S or valinomycin; valinomycin imparted a strong selectivity for potassium over sodium, whereas gramicidin S revealed only a slight selectivity in favor of potassium ions. As indicated in the preceding sections, these findings have been confirmed with bilayers, although Tosteson et al. (69) have reported that gramicidin S was inactive in their system.

Henderson, McGivan & Chappel have recently published a complete and extended account of these liposome experiments (74). They have tested at least one member of each class of macrocyclic antibiotic (with the exception of alamecithin) and distinguished them on the basis of their ability to induce hydrogen ion permeability. The group I antibiotics (valinomycin, the actins, and enniatin A) do not affect proton permeability. They facilitate rapid diffusion of small cations through liposomes, erythrocyte, and mitochondrial membranes with a selectivity sequence and ratio similar to that induced in bilayer membranes (see Table I). Group II antibiotics (the monocarboxylic acids: nigericin (X464), dianemycin, monensin, X206, and X537A) render the membrane freely permeable to protons. These antibiotics induce a rapid cation-hydrogen exchange that has been interpreted as indicating a carrier mechanism in which the antibiotic cannot cross the membrane when it bears a negative charge (cf. nigericin in preceding section). Group III antibiotics (a mixture of gramicidins) cause an intermediate proton permeability; the selectivity sequence (Table I) is similar to the antibiotics in group I.

Concluding comment.—Having spent considerable time on the preparation of this review, the author feels entitled to express one additional "biased" opinion. Numerous studies on mitochondria have given rise to the "repeating unit" theory of membrane structure, which is quite different from the classical "unit membrane" picture. However, as emphasized in many of the references cited, the effect of the macrocyclic antibiotics on bilayer model membranes parallels their action on mitochondria. Therefore, does not this keep open the possibility that some portion of the mitochondrial membrane might also contain phospholipids arranged in bilayer configuration?

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