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INTERACTION OF POLYENE ANTIBIOTICS WITH SINGLE AND MIXED LIPID MONOMOLECULAR LAYERS

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SUMMARY

1. Etruscomycin, amphotericin B, and pimaricin preferentially interact with cholesterol, and not with lecithin, monolayers at low molar ratios of antibiotic/lipid. Similar results have been previously reported for filipin and nystatin. At initial surface pressures above the collapse pressures of the antibiotics, the ability of the polyenes to increase the surface pressure of a cholesterol monolayer is in the order: filipin $\geqslant$ etruscomycin $\gg$ amphotericin B $>$ pimaricin $>$ nystatin. These observations suggest that the ability of the polyene antibiotics to cause membrane damage may be correlated with their affinity for sterol. Derivatives of filipin, which have little or no biological activity, also produce a much weaker interaction with cholesterol monolayers than the parent antibiotic.

2. In mixed monolayers of lecithin and cholesterol, the surface pressure increase obtained with filipin is diminished as the relative content of the phospholipid is raised. This observation supports previous studies indicating that the polyene sensitivity of a membrane may be dependent on the phospholipid/sterol ratio and not on the presence of sterol *per se*.

3. Filipin, nystatin and pimaricin can interact with monolayers of lecithin (in the absence of cholesterol) at high molar ratios of antibiotic/lipid. These observations are consistent with the results of SESSA AND WEISSMANN that some of the polyenes can release markers from liposomes prepared with lecithin alone. Past and present experiments suggest that interaction between the polyenes and membrane phospholipid is probably without significance as regards the antifungal and hemolytic activity of the antibiotics. At high molar ratios, filipin can also increase the surface pressure of oleic acid monolayers. These results are in accord with several reports indicating that addition of fatty acids, as well as phospholipids, to the medium can antagonize the growth inhibitory and lytic properties of the polyenes.

4. Filipin has only a slight effect on the surface pressure of cholesterol acetate monolayers, which is consistent with the view that a free hydroxyl group is necessary for interaction with the polyenes. This conclusion is supported by the finding that filipin can also interact with monolayers of cetyl alcohol at low molar ratios of antibiotic/lipid. The inhibitory influence of urea suggests that hydrogen bond formation may play an important role in polyene–sterol interaction.

INTRODUCTION

Previous investigations have shown that the polyene antibiotic, filipin, has a pronounced effect on the morphology of lecithin–cholesterol dispersions resulting in the production of 'pits'[1]. Pit formation, which was also observed in rat and human erythrocyte membranes obtained by lysis of cells with filipin, was dependent on the presence of cholesterol and did not occur with derivatives of the antibiotic that had little or no biological activity. These observations are consistent with earlier studies which have indicated that the presence of sterols in the cell membrane is a necessary prerequisite for filipin sensitivity[2]. The latter conclusion is also supported by experiments with model systems as discussed below.

The above observations are, however, at variance with the extensive studies of WEISSMANN AND SESSA[3,4]. They have shown that filipin causes the leakage of various marker compounds from liposomes prepared without cholesterol; the addition of cholesterol does not result in an increased release. On the other hand, amphotericin B and nystatin have a negligible effect on liposomes lacking the sterol; with these antibiotics, the incorporation of cholesterol into the liposomes produces a much greater release of marker. The findings of WEISSMANN AND SESSA are significant because there is still some question as to whether all polyene antibiotics act by the same mechanism. Numerous studies with *Saccharomyces cerevisiae*, Neurospora protoplasts, and mammalian erythrocytes have demonstrated that the polyenes differ markedly in the degree of membrane damage which they induce. This aspect has been reviewed in detail by LAMPEN[5] who has divided the antibiotics into two categories. One group consists of polyenes (such as filipin and etruscomycin) which cause a very rapid and extensive damage to the membrane; the second group is composed of antibiotics (such as amphotericin B, pimaricin, and nystatin) whose effects are less pronounced. On the basis of their experiments, WEISSMANN AND SESSA have suggested that filipin, and other Group-1 polyenes, may cause more extensive damage by virtue of their ability to interact with membrane phospholipids, a property which apparently the Group-2 antibiotics do not possess.

It should be emphasized, however, that the polyene antibiotics show a graded series of effects so that the above classification is not, in fact, clear-cut (see DISCUSSION in refs. 5, 6 and 7). On the basis of our previous monolayer[8] and bilayer studies[9], we concluded that all polyenes share the same basic mechanism and that the differences between the antibiotics may be due to varying affinities for sterol. Thus, it has been shown that (1) filipin produces a greater increase in the surface pressure of both cholesterol and ergosterol monolayers than does nystatin and that (2) the survival time of lipid bilayer films containing cholesterol is much shorter in the presence of filipin than in the presence of nystatin. Our previous inability to detect any significant interaction of filipin and nystatin with monolayers, or bilayers, containing only lecithin was obviously in contrast to the observations of WEISSMANN AND SESSA cited above and cast some doubt on the feasibility of using these model systems for studying polyene mechanism. The present investigation with monolayers and the following study with bilayers[10] were undertaken in an attempt to reconcile the above observations.

MATERIALS AND METHODS

Lipids

The synthetic lecithin, (1-stearoyl-2-oleoyl)-α-phosphatidyl choline (18:0/18:1 phosphatidyl choline), was prepared by methods previously described[11]. The purity of this compound and egg lecithin (provided by Dr. H. VAN ZUTPHEN) was routinely examined by chromatography on silica gel plates with chloroform–methanol–water (65:35:4, v/v/v) and diisobutyl ketone–acetic acid–water (40:25:5, v/v/v) as solvent systems. Cholesterol, cholesterol acetate, oleic acid, cetyl alcohol, and dicetyl phosphate were obtained from commercial sources.

Polyene antibiotics and derivatives

The polyene antibiotics were generously donated by the following companies: the Squibb Institute for Medical Research, New Brunswick, N.J. (nystatin, amphotericin B); Farmitalia, Milan, Italy (etruscomycin); The Upjohn Company, Kalamazoo, Mich. (filipin); and Lederle Laboratories, Pearl River, N.Y. (pimaricin). The purity of the antibiotics was 90 % or higher (information supplied by manufacturer). Molecular weights employed in the calculations were: nystatin, 932; amphotericin B, 960; etruscomycin, 700; filipin, 654; pimaricin, 681.

The preparation of perhydrofilipin (mol. wt. 664), irradiated filipin, and saponified filipin was described in an earlier report[12]. Because the product(s) of irradiation and saponification have not been identified, a molecular weight of 654 was assumed. Filipin nonaacetate (mol. wt. 1068) was synthesized by the method of DHAR, THALLER AND WHITTING[13].

Stock solutions of the antibiotics or derivatives were made by dissolving appropriate quantities in redistilled dimethylformamide.

Measurement of surface pressure

Surface pressures were determined in a paraffin-coated glass trough with a conventional Langmuir–Adam film balance. The trough was 58 cm long and 14 cm wide and had a total capacity of 700 ml. All experiments were carried out at room temperature (about 22°).

Monolayers were prepared by spreading either single lipids or lipid mixtures on unbuffered water that had been distilled from alkaline permanganate and redistilled in a quartz still. The initial film area was 560 cm^2. After compression to give surface pressures between 2 and 30 dynes/cm (film area: 140–170 cm^2), 10–50 μl of antibiotic solution were injected beneath the monolayer with an Agla micrometer syringe. A stable pressure change was obtained within 2–3 min. Additional details are described below.

RESULTS

Force–area curves

The force–area curves for amphotericin B, etruscomycin, filipin, nystatin, and pimaricin have been published previously[8]. The force–area curves for the derivatives used in the present investigation are shown in Fig. 1. Monolayers of the polyene antibiotics and derivatives in general have relatively low collapse pressures or produce

detectable surface pressures only at 'unreasonable' area/molecule values. For example, no appreciable surface pressure was observed with pimaricin until the area/molecule approached a calculated value of 8 Å^2 (ref. 8). This value is not in accord with area estimates (100–250 Å^2, depending on orientation chosen) obtained from Stuart models constructed according to the formula proposed recently by GOLDING *et al.*[14]. The above observations are consistent with the fact that all the polyene antibiotics are polyalcohols and are soluble in water at low concentrations. Indeed, only filipin nona-

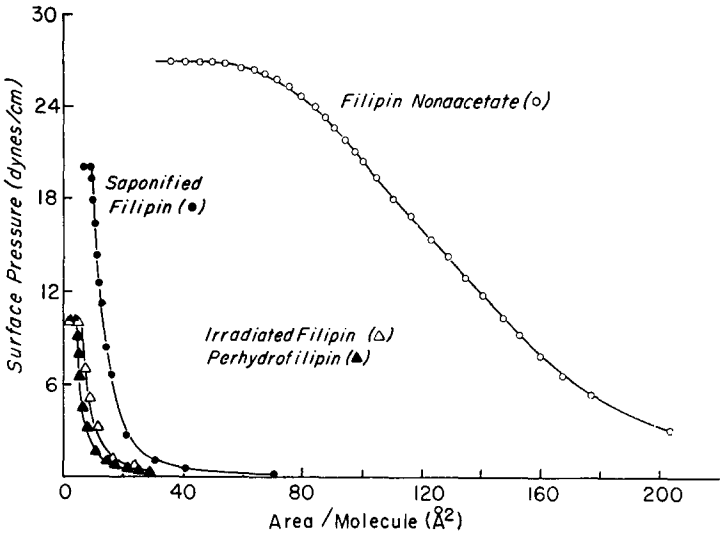

Fig. 1. Force–area curves of filipin derivatives. The following amounts (in μmoles) of the filipin derivatives were spread on a surface area of 560 cm^2, and the change in pressure that resulted upon compression was determined: filipin nonaacetate, 0.040; saponified filipin, 0.028; irradiated filipin, 0.131; perhydrofilipin, 0.005. Identical curves (not shown) were obtained with higher amounts of the derivatives, *i.e.*, 0.066, 0.055, and 0.028 μmole of filipin nonaacetate, saponified filipin, and perhydrofilipin, respectively.

acetate which would be expected to have much less water solubility than the parent antibiotic, gives a 'respectable' force–area curve (Fig. 1). The observed collapse pressures are nevertheless operationally significant as regards all subsequent experiments concerning monolayer penetration. If injection of a compound (polyene or derivative) into the aqueous substrate produces an increased pressure in monolayers which have been previously compressed to surface pressures at, or above, the collapse pressure of the antibiotic, then we interpret this increase as indicating specific interaction with some constituent of the monolayer. This assumption appears validated by experiments with the acetyl derivative of filipin as described in the next section.

Interaction of filipin and derivatives with cholesterol and lecithin monolayers

The effect of filipin and derivatives on the surface pressure of cholesterol and lecithin monolayers is shown in Fig. 2. All the compounds, with the exception of filipin nonaacetate, preferentially interact with the sterol monolayer at initial surface pressures equal to, or greater than, their collapse pressures (indicated by the arrows). The acetyl derivative does not manifest any significant selectivity for cholesterol. The pressure increases obtained with this derivative in all likelihood do not reflect

interaction with either lipid monolayer, but are probably due to the ability of filipin nonaacetate molecules to orient *per se* at the air/water interface (*cf.* discussion in preceding section). Consistent with this conclusion is the observation that only small pressure increases are obtained when the acetyl derivative is injected underneath cholesterol or lecithin monolayers compressed to pressures near the collapse pressure of the compound, about 27 dynes/cm (Fig. 1). For this reason, further experiments with the acetyl derivative were not performed.

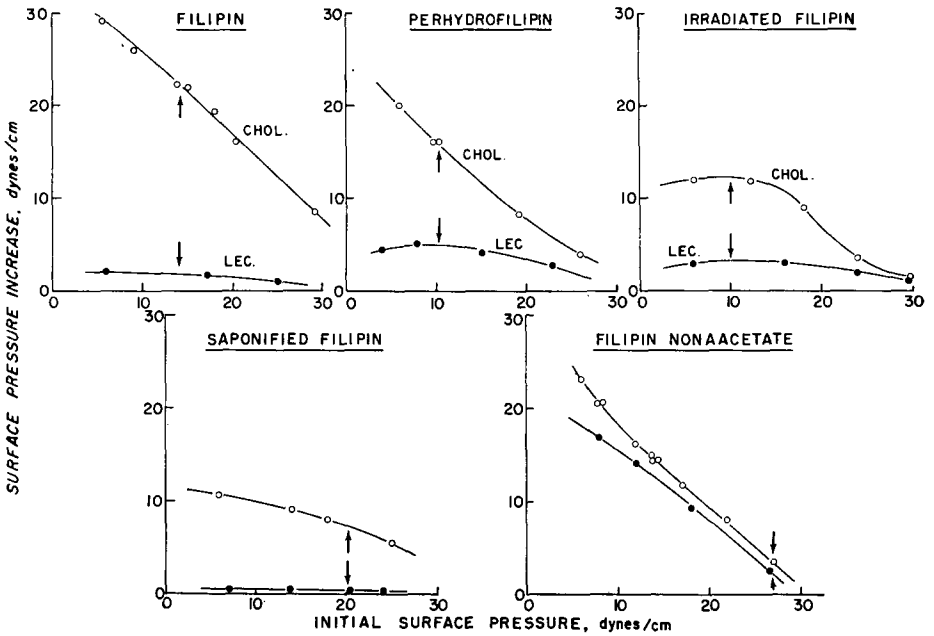

Fig. 2. Interaction of filipin and derivatives with cholesterol and lecithin monolayers. Monolayers were prepared with 0.064 μmole of cholesterol and 0.038 μmole of synthetic lecithin (18:0/18:1 phosphatidyl choline), and compressed to the initial surface pressures shown on the abscissa. The following amounts (in nmoles) of filipin or derivatives were then injected underneath, and the increase in surface pressure was determined as described under MATERIALS AND METHODS: filipin, 6.38; perhydrofilipin, 5.58; irradiated filipin, 6.38; saponified filipin, 5.51; filipin nonaacetate, 6.67. The arrows indicate the collapse pressures as determined from the force–area curves. For additional details, see text.

As demonstrated in an earlier paper[12], perhydrofilipin and the preparations of saponified or irradiated filipin are far less potent hemolytic agents than the parent antibiotic; the irradiated derivative is, in fact, completely devoid of biological activity. Fig. 3 shows that these derivatives produce a much smaller increase in the surface pressure of cholesterol monolayers than filipin. These observations suggest a tentative correlation between the ability of these compounds to interact with cholesterol and their hemolytic potency. However, it should be noted that, as far as the derivatives are concerned, this relationship is not quite perfect. Thus, irradiated filipin, which also has no visible effect on lipid dispersions containing cholesterol[1], nevertheless produces a measurable increase in the surface pressure of the sterol monolayer. The reason for this apparent discrepancy is currently under investigation (contingent on the eventual isolation and characterization of the irradiated products).

Interaction of other polyene antibiotics with cholesterol and lecithin monolayers

We have previously shown that nystatin, like filipin, preferentially interacts with sterol monolayers[8]. These studies have now been extended to include amphotericin B, etruscomycin, and pimaricin (Fig. 4). These polyenes also produce significant

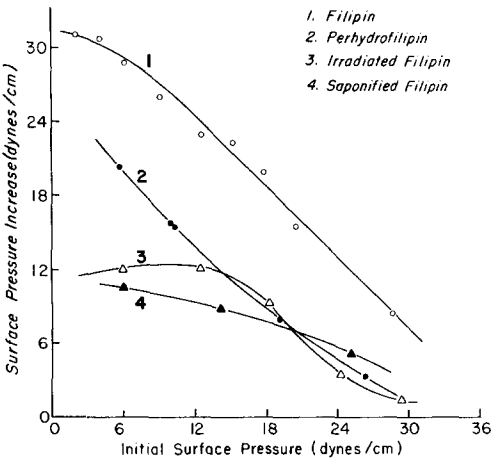

Fig. 3. Comparative interaction of filipin and derivatives with cholesterol monolayers. Data taken from Fig. 2. The molar ratio of compound injected/spread lipid ranged from 0.15 (saponified filipin) to 0.18 (irradiated filipin).

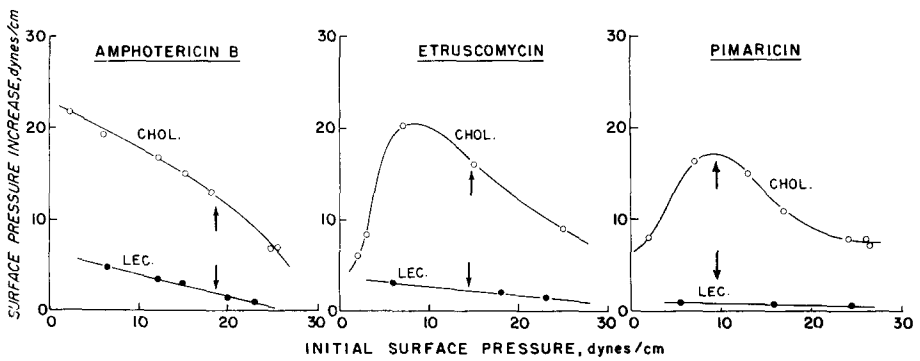

Fig. 4. Interaction of amphotericin B, etruscomycin and pimaricin with cholesterol and lecithin monolayers. Monolayers were prepared with 0.064 μmole of cholesterol and 0.038 μmole of synthetic lecithin (18:0/18:1 phosphatidyl choline) and compressed to the initial surface pressures shown on the abscissa. The antibiotics (6.38 nmoles) were then injected underneath, and the increase in surface pressure was determined as described under MATERIALS AND METHODS.

increases in the surface pressure of cholesterol monolayers at, or above their respective collapse pressures (indicated by the arrows). This interaction is, again, more pronounced with the sterol than with the lecithin monolayers. These experiments, together with the earlier filipin and nystatin data, are summarized in Fig. 5. At initial surface pressures greater than 12 dynes/cm, filipin produces the largest increase in the surface pressure of cholesterol monolayers with etruscomycin, amphotericin B, pimaricin, and nystatin following in this order. This is the order in which the antibiotics are able to cause membrane damage to mammalian erythrocytes or the yeast

cell (see INTRODUCTION). These results thus support our earlier suggestion that the differences in the potencies of the antibiotics may reflect varying affinities of the polyenes for sterols[2,8,9].

Effect of lecithin on the interaction of polyene antibiotics with cholesterol monolayers

The preceding experiments were performed with either pure cholesterol or pure lecithin monolayers. Conclusions based on these studies may have no relevance to natural cell membranes because the latter contain a variety of lipids. Indeed, previous investigations[15] have suggested that the presence of sterols in a natural membrane is a necessary prerequisite for polyene sensitivity, but not a sufficient condition. These studies have indicated that the phospholipid/sterol ratio may be an important factor in determining whether or not a membrane is sensitive to the polyenes. For example,

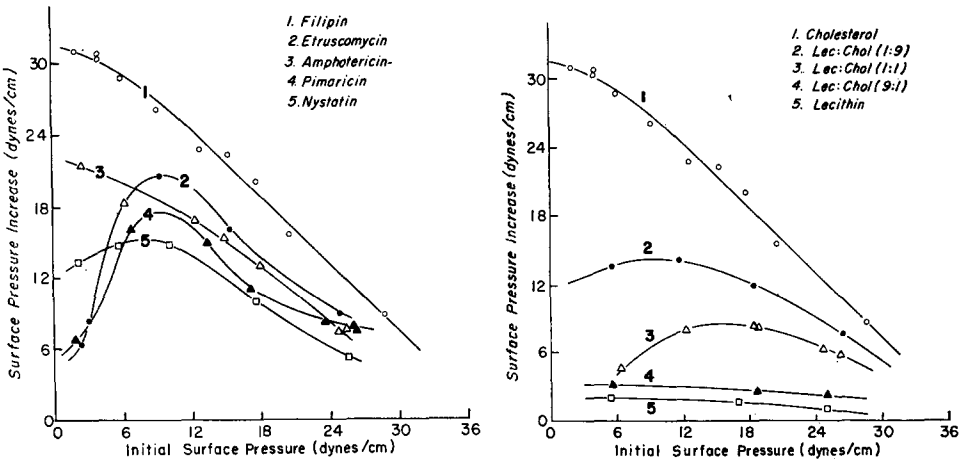

Fig. 5. Comparative interaction of polyene antibiotics with cholesterol monolayers. Data taken from Fig. 4 (present paper) and Figs. 2 and 3 (ref. 8). The molar ratio of antibiotic injected/spread lipid ranged from 0.10 (amphotericin B, etruscomycin and pimaricin) to 0.12 (filipin and nystatin).

Fig. 6. Interaction of filipin with lecithin–cholesterol monolayers. Monolayers were prepared from lipid mixtures containing the following molar ratios of synthetic lecithin (18:0/18:1 phosphatidyl choline) and cholesterol: (1) cholesterol alone, no lecithin; (2) lecithin/cholesterol, 1:9; (3) lecithin/cholesterol, 1:1; (4) lecithin/cholesterol, 9:1; (5) lecithin alone, no cholesterol. After compression to the initial surface pressures indicated on the abscissa, 6.38 nmoles of filipin was injected into the substrate and the increase in surface pressure was determined as described under MATERIALS AND METHODS. The film area ranged from 140 cm^2 (pure cholesterol monolayer) to 162 cm^2 (pure lecithin monolayer) and the molar ratio of filipin injected/spread lipid was kept constant (0.14).

membranes with a high phospholipid/sterol ratio, such as from bacteria (P:S = ∞) or mitochondria (P:S about 40), are not affected by the antibiotics, whereas membranes from fungi (P:S about 8) or erythrocytes (P:S about 1) are extremely sensitive to the polyenes.

The effect of lecithin on the interaction of the antibiotics with cholesterol was examined in detail because this compound is the predominant phospholipid present in the membrane of polyene-sensitive organisms. Fig. 6 shows that filipin produces a much smaller increase in the surface pressure of mixed monolayers containing

cholesterol and lecithin. This phenomenon, which is consistent with the above conclusions, was observed over the entire range of initial surface pressures. It should be emphasized, however, that the reason for this effect is not clearly understood at the moment. Comparison of Curves 1 and 2 indicates that, at 14 dynes/cm, a 10 % dilution of the cholesterol content of the monolayer by lecithin results in a 40 % reduction of the surface pressure increase. It is therefore probable that other factors, in addition to a 'dilution' of the number of 'reactive sites' (sterol molecules), may be involved. Further extensive speculation on this point seems unwarranted because, as discussed previously[2], the physical basis for the surface pressure increase is not yet known. The surface pressure increase may be due to actual penetration of the polyene into the monolayer and/or may reflect accumulation of the antibiotic directly underneath the monolayer with a subsequent spatial reorientation of the sterol molecules.

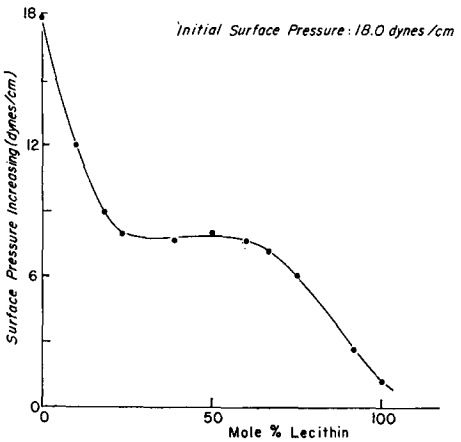

Fig. 7. Interaction of filipin with lecithin–cholesterol monolayers. Filipin (6.38 nmoles) was injected underneath monolayers prepared from lipid mixtures containing appropriate amounts of synthetic lecithin (18:0/18:1 phosphatidyl choline) and cholesterol to give the composition indicated on the abscissa. In this experiment, varying amounts of lipid were spread on the film surface to obtain an initial surface pressure of 18.0 dynes/cm at constant area (140 cm^2). The molar ratio of filipin injected/spread lipid ranged from 0.09 (pure cholesterol monolayer) to 0.14 (pure lecithin monolayer).

The effect of lecithin is further illustrated by Fig. 7. In this experiment, both the initial surface pressure and the film area were kept constant, and the interaction of filipin with monolayers containing various ratios of lecithin/cholesterol was determined. Under these conditions, lecithin does not produce a gradual 'inhibitory' effect but results in a plateau. The existence of this plateau may be consistent with the fact that the phospholipid/sterol ratio does vary to some extent even in membrane systems sensitive to the polyenes.

Effect of antibiotic/lipid ratio

In the experiments described above, the concentration of antibiotic was not varied. Previous studies[12,16] have demonstrated that the rate and extent of erythrocyte hemolysis induced by the polyenes is dependent on the antibiotic/cell (or antibiotic/membrane lipid) ratio and not only on the absolute concentration of the polyene. These observations have prompted us to investigate the effect of the anti-

biotic/lipid ratio on the surface pressure of cholesterol, lecithin–cholesterol, and lecithin monolayers.

Results obtained with filipin are shown in Fig. 8 in which the surface pressure increase is plotted as a function of the ratio: mole antibiotic (injected into the substrate) per mole of lipid present in the monolayer ('spread lipid'). As indicated by Curve 1, the maximum surface pressure increase is obtained at a very low molar ratio of filipin/spread lipid with cholesterol monolayers. The molar ratio necessary to obtain a given surface pressure is increased markedly when lecithin is present in the monolayer. Thus, in order to obtain a rise in surface pressure of 24 dynes/cm, molar ratios of approx. 0.05, 0.55 and 1.75 are required with monolayers containing 0 % (Curve 1), 50 % (Curve 3) and 91 % (Curve 5) lecithin, respectively. These results again demonstrate the inhibitory effect of lecithin on the interaction between filipin and cholesterol.

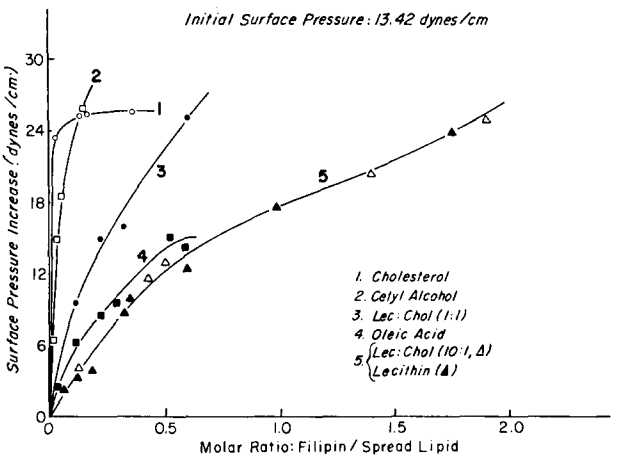

Fig. 8. Effect of filipin concentration on interaction with lipid monolayers. Monolayers were prepared with single lipids (cholesterol, cetyl alcohol, oleic acid, egg lecithin) or lipid mixtures (lecithin–cholesterol; molar ratio, 10:1 or 1:1) and compressed to an initial surface pressure of 13.42 dynes/cm. Varying amounts of the antibiotic were injected into the substrate to give the molar ratio of filipin/spread lipid indicated on the abscissa. The increase in surface pressure was determined as described under MATERIALS AND METHODS.

Filipin does, however, produce an appreciable increase in the surface pressure of monolayers containing lecithin alone at high molar ratios of antibiotic/lipid (Curve 5). These results are thus consistent with the finding by WEISSMANN AND SESSA that the antibiotic causes release of marker compounds from liposomes prepared without cholesterol[3,4]. Our previous inability to detect any significant interaction of the antibiotic with phospholipids was apparently due to the fact that we employed low concentrations of filipin. For example, in the experiment described in Fig. 6, the filipin/lecithin ratio was 0.14 which, as shown in Fig. 8, gives an increase in surface pressure much smaller than that obtained with the cholesterol monolayer. Whether the observed interaction of filipin has any physiological significance as regards polyene mechanism is discussed in detail below.

Results obtained with nystatin and pimaricin, the two other polyenes which have been presently investigated in this manner, are shown in Figs. 9 and 10. Inter-

action of these antibiotics with cholesterol is also inhibited by lecithin although, as is the case with filipin, nystatin and pimaricin can increase the surface pressure of the lecithin monolayer at high antibiotic/lipid ratios. These ratios were not employed under the conditions of the experiments described in Fig. 3 of ref. 8 and Fig. 4 of the present paper.

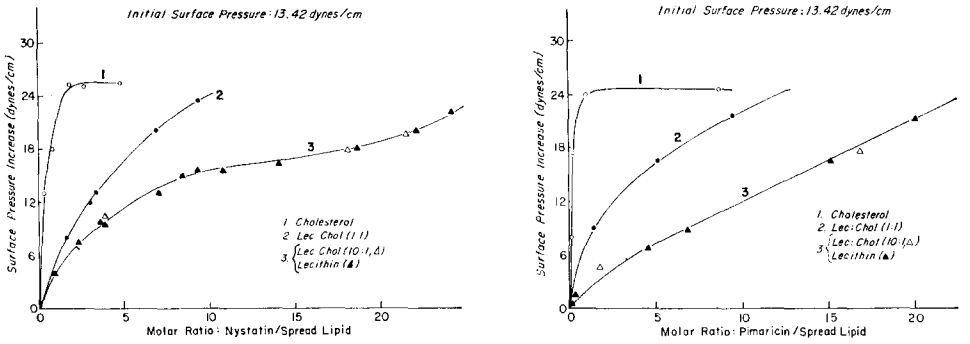

Fig. 9. Effect of nystatin concentration on interaction with lipid monolayers. Procedure identical to that described in legend to Fig. 8.

Fig. 10. Effect of pimaricin concentration on interaction with lipid monolayers. Procedure identical to that described in legend to Fig. 8.

The preceding results again demonstrate preferential interaction of the polyene antibiotics with cholesterol. It must be emphasized that, in these experiments, the maximum surface pressure obtainable (equivalent to the sum of the initial surface pressure *plus* the pressure increase due to injection of the antibiotic) corresponds to the collapse pressure of the lipid monolayer under investigation. This maximum was obtained with cholesterol and not with lecithin monolayers even at the highest antibiotic/lipid ratio (Figs. 8, 9 and 10).

Interaction of polyene antibiotics with lipids other than cholesterol or lecithin

We have also studied the interaction of filipin with several lipids which are not regarded as membrane constituents *per se*. As indicated in Fig. 8, Curve 4, filipin can increase the surface pressure of oleic acid monolayers but it is significant (see below) that this interaction is observed at relatively high molar ratios of antibiotic/lipid. Of all the compounds thus far tested, only monolayers of cetyl alcohol show an appreciable increase in surface pressure at low antibiotic/lipid ratios (Curve 2). Because cetyl alcohol, like cholesterol, contains an hydroxyl function, these results suggest that this group may be necessary for interaction with the polyenes. The experiment described in Fig. 11 supports this conclusion. Comparison of Curve 1 with Curve 5 indicates that esterification of the hydroxyl group in cholesterol has a pronounced inhibitory effect on the interaction with filipin. In fact, surface pressure increases with monolayers of cholesterol acetate were only obtained at initial surface pressures below the collapse pressure of the antibiotic. Similarly, surface pressure increases are less with monolayers of dicetyl phosphate than with monolayers of cetyl alcohol (*cf.* Curves 2 and 4). Lack of any significant interaction between the antibiotic and dicetyl phosphate is consistent with previous observations[1] that the presence of

dicetyl phosphate did not influence the morphological effects of filipin on lipid dispersions.

It is interesting to note that the presence of 5 M urea in the aqueous substrate results in a marked reduction in the surface pressure increase obtained by injecting filipin underneath cholesterol monolayers (*cf.* Curves 1 and 3). It is therefore possible

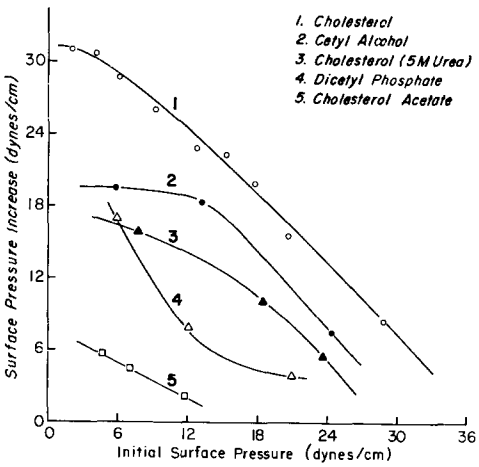

Fig. 11. Interaction of filipin with lipid monolayers. Monolayers were prepared with 0.054 μmole of cholesterol (Curve 1), 0.077 μmole of cetyl alcohol (Curve 2), 0.054 μmole of cholesterol on a subphase containing 5 M urea (Curve 3), 0.060 μmole of dicetyl phosphate (Curve 4), and 0.052 μmole of cholesterol acetate (Curve 5). After compression to the initial surface pressures shown on the abscissa, 6.38 nmoles of filipin were injected and the increase in surface pressure was determined as described under MATERIALS AND METHODS. The molar ratio of filipin injected/spread lipid was 0.12, 0.08, 0.12, 0.11, and 0.12 for Curves 1–5, respectively.

that hydrogen-bond formation may play an important role in polyene–sterol interaction. Additional experiments to determine which groups and configurations of the sterol nucleus are necessary for reaction with the antibiotics are now in progress.

DISCUSSION

It is of considerable interest to compare the present results using lipid monolayers as a model for studying polyene action with the recent experiments of WEISSMANN AND SESSA[4] who have studied the effects of the antibiotics on liposomes. Under their experimental conditions, WEISSMANN AND SESSA found that essentially the same amount of chromate, glucose or phosphate was released by filipin from liposomes prepared with and without cholesterol. This observation is difficult to reconcile with the prevailing evidence indicating that filipin is selectively toxic to those organisms which contain sterol in the cell membrane. For example, it has been previously demonstrated[17] that filipin has no effect on *Mycoplasma laidlawii* cultured in the absence of cholesterol, whereas the antibiotic inhibits growth, and induces lysis, of cells grown in the presence of the sterol. On the other hand, the results reported above clearly indicate that, at low molar ratios of antibiotic/lipid, filipin preferentially interacts with monolayers containing cholesterol. As an explanation for the divergent results obtained with monolayers and liposomes, we suggest that emphasis has to be

placed on the fact that, in the experiments described by WEISSMANN AND SESSA, extremely high concentrations (0.1 mM) of the polyenes were required to obtain an appreciable effect. In their experiments, the molar ratio of filipin/lipid may have been in the range where interaction between the antibiotic and lecithin has been observed, *i.e.* where filipin does not interact preferentially with cholesterol (*cf.* Fig. 8). Unfortunately, there is at the moment no way of determining what percentage of the total lipid, added as liposomes, is present in the bilayer configuration and accessible to the antibiotic.

The polyene antibiotics are amphipathic compounds and it is, therefore, not surprising to find that, under appropriate conditions, these antibiotics can interact with a variety of lipids. For example, in addition to lecithin monolayers, filipin can cause a significant increase in the surface pressure of oleic acid monolayers at high molar ratios of antibiotic/lipid (Fig. 8). This observation is consistent with numerous reports indicating that fatty acids, as well as phospholipids, can antagonize the growth-inhibiting properties of the polyenes. It is apparent, however, that interaction between the antibiotics and phospholipids, *etc.*, cannot account for the selective toxicity of the polyenes because these lipids, unlike sterols, are constituents of both antibiotic-sensitive and -insensitive cells. The physiological significance of results obtained with any membrane model requiring high concentrations of antibiotic and/or lipid to demonstrate an effect, must be interpreted with caution; this restriction applies not only to liposomes but also to monolayers and bilayers.

As noted in the discussion of Fig. 5, there is some correlation between the ability of the polyenes to interact with cholesterol monolayers and the extent of membrane damage which they cause. It is probable that other factors, currently unknown, may also play an important role. Indeed, WEISSMANN AND SESSA have suggested that the more extensive membrane damage which results with filipin is due to interaction of this antibiotic with membrane phospholipid[4]. On the basis of the available evidence, we feel that this assumption is unnecessary and that the present experiments support our earlier contention that all polyenes share the same basic mechanism[2]. We do not mean to imply that polyene–phospholipid interaction does not occur in biological systems under some circumstances. Several laboratories have recently used filipin and other polyene antibiotics as tools for investigating membrane-associated phenomena.

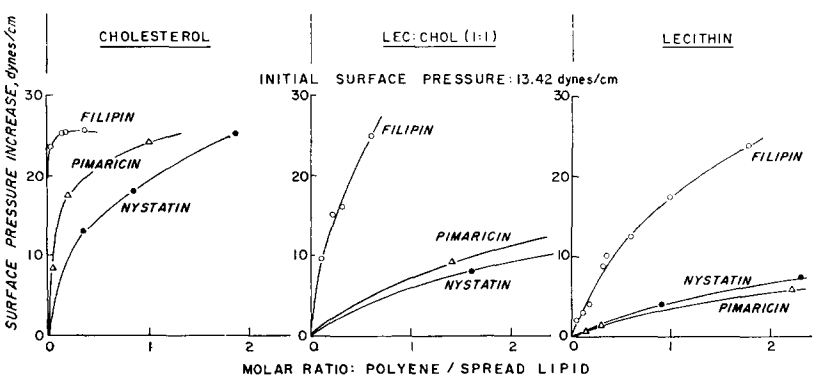

Fig. 12. Comparative interaction of filipin, nystatin and pimaricin with monolayers of cholesterol, lecithin–cholesterol (1:1) and lecithin. Data taken from Figs. 8, 9, and 10.

In some of these studies (see for example, refs. 18 and 19) very high levels of the antibiotics were employed and it is possible that many of the observed effects can be attributed to interaction with phospholipid.

Fig. 12 demonstrates that the correlation between polyene interaction with monolayers and membrane damage still holds with mixed monolayers containing lecithin and cholesterol. Thus, to obtain the same surface pressure increase requires much smaller molar ratios of antibiotic/lipid with filipin than with nystatin; pimaricin occupies an intermediate position. In the case of liposomes, however, WEISSMANN AND SESSA found that filipin and nystatin (0.1 to 1 mM) induce leakage of chromate from lecithin–cholesterol liposomes to the same extent. They also observed that nystatin was more effective than amphotericin B, which, in turn, was more effective than etruscomycin. This is not in agreement with previously published experiments (ref. 16; see also DISCUSSION in refs. 5, 6 and 7) indicating that the hemolytic potency was in the order: filipin $\geq$ etruscomycin $\gg$ amphotericin B $>$ pimaricin $>$ nystatin. In fact, WEISSMANN AND SESSA have emphasized that pimaricin had no effect on the liposomes which is difficult to reconcile with the known biological activity of this antibiotic. The present experiments have shown that pimaricin can interact with monolayers containing lecithin only (Figs. 10 and 12). In this regard, it is interesting to note that, although filipin produces a greater increase in the surface pressure of a lecithin monolayer than either nystatin or pimaricin, the latter antibiotics are essentially equally effective.

We have stressed the importance of the antibiotic/lipid ratio in studies employing model membrane systems because previous investigations have demonstrated that the rate and extent of polyene-induced hemolysis is dependent on the antibiotic/cell ratio[12,16]. In the case of filipin[12], the minimum molar ratio of antibiotic/membrane lipid necessary for complete hemolysis is approx. 0.10. Fig. 8 indicates that, at this molar ratio, filipin interacts preferentially with monolayers containing cholesterol. Similar (unpublished) experiments with nystatin have shown that the minimum molar ratio of antibiotic/membrane lipid necessary for induction of complete hemolysis is approx. 3.0. At this molar ratio, nystatin also interacts preferentially with monolayers containing cholesterol (Fig. 9). Considering the possible sources of error, the agreement between the monolayer and lytic experiments constitutes additional justification for the view that lipid monolayers are appropriate models for studying the mechanism of polyene action.

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