Interaction of a Lytic Polypeptide, Melittin, with Lipid Membrane Systems*

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SUMMARY

To study mechanisms whereby lytic proteins disrupt biomembranes, artificial phospholipid spherules (liposomes) were exposed to melittin, a cationic peptide in which sequences of hydrophobic (Positions 1 to 20) and hydrophilic (Positions 21 to 26) amino acids are unequally distributed. At concentrations above $10^{-4}$ M, melittin, which is the major toxin of bee venom, released marker anions ($\text{CrO}_4^{2-}$) or glucose from the model structures. Release of marker ions was equivalent whether liposomes were prepared with a net negative (dicetyl phosphate) or net positive (stearylamine) charge. Nor was the presence of cholesterol in the model membranes necessary for the action of melittin. Spherules with net negative charges competed approximately 10 times more effectively for melittin's lytic activity on erythrocytes than did spherules with positive charges. Studies with lipid monolayers indicated that melittin had a remarkable affinity for the air-water and more so for the lipid-water interface, for it spread as a film readily from water and penetrated lipid monolayers avidly, irrespective of the surface charge of the lipid film. Negatively stained preparations of liposomes in the electron microscope showed that melittin-treated spherules lost the integrity of their concentric lamellae, which became beaded, frayed, and finally fragmented. These studies indicate that the lytic effects of melittin upon biological membranes may be due to a similar reaction with structural phospholipids. Since ionic interactions between the cationic peptide and charged lamellae were not crucial for lysis, and since melittin has an extraordinary affinity for lipid membranes, it is suggested that the surface activity of melittin and convenient apolar associations between hydrophobic portions of melittin and the acyl chains of phospholipid could account for disruption of the spherules.

Mechanisms by which proteins lyse biomembranes remain unclear largely because neither the structures of the target membranes nor those of the lytic proteins have been rigorously defined. However, by the use of model lipid membranes and considerably purified bacterial hemolysins, it has been shown that proteins such as streptolysin S (1, 2) or staphylococcal $\alpha$-toxin (3, 4) can directly disrupt the lamellar structure of phospholipids in aqueous solutions. It was therefore suggested that lytic proteins act on naturally occurring membranes in a similar fashion. Unfortunately, these larger, lytic proteins have not been isolated in sufficient purity to permit detailed study of primary amino acid sequences, which must precede any attribution of their unique action to a particular structure.

No such problem exists with melittin, which has been purified, resolved from related peptides, and analysed structurally, by Habermann and co-workers (5–8). Melittin is the major component of bee venom, constituting over 50% of its dry weight. The cationic peptide is unusual, in that its amino acids are unequally distributed: Positions 1 to 20 are occupied by largely hydrophobic, and Positions 21 to 26 by hydrophilic, amino acids (Fig. 1). Since the peptide is too small to accommodate itself to a globular configuration, it appears to associate as tetramer micelles in aqueous media: this property accounts for the experimentally obtained molecular weight of approximately 12,000 as against the theoretical molecular weight of 3,400. Thus each melittin chain constitutes a large biological amphipath, the effects of which as a "surface active agent" have been held accountable for its biological properties (6). These include not only hemolysis, but disruption of isolated leukocytes, lysosomes, and mitochondria (9, 10).

If, then, a defined lytic peptide is available, so also are defined models of biomembranes. Phospholipids in the smectic mesophase can be induced to form spherules (liposomes), the permeability properties of which are somewhat similar to biological interfaces (review in Reference 11). The assemblies are, for example, capable of ion discrimination. Cations and divalent anions are retained, while monovalent anions and water diffuse freely: (12) the spherules swell in response to osmotic stimuli (13). In aqueous solutions, liposomes exist as concentric lipid layers with intercalated water compartments in which marker ions or molecules are trapped. X-ray, electron microscopic, and polarized light studies indicate a lamellar substructure, the repeating unit of which is from 55 to 75 A, depending on the

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Melittin

Fig. 1. Amino acid sequence of melittin (6)

particular lipids used (14) and their ionic environment. Since the surface charge and lipid composition of the spherules can be varied at will, and since they respond to a wide variety of lytic molecules (steroids (2), polynens (15), anesthetics (16)), they constitute at least one reasonable model for biological membranes. We have therefore investigated the action of melittin upon liposomes and have found that the peptide released marker ions or glucose from the spherules independently of their surface charge, their content of cholesterol, or the nature of the marker in the aqueous compartments. These functional changes were accompanied by morphological evidence of interaction (negative staining), and the results indicated that melittin could both insert into artificial lipid layers and disrupt their integrity. The former finding was confirmed by studies with lipid monolayers.

MATERIALS AND METHODS

Melittin was obtained through the extreme generosity of Dr. E. Habermann. Phosphatidylcholine from egg yolk was obtained as chromatographically pure from General Biochemicals. Cholesterol was obtained from Sigma, dietyl phosphate and stearylamine from K and K Laboratories (Plainview, New York).

Preparation of Phospholipid Spheres (Liposomes)—Liposomes were prepared by a slight modification of methods previously described in detail (15). In brief, lipids (either lecithin-dietyl phosphate or stearylamine-cholesterol in molar ratios of 7:2:1, or lecithin-dietyl phosphate in the molar ratio of 8:2) were spread from a 0.5 μg per ml solution in chloroform solution to a round bottom flask. By the use of a rotary evaporator, a uniformly thin film was formed. Subsequently 6 ml of the marker solution (either 0.29 μM glucose or 0.145 μM potassium chromate) were added to yield a total lipid concentration of 16.6 μmol per ml. After the lipid film was removed by gentle shaking, the suspension was disrupted by sonic oscillation for 45 sec in a D assigns automatic cleaner (model HD-50, Heat Systems Company, Melville, New Jersey). The milky suspension was permitted to stand for about 2 hours and thereafter carefully layered on top of a Sephadex G-50 column (coarse, 20 cm) (13). The spherules were then eluted from the column with a solution of NaCl-KCl (0.145 M total molarity). Chromate ions and glucose not associated with the spherules were removed by this means. Subsequently, 1-ml samples of spherule suspension were placed into small dialysis sacs to which varying concentrations of melittin in H₂O were added. The sacs were shaken at 37° in tubes filled with 5 ml of NaCl-KCl, 0.145 M. Leakage from the spherules through the sacs into the surrounding fluid was determined at 30 and 60 min. Chromate was determined by its absorbance at 370 nm, glucose by the glucose oxidase method (2).

Hemolytic Assay—Hemolysis was measured by the method of Bernheimer (17) with freshly obtained, heparinized human erythrocytes. In these experiments, phosphate buffer (KCl–K₂HPO₄, 0.14 M total molarity, pH 7.0) was used as aqueous marker instead of the usual markers (glucose, potassium chromate) since this was the solution in which the red blood cells were also suspended. The lipid concentration was decreased to one-fourth of that used for the leakage experiments (4 μmol per ml). The incubated mixture consisted of 0.4 ml of phosphate buffer, pH 7.0, 0.1 ml of the spherule suspension, and appropriate amounts of melittin in a total volume of 0.6 ml. After incubation for 20 min at room temperature, 0.5 ml of human red blood cells (0.7%, v/v, suspension in buffer) was added. The tubes were placed in a water bath for 7 min at 37° and centrifuged for 20 min at 10,000 x g to remove the lipid dispersion and the erythrocytes. Total hemolysis was obtained by adding 10 μg of saponin. The release of hemoglobin was measured by its absorbance at 545 nm.

Monolayer Experiments—Surface activities of the lipids and of melittin itself were studied by techniques which have previously been described (18). Three types of experiments were performed: (a) the capacity was tested of melittin and of the lipids to spread as a film, as was (b) the capacity of melittin to form a film from the subphase, and (c) the ability of melittin to penetrate the lipid film. The trough consisted of a circular crystallizing dish in Pyrex glass (capacity 50 ml), which was divided into two unequal compartments by a fixed barrier annealed across the top. Trough and barrier were coated with paraffin. The larger area, 19 cm², was reserved for the film, whereas the small service area was used for the injection of melittin when film penetration was studied. The subphase, consisting of 0.04 M potassium phosphate buffer, 0.1 M NaCl, pH 7.0, 25°, was mixed with the aid of a magnetic stirrer. This technique for making lipid films has been described in detail previously (19).

In the film penetration studies, lipids (either lecithin-dietyl phosphate-cholesterol or lecithin-stearylamine-cholesterol, in molar ratio of 7:2:1) were spread from a 0.5 μg per ml solution in chloroform-methanol (85:15) until the desired initial film pressure (π_i) was obtained. At this point, "zero time," 50 μg of melittin in 50 μl of water were injected into the subphase with a microsyringe, and the increase in film pressure (Δπ) was recorded as a function of time. In the determination of the surface isotherms, the lipid was spread from 0.5 μg per ml solution in chloroform-methanol (85:15), whereas melittin was applied to the surface from a 1.0 μg per ml solution in distilled water.

Electron Microscopy—For electron microscopy, specimens were negatively stained with 2% ammonium molybdate in 2% ammonium acetate (pH 6.8) and applied to hydrophilic films of carbon-coated Formvar supported on copper specimen mounts. The preparations were examined in a Siemens Elmiskop 1A electron microscope fitted with a decontamination device, with a short focal length objective lens.

RESULTS

Release of CrO₄²⁻ from Negatively and Positively Charged Spheres by Melittin

It is possible to prepare spherules with either a negative or a positive net surface charge by incorporating into the lamellar structures either anionic dietyl phosphate or cationic stearylamine (12). Spherules carrying a net negative charge were exposed to melittin at final concentrations varying from 10⁻⁷ M to 10⁻¹ M. Triton X-100 was also studied, as a relative standard, since, at a final concentration of 0.2%, v/v, the detergent induces maximum release of markers from the spherules; its effect upon release of anions from liposomes has previously been extensively described (2, 20). At concentrations of 10⁻⁴ M (Fig. 2), melittin released significant amounts of divalent anions into
Release of \( \text{CrO}_4^{2-} \) from Spherules Prepared in Absence of Cholesterol

At least some amphipaths, e.g. amphotericin B, nystatin (15) filipins I and III (20), preferentially disrupt liposomes which contain sterol as part of their lamellar composition. To determine whether lytic polypeptides also require sterol for optimum membrane action, liposomes were prepared without cholesterol. Such spherules (Fig. 4) did not respond to melittin with augmented anion release; indeed, the results are quite comparable to those in Figs. 2 and 3. From these experiments, it appeared that the reaction of melittin with liposomes was independent of sterol receptors, resembling in this property those of steroid hormones (2), staphylococcal α-toxin (3, 4), and filipin II (20).

Release of Glucose from Phospholipid-Cholesterol Spherules by Melittin

Since a portion of the melittin molecule contains polar, cationic groups, the possibility remained that these nonpermeant polycations in the medium surrounding the spherules could be responsible for an increased efflux of marker anions. Diffusion of anions across individual lamellae would be due to the incapacity of melittin in the medium to penetrate the liposomes and the requirements for both counter- and psi-bound anions would lead to anion efflux (22). To exclude such a possibility, studies were performed with spherules containing glucose, a nonelectrolyte (Fig. 5). However, glucose was released from liposomes at the same concentrations of melittin which induced

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1 It will be appreciated that in aqueous media of electrolytes direct ionic interactions between charged lipids and peptides are not the only possible ones (21). Indeed, interactions between these and small inorganic solvent ions can readily lead to indirect charge-induced interactions. For convenience, both of these associations will be referred to as “saltlike interactions.”

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**Fig. 2.** Release of \( \text{CrO}_4^{2-} \) from liposomes by increasing amounts of melittin. Spherules prepared with lecithin-dioctyl phosphate-cholesterol in molar ratio of 7:2:1 (16.6 µmoles of lipid per ml). Results are expressed as the percentage of \( \text{CrO}_4^{2-} \) appearing in the dialysate after 30 min of incubation at 37° with Triton X-100 (0.2%). To control samples, 0.2 ml of NaCl-KCl (0.145 M) was added. Mean of three experiments.

**Fig. 3.** Release of \( \text{CrO}_4^{2-} \) from liposomes by increasing amounts of melittin. Spherules prepared with lecithin-stearylamine-cholesterol in molar ratio of 7:2:1 (16.6 µmoles of lipid per ml). Results are expressed as the percentage of \( \text{CrO}_4^{2-} \) appearing in the dialysate after 30 min of incubation at 37° with Triton X-100 (0.2%). To control samples, 0.2 ml of NaCl-KCl (0.145 M) was added. Mean of three experiments.
Fig. 4. Release of CrO$_4^{2-}$ from liposomes by increasing amounts of melittin. Spherules prepared with lecithin and dioleyl phosphatidylethanolamine in molar ratio of 8:2 (16.6 µmoles of lipid per ml). Results are expressed as the percentage of CrO$_4^{2-}$ appearing in the dialysate after 30 min of incubation at 37°C with Triton X-100 (0.2%). To control samples, 0.2 ml of NaCl-KCl (0.145 M) was added. Mean of three experiments.

Fig. 5. Release of glucose from liposomes by increasing amount of melittin. Spherules prepared with lecithin-dioleyl phosphatidylethanolamine-cholesterol in molar ratio of 7:2:1 (16.6 µmoles of lipid per ml). Results are expressed as the percentage of glucose appearing in the dialysate after 30 min of incubation at 37°C with Triton X-100 (0.2%). To control samples, 0.2 ml of NaCl-KCl (0.145 M) was added. Mean of two experiments.

Fig. 6. Release of hemoglobin from human erythrocytes produced by increasing amounts of melittin (micrograms per ml) in the presence or absence of liposomes. Liposomes, prepared either with lecithin-dioleyl phosphatidylethanolamine-cholesterol, or with lecithin-stearoylaminocholesterol in ratio of 7:2:1, were allowed to swell in phosphate buffer, 0.067 M, pH 7.0. After 1 hour liposomes (0.1 ml) were incubated with indicated amounts of melittin for 20 min at room temperature. Thereafter, 0.5 ml of human erythrocytes (0.7%, v/v, suspension in phosphate buffer, 0.067 M, pH 7.0) was added. After incubation at 37°C for 7 min, the suspension was centrifuged for 20 min at 10,000 × g. Hemolysis was calculated as the percentage of total hemolysis induced by saponin-hemolysis in controls or (A$_{540}$ saponin - A$_{540}$ solvent + phospholipid)/(A$_{540}$ melittin - A$_{540}$ solvent + phospholipid) × 100.
μmole per ml could 50% hemolysis be obtained with 11 μg of the peptide. These experiments indicated that negatively charged spherules competed more effectively with erythrocytes (by a factor of 10) for melittin than did positively charged spherules. This suggested not only that ionic interactions of cationic polypeptide and anionic liposomes could be detected, but that these were not crucial for lysis of the target spherules, since liposomes were equally disrupted whether positively or negatively charged. If this increased requirement for melittin (in hemolytic systems with negatively charged spherules) does indeed represent a lytic interaction of melittin with liposomes, then it appeared possible that the crucial step in polypeptide lysis was the insertion of melittin’s hydrophobic residues into lipid layers, although the initial attraction might be saltlike.

**Surface Activity of Melittin**

In order to test the “insertional” hypothesis, namely, whether melittin could penetrate natural or artificial lipid layers, we studied the surface activity of melittin at the air-water interface.

**Surface Isotherm of Melittin**—Melittin spread readily from water and formed a stable film which had the relatively high collapse pressure of 24.5 dynes per cm (Fig. 7); on a weight basis, the water-soluble melittin was markedly more surface-active than the lipid. Indeed, melittin surfaced from a concentration of 1 μg per ml in the aqueous subphase in the absence of lipid and formed a film, the saturation pressure of which (24.5 dynes per cm) was the same as the collapse pressure of the melittin film obtained from spreading. The curves of pressure with respect to concentration and the collapse pressure (about 36 dynes per cm) of the lipid film containing lecithin, cholesterol, and either dicetyl phosphate or stearylamine in the molar ratio of 7:2:1 were similar to those of egg lecithin. In line with a recent observation (18), the lipid did not form a film by spreading from aqueous dispersion, whereas melittin did so readily.

**Penetration of Lipid Monolayers by Melittin**—As observed with other proteins (19), a water surface covered by lipid films (Fig. 8) was penetrated by melittin much more readily than a clean air-water interface. The lipid films were prepared by spreading a mixture of lecithin-dicetyl phosphate-cholesterol, in the same molar ratio as that used for the spherules (7:2:1) on a liquid phase-phosphate buffer. The initial pressure (πi) of the lipid film was varied from 2 to 30 dynes per cm, and it was found (Fig. 8) that at an initial pressure of 2 dynes per cm the injection of melittin produced an increase in the film pressure (Δπ) of 17 dynes per cm within 10 min, which reached a plateau (Δπ) at 25 dynes per cm in 30 min. An increase could indeed be detected at an initial pressure of 10 or 20 dynes per cm, but there was also a considerable increase in pressure. An increase could indeed be detected at an initial pressure of 30 dynes per cm, which is clearly above the collapse pressure of melittin. When stearylamine was substituted for dicetyl phosphate in identical experiments, curves were obtained which were in every way comparable to those shown in Fig. 8; these are omitted for the sake of brevity. Finally, when lecithin alone was used, essentially similar results were again found.

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**Fig. 7.** Surface isotherms of melittin and lipid at an air-water interface. Subphase, 0.04 M potassium phosphate buffer, 0.15 M NaCl, pH 7.0, 25°C. A, •, surface pressure (σ) with respect to concentration in micrograms of melittin spread from water; C, surface pressure (σ) with respect to concentration in micrograms of lipid (lecithin-dicetyl phosphate-cholesterol, 7:2:1), spread from chloroform-methanol, 85:15. B, △, surface pressure (σ) with respect to time curve of melittin film formed from its solution, 1 μg per ml in the subphase, in the absence of lipid.

**Fig. 8.** Interaction of melittin with monolayers of mixed lipid at different initial film pressures (πi). Subphase, 0.04 M potassium phosphate buffer, 0.15 M NaCl, pH 7.0, 25°C. Lipids, lecithin-dicetyl phosphate-cholesterol (7:2:1) spread from chloroform-methanol (85:15). Melittin, 1 μg per ml, i.e. 50 μg of melittin injected into 50 ml of subphase. Ordinate, increase in pressure (Δπ) above the initial film pressure (πi). When dicetyl phosphate was replaced by stearylamine (molar ratio 7:2:1) in the lipid mixture, the film penetration (Δπ) curves could be superimposed upon the ones above.
Electron Microscopy

Spherules were prepared with lecithin-dicetyl phosphate-cholesterol in the usual ratio, at a total lipid concentration of 4 µmoles per ml (which was one-fourth of that used for the leakage experiments), and allowed to swell in water. In Fig. 9 a preparation is shown of liposomes negatively stained with ammonium molybdate. Lipid lamellae intercalated by electron-density aqueous compartments as described by Bangham, Standish, and Watkins (12) are clearly visible. After addition of melittin to the liposomes, the characteristic concentric lamellar arrangement of lipids was no longer evident. Long strands and coalesced sheetlike structures appeared, which seemed to arise from the disruption of the lamellae. Large areas of such sheets appeared as nets of material, possibly due to molecular rearrangements brought about by the presence of melittin (Fig. 9B). At higher magnifications (Fig. 9C), different regions of the preparation showed what appeared to be different degrees of disruption, ranging from lamellae, through flat sheets of material showing many small holes, to the netlike areas made up of threadlike structures of uniform dimensions. Under the same conditions of examination, melittin alone could not be clearly resolved and appeared amorphous. Furthermore, negatively stained preparations of positively charged spherules treated with melittin could not be distinguished from negatively charged liposomes so treated.

Discussion

The experiments presented above document the capacity of melittin to disrupt artificial phospholipid arrays (liposomes and monolayers). If the integrity of biomembranes, at least in part, is governed by lipid-lipid interactions analogous to those of the models, then it is quite likely that the effects of melittin on cellular and intracellular membranes are due to the peptide's interruption of such forces. The structure of liposomes has been discussed elsewhere in detail (11, 23), but their overall integrity is governed both by intermolecular cohesive forces (polar, apolar, hydrogen bonding) and by forces arising from the solvent per se, either set of which could be affected by melittin.

Since melittin in aqueous solutions can be considered a catonic detergent which forms micelles (6), the individual peptide chains will be in constant equilibrium between these and the bulk solution. Thus melittin can disrupt liposomes by one of at least four mechanisms (21, 24, 25). The peptide could (a) overcome polar adhesive forces between phospholipids; (b) disrupt apolar (hydrophobic) cohesive forces between the acyl chains; (c) break cohesive forces imposed by water (as ice) (21) in the region of the charged head groups; or (d) act in classical detergent fashion to form mixed micelles of phospholipid with melittin, in which the individual acyl chains of the liposomes would associate with melittin's hydrophobic portion, the polar groups of phospholipids and melittin facing the bulk solution. The last mechanism would clearly be a more extreme consequence of (b). Whatever cohesive forces may arise from staggered structures of the polar head group of natural membranes (26, 27) recent X-ray studies with pure lamellar bilayers (as in liposomes) indicate that the head groups of phospholipids are fully extended in the presence of water (28). Thus the van-der-Waals should provide little cohesion to the liposome structure, especially in the presence of salt (Na-KCl). Furthermore, the presence of net repulsive forces between long chain anions or cations (stearylamine or dicetyl phosphate) would overcome any polar cohesive forces. Similarly, although the presence of structured water in the vicinity of the polar head groups has been held responsible for cohesion of closely packed lamellae (29), there is little evidence that the head groups of pure lecithins are restricted in their movement (or structured) in the presence of over 16% water (30). Indeed, since the aqueous channels within liposomes are separated by at least 45 Å (13), the free water area is very large relative to any structured "ice" layer.

The major cohesive forces which hold liposomes together are therefore likely to be apolar. It is then also likely that melittin can overcome those forces in the manner of an ionic detergent, after penetrating the outer layers of liposomes. Penetration of monolayers was indeed shown to be avid, proceeding also at high initial values of the peptide pressure (7). Indeed, melittin penetrated lipid films at initial pressures which exceeded the collapse pressure of the peptide. Depending upon the local concentration of reactants, the final structures would consist of micelles containing varying proportions of phospholipid to long chain anion or cation to melittin. If micelles of unlike charge (e.g. mixed micelles of cationic melittin with dicetyl phosphate) were more stable and removed more structural acyl chains from liposomes, then liposomes prepared with dicetyl phosphate should have been more susceptible to melittin disruption. Since this was not found, it is likely that only a few melittin molecules need to insert into the lipid layers, and that transitions from lamellar to micellar forms could arise locally. Since hydrogen bonding is stronger in apolar environments (31), and since proteins can participate in such bonds to a greater extent than simple acyl chains, the single peptide chains may form stronger intra- and intermolecular bonds in the phospholipid milieu than the bonds which the hydrophobic chains of these lipids can form with each other. Indeed, melittin inserted into monolayers below the critical pressure of 34 dynes per cm which was used to separate "detergency" from "penetration" by Pettics and Schulman (32).

In the foregoing, melittin has been considered as an ionic detergent of biological origin. Some of these compounds have previously been shown to interact preferentially with cholesterol in model lipid systems (33, 34). Since the presence or absence of cholesterol made little difference in the disruption of liposomes, melittin clearly does not fall into this group. Nor were charge-induced, ionic interactions between the catonic peptide and the target structures crucial for lysis. Our observation that negatively charged spherules removed more of the hemolytic activity from solutions of melittin can be accounted for by at least two mechanisms. Both anionic and cationic liposomes were disrupted at equal concentrations of melittin. Therefore, amounts of melittin sufficient for lysis entered the lamellae of spherules.

Fig. 9. A, Lecithin-cholesterol-dicetyl phosphate (7:1:2) spherules swollen in water and stained with 2% ammonium molybdate in 2% ammonium acetate (pH 6.8). The concentric lipid bilayers are separated by electron-dense aqueous layers. × 300,000. B, Lecithin spherules prepared as in A, after addition of melittin (10⁻⁴ M). The characteristic concentric lamellar arrangement is no longer evident. Instead long strand and sheetlike structures (arrow) appeared. × 80,000. C, Same preparation as in B, only at higher magnification. Different regions present different degree of disruption, from flat sheets of material showing many small holes, to netlike areas (arrows) made up of threadlike structures of uniform dimensions. × 300,000.
bearing either charge, but negative spherules took up more of
the excess molecules which did not participate in lysis. This
may be a further demonstration that the hemolytic system is
more sensitive in detecting "prolytic" removal of amphipath
from suspension that is the spherule system. Such an obser-
vation has already been made with filipin (20) and reflects
the high lipid to solvent ratio of liposomes relative to erythrocytes.
Alternatively, as Lucy has suggested (24), much of the added
diacyl phosphatidylcholine in liposome suspensions may exist as a
population of anionic micelles which are not necessarily part of
the general liposome population. Were this the case, saltlike
interactions would be detected of melittin with diacyl phosphatidyl-
choline micelles, but these would have no influence upon the amount of
melittin needed for lysis of the formed liposomes.

The interpretation of electron micrography of lipids is difficult.
Although Lucy and Glauert (35) have presented evidence that
lipids dry in salts like ammonium molybdate as if imbedded in
a glass, there is contrary evidence that their exact configuration
varies with water content (36). Fine structural details of control
spherule preparations examined in the above manner do, however, correlate well with results of both optical and
X-ray diffraction studies. Ultrastructural evidence indicates
that melittin induces not only functional, but morphological
changes in spherules of pure lipids. Beading, fraying, coales-
cence, and the appearance of netlike areas were observed when
the peptide disrupted lipid layers. However, these do not
permit conclusions as to whether the new structures were due
to composites of melittin with phospholipids or of phospholipids
alone in a solution containing a cationic detergent such as
melittin. It must be remarked that no such discrete structures
were observed when liposomes were treated with a nonionic
detergent such as Triton X-100 (2), but that both strepto-
lysin S (1) and staphylococcal α-toxin (4) produced new mor-
phological structures which were characteristic of each lysis.

Therefore, although much uncertainty still exists as to the
mechanisms of lipid film penetration by proteins (18) or deter-
gents (32), some results of our monolayer experiments cannot
be overlooked. Namely, melittin is extremely surface-active,
as (a) it spreads readily as a film from water, (b) it forms a film
from its aqueous solutions at a high rate, and (c) it penetrates
lecithin and mixed lipid films (Fig. 8) at a rate and to an extent
which exceed any heretofore described in the literature of bio-
logical surfactants (18, 19). These physical properties of melit-
tin may well account for its ability to penetrate and disrupt
natural and artificial lipid assemblies, and its capacity equally
well to penetrate cationic, anionic, and uncharged (lecithin)
lipid monolayers. Whether melittin interacts with the zwitter-
iconic structure of lecithin may be relevant, but this was not
established. Whatever the exact mechanism involved in disrup-
tion of lamellar lipids by melittin, it is clear that one simple
explanation for the effects of this peptide upon biomembranes
is its capacity to disrupt the three-dimensional structure of
phospholipid arrays.

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