Subphase Exchange Apparatus for Monomolecular Film Studies

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Abstract □ An apparatus for studying monomolecular films, by which the subphase can be changed without disturbing the film, was developed and the effectiveness of the apparatus was studied. Almost 100% of the subphase could be replaced when 1500 ml. of the subphase was exchanged over 20 min. By studying dipalmitoyl lecithin monolayers with and without subphase exchange (the π–A curves in both cases were the same), it was established that the films are not disturbed during the subphase exchange operation. The effect of subphase exchange on human serum albumin films of different concentrations was studied. The films became condensed when a water subphase was exchanged with water, apparently because of loss of some protein from the surface. This effect was more pronounced when films were spread using larger amounts of human serum albumin. The loss of less protein when less human serum albumin was spread was attributed to better spreading. The films also became condensed when a sodium chloride subphase solution was replaced with sodium chloride solution of the same concentration. However, the effect was less pronounced as compared to the water exchange with water. The π–A plots of human serum albumin films, in which the water subphase was exchanged with sodium chloride solution, were superimposable on the π–A plots of films spread on sodium chloride solution without exchange. Films of malate dehydrogenase formed using different amounts of this enzyme were studied after the buffer subphase was changed with a buffer solution of the same concentration. The exchange of this subphase had no effect on the π–A plots of any malate dehydrogenase film.

Keyphrases □ Monomolecular films—apparatus for changing subphase without disturbing film, dipalmitoyl lecithin, human serum albumin, and malate dehydrogenase films □ Films, monomolecular—apparatus for changing subphase without disturbing film □ Dipalmitoyl lecithin films—used to study subphase-changing apparatus □ Human serum albumin films—used to study subphase-changing apparatus □ Malate dehydrogenase films—used to study subphase-changing apparatus

Monomolecular films represent a relatively simple type of membrane model having a well-defined organized structure. They provide a convenient and promising method of studying molecules in a fixed orientation, as well as in a single layer where orientation can be changed by compression of the monomolecular film. As such, they constitute an important model system for the study of many natural phenomena involving surfaces of an oriented array of molecules.

Monomolecular films have been extensively used as structural models of the cell membrane. These model membranes cannot be expected to mimic fully a cell membrane. Their simplicity can also be considered a serious shortcoming to their use as a general model for biological membrane function. What can realistically be expected from studies with model membranes is some insight into the molecular mechanism of specific functions, as well as some knowledge of the function of specific areas within the heterogeneous makeup of biological membranes. Nevertheless, they provide considerable insight into the structure and function of biomembranes.

Monolayer studies involving proteins and enzymes present unique difficulties. For example, estimation of catalytic activity of surface-spread enzymes is difficult because of possible contamination by adsorbed unspread molecules beneath the film or by protein molecules dissolved in the bulk. There is a need for assaying enzymes *in situ*, but the dangers of contaminating the film with unspread globular protein from the subsolution are great (1). Ideally, the assay should be carried out while the molecules are adsorbed at the interface in question without manipulation or transfer of the film (2).

About 25–56% of protein spread on water was reported (3) to escape to the bulk, leaving 75–44% in the surface. Mechanical agitation was applied (3) under the film to assist the molecules adsorbed under the film to escape to the bulk. The amount of protein escaping to the bulk, of course, depends on many factors including the amount of protein applied, method of spreading, and spreading time.

To determine the activity of a surface-spread enzyme, the fraction of the enzyme that is adsorbed just below the surface or that escapes into the bulk phase must first be removed. In previous studies, this was accomplished by removing the surface-spread enzyme from the environment of the subphase either by transferring the enzyme monolayer to metal slides or by removing it with filter paper or a silk net. Alternatively, monolayers have been compressed to a fiber and then transferred to the substrate (3).

To investigate the effects of varying film pressures (varying degree of unfolding) on the activity of surface-spread acetylcholinesterase, a special four-compartment trough was devised (3) to provide a subphase free of dissolved or adsorbed enzyme. The protein was spread on one compartment between two barriers, and vigorous stirring was applied to the underlying solution to remove adsorbed unspread molecules from beneath the film. The film was then transferred by sliding the barriers to another compartment with a clean subphase containing the substrate. The surface pressure was measured in this second compartment using a platinum blade suspended from a tension transducer. When the film was moved, the whole apparatus was shifted so that the blade was always in contact with the film.

A three-compartment trough was utilized in a similar manner (4). The film was spread over one compartment and then was moved to the second compartment, with the help of a movable frame, where the subsolution was vigorously stirred. Then the film was moved to the third compartment onto fresh subsolution by shifting the frame.

A circular, multicompartment, Teflon trough was introduced (5) which allowed the shifting of mono-

layers over different subphases by moving together two barriers resting on the trough edge. A Wilhelmy balance was shifted with the film. The walls separating the compartments were lower than the edges so that the monolayers could be moved from one compartment to the other. The different compartments could be filled or emptied independently.

In all of these methods, the monolayer itself has to be transferred from the subphase on which the film is initially spread. This procedure may disturb the film and introduce additional uncertainties into the study.

The purpose of this study was to develop an apparatus that could be used to free the subphase under a spread film of dissolved constituents or contaminants without moving or disturbing the film. Such an apparatus would be of great use in studying the activity of surface-spread enzymes free of dissolved enzymes. It would also be useful for studying adsorption–desorption of ions and ion-exchange processes using lipid and protein monomolecular films.

EXPERIMENTAL

Materials—Synthetic L-α-dipalmitoyl lecithin[1], procine heart malate dehydrogenase[1] (260 units/mg. as a suspension in 70% ammonium sulfate), and crystalline human serum albumin[2] were used.

The organic solvents used, all spectrograde, were checked for surface-active impurities by compression on the film balance after addition of small amounts of these solvents onto the subphase. About 3 ml. of the solvent was placed on the surface (surface area of trough = 315.0 cm.^{2}) and, after 25 min., the surface area was reduced to 105.0 cm.^{2} by shifting the barrier. No surface pressure was observed for any solvent used. The inorganic chemicals used, all reagent grade, were also checked for surface impurities. Sodium chloride was roasted at 700° for 8 hr. Surface tension measurements of sodium chloride solutions and tromethamine–hydrochloric acid solutions before and after compression showed these solutions to be free of surface-active impurities.

The water used was deionized by passing tap water through a mixed resin bed of a demineralizer[3] and then distilling it in an all-glass still.

Apparatus—The Teflon-coated trough (525-ml. capacity) is removable to facilitate cleaning. The precision lead screw, which drives the reinforced Teflon barrier, allows for changes in surface area of the trough as small as 0.0125 cm. The barrier can be disengaged from the lead screw for rapid sweeping of the surface. The Teflon used for the trough and barrier has an advantage over the traditional wax-coated glass in that it is chemically inert as well as hydrophobic and oleophobic. The surface balance[4] is enclosed in a Plexiglas cabinet to minimize possible surface contamination by dust and grease from the environment.

Surface pressure values were measured by the Wilhelmy plate method (6). The platinum plate, roughened to ensure wetting, was suspended from a torsion balance[5] which can measure surface pressure changes as small as 0.1 dyne/cm.

The described surface balance was modified (Fig. 1) to allow for subphase exchange as required. The liquid used to replace the subphase liquid in the trough was placed in the 2.5-l. separator A, which served as a reservoir. During the operation, the stopper P of the separator was kept tightly closed so that it was air tight, whereas the Teflon stopcock B was kept open. The level of the stem D of the separator was adjusted by raising or lowering the separator A, by adjusting the ring stand supporting the separator, or by raising or lowering the vessel C using the adjustable jack E. The mouth of the vessel C was kept covered by aluminum foil to avoid contamination from the air. The level of the stem D controlled the level of the liquid in vessel C, which, in turn, controlled the level of the liquid in the trough H of the surface balance. The reservoir A and the vessel C worked somewhat like an animal feeder. As soon as the level of the liquid started to drop in vessel C, it was replenished by liquid from reservoir A. This served to maintain the liquid level in C constant, which, in turn, maintained the level in the trough constant. The flow of liquid to the trough could be stopped by closing stopcock F.

The inlet glass tube G was placed in one corner of the trough on the side from which compression was started, and the outlet glass tube I was placed in another corner on the opposite side. The barrier was so positioned that the inlet glass tube G did not interfere with the movement of the barrier during compression. The liquid level was so adjusted that the liquid level in the trough was slightly above the brim of the trough throughout the operation.

To control and maintain a certain flow of liquid through the system (trough), the stopcock J was adjusted and its position was kept fixed afterward. To start or stop the flow of liquid through the system, stopcock L was opened or closed, respectively. To measure the rate of flow, the outflowing liquid from the polyethylene tube K was collected in a graduated vessel M.

In all experiments, the subphase exchange was started 10 min. after spreading the film and the film was compressed 5 min. after stopping the flow. The subphase exchange was completed in 20 min. in all cases. Flow rates were controlled so that either 900 or 1500 ml. was exchanged in this period. At these rates, no visual disturbance of the film was observed. To ensure that the apparatus was free of surface-active impurities, water in the trough was replaced with 2000 ml. of water from separator A and the surface tension was checked at full trough area and after compression to one-third trough area. The apparatus was found to be free of any surface-active contaminants by this method.

Dipalmitoyl lecithin monolayers were studied with and without subphase exchange to establish that there was no film loss during the subphase exchange procedure.

To determine the amount of liquid required to replace the subphase, a 0.01% aqueous solution of benzoic acid in the trough was exchanged with water from separator A. The absorbance of benzoic acid solution samples taken from the trough at different intervals was read at 273 nm., and the concentration of benzoic acid was determined from a standard curve. Percentages of original benzoic acid concentration in the trough *versus* volume of liquid replaced in the trough plots were then constructed for two different flow rates.

Preparation of Spreading Solutions—*Dipalmitoyl Lecithin*—Dipalmitoyl lecithin (22.5 mg., mol. wt. 734) was dissolved in ethanol–hexane (5:95 v/v) to make 50 ml. of solution. In all experiments with dipalmitoyl lecithin, 0.07 ml. of this solution was spread on a tromethamine–hydrochloric acid buffer (pH 7.4) subphase.

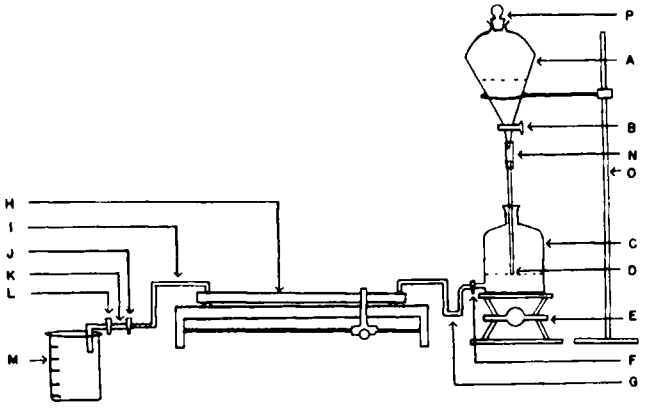

Figure 1—*Diagrammatic representation of the modified surface balance used for subphase exchange. Key: A, separator (2500 ml.) with stem extended by polyethylene tube joint and glass tube (reservoir); B, stopcock; C, 2-l. jar with outlet at bottom; D, glass tube (for liquid level adjustment in vessel C); E, adjustable jack; F, stopcock and polyethylene tubing; G, glass tube; H, surface balance (trough capacity of 525 ml.); I, glass tube; J, stopcock for adjusting flow of liquid; K, polyethylene tube (flexible); L, stopcock for starting or stopping liquid flow; M, graduated beaker for collecting outflowing liquid; N, polyethylene tube joint; O, support stand; and P, stopper.*

[1] Schwarz/Mann Research Laboratories, Orangeburg, N. Y.
[2] Nutritional Biochemical Corp., Cleveland, Ohio.
[3] Bantam.
[4] Frater Instrument Co., Corona, N. Y.
[5] Federal Pacific Electric Co.

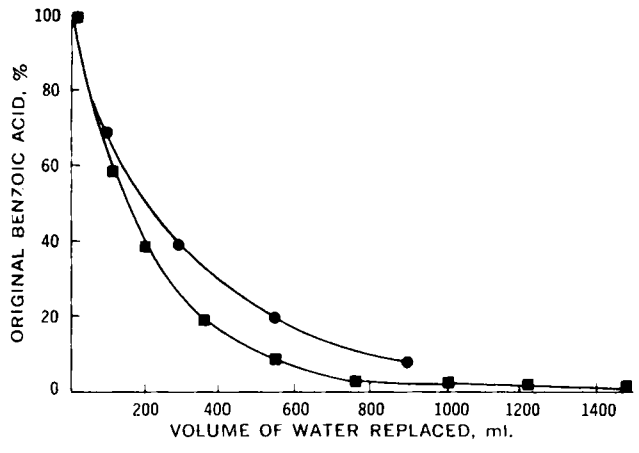

Figure 2—*Volume of water replaced in trough* versus *percentage of the original benzoic acid concentration (0.01% w/v in water) in the trough. Key:* ●, *900 ml. replaced in 20 min.; and* ■, *1500 ml. replaced in 20 min.*

Human Serum Albumin—Crystalline human serum albumin (24.2 mg.) was dissolved in water to make 50 ml. of solution. It was stored in the freezer and was thawed to room temperature immediately before use. In the various experiments, 0.038 and 0.07 ml. of this solution were spread on either water or 10% sodium chloride solution by the Trurnit (8) method.

Malate Dehydrogenase—An appropriate volume of the suspension was dissolved in 5 ml. of the tromethamine-hydrochloric acid buffer solution (pH 7.4) to yield a concentration of 1 mg./ml. This solution was stored in the freezer and thawed to room temperature just before use. In the various experiments, 0.07 and 0.1 ml. of this solution were spread on a tromethamine-hydrochloric acid buffer subphase (pH 7.4) by the Trurnit (8) method. The pH 7.4 for malate dehydrogenase studies was chosen because the highest activity of malate dehydrogenase is reported to occur in the physiological range of pH 7.3–7.5 (7).

In the case of proteins, different volumes of the same spreading solution were delivered to spread films of different protein concentrations.

Procedure—Before spreading the film-former solution, the surface of the subphase was swept several times with the barrier to clean the surface. The surface was checked for cleanliness by taking readings at full trough area and after compressing to one-third trough area. The same reading in both positions ensured that the surface was clean. The platinum plate was then lowered into position and allowed to remain beneath the surface while a known volume of the film-forming substance was spread with a micrometer syringe[6], which can deliver volumes as small as 0.001 ml. accurately. In all experiments, compression was initiated 35 min. after the film was spread, allowing the system to come to "equilibrium" and also ensuring complete evaporation of the spreading solvents for phospholipids. The area available to the film molecules was then reduced in small increments, and surface pressure readings were taken 1 min. after each area change. Compression was usually continued until the film collapsed.

Solutions of dipalmitoyl lecithin were applied onto the subphase by allowing small drops to fall from the syringe held a few millimeters above the surface. The proteins were spread by the Trurnit (8) method. A 5-mm. diameter glass rod was bent so that it could be placed in the trough on its own base. The vertical limb of the glass rod was 40 mm. long and had a hemispherical top. Before spreading, the glass rod was thoroughly washed and then placed in the trough. The protein solutions were applied from the micrometer syringe on the glass rod top at a slow, steady rate. The syringe was always held directly above the top of the glass rod. The glass rod was then washed by applying drops of the same solution being used as the subphase, with the help of a clean dropper. The number of drops was kept constant for all readings. The washing ensured that no protein was left sticking to the portion of the glass rod outside the subphase. The glass rod was so positioned that it could remain in place during the compression cycle.

Two different amounts of each protein were spread to demonstrate the effects of: (*a*) subphase exchange on different protein concentrations, and (*b*) protein concentration on area per unit weight of the protein.

The 35-min. period between spreading and compression also allowed enough time for subphase exchange when required.

All experiments were carried out at room temperature (25 ± 2°).

RESULTS AND DISCUSSION

A plot of the original benzoic acid concentration in the trough *versus* volume of water replaced in the trough is shown in Fig. 2. It was observed that when 900 ml. of water was replaced over 20 min., the benzoic acid concentration in the trough was reduced by 92%. In the first 250-ml. change of subphase, 57% loss in concentration occurred. Figure 2 also shows a plot of benzoic acid concentration *versus* volume of water replaced in the trough when 1500 ml. of water was used to replace the subphase solution over the same 20-min. period; almost 100% (>99%) of the subphase solution was replaced. The major loss in benzoic acid concentration (69%) again occurred with the replacement of the first 250 ml. It is important to note that for a given volume of subphase exchanged, the amount of benzoic acid solution replaced is dependent on the rate of exchange of the subphase; *i.e.*, the rate of benzoic acid loss is greater at a faster rate of subphase replacement. Thus, to achieve reproducible results, it is necessary to work at a fixed rate of exchange of the trough liquid. With the apparatus and technique used, essentially 100% exchange of the subphase solution in the surface balance trough can be achieved.

Figure 3 shows π–A plots of spread films of dipalmitoyl lecithin on water without subphase exchange and of dipalmitoyl lecithin films on water when the subphase was exchanged with 900 and 1500 ml. of water. The π–A plots in all these cases are identical, indicating that there was no leakage of the film under the movable barriers or any disturbance of the film during the subphase exchange operation. These experiments indicate that this apparatus can be used to replace the subphase under an insoluble lipid film without affecting the film characteristics.

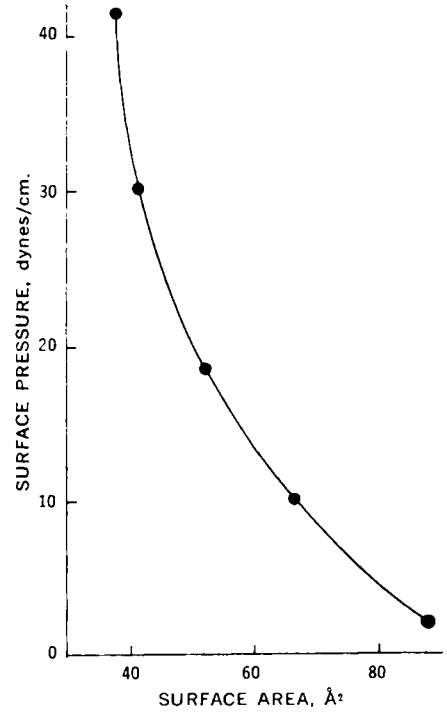

Figure 3—π–A *curves of dipalmitoyl lecithin spread on water. Key:* ●, *no subphase replacement;* ●, *subphase replaced with 900 ml. water; and* ●, *subphase replaced with 1500 ml. water.*

[6] Agla, Burroughs Wellcome.

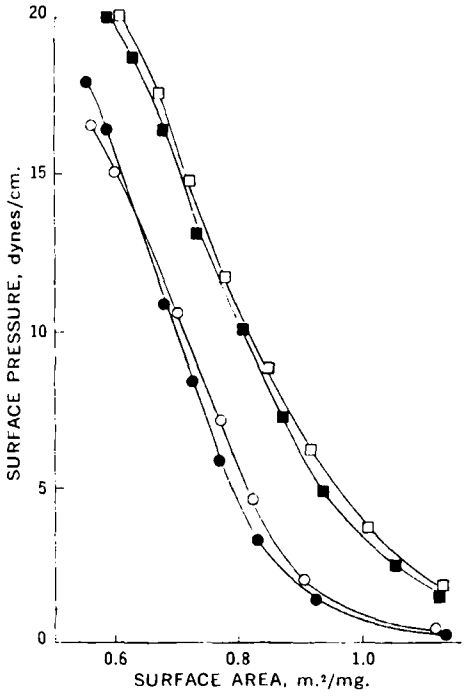

Figure 4-- π-A *curves for 0.0184 mg. human serum albumin. Key:* ○, *spread on water;* ●, *spread on water and subphase replaced with 1500 ml. water;* □, *spread on 10% (w/v) sodium chloride solution; and* ■, *spread on 10% (w/v) sodium chloride solution and subphase replaced with 1500 ml. 10% (w/v) sodium chloride solution.*

In the protein studies, human serum albumin films were spread by the Trurnit (8) method either on water or on 10% sodium chloride solution; in certain experiments, the subphase was exchanged. In all experiments, the human serum albumin formed stable films which gave reproducible π-A plots. Figure 4 shows π-A plots of human serum albumin films (0.0184 mg.) on a water subphase and on a 10% sodium chloride solution subphase. The two plots are parallel, but the film on the 10% sodium chloride subphase has a much larger area per milligram at all surface pressures; *i.e.*, it is more expanded than the film spread on water. The expansion effect was also observed when a larger amount of human serum albumin was spread (0.0339 mg.) on the 10% sodium chloride solution subphase (Fig. 5). These data suggest that more complete spreading occurred with both concentrations of protein in the presence of 10% sodium chloride.

In the case of proteins, the presence of charges on the molecules must be given consideration concerning their ability to spread. According to Crisp (9), better spreading of protein on solutions of high ionic strength can readily be understood if it is recognized that the electrical potential set up by the charged film inhibits further adsorption of additional charged protein molecules at the interface. A decrease in this potential occurs as the concentration of electrolyte in the solution is increased. Charge repulsions between molecules in the plane of the film are reduced also as the ionic strength increases, because with it the dielectric constant of the subphase increases. Cheeseman and Davies (10) attributed the facilitation of spreading and unfolding of protein molecules by salt solutions to the diminution of intramolecular cohesion, because of reduced electrostatic attraction between ionizable groups as the effect of the ionic charges are canceled. Some investigators (11) stated that ions exert their effect by altering the structure of water, thus weakening the tendency of protein to form hydrophobic bonds and so facilitating uncoiling of the protein molecule. Spreading protein on a high salt concentration subphase may simply reduce the solubility of the protein considerably (salting-out effect), thus minimizing the loss of protein into the subphase by diffusion (12). Therefore, salts would be expected to cause a marked increase in surface area of protein films *via* one or all of the mechanisms previously described, although it cannot be said with certainty which is responsible for the observed expansion of human serum albumin films on the salt solution subphase.

Figure 4 shows the effect of change of subphase with water on films of human serum albumin (0.0184 mg.) spread on water. Exchange of the subphase results in a slight shift of the π-A plots to smaller surface areas. The difference, while small, is significant. The same effect is observed when the subphase of human serum albumin films spread on 10% sodium chloride solution is replaced by 1500 ml. of a sodium chloride solution of the same concentration.

The effect of subphase exchange is much more pronounced when a larger amount of the protein is spread. Figure 5 shows the effect of subphase exchange on films of human serum albumin when 0.0339 mg. of the protein was spread. When the subphase of the film spread on water was replaced by water, the π-A profile shifted to smaller areas. This suggests that when the subphase is exchanged, some protein is lost. Cheeseman and Davies (10) postulated that, when proteins are spread on substrates in which they are soluble, an unstable equilibrium may be established. The monolayer proper consists of completely or almost completely unfolded molecules, beneath which exists an adsorbed layer of relatively unmodified molecules. These molecules are held in the interfacial region by salt linkages or hydrogen bonding, and some of this unmodified protein is forced into the subphase. It is possible that this adsorbed layer of relatively less unfolded protein molecules under the monolayer may be swept away and removed as the subphase is replaced by fresh subphase. This would, in turn, result in a reduction in surface area at all given surface pressures.

When the effect of subphase exchange on the two amounts of spread human serum albumin is compared, it can be seen that there is a very small effect on the π-A plots when 0.0184 mg. is spread (Fig. 4) while the effect is quite significant when 0.0339 mg. is spread (Fig. 5). This may be due to better spreading in the former case since the protein molecules under this condition have more area available at the interface for unfolding, allowing relatively more molecules to unfold completely and form a more stable monolayer with fewer unmodified molecules adsorbed under it. In this case, more molecules would be held securely at the interface and thus less protein would be removed by subphase exchange. At higher concentrations of protein, molecules would have less area available for unfolding and thus more molecules would be adsorbed under the film. These adsorbed molecules would be more easily removed when the subphase is exchanged.

Figure 5 also shows the effect of exchanging the subphase with sodium chloride solution when 0.0339 mg. human serum albumin was spread on the sodium chloride subphase solution. This reduction in surface area is less than when the water subphase is changed

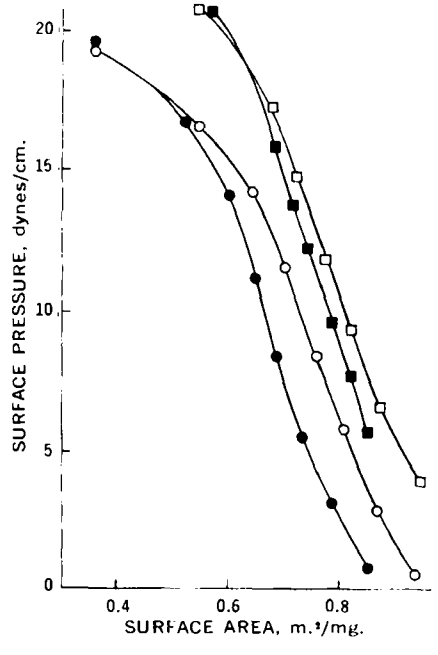

Figure 5-- π-A *curves for 0.0339 mg. human serum albumin. Key:* ○, *spread on water;* ●, *spread on water and subphase replaced with 900 and 1500 ml. water;* □, *spread on 10% (w/v) sodium chloride solution; and* ■, *spread on 10% (w/v) sodium chloride solution and subphase replaced with 1500 ml. 10% (w/v) sodium chloride solution.*

with water. This may be due to the better spreading of the protein on the salt solution as well as to its reduced solubility in the subphase as compared to water. The π–A plots for human serum albumin films are essentially the same when both 0.0339 and 0.0184 mg. of protein are spread (Figs. 4 and 5). This demonstrates that the area per unit weight of the human serum albumin film is almost independent of the amount of protein spread within the range studied.

The effect of exchanging the water subphase with sodium chloride solution on films of 0.0339 mg. human serum albumin is shown in Fig. 6. The π–A plots of the film after the exchange of subphase are superimposable on the π–A plot of 0.0339 mg. human serum albumin spread on 10% sodium chloride solution. The film is expanded as compared to the film on the water subphase. This suggests that when sodium chloride solution is exchanged for the water subphase, the salt either allows the protein molecule in the film to unfold more completely or forces any protein molecule adsorbed under the film into an area determining position at the interface. If the latter is true, these data would also indicate that, in a water subphase, any protein molecule not in an area determining position is adsorbed at or close to the interface rather than dispersed uniformly throughout the solution. If the protein is dispersed uniformly throughout the subphase rather than being primarily in the unstirred layers close to the interface, much of it would be swept away during subphase change and the π–A curves would not be superimposable.

The effect of replacing the subphase with water when 0.0339 mg. of human serum albumin was spread on 10% sodium chloride solution is also shown in Fig. 6. There is a significant reduction in surface area, but the π–A plot is parallel to the π–A plot of the human serum albumin film spread on the sodium chloride solution. However, the π–A plot does not quite coincide with the plot of the film spread on water alone. This may be due to better spreading of the protein initially on the sodium chloride solution subphase, which would tend to reduce the amount of protein lost when the subphase is exchanged. Moreover, since the protein solubility is greater in water than in sodium chloride solution, some protein would be expected to be lost following the exchange. This could account for the reduction in the surface area of the film as shown in Fig. 6.

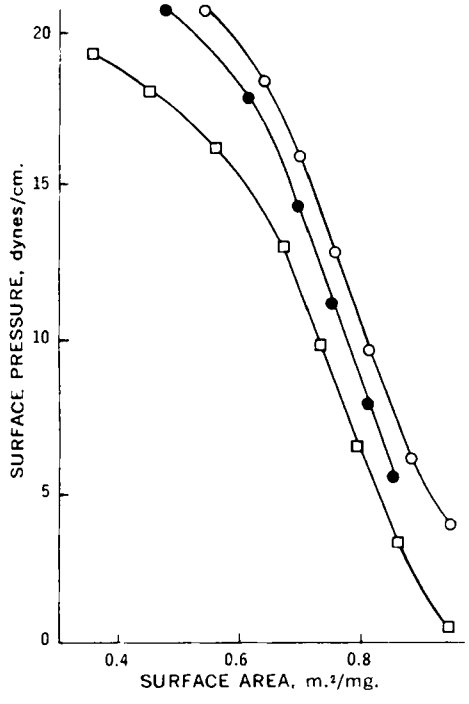

Figure 6—π–A *curves for 0.0339 mg. human serum albumin. Key:* ○, *spread on 10% (w/v) sodium chloride solution;* ○, *spread on water and subphase replaced with 1500 ml. 10% (w/v) sodium chloride solution;* ●, *spread on 10% (w/v) sodium chloride solution and subphase replaced with 1500 ml. water; and* □, *spread on water.*

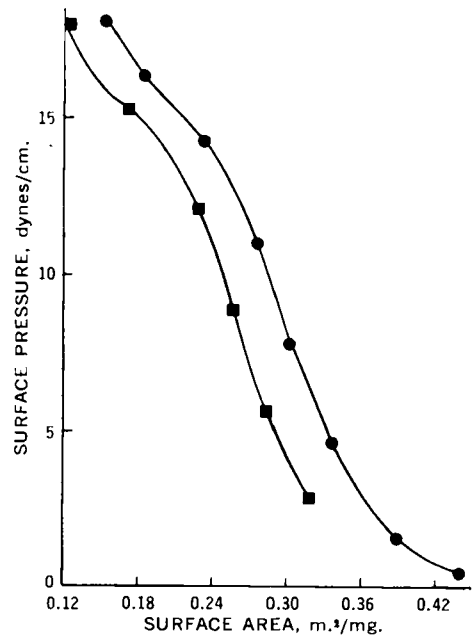

Figure 7—π–A *curves of malate dehydrogenase films on pH 7.4 tromethamine–hydrochloric acid buffer solution. Key:* ●, *0.07 mg. spread on buffer;* ●, *0.07 mg. spread on buffer and subphase replaced with 900 and 1500 ml. buffer;* ■, *0.10 mg. spread on buffer; and* ■, *0.10 mg. spread on buffer and subphase replaced with 900 and 1500 ml. buffer.*

The malate dehydrogenase films were spread on pH 7.4 tromethamine–hydrochloric acid buffer solution. In some experiments, the subphase was exchanged with a buffer solution of the same composition. The malate dehydrogenase films were spread by the Trurnit (8) method and, in all experiments, malate dehydrogenase formed stable films which gave reproducible π–A curves.

Figure 7 shows π–A plots of 0.1 and 0.07 mg. malate dehydrogenase spread on the buffer solution. The π–A plots of the two different amounts of malate dehydrogenase are not superimposable, and the curve representing the larger amount of enzyme spread is more condensed. However, the two plots are parallel, and both show an inflection point at about 15.7 dynes/cm. surface pressure. Beyond this inflection point, at smaller surface areas, the films become less compressible.

The reason why the area per molecule at all surface pressures is greater when a smaller amount of malate dehydrogenase is spread might be due to better spreading of the film under this condition, since more area is available to the molecules and better unfolding and less enzyme loss to the subphase would result. Thus, in the case of malate dehydrogenase films, the area per unit weight of protein is dependent on the amount spread.

Figure 7 also shows the effect of replacing the tromethamine–hydrochloric acid buffer subphase with 900 and 1500 ml. of a buffer solution of the same composition on 0.07 and 0.1 mg. malate dehydrogenase films. The change of subphase has no effect on either concentration of malate dehydrogenase films. This shows that both the 0.1- and 0.07-mg. malate dehydrogenase films are very stable, and no enzyme is lost by replacing the subphase. Thus, it appears that the difference in surface area observed when different amounts of this enzyme are spread is the result of differences in the degree of unfolding only, rather than dissolution or adsorption of protein below the film surface.

The study demonstrates the effectiveness of this apparatus in exchanging the subphase under monomolecular films of lipids and proteins without significantly disturbing the films or modifying their π–A characteristics.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 19, 1973, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104, and the †College of Pharmacy, Rutgers—The State University, New Brunswick, NJ 08903

Accepted for publication July 2, 1973.

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NOTES

Cardiovascular Effects of Digoxin–Phenelzine Interaction in Rabbits

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Abstract □ The potential toxicity of combining phenelzine, an MAO inhibitor, and digoxin, a cardiac glycoside, was studied in rabbits. Cardiovascular responses following concurrent administration of these drugs and the administration of these drugs 20 and 60 min. apart, respectively, revealed that phenelzine and digoxin evoked arrhythmias in about 50% of all rabbits tested without markedly altering the arterial blood pressure. Spontaneous heart rate in these animals was found to be lower than control values. The incidence of arrhythmia decreased as the time interval between the administration of both drugs was increased from 20 to 60 min. An increase in the number of days of pretreatment with phenelzine resulted in the use of a higher dose of digoxin to evoke arrhythmia.

Keyphrases □ Digoxin–phenelzine interactions—cardiovascular effects in rabbits, dose–time relationships □ Phenelzine–digoxin interactions—cardiovascular effects in rabbits, dose–time relationships □ Interactions, digoxin–phenelzine—cardiovascular effects in rabbits, dose–time relationships □ Drug interactions digoxin–phenelzine in rabbits

The administration of digoxin in combination with phenelzine is occasionally employed in clinical practice. However, when an antidepressant drug such as phenelzine, a monoamine oxidase (MAO) inhibitor, is administered concurrently with a cardiac glycoside such as digoxin, an interaction may occur. A digitoxigenin–biogenic amine interaction was reported (1) to play a significant role in the development of digitoxigenin toxicity and lethality. Drugs not belonging to the class of MAO inhibitors but that deplete brain monoamines were found to protect mice and rats against digitoxigenin lethality. On the basis of the preceding information, it can be surmised that digoxin toxicity may be enhanced with concurrent phenelzine administration.

This preliminary investigation was undertaken to determine if concurrent digoxin phenelzine administration would result in enhanced toxicity and if such toxicity was dose related and/or time dependent in terms of the two drugs administered together.

EXPERIMENTAL

Cardiovascular Studies of Phenelzine and Digoxin—Adult, female, albino rabbits, weighing 1.96–4.9 kg., were anesthetized with sodium pentobarbital[1], 25 mg./kg. i.v.; supplemental doses of 0.2 ml. sodium pentobarbital were injected as needed. Carotid arterial blood pressure was recorded using a pressure transducer[2], while heart rate was determined from limb lead II electrocardiographic records. All monitored parameters were recorded on an ink-writing oscillograph[3].

Studies of certain cardiovascular responses to either phenelzine (2.5, 5, and 10 mg./kg.) or digoxin (0.125 and 0.250 mg./kg.) administered individually by jugular vein served as the control basis for comparison with concurrent administration of both drugs. At least six rabbits were used in each experiment. Control readings of spontaneous heart rate and arterial blood pressure were recorded at 10-min. intervals prior to the injection of the first drug. Following each drug administration, readings were made at intervals of 0.5, 1.0, 5.0, and 10.0 min. and then every 10 min. thereafter; three nearly similar readings were obtained before a subsequent dose was administered. Replicate observations were made in the same animal (*i.e.*, 0.125 mg./kg. digoxin was given noncumulatively in the same animal three times before attempting the next dose, 0.25 mg./kg., for the next three trials).

Drug Interaction–Time Course—The following three time-spaced studies of phenelzine and digoxin were conducted: (*a*) drugs concurrently injected intravenously; (*b*) drug administration 20 min.

[1] Abbott Laboratories.
[2] Linear Core P-1000A, Narco Biosystems, Inc., Houston, Tex.
[3] Type PMP-4A, Narco Biosystems, Inc., Houston, Tex.