Interactions of Veratrum Alkaloids, Procaine, and Calcium with Monolayers of Stearic Acid and Their Implications for Pharmacological Action

ABRAHAM M. SHANES and NORMAN L. GERSHFELD

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, United States Department of Health, Education, and Welfare, Bethesda

ABSTRACT The interactions of veratridine, cevadine, veracevine, and veratramine with monolayers of stearic acid show marked differences. Veratridine and cevadine, at concentrations that are known from potential, ionic flux, and other measurements to affect living membranes, react strongly with the film and appear to cause an "interfacial dissolution" whereby both the alkaloid and the stearate leave the surface. Veracevine at the same concentration does not interact with the film. The veratramine reaction is weak, much like that of the local anesthetic procaine. The veratridine and cevadine effects are antagonized by 10^{-3} M Ca^{++}, low pH, and 3.7 and 7.4 $\times$ 10^{-3} M procaine. These differences among the veratrum alkaloids and the antagonisms parallel effects observed in living systems. Such parallelism suggests that similar physical interactions are involved in the stearate film and in natural membranes.

INTRODUCTION

The veratrum alkaloids have been the subject of wide chemical (21) and pharmacological (17) examination. While these compounds have similar chemical structures, the physiological responses evoked by some of these drugs may be quite different. Thus, it has been shown that low concentrations (*ca.* 2×10^{-5} M) of veratridine, cevadine, or their familiar mixture veratrine, depolarize frog nerve with an accompanying enhanced exchange of sodium and potassium across the cell membrane (20, 29, 35). These agents have been referred to as "labilizers" (26, 27) or "unstabilizers" (*e.g.*, reference 28) because they have the additional effect of enhancing the bioelectrical and permeability changes which occur in excitable cells on stimulation or during metabolic inhibition. Veracevine, on the other hand, is pharmacologically inactive at this concentration (17). Veratramine has "stabilizing" properties (26, 27) similar to quinine and quinidine (18); that is, it *reduces*

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the permeability and bioelectrical changes in response to electrical stimuli or to excitatory substances (26, 27).

The veratrine depolarization in nerve appears to be related to the action potential in that both phenomena are accompanied by an increase of the permeability of the cell membrane to ions, especially to sodium (26, 27), and in that a "stabilizer" like procaine will reduce the veratrine effect (28) and block the action potential (2, 3).

Skou has shown that the potency of local anesthetics, alcohols, and other stabilizers in blocking nerve conduction is closely paralleled by their ability to increase the surface pressure of monomolecular films of stearate and of lipid extracts on Ringer's solution (32–34). This parallelism may reflect similarity in the interactions that take place in the films and in the living membrane. It seemed to us that this point of view would be strengthened if labilizers, such as the veratrum alkaloids, could be shown to have different effects than do stabilizers on these films and to exhibit the antagonisms that are now well known with nerve membranes. Exploratory studies with stearate films demonstrated that this is the case (10). In this report additional findings are presented which show not only that the local anesthetics and veratrine may be distinguished with respect to their interfacial properties, but that among veratrum alkaloids, where the differences in chemical structure are more subtle, differences in interfacial effects are observed that are also consistent with different pharmacological actions. The method entails spreading a monolayer of stearic acid on various Ringer's substrates containing the drugs and observing the changes in the physical state of the monolayer brought on by these drugs. Effective concentrations are the same as those employed pharmacologically. This, and the nature of the results, indicate that our findings with the films are related to the mechanism of action of these pharmacological agents in living systems. A preliminary report of these findings has been presented (11).

METHODS

Surface Tension Surface tensions were obtained by the drop-weight method (13) and the empirical corrections of Harkins and Brown (14) were applied. A stainless steel rod (5 cm. in length, 0.5 cm. diameter) was bored coaxially with a 1 mm. diameter hole. One end surface of the tube was ground plane in a lathe. This tube was connected by a short piece of heavy walled rubber tubing to a syringe driven by a micrometer screw, and the entire apparatus was mounted vertically. A tared 2 ml. pycnometer with a neck 0.9 cm. inside diameter was used to collect the drops. Approximately 90 per cent of each drop was rapidly preformed, then allowed to reach full size under the force of gravity. Each drop was collected and weighed; a total of ten drops was used to obtain the average surface tension, the deviation from the mean always being less than 0.5 per cent. All measurements were made at 25° ± 0.5°C.

Surface Pressures A Langmuir type film balance (the Cenco hydrophil balance) was used to measure the surface pressures of the surface films (1, 13). The tray was heavily coated with paraffin and the float assembly modified by using polyethylene end-loops (0.002 in. thick) and an aluminum float coated with a thin layer of teflon.

Reagents All solutions were prepared from water obtained from a quartz still. The concentrations of the various ionic species in the Ca^{++}-free Ringer's solution were: 107×10^{-3} M NaCl, 1.8×10^{-3} M KCl, and a 1.0×10^{-3} M phosphate buffer; for the normal Ringer's solution, 1×10^{-3} M $CaCl_2$ was added (all Merck, reagent grade).

The veratrum alkaloids were provided as the free base by Professor Krayer and were employed without further purification; veratramine was supplied by Dr. Wintersteiner. The melting points of these compounds were for veratramine, 205 to 207°C., for veracevine, 215 to 223°C., for veratridine, 160 to 180°C., and for cevadine, 205 to 207°C. The correct amount of drug was dissolved in a small volume of 0.1 N HCl, neutralized with 0.1 N NaOH, and then added to the buffered Ringer's solution at pH 7.2. The procaine used was the hydrochloride salt (Gaine and Ingram U.S.P.) which readily dissolved in the Ringer's solution.

Stearate films were formed by dripping a benzene solution of stearic acid on the clean, enclosed solution surface of the film balance. From the weight of stearic acid delivered and the total surface area of the tray one is able to calculate the average area occupied by a single stearate molecule (1). The drug interactions were studied by spreading the monolayer on the surface of the Ringer's solution containing the drug or drugs and measuring the surface pressure as a function of the area. All solutions were freshly prepared for each day's experiments to minimize bacterial contamination. For veratrine interactions a fresh solution was used for each run.

Benzene (Malinkrodt, thiophene-free) was prepared free of surface-active contaminants by filtering through a column of silica gel and florisil. Stearic acid was Fisher reagent grade.

Molecular Areas Molecular areas of the drugs were estimated by first constructing, with models of the atoms (22) (scaled 1 cm. = Å), three-dimensional models, and then pencil tracing the plane projections of the models in their various orientations on paper. The total area of each plane projection was obtained with a planimeter, and represented the approximate surface area occupied by the drug in a particular orientation at the air/water interface. Three successive pencil tracings of each model and the subsequent area determinations gave values for the areas which agreed within 5 per cent.

RESULTS

Physicochemical Properties The structural relations between veratridine, cevadine, and veracevine are shown in Fig. 1 (21). This may be further illustrated by the following esterification equations:

Veracevine + tiglic acid = cevadine
Veracevine + veratric acid = veratridine

Fig. 1 also gives the structures of veratramine (21) and procaine. The pK values of the nitrogen groups for these drugs are listed in Table I and indicate that all the compounds have approximately the same base strength.

According to the Gibbs adsorption isotherm the extent to which a solute concentrates in the surface of a solvent, in excess of its bulk phase concentration, varies directly with its ability to lower the surface tension of the solvent (1). In Fig. 2 the surface tension of Ringer's solution is shown as a function of the concentrations of the added drugs. The interesting fact to note is that

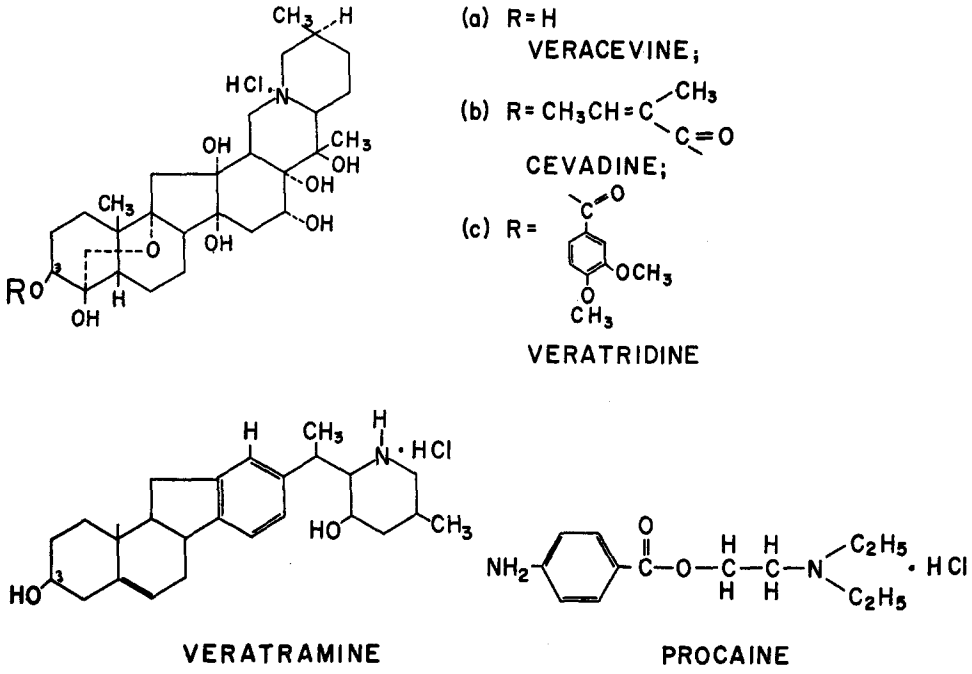

FIGURE 1. Chemical structures of the veratrum alkaloids and procaine.

while veracevine is only slightly surface-active, esterification of a terminal hydroxyl group in the 3-position transforms the compound into the highly surface-active veratridine or cevadine. Veratramine is the most effective of these compounds, while procaine is the least active in lowering the surface tension of Ringer's solution.

Estimates of the dimensions of the alkaloids in the two configurations in which they would be most likely to adsorb at the water/air interface were made from molecular models (22). The cross-sectional areas of the molecules oriented with the long axis vertical (with the hydrophilic nitrogen anchored in the aqueous phase), and with the axis lying horizontal with respect to the surface, are given in Table I.

The observed values given in Table I were measured by a variation of the

TABLE I

PHYSICOCHEMICAL PROPERTIES OF VERATRUM ALKALOIDS AND PROCAINE

Drug	pK (16)	Areas of projection at the *a/w* interface from molecular models (20)		Observed molecular areas at the *a/w* interface, by various methods			Possible molecular orentations at the *a/w* interface H = Horizontal; V = Vertical		
		Horizontal	Vertical	1*	2‡	3‡	1*	2‡	3‡
Veracevine	8.85	80 Å²	45 Å²		§	§			
Veratridine	8.85	130 Å²	45 Å²	85 Å²	110 Å²	42 Å²	H	H	V
Cevadine	8.85	100 Å²	45 Å²	80 Å²	90 Å²	42 Å²	H	H	V
Veratramine	∽10‖	76 Å²	35 Å²	70 Å²		60 Å²	H		H
Procaine	9.05	67 Å²	21 Å²	60 Å²	§	§	H		

* Estimated from shoulder in F-A stearic acid curve (see text).

‡ From F-A measurements of insoluble monolayers of the alkaloids (8). Larger values (column 2) represent areas at which an inflection in the F-A curve appears; smaller values (column 3) represent areas at which film collapse is observed. Veratramine shows no inflection point, only film collapse at 60 Å², indicating a slight tilt of the molecule from the horizontal.

§ Compound does not form stable monolayers at the *a/w* (air/water) interface (8).

‖ Estimated from piperidine as a model.

F-A (surface-pressure area) technique, the details of which are presented elsewhere (8). Briefly, the method is to spread the drugs as insoluble monolayers and observe the F-A relations. An inflection appears in these curves which coincides with the area of the alkaloid horizontally oriented in the air/water interface. Film collapse occurs at the smaller areas, corresponding

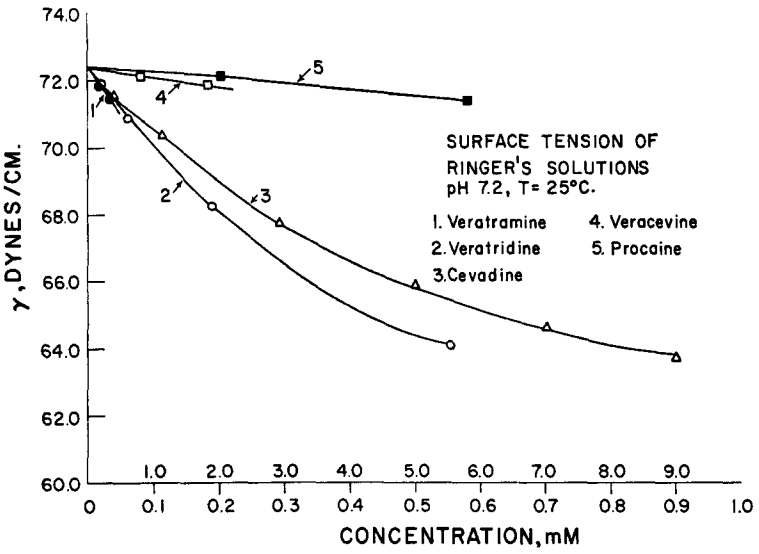

FIGURE 2. Lowering of the surface tension, γ, of Ringer's solutions by the pharmacological agents. Curves 1, 2, 3 lower scale, curves 4, 5 upper scale of abscissa.

to the vertical orientation of the molecule. These results indicate that while veratramine will orient only horizontally in the air/water interface, veratridine and cevadine are able to assume a vertical orientation as well.

Interactions with Monolayers of Stearic Acid. Stabilizers The properties of monolayers of stearic acid on water substrates are well known (1, 13). For this reason, and because they reflect the charge and lipoid character of nerve

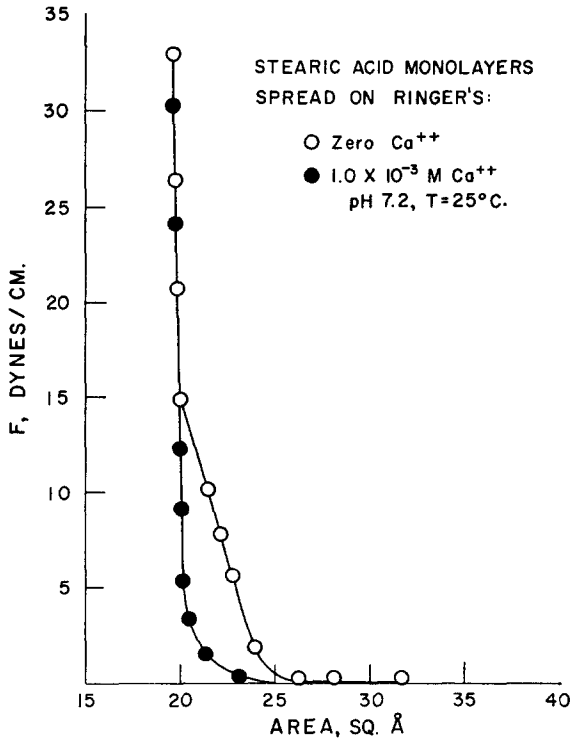

FIGURE 3. F-A relation for monomolecular films of stearic acid on Ca^{++}-free and normal Ringer's substrate solutions.

lipid monolayers, which seem to react to local anesthetics (33) and to narcotizing alcohols (34) in a manner similar to the cell interface, stearate monolayers were explored as a model to demonstrate physical interactions that may occur between drugs and the cell membrane. Fig. 3 is the F-A isotherm of the stearic acid monolayer at 25°C. on substrates of Ca^{++}-free and normal Ringer's solution. The Ca^{++}-free system shows two linear regions, indicative of the liquid-solid and solid condensed states which extrapolate at zero surface pressure to about 24 $Å^2$ and 20 $Å^2$, respectively. The smaller area corresponds to that of stearic acid oriented almost vertically to the air/water interface, with the carboxyl group embedded in the water phase (25). When 1.0 mM Ca^{++} is added to the substrate, the film in the liquid-solid state is further

condensed to the solid state. This phase condensation is associated with a more compact adlineation of the stearate molecules in the film and is brought about by the formation of the calcium stearate salt (19).

When the local anesthetic procaine is added to the Ca^{++}-free Ringer

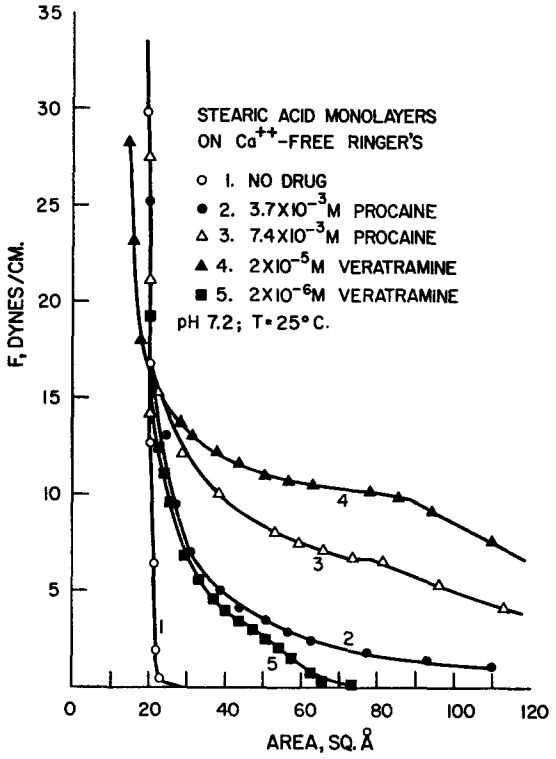

FIGURE 4. The effects of procaine and veratramine on the F-A curve for stearic acid monolayers on Ca^{++}-free Ringer's substrate solutions.

substrate, some profound changes in the stearate F-A curve are observed and these are shown in Fig. 4. At a concentration of 3.7×10^{-3} M (curve 2) there is an increase in the surface pressure for the attenuated film. This may be taken to mean that procaine interacts with the stearate film for neither the procaine-Ringer solution alone nor the stearate monolayers on Ca^{++}-free Ringer's solution give rise to a surface pressure at these areas. As the film area decreases the surface pressure increases, and the curve approaches the steep curve for stearate and its limiting area of 20 $Å^2$.

The distribution of procaine between the bulk solution and the surface phase will depend on the surface activity of the drug and its interactions with the insoluble monolayer. The Gibbs adsorption isotherm (1) gives the concentration of procaine at the air/water interface (increasing with increasing concentration in the bulk solution); but when an insoluble monolayer is added to the surface, interactions between the

surface-active solute and the monolayer are manifested by an increase in the adsorption of the solute at the interface. In the case of procaine and the stearate film these interactions may be of several types: (*a*) ion-ion, or coulombic interactions, between the $—COO^-$ group of the stearate molecule and the dissociated form of the anesthetic; (*b*) ion-dipole, between the $—COO^-$ in the film and the undissociated form of the drug; and (*c*) van der Waals forces between the non-polar moieties of the film and anesthetic molecules. Interaction types (*a*) and (*b*) are strongly pH-dependent, the relative proportions of undissociated to dissociated procaine being determined by the pK of the basic group of the drug and the pH of the solution. Since the undissociated molecule is more surface-active than the cation (31), this, too, must be taken into consideration in pH studies of this system.

To explain the F-A relation observed in Fig. 4 for the procaine-stearate system, the following mechanism is suggested as consistent with the available facts. As the surface area of the stearate monolayer is decreased, the nature of the surface changes from one which was principally an air/water interface in the attenuated films to one which is predominantly a monolayer/water interface in the compact films. For the latter, interactions between the monolayer and procaine increase, with a concomitant increase in surface pressure. The surface pressure is now a composite of the lateral forces exerted by the stearate and procaine molecules at the interface.

The orientation of procaine with respect to the stearate film may be either that of an adsorbed "subfilm" *beneath* the monolayer, or that of a penetrant *between* the stearate molecules in the monolayer; the extent to which both contribute to the structure of the surface film cannot be determined solely from F-A measurements, but it may be possible to ascertain the structure from surface potential studies (1). Since the limiting molecular area of these films is 20 Å^2—that of stearate alone—this indicates that the adsorbed subfilm is highly compressible and that any molecules which have penetrated the monolayer can be squeezed from the surface.

When the procaine concentration in the substrate is raised to 7.4×10^{-3} M, there is a further increase in the surface pressure of the attenuated film (Fig. 4, curve 3). There is an additional feature that appears in this curve that is not observed at the lower procaine concentration. At 80 Å^2, a "shoulder" occurs followed by a flattening of the curve as the stearate film is compressed. If the procaine concentration is raised further, the entire curve is shifted to higher surface pressures, but the shoulder still occurs at about 80 Å^2 (data not shown, but see Skou (32)).[1]

The increase in surface pressure observed when the procaine concentration is raised to 7.4 mM in the substrate reflects an increase in the amount of the drug which participates in the formation of the subfilm and in the penetration of the monolayer. Moreover, the critical area at which the shoulder occurs (80 Å^2) appears explainable if it is assumed that the extent of penetra-

[1] Skou further found that the area at which the shoulder appears was shifted when different local anesthetics were in the substrate (32), the position shift corresponding to a change in the molecular weight of the drug.

tion is limited to the formation of an equimolar mixed film of procaine and stearate.

Thus, when a surface pressure above 1 dyne/cm. is observed in the mixed stearate-procaine film, the stearate molecule is probably oriented vertically in the surface. This follows from Fig. 3, for at pressures above 1 dyne/cm. the stearate molecule has already assumed its vertical orientation. If the area occupied by stearate (20 Å^2) is subtracted from the average area per stearate molecule at the shoulder (80 Å^2), the difference represents the surface space occupied by procaine. The 60 Å^2 so obtained is very close to the area a procaine molecule would occupy if it were oriented horizontally at the air/water interface; *i.e.*, 67 A^2 as estimated from molecular models (Table I).

As the surface area is decreased below 80 Å^2 the close packed mixed film will be unable to withstand the force of compression and the drug will be squeezed from the surface into the procaine subfilm. This is reflected in the observed flattening of the curve. The shoulder in the curve then appears as the result of film penetration by procaine and the subsequent formation of an equimolar mixed film of procaine and stearate with the former oriented almost horizontally and the latter almost vertically to the plane of interface.

The area at which the shoulder occurs should depend only on the available surface space and hence be unaffected by further increases in procaine concentration. This is actually observable in Skou's figures (32).

Labilizers The F-A curves observed when veratridine, cevadine, or veracevine are added to the Ca^{++}-free Ringer substrate are shown in Fig. 5. The presence of 2×10^{-5} M veracevine in the Ca^{++}-free Ringer (curve 1) has no apparent affect on the F-A relation for the stearate film. This result and the observed weak surface activity of veracevine (Fig. 1) are good indications that at this concentration little if any interaction occurs between this alkaloid and the stearate monolayer.

On the other hand, cevadine (Fig. 5, curve 2) and veratridine (Fig. 5, curve 3), at concentrations of 2×10^{-5} M, each interact with the attenuated stearate film much like procaine at 7.4 mM (Fig. 4, curve 3). There is, for example, the elevated surface pressure, which increases gradually as the film is compressed. The surface pressure levels off—the shoulder appearing at 105 Å^2 and 100 Å^2 for veratridine and cevadine, respectively—and remains fairly flat until about 40 Å^2. The fact that veratridine yields a slightly higher surface pressure than cevadine is a reflection of the slightly greater surface activity of the former (see Fig. 2).

However, unlike with the local anesthetics, the curves do not approach the area for stearate, *i.e.* 20 Å^2, as a limit but, rather, they cross the stearate curve and approach asymptotically the value of about 15 Å^2 in the steep portion of each curve. These small limiting areas do *not* represent a film collapse process (13); *i.e.*, no evidence was present of film striations or a

sudden and rapid decrease in the surface pressure. However, these films are unstable, being marked by a slow decrease in surface pressure at each compression, especially at the smaller areas, and this decrease is not reversible. To obtain reproducible results 2 minutes were allowed to elapse between each reading. If longer time intervals are chosen, the limiting area is less than 15 Å^2, indicating a continuing process of surface film degradation.[2]

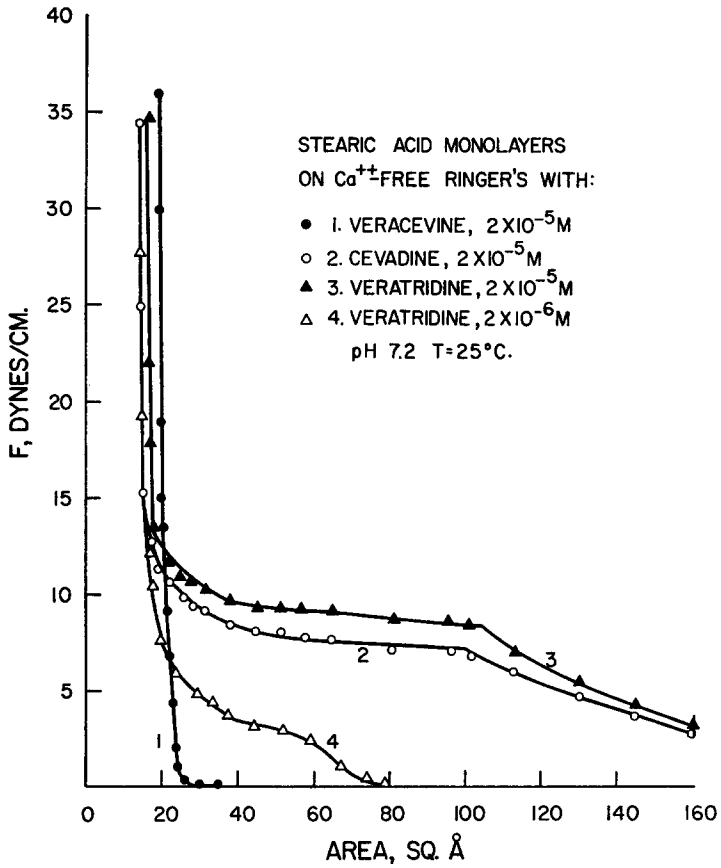

FIGURE 5. The effects of veracevine, cevadine, and veratridine on the F-A curve for stearic acid.

An important feature of the veratrum alkaloids is the possible orientations these compounds can assume at the air/water interface. It has been shown (8) that veratridine and cevadine orient vertically as well as horizontally in the air/water interface (see Table I). The transition from the horizontal to the vertical orientation may be ex-

[2] If, for example, the area of the stearate film is maintained at 17 Å^2 with 2 × 10^{-5} M veratridine in the substrate, the surface pressure (2 minutes after compression) is 20 dynes/cm., after 20 minutes the surface pressure has fallen to 14 dynes/cm., and continues to decline, but less rapidly for several hours. This phenomenon is not observed for stearic acid films on drug-free or procaine-containing substrates at comparable surface pressures.

pected when a film containing either veratridine or cevadine is compressed to surface pressures of about 6 dynes/cm. (8). This provides an adequate basis to account for the effects of the labilizers in stearate films at high compression.

Thus, the "veratrinic" compounds, *i.e.* veratridine and cevadine, like procaine, also penetrate the insoluble stearate monolayer, as evidenced by the shoulder in the F-A curve at about 105 Å^2. For reasons such as were given for procaine, this area represents an orientation of the veratrinic compounds that is almost horizontal in the plane of the interface. The values of 80 and 85 Å^2 would indicate there is some tilt away from the horizontal (see Table I). But, unlike procaine, where further compression of the film "squeezes" this drug from the surface, it may be proposed that the leveling off of the curve for the veratrinic compounds now also reflects the reorientation of these compounds from the horizontal to the vertical in the surface film. In consequence, there will arise van der Waals forces between the hydrocarbon chains of the stearate and the alkaloid molecules by virtue of improved conditions for contact. Moreover, these interactions will resist the expulsion of the veratrinic compound from the interface.

Another consequence of the reorientation may be anticipated. It has been reported (12) that when a bulky, irregularly shaped molecule penetrates a regularly ordered surface film such as stearic acid, the lateral cohesive forces between the stearate molecules which are responsible for the insolubility and stability of the film are disrupted; a process of "interfacial dissolution" occurs whereby both the film and penetrant molecules are desorbed from the surface. This, then, may underlie the instability of the highly compressed stearate films caused by veratridine and cevadine.

Veratramine This alkaloid has stablilizing properties in muscle, provided it is used below a critical concentration range; at higher concentrations it behaves as a labilizer (18). A seemingly parallel phenomenon exists in the interactions of veratramine with stearic acid monolayers.

Veratramine at a concentration of 2×10^{-5} M in a Ca^{++}-free Ringer's substrate interacts with the stearate monolayer very much like the veratrinic compounds cevadine and veratridine (Fig. 4, curve 4). Characteristically for these labilizers, a shoulder appears in the F-A curve at 90 Å^2; at smaller areas, the curve crosses that for stearate indicating that veratramine at this concentration does indeed behave as a labilizer. The shoulder at 90 Å^2 for veratramine suggests a cross-sectional area for veratramine of 70 Å^2, very close to the 76 Å^2 predicted for the molecule oriented horizontally at the interface (see Table I).

If the concentration of veratramine in the substrate is reduced tenfold to 2×10^{-6} M, two important features of the F-A curve (Fig. 4, curve 5) are to be noted (*a*) the shoulder has disappeared, and (*b*) the steep portion of the curve now coincides at small areas with the curve for stearic acid. This behavior is very similar to that of procaine (Fig. 4, curve 2) and appears to be characteristic of stabilizers.

Since labilizer action on stearate films connotes a *vertical* orientation of the penetrating molecule, while stabilizer action is consistent with a *horizontal* orientation of the molecule, we must consider those elements which influence the orientation of veratramine to understand how this molecule can act as a labilizer at high concentrations and a stabilizer at low concentrations. It has been established that for the distribution of hydrophilic groups on veratramine this molecule would of necessity orient horizontally at the air/water interface (8). This is by virtue of the strong H-bonding character of these polar groups with water. The horizontal orientation may be changed if the H-bond which exists between the OH group in the 3-position and water is weakened, allowing this end of the molecule another degree of freedom and thereby permitting the molecule to orient vertically in the interface.

At high concentrations of veratramine this situation may exist by competitive *inter*molecular H-bonding between OH groups on adjacent veratramine molecules. At low concentrations of veratramine this effect would be much less likely and the expected horizontal orientation would prevail. It is interesting to note that just such a concentration dependence of molecular orientation has been observed with dye molecules (11*a*) whose structure with respect to the distribution of polar groups on the molecule is similar to veratramine.

Unlike veratramine, veratridine (which we shall consider as a typical veratrinic compound) retains its ability to disrupt the stearate film (Fig. 5, curve 4) even at concentrations as low as 2×10^{-6} M. Moreover, the shoulder still persists in the F-A curve though shifted to smaller areas (60 Å^2).

Biological Features Duplicated by the Monolayer The results described so far indicate that the interactions between stearate films and stabilizers and labilizers may be useful as a basis for screening compounds for these characteristics. But possibly of greater importance are the indications that these interactions are similar to or perhaps the same as those involved in the pharmacological action of stabilizing or labilizing agents. As has already been mentioned, the concentrations at which the effects are obtained correspond to those active in nerve and muscle and, unlike studies with collodion membranes (6), an important difference is observable in the action of stabilizers and labilizers.

Additional characteristics of the interaction between "veratrine" or its alkaloids and nerve are duplicated by the stearate monolayer. Thus, in nerve (*a*) the depolarizing action of veratrine and veratridine is reduced by lowering the pH (20, 35), (*b*) elevated Ca^{++} in the medium antagonizes veratridine depolarization (7), and (*c*) the local anesthetic procaine also reduces or prevents veratrine depolarization (7, 29), and the associated exchange of sodium and potassium ions (9, 28).

For comparison with the biological effects, the drug interactions with the stearate films were examined at low pH and with calcium in Ringer's solutions. The results are shown in Fig. 6. Veratridine, at a concentration of

2×10^{-5} M, was chosen as a typical veratrinic compound to illustrate the desired effects; cevadine, or the mixture—veratrine—may also be used, but essentially the same results are observed. In curve 3, Fig. 6, the Ca^{++} level in the substrate is 1×10^{-3} M; curve 4 is the result of lowering the pH of the Ca^{++}-free Ringer's substrate to 3.0. In both cases three striking changes have

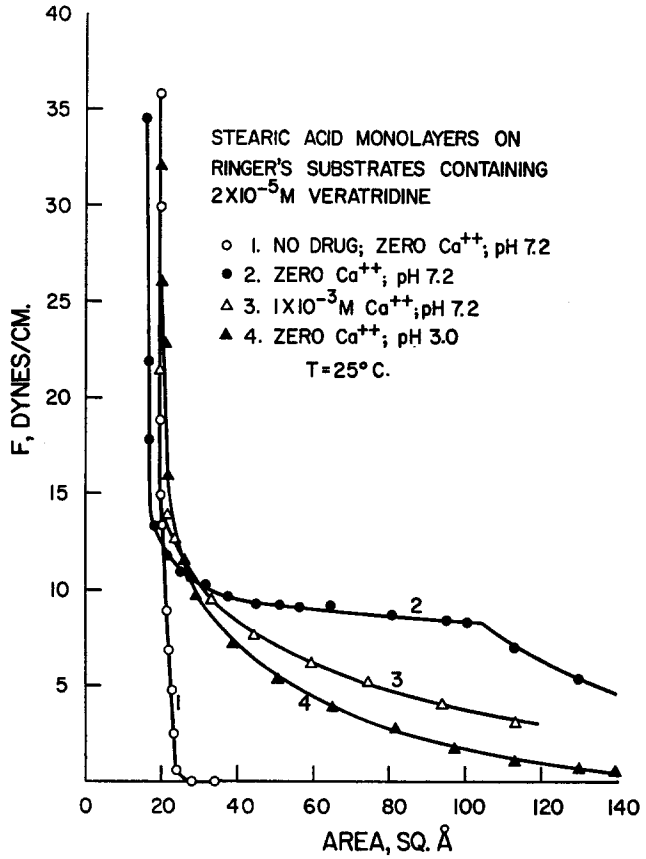

FIGURE 6. The antagonism of the veratridine-stearate interaction by low pH and Ca^{++}.

occurred in the F-A curve: (*a*) There is a decrease in the surface pressure over almost the entire range of surface areas pointing to a decrease in drug-stearate interactions; (*b*) the shoulder in the curve has disappeared, indicating its dependence on electrostatic interactions as well as the possible decrease or absence of film penetration by the drug; and (*c*) film instability also is absent so that the limiting area (20 $Å^2$) for stearate is obtained on compression of the film.

These striking results have their common basis in the fact that the ion-ion interactions between the carboxylate and cationic amine have been reduced.

Ca^{++} has been shown to form the calcium stearate salt (Fig. 3) (19), and lowering the pH will repress the dissociation of the carboxyl group (at pH 3, less than 1 per cent of —COOH, with a $pK = 5$, will be dissociated). In both cases the accessibility of the carboxylate group has been diminished. That lowering the pH is more effective than raising the Ca^{++} level in re-

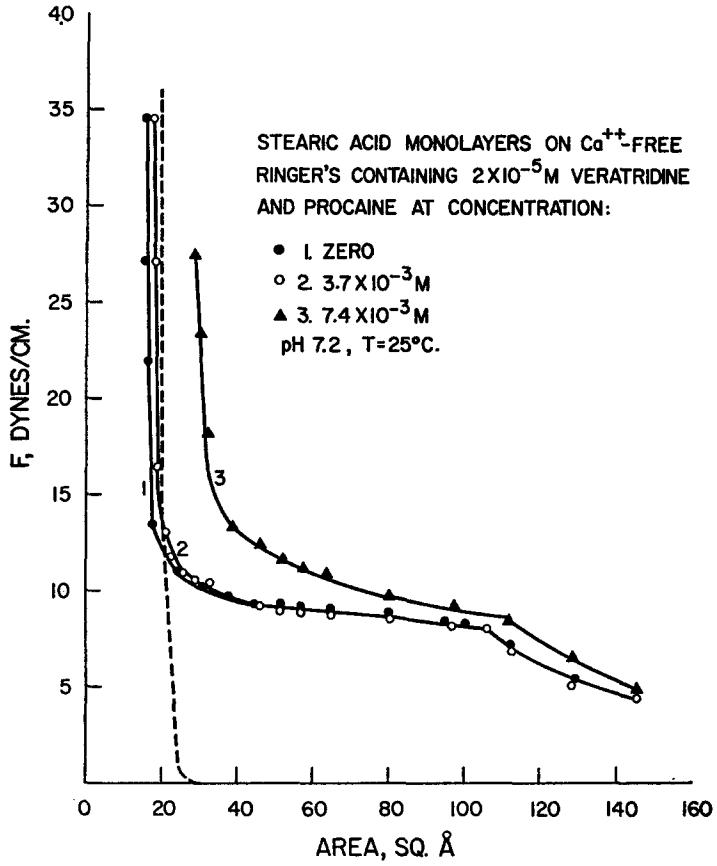

FIGURE 7. Procaine antagonism of the veratridine-stearate interaction. Dotted curve F-A curve for stearic acid on Ca^{++}-free Ringer's solution.

ducing the surface pressure is due at least in part to the fact that the surface activity of veratrinic compounds is also reduced at low pH (about −0.5 dyne/cm. at pH 3); this in turn is reflected in a lowering of the "surface excess" (1). It has already been shown that Ca^{++} does not interact with the veratrine alkaloids in any way that would reduce their surface activity (10). Thus, we see that Ca^{++} or low pH alters the nature of the stearate film so as to inhibit film penetration by the drugs and the associated instability.

The effect of procaine on the veratridine-stearate film interaction is shown in Fig. 7. Very little is seen to happen to the veratridine-stearate F-A curve

at large areas when Ringer's substrate contains procaine at a concentration of 3.7×10^{-3} M. The shoulder still appears at 105 Å^2, and the surface pressure at large areas is identical with that of the veratridine-stearate F-A curve observed in the absence of procaine. These are strong indications that procaine, at this concentration, has not itself penetrated the film and has not altered the penetration of the film by veratridine. However, a somewhat larger limiting area (about 18 Å^2) is observed when the film is compressed. The fact that the limiting area is now increased, but is still smaller than the stable stearate films formed in the absence of veratrine (20 Å^2), indicates a reduction of the instability of the veratridine-stearate system.

If the procaine concentration is raised to 7.4×10^{-3} M, additional marked effects are observed in the F-A curve: (*a*) there is an increase in the surface pressure for the entire curve; (*b*) the shoulder is shifted from 105 to 110 Å^2, indicating that some procaine in addition to veratridine has penetrated the monolayer; (*c*) the pressure at which film collapse occurs is reduced from about 35 dynes to about 27 dynes/cm., and (*d*) the limiting area is increased to about 30 Å^2, showing that there is a marked increase in film stability.[3]

These phenomena may be interpreted in terms of a considerable adsorption of the anesthetic by the monolayer, resulting in the formation of a procaine subfilm beneath the stearate monolayer. This procaine subfilm may have two effects: (*a*) it may reduce the contact of the stearate film with the water substrate and thereby further decrease the film's solubility, and (*b*) it may act to shore up the stearate superstructure which veratridine would otherwise disrupt. In either case, the net effect is to "stabilize" the stearate-veratridine mixed film by decreasing the rate of desorption of the veratridine-stearate complex. Moreover, the larger limiting area (30 Å^2) and the lower collapse pressures would be more in keeping with a film whose structure is composed of veratridine and stearate. It appears that the procaine subfilm permits the penetration of veratridine into the stearate monolayer (as evidenced by the shoulder in the F-A curve at 110 Å^2), but it also acts to reduce the rate of desorption of the veratridine-stearate complex from the film.

DISCUSSION

The results which have been obtained suggest that the diverse pharmacological behavior of the veratrum alkaloids is reflected by the interaction of these drugs with the stearic acid film. The veratrinic compounds, veratridine and cevadine, interact most strongly by penetrating the film and rendering it unstable, veratramine less strongly as reflected by its being squeezed from the

[3] Preliminary observations indicate that the veratridine-stearate system is 40 per cent more stable (*i.e.*, the force declines at 60 per cent the usual rate) when 7.4×10^{-3} M procaine is added to the substrate. This value was obtained by comparing the relative change of surface pressure per unit time over a wide range of film areas (80 to 40 Å^2) for the veratridine-containing substrates, with and without the local anesthetic. More quantitative studies remain to be carried out.

surface upon compression of the film, and veracevine least strongly because it lacks the surface activity necessary to concentrate at the interface. A remarkable feature of these results is that relatively small changes in the chemical structure of the alkaloids are sufficient to alter the response of the stearate film to the drug. Also, the effective concentrations in the substrate are those active pharmacologically, and the same antagonisms by stabilizers, calcium, and low pH, familiar with living systems, are apparent with the film.

The cell membrane has been variously described (4, 5, 23, 24). One general picture that emerges is that of a thin structure, perhaps 100 Å thick, containing various lipid materials organized in a liquid crystalline state of aggregation and stabilized by a network of protein. It is the lipid component of the membrane that the stearic acid monolayer may resemble at least in part. Although the stearate monolayer can only approximate the lipid phase of the biological membrane "*chemically*," it may nevertheless closely duplicate the *physical state* of aggregation. As a result of compression, the stearate film changes from the gaseous to the liquid, liquid crystalline, and crystalline states of aggregation, successively (25). The F-A curves permit one to observe the interactions of the drugs with the film in all these different states of aggregation.

The liquid crystalline region for the stearate films occurs between 20 and 25 $Å^2$. Veratridine and cevadine can disrupt this film structure while veratramine and the local anesthetics primarily adsorb to the subsurface of the film and enhance the film's superstructure.

If it is assumed that just such processes occur in the biological membrane, then one may infer that the action of the veratrinic compounds is to increase the passive ionic fluxes by partially disrupting the membrane structure, perhaps by removal of one or more lipids of the membrane. The reversibility of this effect in the biological systems would then be attributable to the replenishment of these lipids from intracellular stores. On the other hand, veratramine, the local anesthetics, and calcium may be considered to antagonize the veratrinic effect by maintaining the membrane structure either by their ability to prevent or slow down the removal of the lipids and thereby favoring their replacement from the internal reservoir or by replacing the lipid removed. It may be possible to test these views by exposing excitable cells to depolarizing concentrations of veratrine and then searching for a lipid in the bathing medium that does not appear under other depolarizing conditions.

Additional pharmacological data suggest that the mechanism of drug action observed with stearate films is pertinent to biological systems. For example, it is well known that a local anesthetic, when applied directly to the frog nodal membrane, blocks the action potential in less than 1 second of

application (15); this and other observations, recently reviewed (26), suggest a very superficial site of action, in keeping with the idea of interaction with the surface of the membrane. On the other hand, the effects of veratridine application to small nerve bundles develop more slowly than with KCl (35). This, too, is in keeping with the suggested mode of action, for the time delay is ascribable to the nature of the disruptive processes rather than to the time for diffusion of the drug into the membrane. And, finally, the protective action afforded by low pH, calcium, and procaine against the action of veratrine in living systems is reflected in our findings with the stearate monolayer. In this connection it is important to note, as observed by Skou with local anesthetics (30), that raising the pH has the additional effect of increasing the surface activity of alkaloids; the rise in their concentration at the interface, indicated by this, must thereby increase their effectiveness. At the pH at which the free base exists, presumably ion-dipole interactions occur instead of the ion-ion type.

In summary, then, the similarity of the stearic acid film to the biological membranes with respect to (*a*) its sensitivity, (*b*) its ability to distinguish between stabilizers and labilizers, and (*c*) its ability to demonstrate well known antagonisms, leads us to the tentative conclusion that it contains important elements pertinent to the mechanism of action of pharmacological and physiological agents for which cellular membrane effects of a physiochemical nature are well established (26, 27). Certain of these elements were implied by earlier studies that called attention to lipid solubility or to surface activity as important factors in the action of physiological and pharmacological agents. The present research focuses attention on more specific details, such as (*a*) surface activity, which determines the availability of an agent at the interface between the medium and the living membrane; (*b*) interactions with and within the membrane involving coulombic (ion-ion or dipolar) and van der Waals forces; (*c*) the dimensions of the membrane and foreign molecules or ions; and (*d*) the orientation of membrane components, governed by the preceding factors and by the presence of hydrophilic (polar) and hydrophobic (non-polar) groups. It is desirable to extend such studies to a wider variety of agents and to more complex lipoidal systems.

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Note Added in Proof.—The work and manuscript of reference 8 below were undertaken as a direct consequence and after completion of the research and manuscript of the present paper. Through an oversight, reference to the present joint effort was ommited.

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