Antagonism of Veratrine by Calcium Ion in Monolayers of Stearic Acid

Abstract. Force-area diagrams for monolayers of stearic acid on Ringer's solution demonstrate a competition between veratrine and calcium for carboxyl groups of the films. The competition occurs at customary concentrations. Local anesthetics act quite differently. The interactions suggest those observed less directly in living cells and therefore indicate that such surface films may serve as models for the study of drug and ion effects.

The alkaloids of veratrine—veratridine and cevadine, and their familiar mixture, "veratrine"—are of interest for the study of cellular properties, particularly because of the marked changes they produce in ion permeability and in bioelectrical phenomena (*1*). Thus, the application of 2.0 mg of veratrine in 100 ml of frog Ringer's solution to frog sciatic nerve causes an increase in exchange of Na^+ and K^+ of approximately 1.5 μmole/g hr. The effect is reduced or eliminated if cocaine is added to the solution; calcium probably also reduces the ionic exchange, for, like cocaine (*2*), it prevents, at higher concentrations, depolarization by such alkaloids. From bioelectric observations of repetitive activity, in which an antagonism between Ca^{++} and veratrine was noted, it has been suggested that calcium and veratrine compete for sites on the cell surface (*3*). While no direct evidence is available to support this idea, we can show, through a simple model system, that such antagonism is entirely feasible.

We chose a monomolecular film of stearic acid as our model of the cell membrane because (i) it may have charge and lipoidal characteristics similar to those of biological membranes and (ii) it has been well characterized under a variety of conditions (*4*). This film was also of special interest because of the relationship that has been established for local anesthetics between their blocking potency of local anesthetics and in frog nerve and their effect on the spreading pressure (*5*; see p. 139, reference 1); the significance of stearic acid films as membrane models would be greatly enhanced if, unlike rigid membranes (*6*), these films react differently to different veratrine alkaloids. Indeed, they do react differently.

Stearic acid films were spread from benzene on substrates of Ringer's solution with and without calcium buffered to *p*H 7.2; veratrine (Penick and Co.) at a concentration of 1:75,000 was dissolved in the Ca^{++}-free and normal Ringer's solution (107 mmole of NaCl, 1.6 mmole of KCl, and 1.0 mmole of $CaCl_2$ per liter, and an all-sodium Sørenson buffer which added 1.7 mmole of sodium per liter). This concentration of veratrine was sufficient to increase the Na^+ and K^+ exchange in toad sciatic nerve to about 1.0 μmole/g hr (*7*).

A commercial Langmuir-type film balance was used to record the spreading pressure (force) at each molecular area (*4*) at room temperature. The molecular areas were calculated from the amount of stearic acid that was initially spread on the surface and from the surface area at each compression.

Figure 1, in which the force in dynes per centimeter is plotted against the average area occupied by a stearic acid molecule in the air-solution interface, shows the results of our experiments. Curve *A* shows the force-area relation for stearic acid on Ca^{++}-free Ringer's. The two linear regions, when extrapolated to zero force, give the limiting areas 20.5 A^2 and 24.2 A^2 for the solid and liquid-solid phases, respectively (*8*). The smaller limiting area corresponds to the cross-sectional area of the stearic acid molecule oriented with the principal axis almost vertical to the surface and with the —COOH group embedded in the substrate. When Ca^{++} is added to the substrate, the liquid-solid phase is condensed to the solid phase (curve *A'*). This phase change is apparently due to the formation of calcium stearate, which causes a more rigid alignment of the molecules in the stearate film (*8*).

If 1:75,000 (1.3 mg/100 ml) veratrine is added to the calcium-free Ringer's substrate, curve *B* results. Two striking features are immediately evident: (i) in the attenuated film (that is, at large areas) a spreading pressure of 8 to 10 dyne/cm, which is maintained over a wide range of areas, is observed; (ii) compression of the film yields a limiting area of approximately 17 A^2, considerably smaller than that for stearic acid. These films are quite unstable, particularly in the steep region of the curve, and the force decreases markedly with time. To obtain reproducible results, 2 minutes were allowed to elapse between compressions of the film. Re-expansion of the compressed films indicated that the compression was not reversible.

Curve *B'* (Fig. 1) is the force-area curve of stearic acid spread on normal Ringer's solution with 1.3 mg of veratrine per 100 ml added. At large areas a force of 6 to 7 dyne/cm is observed, but when the film is compressed, the curve approaches that for stearic acid, and the limiting area of about 20 A^2. No film instability was observed. This behavior is quite distinct from that of the local anesthetic procaine with regard to film instability. The limiting area for stearic acid films on Ca^{++}-free Ringer's solution containing 3.7 mmole of procaine per liter is 20.5 A^2, and no film instability is observed (*5, 7*).

It appears that veratrine interacts with the stearic acid membrane in at least two ways. At a concentration of 1.3 mg/100 ml, veratrine lowers the surface tension of Ringer's solution about 1 dyne/cm (*7*); because of this surface activity, the alkaloid can concentrate in the interface. Since veratrine is a moder-

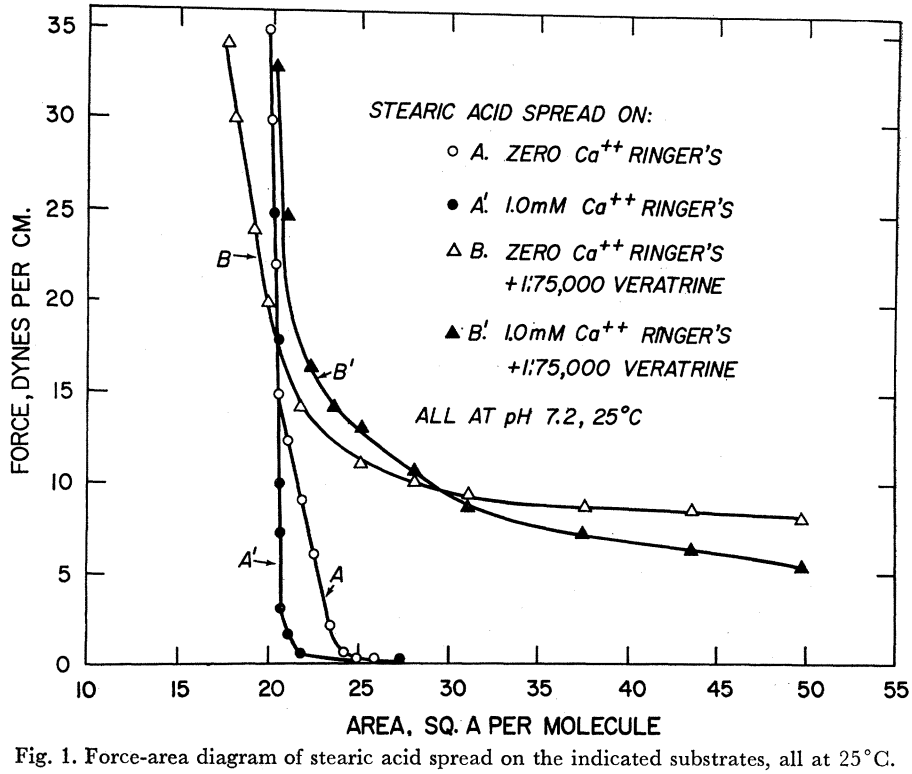

Fig. 1. Force-area diagram of stearic acid spread on the indicated substrates, all at 25°C.

ately strong base with a pK_b of 5.2 (*9*), an acid-base reaction between stearic acid and the basic nitrogen group of the drug may occur. The accessibility of the carboxyl group should strongly determine the extent of this interaction. Harkins (*8*) has reported that for mixed films of stearyl amine and stearic acid an acid-base interaction does indeed take place.

In addition to ion-ion interactions, interactions resulting from short-range van der Waals forces between the film and drug molecules, are also present; by their very nature, these interactions depend on the proximity to each other of the molecules in the surface film. Consequently, when the film is attenuated, the acid-base reaction should predominate, but as the film is compressed and the molecules are aligned, the van der Waals forces become increasingly prominent.

The problem of film instability can best be explained by postulating a process of "interfacial-dissolution" which sometimes occurs in films whose regular arrangement is disrupted when an irregularly shaped molecule penetrates the film (*10*). The lateral cohesion arising from the van der Waals forces, which enhance film stabilization in the case of the stearate films, is disrupted when veratrine (a bulky and irregularly shaped entity) is the penetrating molecule. The facts that no signs of film collapse (that is, film striations and an abrupt decrease in film pressure) were observed and that a film pressure of about 35 dynes was obtained on rapid compression of the film are other indications that this is a true "dissolution" process. Consequently, both veratrine and stearate leave the interface, perhaps as the salt, and are dissolved in the substrate. This process also accounts for the small limiting area as well as the instability of the film. When Ca^{++} is added to the substrate, the carboxyl groups are tied up as calcium stearate and the acid-base reaction cannot occur (*11*). This is also reflected in the lowering of the spreading pressure in the attenuated film. The fact that the limiting area for stearate is obtained on the Ca^{++} substrate indicates that here only the van der Waals forces predominate, and they are too weak to prevent the veratrine from being squeezed from the film.

The Ca^{++} antagonism for veratrine, then, is the competition for the acid groups of the film and is influenced by the relative interaction energies of these competing agents. One should also find that the antagonism is influenced by the Ca^{++} concentration, *p*H, and temperature. Evidence is already available for nerve that at low temperature (*12*) and *p*H (*13*) veratridine depolarization is weaker (*14*).

These findings provide further evidence that monolayers of stearic acid are affected by drugs and ions at physiological and pharmacological concentrations and exhibit responses and antagonisms that are sufficiently distinctive to suggest interactions related to biological effects. These and related model systems certainly merit further exploration (*15*).

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14. A paper describing these conditions, as well as the significance of film instability, is in preparation.
15. We are indebted to W. A. Zisman and L. Jarvis for the loan of the film balance and for introducing one of us (N. L. G.) to the film balance technique.

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Metabolically Induced Precipitation of Trace Elements from Sea Water

Abstract. The presence of organic material in some manganese-rich nodules suggests that the nodules are of biological origin. This suggestion leads to the proposal that in sea water, heavy metals get protection from hydrolysis by organic complexing; parts of these complexes become metabolized, the remaining parts then precipitate. These processes would explain a number of interesting observations.

Two recent detailed papers (*1, 2*) discuss from the physicochemical standpoint the genesis of the commonly found (*3*) manganese-rich deposits on the sea floor. Adequate consideration has never been given, however, to the possibility that these deposits stem from biological processes.

I have recently been examining some typical manganese-rich nodules from the Blake Plateau (collected in August 1957 at latitude 30° 51′N, longitude 78° 27′W in about 400 fathoms) and have succeeded in finding in them a fraction of a percent of organic material. A crushed and ground nodule, when rinsed with distilled water (*p*H 7), yields a bright yellow solution with *p*H about 4. If this solution is acidified, the color is eliminated. Ethyl acetate extracts from this colorless solution a fraction which can be recovered, by extraction into a weakly ammoniacal solution, and which then becomes yellow. Paper chromatography of this solution reveals at least three components. Extraction of the nodule with 7*N* NH_4OH yields further organic material which can be charred by heating it in an open dish. Some material having free amino groups has been detected with Ninhydrin. A further organic fraction can be extracted with 95 percent ethyl alcohol.

This organic material might have been adsorbed from sea water during the inorganic process of deposition of the trace elements, or alternatively, it could be accounted for directly if bottom-dwelling organisms are the agents by which the nodules are made. A nonphotosynthetic organism would be required for the latter interpretation, since manganese-rich deposits have been found throughout the depths of the oceans. A nonchemosynthetic organism also seems to be required by the fact that the deposits are found at all depths where adequate oxygen is available. Food would have to be derived from the several milligrams per liter of organic material that are present in sea water at most depths. The composition of this material is poorly known, but tyrosine analysis of hydrolyzed sea water assays this amino acid at a fraction of a milligram to a few milligrams per liter (*4*); many others should be present in comparable amounts. These amino acids and protein-derived compounds could serve as a valuable part of the nutrients of a great variety of organisms; if they were an important part, slow growth would be required for life on this starvation diet.

It is well known that by various types of chemical bonding, amino acids, peptides, and proteins form complex molecules with numerous metallic ions (*5*). Some of these complexes are sufficiently stable that the usual properties of the metallic ions in solution become effectively masked, and it is then impossible to treat their chemistry by the usual rules of solubility, *p*H sensitivity, and so forth, that apply to the simple inorganic ions in dilute solution. Many of these complexes are so stable that it seems quite certain that they must exist in sea water if the proper ingredients are available.

Unfortunately, not enough is yet