The Displacement of Calcium Ions from Phospholipid Monolayers by Pharmacologically Active and other Organic Bases

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1. The binding of $^{45}\text{Ca}^{2+}$ to a monolayer of phosphatidylinositol at the air-water interface was maximal when the separation of the phospholipid head groups approximated to the diameter of a hydrated Ca^{2+} ion. 2. The displacement of Ca^{2+} adsorbed on monomolecular films of phosphatidylinositol by a series of drugs (both narcotic and excitatory) and other organic bases was related to the ability of the bases to penetrate into the film. 3. With films of phosphatidylinositol at constant area, and at an initial surface pressure of 10 dynes/cm., the displacement of Ca^{2+} by increasing concentrations of the local anaesthetic, tetracaine, was linearly related to the change in surface pressure ($\Delta\pi$) caused by the penetration of the drug. 4. $\Delta\pi$ and the displacement of Ca^{2+} showed a related fall when the initial surface pressure of the phosphatidylinositol film was increased from 4 to 40 dynes/cm. both at a constant bulk tetracaine concentration and when this latter concentration was adjusted to keep it at a constant ratio to the surface density of phosphatidylinositol molecules. 5. The displacement of Ca^{2+} from phosphatidylinositol films by cetyltrimethylammonium ions was directly compared with the surface concentration of the base in the film, measured by using labelled base and a surface-radioactivity technique. 6. The ability of a series of straight-chain aliphatic amines to displace Ca^{2+} from phosphatidylinositol films increased with the number of carbon atoms up to C_{12} . However, there was a marked jump in the displacing activity after hexylamine, and this could probably be correlated with the carbon chain's being of sufficient length to just reach the hydrophobic fatty acid chains of the orientated phospholipid molecules with the charges on both substances in juxtaposition.

It is now generally assumed that calcium plays an important role in controlling the excitability of nervous tissue. Lowering the calcium concentration results in an increase in the permeability of biological membranes and produces a spontaneous firing of neurones (e.g. Frankenhaeuser & Hodgkin, 1957; Ames, Tsukada & Nesbett, 1967).

We have carried out an investigation of the mode of binding of $^{45}\text{Ca}^{2+}$ to monomolecular films of the complex lipids found in nerve-cell membranes (Hauser & Dawson, 1967). The acidic phospholipids readily bound Ca^{2+} , the adsorption being largely independent of the chemical nature of the phospholipid, and controlled by coulombic forces directly related to the net excess of negative charge on the lipid molecule. The adsorbed Ca^{2+} on such films could be displaced readily by relatively high concentrations of univalent metal cations such as Na^+ or K^+ . The starting point of this investigation

was the observation that certain pharmacologically active bases were several hundred times more effective at displacing Ca^{2+} from acidic phospholipid monolayers than Na^+ or K^+ . Because of the idea often put forward that these substances act by interfering with or mimicking the effect of Ca^{2+} on the nerve-cell membrane (e.g. Feinstein, 1964; Feinstein & Paimre, 1966; Rogeness, Krugman & Abood, 1966; Blaustein, 1967), the displacing effect was investigated in some detail. The displacement of Ca^{2+} by organic bases at low concentration is probably due to their ability to penetrate into the phospholipid film so that their basic groups are more effective in neutralizing the negative field around the phospholipid polar groups than if they are acting as simple gegenions.

EXPERIMENTAL

The apparatus and techniques used for determining the amount of $^{45}\text{Ca}^{2+}$ adsorbed on a monolayer by surface-radioactivity measurements have already been described

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(Hauser & Dawson, 1967). However, a flow counter with a large window (6.5 cm. $\times$ 1.2 cm.) covered with a sheet of 6 μ -thick polyethyleneterephthalate plastic (Melinex; I.C.I.) gave better detection of the weak β -emission from $^{45}\text{Ca}^{2+}$ (Dawson, 1968). As the phospholipid films used in the present work were mainly below collapse pressure, the slow loss of surface radioactivity after maximum adsorption of $^{45}\text{Ca}^{2+}$ was less of a problem than in our previous investigation (Hauser & Dawson, 1967). In general, a known amount of phospholipid was spread as a monolayer on the trough filled to the brim with doubly distilled water, pH 5.5 (72 ml.); $^{45}\text{CaCl}_2$ (0.0154 μ mole; 3.5 μC) (The Radiochemical Centre, Amersham, Bucks.) was added to the trough and the film was compressed with a barrier to the pressure required. After the surface radioactivity reached equilibrium the displacing substance was added to the bulk phase and distributed throughout the trough by magnetic stirring. When displacement was complete the surface pressure was recorded, and another sample of the solution of displacing agent was added to the trough. In this way plots were constructed of the surface radioactivity and the change in surface pressure ($\Delta\pi$) against concentration of displacing substance, so that from these the amount of the displacing substance required to produce 50% displacement of Ca^{2+} and the corresponding $\Delta\pi$ could be deduced.

Phospholipids. Phosphatidic acid was prepared by the method of Palmer & Dawson (1968), and phosphatidylinositol was prepared from frozen peas by a procedure evolved in this Laboratory by Dr A. Sheltawy and based on methods described by Rouser, Kritchevsky, Heller & Lieber (1963) and Ansell & Hawthorne (1964). Thin-layer chromatography and alkaline degradation indicated that both were pure. Dicytlyphosphoric acid was a commercial sample (Albright and Wilson Ltd., London, W. 1).

RESULTS

Binding of Ca^{2+} to a phosphatidylinositol film at various surface pressures. Fig. 1 shows the effect of varying the surface pressure of a phosphatidylinositol monolayer orientated at the air-water interface on the binding of Ca^{2+} , measured by the surface-radioactivity technique. With increasing surface pressure there was a linear increase in the surface concentration of phosphatidylinositol molecules. However, the surface concentration of Ca^{2+} , $[\text{S}]_{\text{Ca}}$, was not proportional to the equivalent concentration of phospholipid molecules, $[\text{S}]_{\text{PL}}$ (Fig. 1a). The ratio $[\text{S}]_{\text{PL}}/[\text{S}]_{\text{Ca}}$ reached a minimum of 3.7:1 between 20 and 30 dynes/cm. pressure (Fig. 1b), the affinity of the Ca^{2+} for the film falling off at lower and higher pressures. However, the absolute value of this ratio is fixed by the concentration of Ca^{2+} in the subphase. In this instance, a saturating concentration of Ca^{2+} was not used because of limitations in the surface-radioactivity technique (Hauser & Dawson, 1967). Each point plotted in Fig. 1(a) represents the surface concentration of Ca^{2+} as determined by the amount of Ca^{2+} added to the system and the adsorption isotherm for Ca^{2+} at the particular surface pressure of phosphatidylinositol examined.

Fig. 1. Variation of adsorption of Ca^{2+} on a phosphatidylinositol film with surface pressure. (a) Surface concentration of phosphatidylinositol (●) and Ca^{2+} (○). (b) Ratio between the surface concentration of phosphatidylinositol and Ca^{2+} (Δ), and the calculated average spacing between the negative charges on the phospholipid head groups (▲).

Table 1. Comparison of displacement of Ca^{2+} from monomolecular films of acidic phospholipids by Na^{+} and by tetracaine

Displacing agents were added to the bulk phase in the Langmuir trough.

Film	Pressure (dynes/cm.)	Na^{+} added for 50% displacement ($\mu\text{g. ions}$)	Tetracaine added for 50% displacement (μmoles)
Dicytlyphosphoric acid	49.5	150	3.8
Phosphatidic acid	42.5	126	0.43
Phosphatidylinositol	42.5	90	0.30

At low pressures it is possible that the wide spacing of the phospholipid molecules inhibited two-point electrostatic attachment of Ca^{2+} (Hauser & Dawson, 1967) to the anionic sites on the film, and the close spacing at higher pressures might have prevented the penetration of the hydrated Ca^{2+} ion between the anionic head groups of the phospholipid molecules. Thus calculation from the force-area curves of phosphatidylinositol showed that the separation of negative sites on the interface approached the diameter of a hydrated Ca^{2+} ion (9.6 $\text{\AA}$) at a point when Ca^{2+} binding was maximal (Fig. 1b).

Displacement of Ca^{2+} from monolayers. When

Table 2. Displacement of Ca²⁺ from monolayers of phosphatidylinositol by various bases

The films were at an initial pressure of 10 dynes/cm. Other details are given in the Experimental section.

Substance	Amount required to displace 50% of Ca ²⁺ (μmoles)	Increase in surface pressure (Δπ) at 50% displacement (dynes/cm.)
Drugs		
Adrenaline hydrochloride	3.55	1.7
Amphetamine sulphate	1.1	3.7
Atropine sulphate	2.04	9.3
Brucine sulphate	0.48	1.5
Chlorpromazine hydrochloride	0.027	8.3
Cocaine hydrochloride	0.71	4.4
Dibucaine hydrochloride	0.022	4.8
Meprobamate	32.4	4.0
Orphenadrine hydrochloride	0.125	9.1
Procaine hydrochloride	0.75	1.95
Promethazine hydrochloride	0.075	11.8
Tetracaine hydrochloride	0.1	3.5
Tyramine hydrochloride	1.92	1.1
Strychnine hydrochloride	0.92	4.2
Amino acids		
Phenylalanine	23.7	1.4
Cysteine	89.0	0
Histidine hydrochloride	4.4	0.9
Arginine hydrochloride	7.8	0.9
Miscellaneous		
2-Aminoethylbenzene hydrochloride	2.93	4.1
1-Aminoethylbenzene hydrochloride	3.9	4.0
Trimethylamine hydrochloride	40.6	1.7
Tetramethylammonium bromide	48.7	1.6

Ca²⁺ was adsorbed on a monomolecular film of an acidic phospholipid it was found that many pharmacologically active water-soluble bases were far more effective agents than Na⁺ or K⁺ ions in causing a displacement of the bivalent ion. Thus the local anaesthetic, tetracaine, displaced Ca²⁺ from high-pressure monolayers of phosphatidic acid and phosphatidylinositol in concentrations (Table 1) that were several hundred times lower than those expected if it were acting as a simple water-soluble univalent gegenion such as Na⁺. Table 2 shows that there was considerable variation in the concentrations of a series of basic compounds required to cause a 50% displacement of Ca²⁺ from phosphatidylinositol films. Many organic bases, including both anaesthetic and stimulatory drugs, and basic amino acids were effective. In nearly all instances the substances caused an increase in the surface pressure (Δπ) of the film, suggesting that their

Fig. 2. Displacement of Ca²⁺ (●) and increase in surface pressure (Δπ) (○) caused by adding tetracaine (0.1 μmole) to the bulk phase below phosphatidylinositol monolayers at various surface pressures.

effectiveness in causing displacement of Ca²⁺ might be connected with their ability to penetrate into the phospholipid film. However, as might be expected from agents of such diverse chemical structure, there was no simple connexion between displacement and penetration as judged by the Δπ value.

The relationship between penetration and Ca²⁺ displacement was then examined in more detail in a series of experiments with the local anaesthetic tetracaine.

Displacement of Ca²⁺ with varying initial surface pressure of the monolayer. Increasing the pressure of a phosphatidylinositol film caused a fall in the percentage of Ca²⁺ displaced by adding a constant amount of tetracaine (0.1 μmole) to the bulk buffer phase (Fig. 2). At the same time the increase in surface pressure (Δπ) on the addition of tetracaine rose to a maximum at an initial film pressure of 4–6 dynes/cm. and then fell steadily with increasing starting pressure of the phosphatidylinositol film (Fig. 2). In Fig. 3 the displacement of Ca²⁺ from a phosphatidylinositol monolayer produced by adding tetracaine (0.1 μmole) is plotted against the increase in surface pressure caused by penetration of the drug. There was an approximately linear relationship between these two variables at starting film pressures above 4 dynes/cm., but at lower starting pressures the relationship no longer held.

In the experiments with a constant amount of tetracaine (Figs. 2–3), the ratio between the bulk concentration of tetracaine and the surface concentration of phosphatidylinositol decreased as the starting pressure of the film was increased. Fig. 4 shows an experiment in which the ratio of bulk tetracaine concentration to the surface concentration of phosphatidylinositol was kept constant.

Fig. 3. Increase in surface pressure ($\Delta\pi$) plotted against the displacement of Ca^{2+} produced by adding tetracaine ($0.1 \mu\text{mole}$) to the bulk phase below phosphatidylinositol monolayers at various starting pressures (indicated, in dynes/cm., by the numbers in parentheses).

Fig. 5. Plot of Ca^{2+} displacement against the increase in surface pressure ($\Delta\pi$) obtained when increasing amounts of tetracaine were introduced into the bulk phase below phosphatidylinositol monolayers at a constant initial starting pressure (10 dynes/cm.).

Fig. 4. Displacement of Ca^{2+} (●) from phosphatidylinositol monolayers at various initial starting pressures produced by adding tetracaine to the bulk phase. The amount of tetracaine added (○) was adjusted so that its concentration was approximately proportional to the surface concentration of phosphatidylinositol molecules calculated from the force-area curve.

Fig. 6. Langmuir-type plot of the amount of tetracaine added to the trough against this amount divided by the increase in surface pressure ($\Delta\pi$) when the drug is introduced below a monolayer of phosphatidylinositol at 10 dynes/cm.

There was a linear fall in the displacement of Ca^{2+} as the initial starting pressure was increased.

Displacement of Ca^{2+} at constant initial surface pressure of the monolayer. In a further series of experiments the starting pressure of a phosphatidylinositol film was kept constant and the amount of tetracaine added to the bulk phase was varied. The displacement of Ca^{2+} from films of phosphatidylinositol at a starting pressure of about 10 dynes/cm. was directly related to the increase

in surface pressure ($\Delta\pi$) on the addition of tetracaine (Fig. 5). If $\Delta\pi$ is used as a measure of the surface concentration of tetracaine, the penetration isotherms were all of the Langmuir form $\Delta\pi = (K_1[\text{T}]_B)/(K_2 + [\text{T}]_B)$ where $[\text{T}]_B$ is the bulk concentration of tetracaine (Pethica, 1955). Thus a plot of $[\text{T}]_B/\Delta\pi$ against $[\text{T}]_B$ is linear (Fig. 6).

Fig. 7. Displacement of Ca²⁺ (●) and the change in surface pressure ($\Delta\pi$) (○) caused by the penetration of cetyltrimethylammonium ions (CETA⁺) into a film of phosphatidylinositol at an initial starting pressure of 10 dynes/cm. Δ , Theoretical points for the displacement calculated from the mass-equilibrium equation (see the Discussion section).

Displacement of Ca²⁺ from phosphatidylinositol monolayers with cetyltrimethylammonium bromide. The availability of [1-¹⁴C]cetyltrimethylammonium bromide led us to investigate more directly the relationship between the displacement of Ca²⁺ from a phosphatidylinositol film and the concentration of displacing agent in the surface monolayer of phosphatidylinositol. The surface concentration of cetyltrimethylammonium ions was computed for a series of phosphatidylinositol films at pressures of 9–12 dynes/cm. by measuring the surface radioactivity after the addition of various amounts of the base to the bulk phase. Fig. 7 shows that, as the surface concentration of cetyltrimethylammonium ions increased, $\Delta\pi$ increased non-linearly, the values obtained in different experiments having acceptable reproducibility. Equivalent experiments were then performed in which Ca²⁺ was displaced from a series of phosphatidylinositol monolayers by unlabelled cetyltrimethylammonium bromide. Thus a direct comparison of the Ca²⁺ displacement

Fig. 8. Displacement of Ca²⁺ and surface-pressure changes caused by introducing a series of straight-chain aliphatic amines (hydrochlorides) into the bulk phase below phosphatidylinositol films at initial pressures of 10 dynes/cm. The logarithm of the concentration of amine required to produce 50% displacement of Ca²⁺ (●) and the equivalent increase in surface pressure ($\Delta\pi$) (○) are plotted against the number of carbon atoms in the amine molecule.

from phosphatidylinositol films and the surface concentration of displacing agent was made (Fig. 7).

Displacement of Ca²⁺ from phosphatidylinositol monolayers by homologous series of aliphatic amines. The effect on Ca²⁺ displacement from phosphatidylinositol monolayers of systematically increasing the hydrophobic part of the displacing molecule was investigated by using a homologous series of straight-chain aliphatic amines. The curve relating the change in surface pressure produced by adding sufficient amine to cause 50% displacement of Ca²⁺ ions and the number of carbon atoms in the molecule of the displacing agent (Fig. 8) showed a clear discontinuity between C₆ and C₇ (*n*-hexylamine and *n*-heptylamine). A plot of the logarithm of the concentration of amine required to produce half-displacement of Ca²⁺ from the phospholipid monolayer against amine carbon number produced a constant slope between C₂ and C₁₂, with again a clear lateral shift between C₆ and C₇ (Fig. 8). Increasing the chain length of the amine beyond C₁₂ did not increase its ability to displace calcium from the monolayer, but rather surprisingly the $\Delta\pi$ value for 50% Ca²⁺ displacement fell.

DISCUSSION

It has been shown that local anaesthetics such as procaine and tetracaine can combine with phospholipids and prevent the lipid solubility that the

phospholipids confer on Ca^{2+} ions in a two-phase solvent system (Feinstein, 1964; Blaustein, 1967). The present results show that very low concentrations of certain pharmacologically active bases can displace Ca^{2+} ions adsorbed on monolayers of acidic phospholipids at the air-water interface. Thus, for example, chlorpromazine at a concentration of $0.4 \mu\text{M}$ in the Langmuir trough displaces 50% of the calcium adsorbed on a monomolecular film of phosphatidylinositol. Whether such films are suitable models for the anionic binding sites of calcium on the nerve-cell membrane is a matter for conjecture. Certainly phosphatidylinositol and the metabolically related polyphosphoinositides are components of these membranes (Eichberg, Whittaker & Dawson, 1964; Eichberg & Dawson, 1965). It is likely that the negatively charged head groups on the phospholipid molecules would be orientated on the surface of the organized lipid microstructures (e.g. dimolecular leaflets) that probably exist in membranes, and thus act as centres of electrostatic interaction. However, the extent to which this potentiality for calcium binding is modified by membrane protein is not known. There has recently been a great tendency to invoke phospholipids as membrane models with scant consideration for the role played by the protein component.

All the base molecules that effectively displace calcium from a phosphatidylinositol film possess two characteristics: first, their basic group or groups are fully or at least partially ionized at the pH used; secondly, when introduced into the Langmuir trough they cause an increase in the surface pressure of the film.

The most likely explanation of the rise of surface pressure on addition of the displacing base is that part of the base molecule penetrates and physically occupies the available spaces in the monolayer. If surface adsorption of the base molecules below the film occurred, this would tend to decrease both the kinetic and the electrical energy of the system and hence contract the film and decrease the surface pressure (Phillips, 1955). This penetration need not necessarily extend to the hydrophobic 'tail' region of the phospholipid monolayer, and it is clear that limited penetration into the hydrophilic head-group region does occur with certain basic drugs such as procaine (Gershfeld, 1962). As discussed below, this also applies to the short-chain aliphatic amines used in the present investigation.

The ability of tetracaine to cause a displacement of calcium from a phosphatidylinositol film seems clearly related to its capacity to cause an increase in the surface pressure ($\Delta\pi$). Both at constant film pressure (10 dynes/cm.) with increasing concentration of tetracaine and at various film pressures (4–40 dynes/cm.) with a constant concentration of

tetracaine there is a simple direct linear relationship between calcium displacement and $\Delta\pi$. Below initial film pressures of 4 dynes/cm. this relationship is lost, the $\Delta\pi$ curve for the concentration of tetracaine used showing a maximum at 4 dynes/cm. (Fig. 2). At low initial starting pressures, the tetracaine still displaces Ca^{2+} ions from the film (Figs. 2 and 3), but the penetration shows as a minimal increment in the surface pressure compared with that observed at higher pressures. At these low pressures the film would be in the liquid expanded state with plenty of space available between the widely spaced phospholipid molecules. The penetration of tetracaine could occur without an appreciable increase in the kinetic term of the equation of the state of the monolayer, more space being made available in the film by the hydrophobic 'tails' of the phosphatidylinositol passing to a more vertical orientation to the interface. At higher starting pressures the tetracaine penetration would exert its full spacing potentialities, but the amount of penetration would fall as the pressure was increased owing to the increased energy barrier preventing access to the more closely packed phospholipid film. The penetration of tetracaine may be based on a two-point electrostatic attachment of the two electropositive centres existing in the molecule with two phospholipid head groups, as envisaged by Feinstein (1964). By making an assumption about the size of the tetracaine molecule, it is possible to calculate from a force-area curve of a phosphatidylinositol film the surface concentration of tetracaine representing a given value of $\Delta\pi$. Such a computation shows that at the surface concentration of tetracaine required to cause complete displacement of calcium the tetracaine/phospholipid surface concentration ratio is very approximately 1:2, implying two-point electrostatic attraction of the drug. With widely spaced phospholipid films this two-point attachment by itself could cause an electrostatic contraction of the film, so that $\Delta\pi$ would be lower than that observed with more closely packed films. It is doubtful if the C_4 hydrophobic chain of tetracaine would be long enough to penetrate to the hydrophobic fatty acid region of the phospholipid film. Nevertheless it could still have an affinity for the film as is shown by the increased penetration of aliphatic amines into the film as their chain length is increased from C_1 to C_6 , as is discussed below.

In these experiments with tetracaine it was not possible to measure directly the surface concentration of tetracaine in the film, on which the displacement of calcium ultimately depends. Presumably this concentration is related to $\Delta\pi$ by the Gibbs adsorption isotherm (Pethica, 1955). A more direct comparison was possible by the use of labelled cetyltrimethylammonium ions, which

allowed the penetration to be measured in terms of the increment of surface radioactivity produced, assuming that such a long-chain amphiphile would entirely penetrate into the film rather than being adsorbed on the surface without penetration. The penetration of cetyltrimethylammonium ions into the film determined experimentally by this technique agreed closely with the surface concentration calculated from the Gibbs adsorption isotherm (Pethica, 1955), even though no specific electrostatic term was introduced into the equation.

The experimental curve relating the displacement of calcium to the surface concentration of cetyltrimethylammonium ions (Fig. 7) shows that complete displacement would occur when the concentration of cetyltrimethylammonium ions in the film approached that of the phosphatidyl-inositol (10.6×10^{13} molecules/cm.²): in other words, on complete neutralization of the anionic sites of the film.

The form of the curve relating calcium displacement to the surface concentration of cetyltrimethylammonium ion can in fact be calculated from the mass-equilibrium equation:

$$K = [\text{Ca}]_L/[\text{L}]_f[\text{Ca}]_f$$

where $[\text{Ca}]_L$ is the calcium combined with lipid, $[\text{Ca}]_f$ the free calcium and $[\text{L}]_f$ the free lipid on the surface.

$$[\text{L}]_f = [\text{L}]_s - [\text{CETA}^+]_s$$

i.e. the surface concentration of phosphatidyl-inositol, $[\text{L}]_s$, minus that of the cetyltrimethylammonium ions, $[\text{CETA}^+]_s$. This curve, shown in Fig. 7, closely approximates to that determined experimentally.

The experiments measuring the displacement of calcium by aliphatic amines clearly show that as the chain length of the amine is increased from C₂ to C₈ there is an increased ability to displace calcium. The logarithm of the concentration of amine required to produce 50% displacement when plotted against the number of carbon atoms in the amine shows a constant slope (Fig. 8). A linear relationship is also often found in any homologous series between the logarithm of the distribution coefficients between two immiscible phases and the number of carbon atoms in the molecule. As pointed out by Ferguson (1939), this simply means that there is a constant increment for each successive homologue in the partial molar free-energy difference between the standard states in the two phases. In the present experiments, the displacement plot shows a lateral shift between C₆ and C₇, indicating that heptylamine is more efficient at producing displacement of calcium than could be predicted from the behaviour of the C₂–C₆ amines. This is

reflected in discontinuity in the curve relating $\Delta\pi$ at 50% calcium displacement to the number of carbon atoms in the amine. Calculations and the building of molecular models show that, if it is assumed that the protonated amino group is at the level of the negatively charged phosphate on the phospholipid head group, the C₇ chain atoms would just reach into the hydrophobic fatty acid chains of the phospholipids, assuming a vertical orientation of the head group. Presumably this might assist the penetration of the amine into the film. Clearly, however, assisted penetration must occur with the shorter-chain amines without this type of hydrophobic bonding. The curve relating $\Delta\pi$ at 50% calcium displacement to the number of carbon atoms is clearly a complex one and cannot be explained adequately until more information is obtained about the surface concentration of amine at each chain length and the way the amines are positioned in the interface.

It is perhaps premature to try to relate the present findings, obtained in a model system, to the action of local anaesthetics in the intact animal. Certainly such anaesthetics would effectively liberate calcium bound to a phospholipid–water interface on a cell membrane, and it is known that they increase the calcium lost from excitable tissue such as nerve fibre or muscle (Kuperman, Altura & Chezer, 1968). However, it is by no means apparent that calcium and the local anaesthetics have a similar site of action on the nerve cell (Shanes, Freygang, Grundfest & Amatniek, 1959). If, as suggested by Feinstein (1964), Feinstein & Paimre (1966) and Blaustein (1967), the displacement of calcium from phospholipid surfaces is of importance in the development of anaesthesia, it needs to be explained why excitatory drugs such as brucine, strychnine or amphetamine or certain non-pharmacologically active bases are equally effective in removing calcium from such interfaces.

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