The Action of Anesthetics upon Interfaces

On the Mechanism of Anesthesia

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The different theories which try to explain the anesthetic action can be divided into two groups, those which in some way try to find a correlation between surface activity and anesthetic action and those which do not. Theories of the first type are those of Traube [1] and Warburg [2]. Many authors [3–7] have discussed the relation between the surface activity at the interface air/water and the anesthetic action. None of these authors have found a general correlation between the surface and the anesthetic activity.

A theory of another type is that of Meyer [8] and Overton [9] who have found a good correlation between the physiological action and the distribution coefficient at oil/water. »Narkose tritt ein wenn ein beliebiger chemisch indifferenter Stoff in einer bestimmten molaren Konzentration in die Zellipoide (richtiger: die lipoiden Alkohole der Zellsubstans) eingedrungen ist. Diese Konzentration ist von der Tier- oder Zellart abhängig» (Meyer and Hemmi [10]). *The permeability theory* advocated originally by Höber and Lillie states that stimulation causes an increase in the permeability of the plasma membrane and this is the primary cause of exitation, and that anesthetics would inhibit exitation by preventing an increase in permeability. *A calcium release theory* is put forward by Heilbrunn [11] (further information about this theory is given in the discussion). Finally, Seelich [12] used an interesting model to which we will return later. He studied the action of anesthetics upon an adsorbed layer of ergosterol at the interface water/paraffin oil.

Since very little is known about the chemical structure of cell membranes it is impossible to make an exact interpretation of the phenomena connected with the action of anesthetics at these membranes. This is also the reason why every model is necessarily incomplete. Norris [13] has made a summary of what we know about the cell membrane. It has a low tension about 0.1 dyn and its

probable constitution (Danielli [14]) is that of two monomolecular layers of lipids at which proteins are adsorbed. Warburg suggests that some of the molecules with enzymatic effect, adsorbed at cell interfaces, are necessary for the transport of impulses.

The problems connected with the action of anesthetics after injection or inhalation in the body where the distribution to the vital parts of the nerve is of importance, are very complicated. In this paper the problems are limited to those which are connected with the single cell.

We had a great many local anesthetics and related compounds synthesized by Löfgren and co-workers at our disposal. It was therefore an attractive task to test if measurements in conformity with those described in the papers mentioned above could give any positive results. Many of the compounds studied are closely related to a new, available, clinically tested, local anesthetic [16], i. e. ω-diethylamino-2,6-dimethylacetanilide.

$$CH_3$$
$$—NH—CO—CH_2—N\Big\langle {C_2H_5 \atop C_2H_5}$$
$$CH_3 \qquad Xylocaine$$
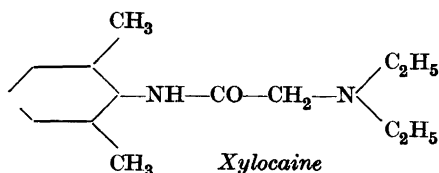
SURFACE ACTIVITY AT VARIOUS INTERFACES

The only conditions which produce correlation between surface and physiological activity are those in homologous series. Any physical property in one homologous series is likely to give such a correlation and positive results received in this way tell nothing about the mechanism of anesthesia. Miescher [15] found a maximum of surface activity in the percaine series. The same phenomenon was found by us in homologous series of the type ω-alkylamino-acet-*o*-toluidide * and ω-alkylamino-acet-*vic.-m*-xylidide ** (Fig. 1). In the diagram the surface tension of 1.47 mC solutions in Sörensen buffer at pH = 7.4 is put against the number of C-atoms in the alkylamino groups; γ_0 is the surface tension of the pure 0.25 C buffer and γ is the surface tension of the solutions.

The uncharged form of organic protolytes are more strongly adsorbed than the corresponding ion (Adam [18], Gardner and Semb [19]). Anomalies like maxima or minima in surface activity for protolytes in homologous series could therefore depend on a variation in pK_a. The pK_a values for the ω-alkylamino-acet-

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* Substances made by Löfgren [14].

** Substances made by Löfgren and Widmark [17].
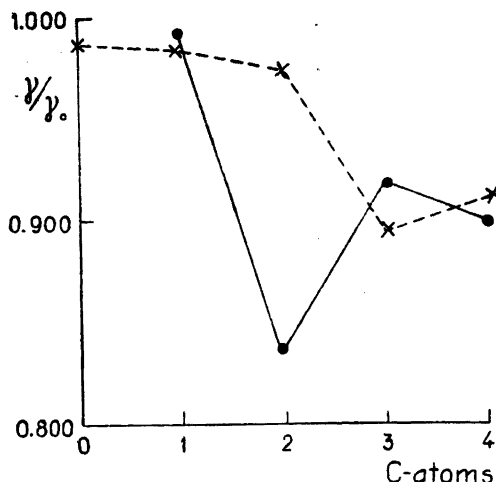
Fig. 1. Surface tensions of equimolar solutions of homologues in the series ω-alkylamino-acet-orto-toluidides (×— — —×) and ω-alkylamino-acet-vic.-m-xylidides (●———●).

vic.-m-xylidides are in order from the methylamino to the butylamino derivative 7.992, 8.075, 8.012 and 8.036 (Löfgren and Östlund, unpublished). If the base is the most surface active these values do not explain the maximum value in surface activity for the ethyl derivative because here the base concentration must be the lowest in the series. The explanation to this maximum value must be that the molecules are orientated at the surface and that a new methylene group added to one derivative is not equally situated in relation to the surface as the preceding one.

Since it is impossible to predict anything about the way these molecules orientate at the cell interface, it is impossible to infer anything about the mechanism of anesthesia through comparisons with the anesthetic effect in the same series. Moreover, the activities in the alkylamino-acet-vic.-m-xylidide series measured as minimal effective concentrations (action on frog sciatic nerve at pH = 7.4) show neither maxima nor minima. The activities increase with increasing molecular weight.*

The importance of selecting the right model for testing the correlation between surface and physiological activity appears from the following results. The surface activity at the interface air/water for some common local anesthetics is compared to the interfacial activity at the interface benzene water. Column I in Table 1 gives the surface tension in dynes/cm of 1.46 mC solutions of the anesthetics in 0.25 C Sørensen buffer at pH = 7.4. Column II gives the surface tension in dynes/cm in the interface benzene/water if 0.5 mmoles

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* L. Ehrenberg at this laboratory, unpublished.

of anesthetic are distributed to equilibrium between 50 ml benzene and 25 ml
0.25 C Sørensen buffer at pH = 7.4.

Table 1. Surface tensions of equimolar solutions at I air/water and II benzene/water.

	I	II
Novocaine	70.0	25.7
Tutocaine	67.0	24.4
Benzocaine	63.5	28.4
Pantocaine	56.5	25.7
Cocaine	54.7	25.7
Percaine	49.6	26.3
Pure buffer solution	74.4	30.5

The two interfaces give different orders of the values if the anesthetics
are ordered after increasing surface activity. It is obvious that it is impossible
to estimate the anesthetic effect through measurements of the surface tension
at an *arbitrary* interface, as for instance air/water.


ACTION OF ANESTHETICS UPON ADSORBED LAYERS

The Warburg theory points out that anesthetics, because of their surface activity,
would *displace*, other molecules necessary for the transport of impulses, — a theory
which is criticized by Seelich (*l. c.*). As far as that a displacement is very unlikely, we
agree. Furthermore, it is not probable that inhibition of some enzymatic effect directly
causes anesthesia. Seelich believes that a dehydration of the cell membrane is responsible
for the anesthesia. His reasoning about variations of permeability is not in agreement
with modern conceptions (Heilbrunn [20]). Seelich mentions that it is possible that besides
the dehydration, even a variation in the adsorption equilibrium for some enzymes
adsorbed at the cell membrane might be responsible for the anesthesia.

Seelichs model contains a liquid/liquid interface which has a low inter-
facial tension. A 0.05 % solution of ergosterol in paraffin oil is one of the
phases and water the other. Ergosterol is strongly adsorbed at the interface,
and (a) in Fig. 2 shows the variation of the interfacial tension with time until
equilibrium is reached. The equalities between this interface at equilibrium
and the cell interface are naturally only of physical character.

If the anesthesia is correlated to an alteration in adsorption equilibriums
for molecules at the cell membrane, Seelichs's model is useful. Thus we decided
to test what effect some of our local anesthetics would have on the adsorbed
ergosterol layer. Seelich tested chloroform and the five first alcohols in the
aliphatic series.

Fig. 2. a) ×————× The interfacial
tension — time curve caused by the ad-
sorbtion of ergosterol at the interface
paraffin oil/buffer solution.
b) ●— — —● The similar curve if the
oil phase contains propanol.
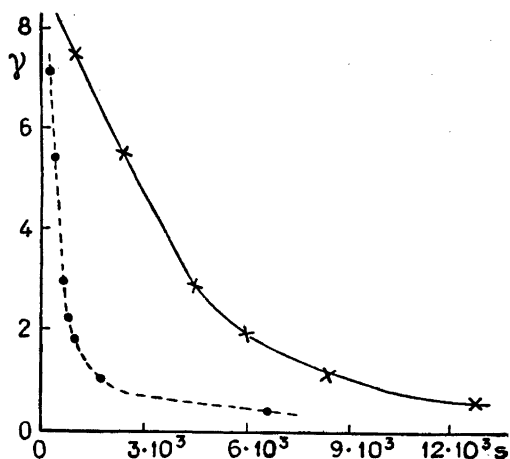
PRELIMINARY EXPERIMENTS

1. Adsorption of ergosterol at the interface oil/water

a) 20 ml of paraffin oil with 0.05 % ergosterol is poured over 20 ml Søren-sen buffer (0.25 C, pH = 7.4). The interfacial tension is put against time and thus we get (a) shown in Fig. 2.

b) 20 ml of paraffin oil with 0.05 % ergosterol and 0.3 ml n-propanol is poured over 20 ml Sørensen buffer (0.25 C, pH = 7.4). Time and interfacial tension, — see (b) in Fig. 2.
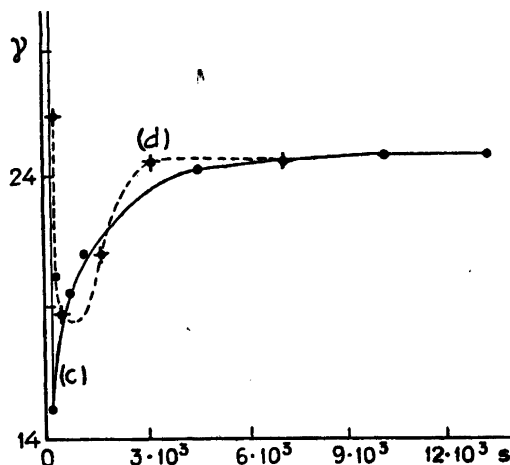

Fig. 3. c) The interfacial tension —
time curve showing the effect when pro-
panol is distributed from the water phase
to the oil phase.
d) The similar curve for the opposite
direction of the distribution.

The interfacial tension is expressed in dynes/cm and the time in seconds. Time is zero at the moment of overpouring.

It is important for the following that we are able to establish that (a) and (b) are going against the same value (about 0.5 dynes/cm) when time increases.

2. Propanol at the interface pure paraffin oil/ Sørensen buffer (0.25 C, pH = 7.4)

c) 20 ml pure paraffin oil is poured over 20 ml Sørensen buffer (0.25 C, pH = 7.4) and then 0.3 ml propanol, dissolved in 3 ml of water, is injected into the water phase. Time and interfacial tension are put against each other in (c) in Fig. 3.

d) 20 ml pure paraffin oil containing 0.3 ml propanol is poured over 23 ml Sørensen buffer (0.218 C, pH = 7.4). Time and interfacial tension, — see (d) in Fig. 3.

The interfacial tension is expressed in dynes/cm and time in seconds. Time is zero at the moment of overpouring in (d).

The initial interfacial tension ((c) and (d)) between pure paraffin oil and buffer solution was 33 dynes cm; (c) and (d) reach the same (equilibrium) value (24.6 dynes/cm) when time increases, which was to be expected. (c) shows that the propanol concentration in the layers next to the interface is abnormally high immediately after the injection, and that equilibrium is reached through diffusion *from* the interface. The minimum value of the interfacial tension in (c) is unknown because the tension was already increasing between the two first points which we were able to determine.

If the propanol comes from the oil phase as in (d), the course is in point of principle the same, but the minimum value is not so low as in (c). The propanol concentration in the layers next to the interface does not reach such high values as during the course (c).

3. Action of propanol upon ergosterol adsorbed at the interface paraffin oil/buffer solution

After ergosterol is adsorbed to equilibrium (see Fig. 2 (a)) at the interface paraffin oil + 0.05 % ergosterol/Sørensen buffer (0.25 C, pH = 7.4), 0.3 ml propanol is injected into the waterphase (before the injection the propanol was dissolved in a few ml of the water phase).

The interfacial tension after the injection is put against time in Fig. 4. Time is zero at the moment of injection and γ is expressed in dynes/cm.
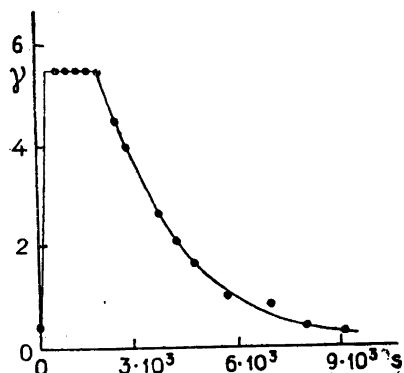
Fig. 4. Interfacial tension — time curve showing the action of propanol on an interface paraffin oil/buffer solution at which ergosterol is adsorbed, — the propanol is injected into the water phase.

The increase of the surface tension is caused by the *first* high propanol concentrations in the layers next to the interface. The ergosterol film at this stage is partially dissolved by these layers. That the propanol concentrations in these layers (immediately after the injection) are high, is clearly indicated by (c) of Fig. 3. When the high propanol concentrations are levelled, the interfacial tension in Fig. 4 decreases to a value very close to that of the injection point (0.5 dynes/cm). Fig. 2 also shows that the equilibrium values with and without propanol in the oil phase are almost the same. Injection of propanol in the oil phase (before the injection the propanol was dissolved in a few ml of the oil phase) gave no alteration in the interfacial tension. It is obvious that the concentrations of propanol in the layers next to the interface, corresponding to the lowest values in (d) of Fig. 3 are not high enough to effect any decrease in the (two dimensional) ergosterol concentration at the interface. Our interpretation of the decrease of the interfacial tension, *e. g.* in Fig. 4 is not in coincidence with Seelich's. He believes that evaporation of the anesthetic causes the decrease which apparently is impossible.

EXPERIMENTS WITH LOCAL ANESTHETICS AND RELATED COMPOUNDS

The same effect as in Fig. 4 was received if 0.176 mmoles LL 31 * was injected in the same way as the propanol (Fig. 6). In an experiment with the same model the distribution of LL 31 between paraffin oil + 0.05 % ergosterol and Sørensen buffer was studied; the quotient C_{oil}/C_{water} was determined at various times after the injection of 0.176 mmoles in the water phase. The result is illustrated in Fig. 5.

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* Trivial name for ω-diethylamino-2,4,6-trimethylacetanilide, an available, new local anesthetic (N. Löfgren *l. c.*).
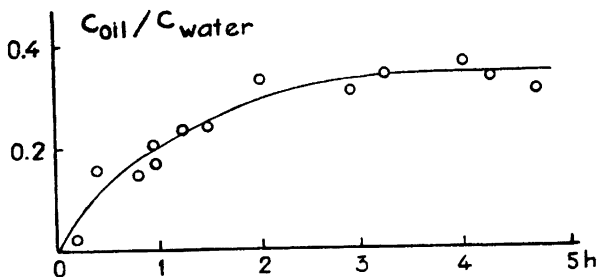
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Fig. 5. Distribution of a local anesthetic (LL 31) between paraffin oil with ergosterol and buffer solution.

A comparison between Figs. 5 and 6 indicates that the effect in Fig. 6 is maintained as long as the value of C_{oil}/C_{water} perceptibly differs from the distribution coefficient (*i. e.* the quotient at equilibrium).

Fig. 7 shows the effect of 0.176 mmoles of a) ω-diethylaminoaceto-cyclohexylamide, b) LL 31, c) Xylocaine and d) ω-diethylaminoacetanilide injected in the same way as in Fig. 4.

Xylocaine produced a complication because after twenty minutes we could observe a precipitation in the interface. The interfacial tension once raised, remained unchanged even after 24 hours.

APPARATUS AND FORMULAS

For the measurements of surface and interfacial tension the ring method with du Noüy tensiometer was used. Temperature was kept constant in the solutions with a water thermostat at 25° C. The vessel with the solutions was attached to a metal stand which could be raised or lowered by screws. In this way the vessel was in contact with the thermostat during measurements.
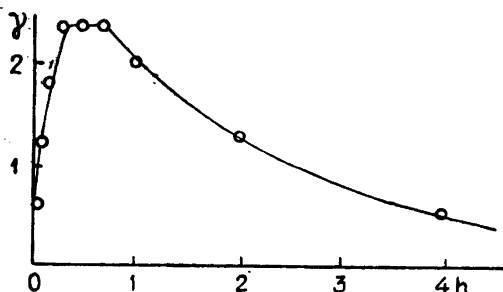

Fig. 6. Interfacial tension — time curve showing the action of a local anesthetic (LL 31) on an interface paraffin oil/-buffer solution at which ergosterol is adsorbed, — the anesthetic is injected into the water phase.

Fig. 7. Interfacial tension — time cur-
ves showing the action of various local
anesthetics on an interface paraffin oil/
buffer solution at which ergosterol is
adsorbed, — the anesthetics are injected
into the water phase.
a) ω-diethylamino-acetocyclohexy amid.
b) LL 31.
c) Xylocaine.
d) ω-diethylamino-acetanilide.
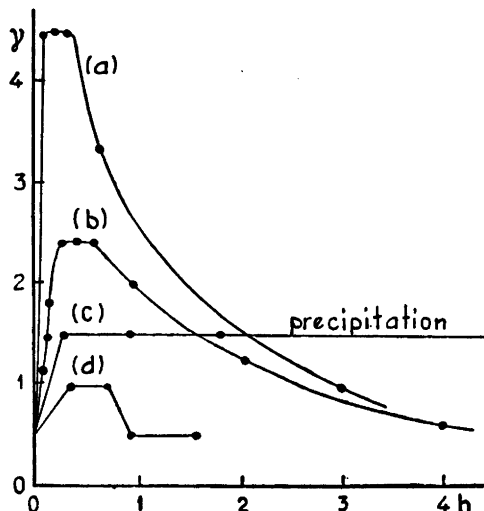
The injections were performed with a common syringe for medical use. After the in-
jection, buffer solution from the water phase was sucked up and injected again three
times. In that way we got a homogeneous solution of the anesthetic in the waterphase
before the measurements began.

The surface and interfacial tensions were obtained according to the equation

$$\gamma = \frac{P'}{4 \pi R} \cdot F$$

given by Harkins and Jordan [21]. In this equation γ is the surface or interfacial tension in
dynes/cm, P' is the maximum pull on the ring in dynes, R is the radius of the ring in
cm. F is a correction factor and is determined by the relation:

$$(F-a)^2 = \frac{4b}{\pi^2 R^2} \cdot \frac{P}{D-d} + C$$

given by Zuidema and Waters [22].

P = maximum pull on the ring in dynes/cm.

D and d = densites of the lower and upper phases respectively.

R = radius of the ring.

C = a constant which depends upon the ratio r/R (where r = radius of the
wire of the ring) in the following manner.

$$C = 0.04534 - 1.679 \frac{R}{r}$$

$a = 0.7250$ and $b = 0.0009075$ are universal constants for all rings.

DISCUSSION

Traube's and Warburg's theories about anesthetics as compounds acting because of their surface activity (surface phenomenons governed by the Gibb's adsorption law) are criticized by Meyer and Hemmi (*l. c.*).

They point out that if an anesthetic should be surface active at the interface cell membrane/water the interfacial tension anesthetic/water must be lower than the interfacial tension at the membrane *in vivo* (lower than about 0.1 dynes/cm). For hydrophobic general anesthetics (methane for instance) this is impossible. Local anesthetics always have polar (hydrophilic)-nonpolar structures. But in spite of this fact, it is highly improbable that any interface local anesthetic/water would have an interfacial tension lower than that of the membrane in vivo.

The lipid-solubility law given by Meyer and Overton is the only one that gives a mathematical formulation that is applicable. If oleyl alcohol is used as a model compound (Meyer and Hemmi, *l. c.*), the law has validity for general narcotics. Plotting the logarithms of the minimal effective concentrations against the logarithms of the distribution coefficients inverse proportionality is obtained —. However, this law gives no explanation of the *mechanism* of anesthesia.

The permeability theory is criticized by Heilbrunn [23] who emphasizes that the magnesium ion which has anesthetic effect certainly lowers permeability, but the calcium ion that lowers permeability still more, is able to break off a magnesium anesthesia. Moreover, recent studies of numerous anesthetics have shown that organic anesthetics do not always lower permeability. These facts show that Seelichs' (*l. c.*) theory is improbable because it is founded on the hypothesis that a lowering of permeability would be followed by anesthesia.

The Heilbrunn [24] calcium-release theory says that stimulation causes a release of calcium from the cell membrane and this calcium then causes a clotting or gelation of the interior protoplasm. The peculiar inhibiting action of anesthetics is due to a preventing effect on the clotting reaction.

After having described some results from measurements of salt potentials of resting muscles and nerves Höber [25] says: »Taking into account these various observations on the salt potentials and further referring to the aforementioned effects on excitability of muscle and nerve, *it is suggestive to interpret the bioelectric phenomena as surface effects taking place in the plasma membrane.* — The ionic effects on the potential suggest the presence of a colloidal membrane which shows both hydrophilic and hydrophobic attributes.» — Furthermore, by studying the birefringense of the cell membrane, it has been shown that there must be a strong organisation of the molecules in the plasma membrane (see Heilbrunn [26]).

From experiments by Kato [27] we are able to draw some important conclusions about the site of action of anesthetics on nerve tissue. Tasaki and Kato found that when nodes of Ranvier (of a single nerve fibre) are exposed to relatively dilute (though over the minimal effective concentration) narcotising solutions (such as urethane and cocaine) *anesthesia is induced after less than*

a quarter of a second. It seems to us that *the immediate effect shows that the mechanism of anesthesia is a surface course.* Moreover, when *Ranvier's* nodes are exposed to Ringer solution containing an anesthetic (urethane), the conc. of which is below the minimal effective conc. necessary for complete anesthesia, the alteration of the threshold of the stimulating current occurs abruptly. The effect of anesthetics on adsorbed lipids measured by us was also almost immediate (see Fig. 4).

If we assume, which is reasonable, that *the transport of impulses is dependent on a special organisation of molecules and metal ions at the cell membrane,* we are able to propose that *a disturbing of the equilibrium sites of these molecules and ions may cause the phenomena of exitation and anesthesia.* The physical difference between these is likely to be a difference in the nature and degree of the disorder caused.

In our experiments with ergosterol adsorbed at the interface paraffin oil/water, we found a marked alteration in the adsorbtion equilibrium of the film, when a narcotic was injected in the water face. *The effect was marked only before equilibrium distribution for the anesthetic was reached* (Figs. 5 and 6). The equilibrium value for the adsorbtion of the lipid molecules returns to a value very close to the original value when distribution equilibrium is reached for the anesthetic. A similar course is likely to take place at the cell membrane, *the equilibrium state of the impulse conducting organised layer* (built by lipo-proteins and metal ions) *is likely to be altered when an anesthetic is distributed to the lipid bulk phase* (about this phase, — see *e. g.* Höber [28]).

For general anesthetics the disturbing effect must be ruled by the value of the distribution coefficient lipid/water. Immediately after the injection, layers with high concentration of the anesthetic in the oil phase, develop near the interface (see Fig. 3) *because of a stopping effect of the viscous oil.* The dissolving power of these layers on the adsorbed interfacial layer must be stronger, the stronger the attracting forces is between the anesthetic and the adsorbed interfacial lipid molecules. The distribution coefficient lipid/water for the anesthetic is a measure of the attracting forces lipid-anesthetic in relation to the attracting forces water-anesthetic. Thus it is very *probable* that the distribution coefficient is a measure of the attracting forces between the adsorbed lipid layer and the anesthetic. The former reasoning is probably applicable to the cell in vivo and thus seems to be the reason for the known parallelism (see *e. g.* Meyer and Hemmi, *l. c.*) between the anesthetic effect and the distribution factor oil/water for general anesthetics.

For the local anesthesia it is impossible to get a mathematical relation based on the Meyer-Overton rule. (Löfgren-Ehrenberg, — unpublished). The distribution coefficient seems to have some significance but is not dominating.

Local anesthetics probably have a more specific way of action. These compounds always have one lipophilic and one hydrophilic part (aromatic residue — intermediate chain—amino group).

Rideal *et al.*, have made fundamental experiments with »penetration» of surface films. It is especially a lipophilic-hydrophilic construction that constitutes a »penetrant», Rideal [29] considers that such compounds have a strong physiological action. According to Schulman and Rideal [30] two types of complexes can be identified »those formed by association of a submerged polar group in the material injected, and those in which a subsequent penetration of the hydrophobic portion of the film by the hydrophobic portion of the material injected takes place. This subsequent stage is termed film penetration, and the stability of the resulting mixed films are shown to be due to molecular association.»

In our experiments the effect of local anesthetics was similar to that of general anesthetics. Even here we must propose a development of layers with high concentrations of the anesthetic in the oil phase next to the interface, and a dissolving effect of these layers on the adsorbed lipid layer at the beginning of the distribution of the anesthetic. Even here it seems reasonable to presume that a similar course in vivo causes anesthesia. The reason why it is impossible to get a mathematical relation based on the parallelism between distribution coefficient and anesthesia is that the distribution coefficient, because of the peculiar nature of a local anesthetic, is much less probable to be a measure of its power to attract lipid molecules. We must assume that local anesthetics act in a more specific way than general anesthetics, perhaps with forces like those described by Rideal *et al.* (*l. c.*) which are established at »penetration».

Metal ion anesthesia can be understood if we make one assumption, namely that a calcium ion-lipo-protein complex is responsible for the transport of impulses. If magnesium ions are injected, calcium is displaced at the lipids by magnesium (as at a permutite filter) and anesthesia is established. The inverted course represents the inhibition of magnesium anesthesia by calcium. Organic anesthetics affects the lipoproteins as is described above and thus disturb the metal ion-lipoprotein complex and cause anesthesia.

SUMMARY

Immediately after injection, the anesthetic dissolves in the layers of the lipid bulk phase of high viscosity next to the water phase and these layers are now at the highest concentration of anesthetic. The lipids adsorbed at the cell membrane which are organised and together with metal ions responsible for the transport of impulses, dissolves in the aforementioned bulk layers, and the organisation of the adsorbed lipid layer is altered. This alteration is respon-

sible for anesthesia. For general anesthetics this theory is in accordance with the known parallelism between distribution coefficients and minimal effective concentrations.

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