The penetration of local anesthetics into phosphatidylcholine monolayers

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Abstract The penetration of tetracaine into monolayers of phosphatidylcholine and trioctanoin at different surface pressures, and the penetration of dibucaine, tetracaine, butacaine, lidocaine, and procaine into monolayers of didecanoylphosphatidylcholine at $\Pi = 10$ mN/m was determined by the use of a modified Gibbs adsorption equation. These data were shown to fit a geometric model and compared favorably with data determined by a method based on the geometric model. The penetration of tetracaine into phosphatidylcholine monolayers was pressure dependent. At $\Pi = 10$ mN/m, the local anesthetics penetrate into a phosphatidylcholine monolayer in the order: dibucaine > tetracaine > butacaine > lidocaine > procaine. This correlates with their potencies in blocking nerve conduction and inhibiting phospholipase A_2.

Supplementary key words dibucaine · tetracaine · butacaine · lidocaine · procaine

Because of their surface-active properties, local anesthetics of the procaine type (Scheme 1) are thought to function through interactions with phospholipids in nerve membranes. Skou (1, 2) first demonstrated a correlation between the potency of local anesthetics in blocking nerve conduction and their penetration into lipid monolayers. Since then, many studies have been made on the penetration of local anesthetics into lipid monolayers and bilayers (reviewed by Papahadjopoulos (3)). The penetration of local anesthetics into negatively charged monolayers and bilayers has been studied (4, 5). With negatively charged lipids there is evidence for strong electrostatic interaction and competition between calcium ions and the anesthetics for binding to the lipid. Interaction of local anesthetics and zwitterionic phosphatidylcholine (PC) in the absence of a net negative charge was reported not to occur (6). Thus, the interaction was thought to be primarily electrostatic rather than hydrophobic. Recently, however, Cebrón (7) and Fernández and Cebrón (8) showed by NMR studies that local anesthetics with a hydrophobic tail attached to the nonpolar aromatic end (dibucaine and tetracaine) do indeed penetrate into zwitterionic PC bilayers. These studies and a recent study by Giotta, Chan, and Wang (9) using spin-labeled local anesthetics with PC liposomes, show that hydrophobic forces are also important in the binding of local anesthetics to lipid membranes.

Local anesthetics have been shown to inhibit phospholipase A_2 and other lipolytic enzymes in various membrane systems (10–12). In order to study this inhibition in simple monolayer systems, quantitative data on the penetration of local anesthetics into monolayers would be quite useful. This study began in the hope of obtaining such data, which would subsequently be used in a study of local anesthetic inhibition of phospholipase A_2 action on PC monolayers. The penetration of an insoluble monolayer by a soluble surfactant was studied by Pethica (13) for the penetration of sodium dodecyl sulfate into cholesterol monolayers. A theory based on the Gibbs adsorption isotherm was developed and shown to apply in that system. McGregor and Barnes (14) recently reviewed this treatment and compared it with a pure geometric model. In view of these successful studies, the Gibbs adsorption theory as modified by Pethica was applied to local anesthetic penetration of PC monolayers. The results of this study are presented here.

MATERIALS AND METHODS

Materials

Synthetic 1,2-didecanoyl-*sn*-glycero-3-phosphorylcholine and 1,2-dioleoyl-*sn*-glycero-3-phosphorylcholine were generous gifts from the laboratories of Professors G. H. de Haas and L. L. M. van Deenen, respectively. Trioctanoin was obtained from

Abbreviations: PC, phosphatidylcholine; didecanoyl PC, 1,2-didecanoyl-*sn*-glycero-3-phosphorylcholine; dioleoyl PC, 1,2-dioleoyl-*sn*-glycero-3-phosphorylcholine.

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Supelco, Inc., Bellefonte, Pa. Purity of the lipids was checked by thin-layer chromatography. Dibucaine, tetracaine, lidocaine, and procaine were supplied as the hydrochlorides by the Onderlinge Pharmaceutische Groothandel, Utrecht. Butacaine hemisulfate was obtained from Sigma Chemical Co., St. Louis, Mo. Other chemicals were of reagent grade. Fresh solutions of lipids (about 1 mM) were prepared in redistilled benzene and used for the spreading of monolayers. Distilled water was used for the preparation of all solutions.

Methods

A polypropylene trough (15 cm × 5 cm × 1 cm) equipped with two magnetic stirrers (in stirring wells) and a glass thermostating coil was used for preparing monolayers. Surface pressure was measured by the Wilhelmy plate method using a platinum plate attached to a Beckman electrobalance. When changes in surface area were measured, a Teflon barrier was used and the edges of the trough were lightly waxed with paraffin to prevent film leakage. Local anesthetics (in buffer solution) were injected underneath the monolayer and stirring was continued for several minutes to insure complete mixing. An equal volume of subphase was then removed and stirring was discontinued while the surface pressure was measured. The subphase solution for these experiments contained 0.1 M NaCl, 5 mM $CaCl_2$[2], and 5 mM Tris (pH 7.0) and was thermostated at 25°C. The surface densities of insoluble monolayer species were determined from the pressure–area isotherms for didecanoyl PC (15), dioleoyl PC (16), and trioctanoin (17) at 25°C, or (at low pressures) by carefully measuring the amount of standardized lipid solution applied.

RESULTS

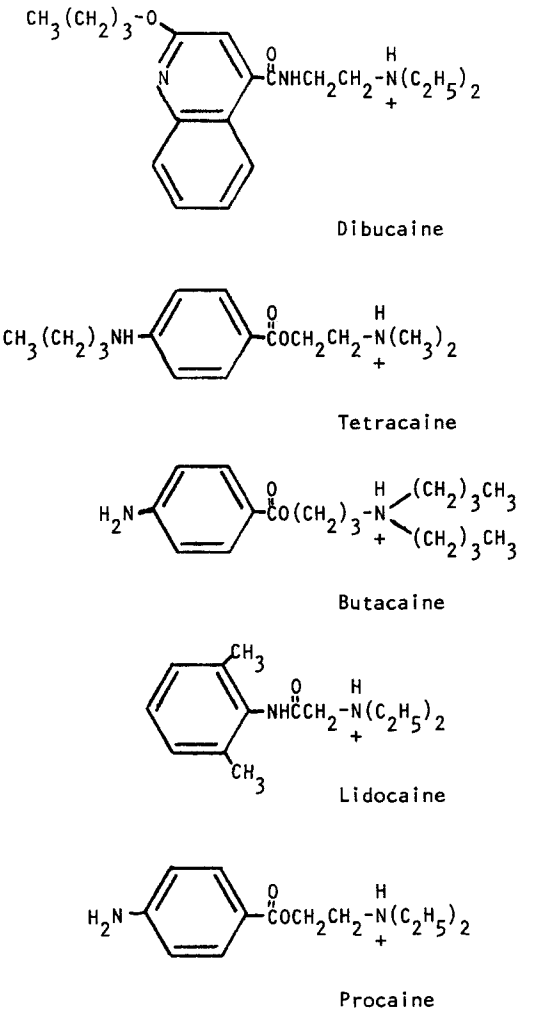
Determination of local anesthetic penetration by the Gibbs equation

In order to illustrate the general derivation of surface concentration, the determination of one point of dibucaine penetration into didecanoyl PC will be discussed. A monolayer of didecanoyl PC was spread at a pressure[3] of 0.75 mN/m (0.073 molecules/nm²). Measured amounts of dibucaine hydrochloride solution were added under the monolayer and the surface pressure was measured after each addition. Equilibrium was reached after a few minutes as evidenced by a stable pressure with respect to time. A plot was then made of the surface pressure increase ($\Delta\Pi$) versus log dibucaine concentration in the subphase (**Fig. 1**). The linear portion of this plot shows saturation adsorption. The slope of this linear portion, $d\Delta\Pi/d\log c$, was then used in a modified Gibbs equation (13) to calculate surface concentration:

$$\Gamma = \frac{d\Delta\Pi/d\log c}{2.303\Phi RT}$$

where Γ = surface concentration of anesthetic; $\Phi = A_{PC}/(A_{PC} - \bar{A}_{PC})$; A_{PC} = total surface area/number of PC molecules; and $\bar{A}_{PC}$ = partial molecular surface area of a PC molecule (the area actually occupied by a PC molecule).

The value of $\bar{A}_{PC}$ is not known, but can be assumed



Scheme 1. Structures of local anesthetics in the ionic forms which exist at pH 7 (pK_a's for procaine, 9.05 and 2.2 (6)).

[2] Although calcium ions appear to have no effect on penetration, 5 mM $CaCl_2$ was included in the subphase for these experiments since kinetic studies of local anesthetic inhibition of phospholipase A_2 require this concentration of calcium ions.

[3] 1 milliNewton/meter = 1 dyne/cm.

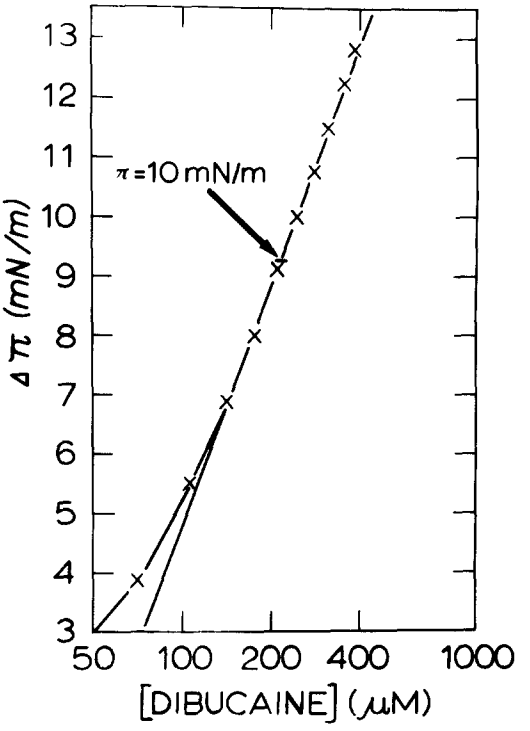


Fig. 1. Plot of ΔΠ versus log concentration dibucaine. Didecanoyl PC monolayer at an initial pressure of 0.75 mN/m (0.073 molecules/nm²). Subphase: 0.1 M NaCl, 5 mM $CaCl_2$, 5 mM Tris pH 7.0, T = 25°C.

to equal the molecular area of PC in a pure PC monolayer at the same pressure (for didecanoyl PC at Π = 10 mN/m, $\bar{A}_{PC}$ = 0.806 nm²/molecule), provided the monolayer is near to or in the liquid condensed region (at a relatively low compressibility) and the subphase contains excess electrolyte (13). Starting at different initial surface pressures, the surface concentrations of dibucaine in a PC monolayer at 10 mN/m and different subphase concentrations can be similarly determined. **Fig. 2** shows the penetration of tetracaine into trioctanoin and PC at different pressures and into a clean air/water interface. Although penetration into PC at the two pressures shown appears to reach a plateau, the data do not warrant such a conclusion. This effect may be due to a change in the value of $\bar{A}_{PC}$ at high surface concentrations of tetracaine. **Fig. 3** shows the penetration of various anesthetics into didecanoyl PC monolayers at 10 mN/m. Varying the calcium ion concentration between 0 and 15 mM had no significant effect on the penetration of tetracaine into a PC monolayer (Fig. 3). The partial molecular area of anesthetic, $\bar{A}$, can be calculated as follows: $\bar{A} = (1/\Phi)\ (1/\Gamma) = 2.303\ RT/(d\Delta\Pi/d\log c)$ where $1/\Phi = (A_{PC} - \bar{A}_{PC})/A_{PC})$, which is the fraction of "free space" in the monolayer available to penetration by anesthetic. These areas are shown in **Table 1** for the penetration of local anesthetics into PC monolayers and are compared with the saturation areas at an air/water interface (determined in a similar manner by the Gibbs equation, and measurements of surface pressure as a function of subphase concentration of anesthetic in the absence of a PC monolayer).

Determination of local anesthetic penetration by a geometric model

McGregor and Barnes (14) have presented a geometric model for monolayer penetration: $\Gamma = \Gamma_w - \bar{A}_{PC}\Gamma_w(1/A_{PC})$, where Γ_w = surface concentration of anesthetics at a stable-monolayer free interface. If this model holds, a plot of Γ versus $1/A_{PC}$ should give a straight line from which the values of $\bar{A} = 1/\Gamma_w$, and $\bar{A}_{PC}$ can be determined. The values of surface concentrations of various anesthetics obtained by the Gibbs-Pethica method are plotted versus $1/A_{PC}$ in **Fig. 4**. Lines were drawn through these points based on $\bar{A}_{PC}$ = 0.806 nm²/molecule and the various partial molecular surface areas of the local anesthetics shown in Table 1. The points for tetracaine seemed to fall along two straight lines, one for sur-

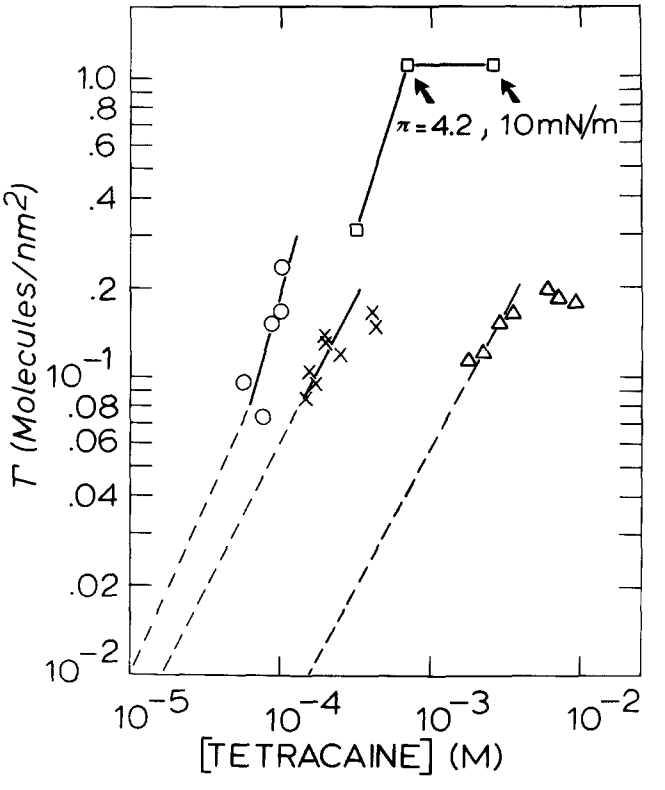


Fig. 2. Tetracaine penetration into different monolayers at different pressures. Log–log plot, determined by the Gibbs-Pethica method. Trioctanoin (Π = 12 mN/m), ○; didecanoyl PC (Π = 10 mN/m), ×; dioleoyl PC (Π = 26 mN/m), △; tetracaine adsorption at an air/water interface, □. Dotted lines are extrapolations to zero of non-log plots. Conditions described in Fig. 1.

face concentrations obtained at tetracaine subphase concentrations below 200 μM ($\bar{A}$ = 0.952 nm²/molecule) and another for those obtained at subphase concentrations above 200 μM ($\bar{A}$ = 1.17 nm²/molecule).

Since the fit to a geometric model (Fig. 4) seemed fairly good, the penetration of anesthetic into PC monolayers was also determined by the area expansion of a PC monolayer with increasing subphase concentration of anesthetic at constant surface pressure. A didecanoyl PC monolayer was spread at 10 mN/m and anesthetic added underneath. After each addition the monolayer was expanded to maintain the pressure at 10 mN/m. Values for the area increase, ΔA, at various subphase concentrations of anesthetic were used to calculate surface concentration in the equation (14) $\Gamma = \Gamma_w \ \Delta A/A_t$, where ΔA = increase in surface area, A_t = total surface area after the addition of anesthetic, and $\Gamma_w = 1/\bar{A}$ (values of $\bar{A}$ obtained from Table 1). Values for anesthetic penetration determined in this manner are also shown in Fig. 3.

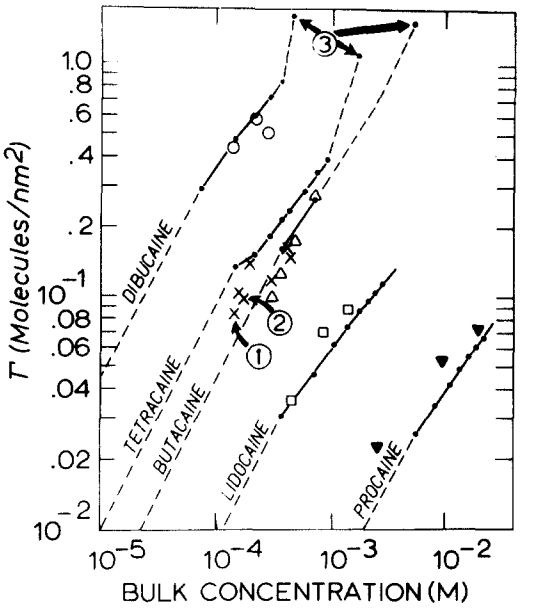


Fig. 3. Penetration of different anesthetics into a didecanoyl PC monolayer at Π = 10 mN/m. Log–log plot. Points determined by Gibbs-Pethica method: dibucaine, ○; tetracaine, ×; butacaine, △; lidocaine, □; procaine, ▼. Points connected by solid line determined from geometric model. Dotted lines are extrapolations to zero of non-log plots and to the surface concentration at a clean air/water interface (3). Conditions same as for Fig. 1, except for points 1 and 2 which were determined at 0 and 15 mM $CaCl_2$, respectively.

DISCUSSION

The molecular surface areas (saturation areas[4]) of local anesthetics at air/water interfaces shown in Table 1 are a measure of the surface activity of these compounds. The saturation areas become smaller with increasing hydrophobicity in the nonpolar portion of the molecule. This is counteracted, however, by the electrostatic repulsion of the charged portion of the molecule that acts to increase the area. The subphase concentration at which saturation adsorption is reached is also a function of the surface activity.

Butacaine seems anomalous in its behaviour since its saturation area is less than that of tetracaine while the concentration required to reach saturation adsorption is greater than that for lidocaine. This can be rationalized by considering that, while its nonpolar end is less hydrophobic than that of tetracaine, the presence of the two bulky and hydrophobic butyl groups on the charged amine moiety probably suppress protonation and thus the charge, so electrostatic repulsion between butacaine molecules becomes less than with other anesthetics. Thus, the area of butacaine at an air/water interface would be less than one would predict considering only the hydrophobic portion of the molecule in comparison with the other anesthetics. Lidocaine and procaine have quite large surface areas at the air/water interface. This is due to the low hydrophobicity of the molecules, so that electrostatic repulsion of the charged portion of the molecule becomes the dominant factor in determining the molecular area. When these anesthetics penetrate a stable neutral monolayer their surface densities are much less, so electrostatic repulsion becomes much less important or negligible. The partial molecular surface areas of local anesthetics in PC monolayers (Table 1) are thus more a measure of the hydrophobic interactions between anesthetic and PC molecules.

The Gibbs-Pethica treatment for anesthetic penetration into PC monolayers assumes that the partial molecular area of the PC molecule is approximately equal to the area of PC in a pure PC monolayer at the same pressure. This appears to be justified by the fact that saturation adsorption is reached in the penetration of anesthetic molecules, and plots of Γ versus $1/A_{PC}$ (Fig. 4) extrapolate to the area of PC molecules in a pure PC mono-

[4] The slope of the log concentration versus Π curve for adsorption at an air/water interface becomes linear at a certain subphase concentration. At this point saturation adsorption has been reached, where the surface concentration remains constant upon further addition of surface active material. The surface pressure continues to increase (surface tension decreases) due to the fact that it becomes easier to bring surface active material to the interface from a progressively more concentrated subphase (16).

TABLE 1. Surface areas of anesthetics

Local Anesthetic	Partial Molecular Surface Area	
	Air/Water	PC/Water[b]
	nm²/molecule	
Dibucaine	0.63 (0.4 mM)[a]	0.66
Tetracaine	0.92 (0.8 mM)	0.952–1.17
Butacaine	0.63 (3.0 mM)	0.99
Lidocaine	4.07 (1.0 mM)	1.54
Procaine	7.0 (16. mM)	2.15

[a] Concentration required for saturation adsorption.
[b] Didecanoyl PC at Π = 10 mN/m.

layer at 10 mN/m. The linear fit of the Gibbs-Pethica data to a geometric model (Fig. 4) also seems to justify determination of penetration by area expansion at constant pressure. Of course, the partial molecular areas of each species must first be determined by the Gibbs-Pethica method. There is fairly good agreement between penetration calculated by both methods (Fig. 2). The area expansion method has the advantage that many points can be determined with one film in a single experiment, while the Gibbs-Pethica method requires a separate experiment for each point.

With tetracaine, a discontinuity in penetration and partial molecular area is seen in the region of 200–300 μM subphase concentration. In this region, at a surface concentration of about 0.17 molecules/nm², the molar ratio of tetracaine to PC is 1:6.

With hexagonal packing the average distance between the tetracaine molecules would be slightly less than two PC molecular diameters. As the surface concentration increases above this value the average distance between tetracaine molecules would become much less. It could be that, at this point, electrostatic repulsion between tetracaine molecules becomes significant and the tetracaine molecular area increases.

The penetration of tetracaine into various stable monolayers at different pressures (Fig. 2) shows the effects of surface pressure and polarity of the monolayer on penetration. As one would expect, increasing surface pressure greatly reduces the extent of penetration at a particular subphase concentration. This effect would be expected to be even greater for the less hydrophobic anesthetics. This would explain the fact that no penetration of butacaine, lidocaine, or procaine is seen with PC liposomes (7, 8) where the packing of lipid molecules probably corresponds to that in a monolayer at a surface pressure greater than 40 mN/m (18). Penetration of tetracaine into trioctanoin monolayers was somewhat greater than into PC monolayers. This may be an effect of surface polarity, PC being more polar than trioctanoin. This effect was also seen in the saturation adsorption of sodium undecylsulfate at various organic solvent/water interfaces (19). The saturation adsorption of surfactant was dependent on the polarity of the organic solvent, decreasing with increasing solvent polarity.

The abilities of various local anesthetics to penetrate didecanoyl PC monolayers (Fig. 2) correlate with their potencies in blocking nerve conduction (12), inhibiting phospholipase A_2 (10), and affecting other membrane properties (3). Calcium ions do not antagonize this penetration, consistent with the fact that calcium ions do not bind to PC under these conditions (20, 21).

The penetration of local anesthetics into negatively charged interfaces is greatly enhanced by electrostatic interactions. For example, Hauser and Dawson (1968) reported 50% displacement of calcium ions from a phosphatidyl inositol monolayer at a pressure of 13.5 mN/m with 1.4 μM tetracaine. This concentration is at least two orders of magnitude less than that required to penetrate PC monolayers at 10 mN/m. Furthermore, Cebrón (7) showed that even the less hydrophobic anesthetics (butacaine and procaine) readily penetrate phosphatidyl serine liposomes, but not PC liposomes.

These data on the penetration of local anesthetics into PC monolayers should be quite useful in studying the effects of these anesthetics on membranes, particularly in well-defined artificial membrane systems. It would also be useful to have quantitative data on the penetration of local anesthetics into charged membranes. This is much more difficult to evaluate, however, due to the additional electro-

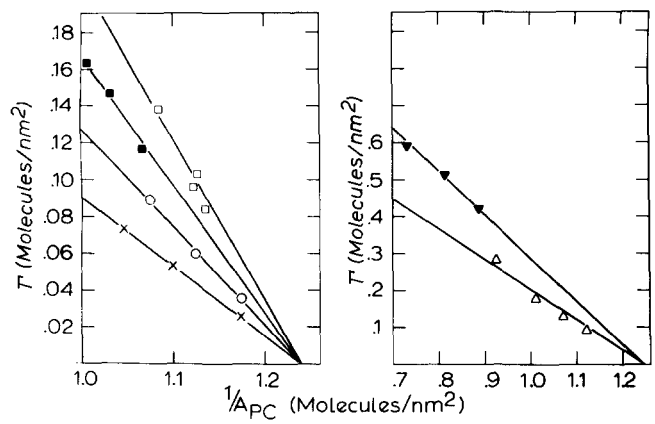


Fig. 4. Fit of penetration data to a geometric model. Plot of Γ versus $1/A_{PC}$; data obtained by Gibbs-Pethica method. Tetracaine, □ for points obtained at concentrations < 200 μM; ■ for points obtained at concentrations > 200 μM; lidocaine, ○; procaine, ×; dibucaine, ▼; butacaine, △. Conditions same as in Fig. 1, Π = 10 mN/m.

static term that would have to be introduced into the Gibbs adsorption equation.

This study was carried out during a sabbatical leave from St. Olaf College and was supported by Grant NS 11777-01 from the National Institute of Neurological Diseases and Stroke, U.S. Public Health Service. The author thanks Professor L. L. M. van Deenen for his generous hospitality and encouragement during the course of this research.

Manuscript received 28 October 1975; accepted 5 April 1976.

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