ting transition are not sensitive to small changes in T. This insensitivity is expected. By the time enough water has been added to the CH_3OH-C_6H_{12} mixture to induce the wetting transition at the air-liquid interface, the T_c of the two liquid phases has been raised to about 75°C (*11*). Then small fluctuations in ambient T will not significantly change the "distance" from the critical line.

We have observed several other transitions from complete to incomplete wetting starting with CH_3OH-C_6H_{12} mixtures. As water is added, the CH_3OH-rich phase will cease to wet glass completely. Instead of raising T_c by adding water, we have also raised it by adding NaOH or NaI; as we expected, complete wetting of the fluid-air interface by the CH_3OH-rich phase ceased. After the addition of sufficient water to cause dewetting at the vapor interface, the addition of acetone, which is known to lower T_c (*10*), restores wetting. Wetting and dewetting of the glass walls of the cuvette were also observed.

Our experiments leave open an important issue. Two recent phenomenological descriptions of the first-order phase transition from complete to partial wetting have been given (*3, 14*). In one description (*3*), the transition we have observed is merely the end point of a manifold of interface phase transitions. If this description is correct, a study of, for example, the interface between the vapor and a C_6H_{12}-rich liquid should reveal discontinuous transitions from low to high adsorption of CH_3OH accompanied by discontinuities in the first derivatives of the tension of this interface. These phenomena are predicted to occur at saturated vapor pressure along a surface in the space of three thermodynamic variables: temperature and mole fractions of water and CH_3OH. This surface is anchored at the line of three-phase coexistence, which happens to pass through the point: $T = 22°C$, $X_{H_2O} = 0.020$, $X_{CH_3OH}/X_{C_6H_{12}} = 0.53$. Experimental observations of the predicted manifold of interface transitions in the absence of three coexisting phases would be most interesting.

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References and Notes

1. T. Young, *Philos. Trans.* **95**, 65 (1805).
2. E. B. Butler, *J. Phys. Chem.* **67**, 1419 (1963).
3. J. W. Cahn, *J. Chem. Phys.* **66**, 3667 (1977).
4. C. Warren and W. W. Webb, *ibid.* **50**, 3694 (1969).
5. W. Rathjen and J. Straub, in *Proceedings of the Seventh Symposium on Thermophysical Properties*, A. Cezairliyan, Ed. (American Society of Mechanical Engineers, New York, 1978), p. 839.
6. S. Fisk and B. Widom, *J. Chem. Phys.* **50**, 3219 (1969).
7. A. N. Campbell and S. C. Anand, *Can. J. Chem.* **50**, 1109 (1972).
8. D. T. Jacobs *et al.*, *Chem. Phys.* **20**, 219 (1977).
9. E. L. Eckfeldt and W. W. Lucasse, *J. Phys. Chem.* **47**, 164 (1947).
10. V. E. Leibnitz, H.-G. Konnecke, S. Niese, *J. Prakt. Chem.* **4**, 278 (1957); E. R. Washburn and H. C. Spencer, *J. Am. Chem. Soc.* **56**, 361 (1934); A. N. Campbell and S. C. Anand, *Can. J. Chem.* **50**, 479 (1972).
11. D. C. Jones and S. Amstell, *J. Chem. Soc.* **1930**, 1316 (1930).
12. R. B. Heady, thesis, Massachusetts Institute of Technology (1969); _____ and J. W. Cahn, *J. Chem. Phys.* **58**, 896 (1973).
13. R. E. Dehl of the Polymer Science and Standards Division of the National Bureau of Standards assisted with these measurements.
14. B. Widom, in *Statistical Mechanics and Statistical Methods in Theory and Application*, U. Landman, Ed. (Plenum, New York, 1977), p. 33. This report does not consider the question of whether the complete-to-partial wetting transition is the end point of a manifold of interface transitions. If this question were considered, the same conclusions as were presented in (*3*) may well have been reached.
15. We thank B. Stephenson for stimulating comments and experimental assistance; Dr. R. Dehl for advising us concerning ellipsometry; J. Gallagher and L. Ketron who assisted with the photography; and Prof. B. Widom who made numerous helpful suggestions. This work has been supported in part by the NASA Lewis Research Center under contract C-62861.

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How Electric Fields Modify Alkane Solubility in Lipid Bilayers

Abstract. *The planar lipid bilayer membrane is assumed to be in osmotic equilibrium with the surrounding Plateau-Gibbs border (annulus) and entrapped microlenses. An electric field applied across the membrane raises the chemical potential of the alkane in the bilayer, causing it to shift from the bilayer to the annulus and microlenses. This shift results in a decrease in thickness.*

Accurate description of bulk solution properties in molecular terms is a long-standing problem of physical chemistry. The problem is a difficult one because little is known about the behavior of solutions in volumes of molecular dimensions. Much could be learned if a "slice" of a solution a few molecules thick could be isolated and scrutinized. While this is not possible in a literal sense, the black lipid film formed in aqueous media (*1*) is an excellent approximation to such a slice in that it is two molecules thick and may have dissolved in it alkane or related molecules. The black lipid film, or planar lipid bilayer membrane, forms spontaneously in the manner of soap films when a solution (~ 1 percent) of surface active lipid in an alkane solvent is spread across an aperture separating two aqueous phases. The resulting film consists of a lipid bilayer saturated with alkane surrounded by a Plateau-Gibbs border (annulus) of the bulk solution. Bulk solution is also dispersed as microscopic lenses within the bilayer. The optical reflectance of the bilayer is very small and hence the films are "black."

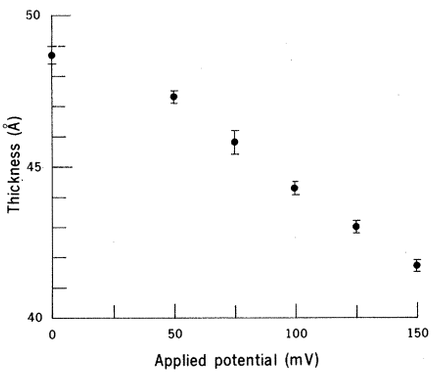

Fig. 1. Thickness of black lipid bilayer membranes as a function of applied potential. The bilayers were formed at 20°C from GMO and *n*-decane (10 mg/ml) in 0.1*M* NaCl solutions. Thickness (δ_B) is calculated from measurements of specific geometric capacitance C_g (Table 1) as described in (*19*).

I report here a mechanism for the modification of the solubility of alkanes in the bilayer by applied electric fields. The mechanism leads to a better understanding of the relation between the gross behavior of a solution and the solution's microscopic properties. Understanding the behavior of alkane-in-bilayer solutions is of particular importance in membrane biology, since biomembranes are essentially "two-dimensional solutions" consisting of hydrophobic proteins dissolved in a bilayer [reviewed in (*2*)].

Electric fields cause large decreases in the thickness of black lipid bilayer membranes (*3–5*). An example of this effect for bilayers formed from glyceryl monooleate (GMO) and *n*-decane is shown in Fig. 1. The thickness change apparently results from a shift of the alkane from the bilayer into the annulus and microlenses (*5*). The shift is generally attributed (*3–5*) to an electrostrictive pressure rise (P_V, dynes per square centimeter) in the bilayer given by

$$P_V = \left(\frac{\epsilon_0 \epsilon_B}{2\delta_B{}^2} V^2\right) \times 10^7 \qquad (1)$$

where $\epsilon_0 = 8.85 \times 10^{-14}$ farads per centimeter, ϵ_B is the dielectric coefficient of

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Table 1. Estimates of the fraction f_{ae} of acyl chains extended into the midbilayer zone of decane in GMO/*n*-decane bilayers.

V (mV)	C_g* (μF/cm^2)	f_{ae}†
0	0.3815 ± 0.0031	0.0042
50	0.3934 ± 0.0018	0.0040
75	0.4071 ± 0.0041	0.0039
100	0.4219 ± 0.0019	0.0038
125	0.4335 ± 0.0009	0.0038
150	0.4500 ± 0.0010	0.0039

*Specific geometric capacitance of the bilayer measured as described in (*19*). The standard errors of measurements on three membranes are indicated. The values of δ_B and $X_A{}^b$ shown in Figs. 1 and 2 were estimated from these values of C_g. †Calculated as described in the text.

the bilayer, δ_B is the thickness in centimeters, V is the applied potential in volts, and 10^7 is a conversion factor. This electrostriction-induced thinning process is analyzed in detail in this report.

Assume the lipid bilayer is in equilibrium with the surrounding annulus and entrapped microlenses and that an electrical potential difference V is maintained across the system. Because the lenses and annulus are much thicker than the bilayer, the pressure rise in these structures caused by V is negligible relative to the rise in the bilayer. Therefore, the alkane molecules in the bilayer are subjected to a pressure P_V not experienced by those in the annulus and microlenses (*6–8*). The chemical potential of the alkane in the bilayer ($\mu_A{}^b$) must equal the chemical potential in the annulus ($\mu_A{}^a$) at equilibrium. Since $(\partial\mu/\partial P)_T = \bar{v}$ (*9*), one may write

$$RT\ln\gamma_A{}^bX_A{}^b + \int_0^{P_V} \bar{v}\, dP = RT\ln\gamma_A{}^aX_A{}^a \quad (2)$$

where R is the gas constant, T is absolute temperature, $\bar{v}$ is the partial molar volume of the alkane in the bilayer, the γ's are activity coefficients, and the X's are mole fractions. The alkane is assumed to be uniformly distributed in both the bilayer phase and the annulus phase. However, as will be shown later, the alkane is probably not uniformly distributed in the bilayer. The contribution of the thickness-dependent van der Waals forces to P (*4, 10, 11*) will be ignored. Equation 2 may be rewritten (assuming $\bar{v}$ = constant) as

$$\gamma_A{}^bX_A{}^b = \gamma_A{}^aX_A{}^a \exp\left(-\frac{P_V\bar{v}}{RT}\right) \quad (3)$$

where $\gamma_A{}^aX_A{}^a$ must be constant regardless of what happens in the bilayer, since the mass of the annulus and microlenses is many orders of magnitude greater than that of the bilayer. It is thus apparent from Eq. 2 or 3 that when P increases, the mole fraction $X_A{}^b$, and consequently the bilayer thickness, must decrease (*12*). The mole fraction of decane in GMO/*n*-decane bilayers as a function of applied potential is shown in Fig. 2. From Fig. 2, $X_A{}^b$ decreases from 0.581 ± 0.004 to 0.529 ± 0.002 as V increases from 0 to 100 mV, corresponding to a pressure rise of approximately 5×10^4 dyne/cm^2 or 0.05 atm. Unfortunately, Eq. 3 suggests that a pressure of this size is too small to have such a large effect on composition. Measurements on GMO/*n*-decane dispersions (10 mg/ml) by vapor pressure osmometry indicate that $\gamma_A{}^a = 1.0$ and $X_A{}^a = 0.997$ (*13*) and consequently $\gamma_A{}^b$ must equal 1.72 to satisfy Eq. 3 for $V = 0$. If $\gamma_A{}^b$ remains constant and $\bar{v}$ has its bulk value of 195 cm^3, the pressure required to change $X_A{}^b$ to its 100-mV value is calculated to be 1.1×10^7 dyne/cm^2 or 11 atm. There is thus a discrepancy of three orders of magnitude between the actual pressure and the pressure required by Eq. 3.

Observation and theory can be reconciled in three ways. First, $\bar{v}$ may be much larger than assumed. This is unlikely, though, because a value on the order of 10^4 cm^3 would be required. Second, $\gamma_A{}^b$ may be strongly dependent on $X_A{}^b$. The data would fit $\gamma_A{}^b$ changed from 1.72 at 0 mV to 1.88 at 100 mV. The third possibility, which is partially related to the second, is that the alkane is not uniformly distributed across the bilayer but tends to form a separate phase in the bilayer midplane. Several experiments are consistent with this last idea. First, the activity of alkane in the bilayer shows very large positive deviations from ideal behavior, consistent with a tendency toward phase separation (*14*). Second, the enthalpy and entropy of solution of decane in the bilayer are nearly zero (*13, 15*). A phase separation of decane normal to the bilayer would explain this result. Third, Brooks *et al.* (*11*) found that calculations of van der Waals forces across GMO/alkane bilayers agree best with experiment if a separate alkane phase in the bilayer midplane is assumed. Thus, a phase separation seems reasonable. How this hypothesis reconciles observation and experiment is demonstrated as follows.

Visualize a three-layer "bilayer" consisting of GMO acyl chains/*n*-decane/GMO acyl chains [see (*16*), however]. Assume that, on the average, a fraction f_{ae} of the acyl chains are extended at any instant into the decane layer to control its chemical potential. Let $\tilde{X}_A{}^b$ be the mole fraction of decane in the layer, given by $\tilde{X}_A{}^b = n_A/(n_A + f_{ae}n_{AC})$, where n_A

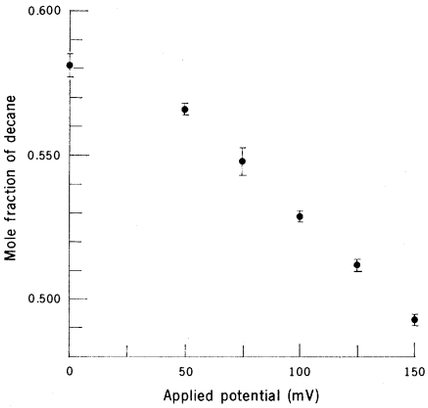

Fig. 2. Apparent mole fraction of *n*-decane in GMO/*n*-decane bilayers as a function of applied potential. Mole fraction ($X_A{}^b$) is calculated from measurements of specific geometric capacitance C_g (Table 1) as described in (*19*).

is the number of decane molecules per square centimeter of bilayer and n_{AC} is the number of acyl chains. Assume for simplicity that the decane behaves ideally in both the decane/acyl chain mixture and the annulus so that $\tilde{\gamma}_A{}^b = \gamma_A{}^a = 1$. Equation 3 can then be written

$$\tilde{X}_A{}^b = \frac{n_A}{n_A + f_{ae}n_{AC}} = X_A{}^a \exp\left(-\frac{P_V\bar{v}}{RT}\right) \quad (4)$$

At 20°C, $n_{AC} = 5.28 \times 10^{14}$ cm^{-2} (*17*). From the data in Fig. 2, n_A is calculated to be 7.32×10^{14} cm^{-2}. Assuming $\tilde{X}_A{}^b = X_A{}^a = 0.997$ for $V = 0$, f_{ae} is found to be 0.0042. That is, at any instant only 0.4 percent of the acyl chains need be extended into the decane phase. This result conforms closely to the notion of Andrews *et al.* (*4*) that only a small fraction of the acyl chains need be extended at any instant to stabilize the bilayer thickness. Now assume that a 100-mV potential is applied and that f_{ae} and $X_A{}^a$ remain constant. Using Eq. 4, n_A is found to decrease from 7.32×10^{14} to 6.49×10^{14} cm^{-2}. This corresponds to an apparent $X_A{}^b$ of 0.551 and a thickness of about 46 Å. The agreement with the data of Figs. 1 and 2 is surprisingly good. Indeed, it is good enough to assume that the basic mechanism is correct. The results of calculations of the f_{ae} at each potential necessary to achieve exact agreement are shown in Table 1; f_{ae} is about 0.004 in all cases.

This picture of acyl chains extended into the midbilayer decane phase to control decane activity may not be completely accurate. Any molecules dissolved in the central phase will affect decane activity. For example, there may well be a few GMO molecules dissolved (polar group and all) in this region. In ad-

dition, the midbilayer decane phase probably does not behave ideally ($\tilde{\gamma}_A{}^b \neq 1$) as assumed in the analysis. In such circumstances $\tilde{\gamma}_A{}^b$ must differ from 1.0 to maintain parity of the decane activity in the bilayer and annulus as the amount of decane mixing with the acyl chains increases.

The dependence of bilayer thickness on voltage decreases as the length of the alkane used in the formation of the bilayer increases. Thus, for GMO/hexadecane membranes, the change in thickness accompanying a 150-mV potential is not detectable (*4*). A simple explanation of this effect is that the longer alkanes mix more thoroughly with the acyl chains than do the shorter ones. In such cases, $\tilde{X}_A{}^b$ would tend to become equal to $X_A{}^b$ and, by virtue of Eq. 3, a significantly larger potential would be required to achieve a given fractional change in $X_A{}^b$. However, since $X_A{}^a$ remains on the order of 0.995 even for hexadecane, more thorough mixing would demand that $\tilde{\gamma}_A{}^b$ be significantly greater than 1 to keep the alkane activity in the bilayer equal to that in the annulus. There is evidence in support of this hypothesis. I demonstrated earlier (*15*) that the enthalpy of solution (ΔH) of *n*-hexadecane in GMO/hexadecane bilayers is large and positive (4 kcal/mole). Since, in general, $\ln \gamma \propto H/T$ (*18*), it follows that $\tilde{\gamma}_A{}^b > 1$.

These results and conclusions indicate the difficulties inherent in making assumptions about the molecular meaning of the activity coefficient. Often, for example, $RT\ln\gamma$ is interpreted as due to differences in solute-solute and solute-solvent interaction energies. It is now clear, however, that the interpretation of γ depends on knowledge of the structure of the environment surrounding the solute. Thus, in this report, the gross activity coefficient ($\gamma_A{}^b$) of the decane in the bilayer is ~ 1.7, while if the assumption of a microscopic phase separation is made, $\gamma_A{}^b \simeq 1$ in the separate phase is adequate to explain the results.

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References and Notes

1. P. Mueller, D. O. Rudin, H. T. Tien, W. C. Wescott, *Circulation* **26**, 1167 (1962); H. T. Tien, *Bilayer Lipid Membranes (BLM)* (Dekker, New York, 1974); R. Fettiplace, L. G. M. Gordon, S. B. Hladky, J. Requena, H. P. Zingsheim, D. A. Haydon, *Methods Membr. Biol.* **4**, 1 (1975).
2. S. J. Singer and G. L. Nicolson, *Science* **175**, 720 (1972).
3. A. V. Babakov, L. N. Ermishkin, E. A. Liberman, *Nature (London)* **210**, 953 (1966); D. Rosen and A. M. Sutton, *Biochim. Biophys. Acta* **163**, 226 (1968); S. H. White, *Biophys. J.* **10**, 1127 (1970); D. Wobschall, *J. Colloid Interface Sci.* **40**, 417 (1970); J. M. Crowley, *Biophys. J.* **13**, 711 (1973); R. Benz, O. Fröhlich, P. Läuger, M. Montal, *Biochim. Biophys. Acta* **394**, 323 (1975).
4. D. M. Andrews, E. D. Manev, D. A. Haydon, *Spec. Discuss. Faraday Soc. No. 1* (1970), p. 46.
5. J. Requena, D. A. Haydon, S. B. Hladky, *Biophys. J.* **15**, 77 (1975).
6. The pressure P_V is that acting at the surface of the bilayer. I assume this pressure is isotropic throughout the bilayer thickness. However, the pressure is probably not isotropic, as can be seen from the Bakker formula (*7*, *8*) for interfacial tension γ_t

$$\gamma_t = \int_{z_1}^{z_2} [P_l{}^N - P_l{}^T(Z)]dZ$$

where $P_l{}^N$ is the component of the interior pressure normal to the bilayer (Z direction), $P_l{}^T(Z)$ is the component tangential to the bilayer (normal to Z), and $Z_2 - Z_1$ is the bilayer thickness. Properly, the pressure tensor for the bilayer must be known before the electrostrictive pressure effect can be completely and accurately described. I believe the basic physical mechanism of compression can be understood by assuming an isotropic pressure. A more complete discussion of the anisotropy in black films is given by D. M. Andrews [thesis, Cambridge University (1970), p. 146].
7. G. Bakker, *Theorie de la couche capillaire plane les Corps purs* (Gauthier-Villars, Paris, 1911), p. 74.
8. R. Defay, I. Prigogine, A. Bellemans, D. H. Everett, *Surface Tension and Absorption* (Longmans, Green, London, 1966), p. 141.
9. This is a general result from thermodynamics. See, for example, W. J. Moore, *Physical Chemistry* (Prentice-Hall, Englewood Cliffs, N.J., ed. 2, 1955), pp. 116–118.
10. J. Requena, D. F. Billett, D. A. Haydon, *Proc. R. Soc. London Ser. A* **347**, 141 (1975); J. Requena and D. A. Haydon, *ibid.*, p. 161.
11. D. E. Brooks, Y. K. Levine, J. Requena, D. A. Haydon, *ibid.*, p. 179.
12. Equations 2 and 3 may appear to be incomplete, since the surfactant molecules, GMO in this case, are also subjected to the pressure P_V. O. Alvarez and R. Latorre [*Biophys. J.* **21**, 1 (1978)] measured the electrocompression of GMO membranes containing negligible amounts of alkane and found only a 0.04 percent change in thickness for a 100-mV potential. This means that the area per GMO (the interfacial concentration) can change by no more than this amount. Compared to the alkane shift effect, this change in concentration is negligible and I need not include equations for GMO [see also (*5*)].
13. S. H. White, unpublished data.
14. ———, *Biophys. J.* **25**, 9a (1979).
15. ———, *Nature (London)* **262**, 421 (1976); *Ann. N.Y. Acad. Sci.* **303**, 243 (1977).
16. The suggested phase separation is not viewed as complete; that is, the acyl chains are not viewed as containing no decane. Unpublished observations in my laboratory suggest that some decane must be dissolved in the acyl chain region. Essentially, under the saturation equilibrium conditions used for the present experiments, decane is distributed between the central decane phase and the acyl chain phases. The final amount and the distribution of decane in the bilayer depend on the activity of the decane in the annulus.
17. S. H. White, *Biophys. J.* **23**, 337 (1978).
18. G. N. Lewis and M. Randall, *Thermodynamics* (McGraw-Hill, New York, ed. 2, 1961), p. 392.
19. S. H. White, *Biophys. J.* **15**, 95 (1975); ——— and T. E. Thompson, *Biochim. Biophys. Acta* **323**, 7 (1973); S. H. White and D. N. Blessum, *Rev. Sci. Instrum.* **46**, 1462 (1975).
20. I thank J. Lowe and C. Kiang for their excellent technical assistance and the referees of this report for helpful remarks. Supported by grants from the National Institutes of Health, National Science Foundation, and Guggenheim Multiple Sclerosis Foundation. The support of an NIH research center development award is gratefully acknowledged.

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Lipid Barrier to Water Exchange in Reptile Epidermis

Abstract. *Extraction of lipids from the shed epidermis of the terrestrial snake* Elaphe obsoleta obsoleta *increases cutaneous water loss in vitro as much as 15-fold. Partial denaturation of epidermal keratin without lipid extraction increases cutaneous water loss only twofold. Histological observations and thin-layer and gas-liquid chromatography of the lipid extracts indicate a complex mixture of polar and neutral lipids predominantly in the mesos layer of the cornified epidermis. Comparative measurements of cutaneous water loss in other species of snakes and a lizard show that permeabilities differ naturally but are essentially identical after lipid extraction. These findings establish the importance of lipids in the permeability barrier of reptilian skin and suggest that keratin or scale morphology are of nominal importance in limiting water exchange.*

One of the important roles of the epidermis is to limit the exchange of water and electrolytes between the organism and its environment. In this context, reptiles have attracted interest because they possess scales and may have very low rates of cutaneous water loss (CWL) (*1*). It had been assumed that reptilian CWL is negligible (*2*), but recent research demonstrated that there is considerable interspecific variability of CWL and that species exposed to more desiccative conditions have lower rates (*1*). The skin properties underlying these differences are not known; speculations frequently concern quantitative aspects of keratinization and the size and pattern of scales (*3*). Although keratinized tissues were shown to be water permeable (*4*), current data do not elucidate the mechanisms determining skin permeability of reptiles. We examined the functional role of epidermal lipids and found that they are critical to a water barrier function.

Squamate reptiles (lizards and snakes) produce unusual stratified epidermal generations periodically during a shedding cycle (*5*). We investigated the properties of squamate epidermis by using intact sheets of naturally shed skin (outer epidermal generation). We determined gravimetrically the rates of water movement across small patches of skin shed by snakes and a lizard. Skin specimens were laid across a fine nylon net stretched across the open top of a small plastic vial containing a water-saturated wick (*6*). Evaporative CWL rates were determined by periodically weighing (to the nearest 10 μg) the vials, which were

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